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Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease


ABSTRACT: The aim of this study was to examine the nature of fibre-type redistribution in relation to fibre metabolic profile in the vastus lateralis in chronic obstructive pulmonary disease (COPD) and COPD subtypes.

Fifteen COPD patients (eight with emphysema stratified by high-resolution computed tomography) and 15 healthy control subjects were studied. A combination of myofibrillar adenosine triphosphatase staining and immunohistochemistry was used to identify pure, as well as hybrid fibre types. For oxidative capacity, fibres were stained for cytochrome c oxidase and succinate dehydrogenase activities, and glycogen phosphorylase for glycolytic capacity.

The proportion of type-I fibres in COPD patients was markedly lower (16% versus 42%), especially in emphysema, and the proportion of hybrid fibres was higher (29% versus 16%) compared to controls. The proportion of fibres staining positive for oxidative enzymes was lower in COPD patients, which correlated with the proportion of type-I fibres. In COPD oxidative capacity was lower within IIA fibres.

The authors conclude that fibre-type transitions are involved in the fibre-type redistribution in chronic obstructive pulmonary disease. Low oxidative capacity is closely related to the proportion of type-I fibres, but an additional reduction of oxidative enzyme activity is present within IIA fibres. Fibre-type abnormalities may be aggravated in emphysema.


One of the hallmarks in chronic obstructive pulmonary disease (COPD) patients is a progressively reduced exercise capacity. Besides lung failure, skeletal-muscle dysfunction also contributes to exercise intolerance [1, 2]. Both biochemical and morphological changes in peripheral skeletal muscles of COPD patients have been described [3], but it is unclear how these alterations develop. Abnormal fibre-type proportions have been found in the quadriceps femoris in COPD [4–6], but in these studies fibres were only classified as either type I, IIA or IIX on the basis of myofibrillar adenosine triphosphatase (mATPase) staining. In the current study, mATPase and immunohistochemical identification of fibres were combined, which allowed detection of hybrid fibres (fibres expressing multiple myosin heavy chain (MyHC) isoforms), providing useful information on the mechanism underlying fibre-type redistribution in COPD.

Data with respect to metabolic enzyme activities in the quadriceps femoris in COPD are not consistent. Glycolytic enzyme activity may or may not be increased [7, 8] and oxidative enzyme activities are reduced [7, 8]. However, SAULEDA et al. [9] found that the activity of cytochrome c oxidase (COX), an enzyme involved in the oxidative energy metabolism, was increased. Therefore, the enzyme profile within the identified fibre-type categories has been studied. COX is a mitochondrial enzyme involved in oxidative phosphorylation. Succinate dehydrogenase (SDH) is also a mitochondrial enzyme and exerts its catalytic action in the citric acid cycle. Glycogen phosphorylase (GlyP) is involved in glycogenolysis and mobilizes glucose-1-phosphate from glycogen storage for glycolysis. Hence, COX and SDH were used as markers for oxidative energy metabolism and GlyP was used as a marker for glycolytic energy metabolism in this study.

Using gel electrophoresis techniques a lower type-I and a higher type-II MyHC isoform content was found in the quadriceps femoris of COPD compared to healthy subjects [10, 11]. In these studies the MyHC type-I content was positively related to the carbon dioxide diffusing capacity of the lung (DL,CO) and the forced expiratory volume in one second (FEV1). On the one hand, the relationship with DL,CO suggests that the loss of type-I fibres is more pronounced in patients with emphysema and on the other
hand, the relationship with FEV1 suggests that the loss is more pronounced in patients with severe COPD. In the current study, a further insight into muscular changes in patients with and without emphysema stratified by high-resolution computed tomography (HRCT) is provided.

The aims of this study were: 1) establish whether fibre-type redistribution in COPD occurs through gradual shifts in intracellular MyHC isoform content, which would result in a greater proportion of hybrid fibre types in patients; 2) provide further insight into the behaviour of metabolic enzymes (oxidative and glycolytic) and their relationship to the fibre-type distribution; 3) establish if abnormal fibre-type distribution is aggravated in emphysema patients compared to nonemphysema patients.

Materials and methods

Study population

A group of 15 patients with moderate-to-severe airflow obstruction and 15 healthy age-matched volunteers were studied (table 1). All patients had COPD according to American Thoracic Society (ATS) guidelines [12] and chronic airflow limitation, defined as measured FEV1 <70% of reference FEV1. Patients also had irreversible obstructive airway disease (<10% improvement of FEV1 predicted baseline after β2-agonist inhalation). They were in a clinically stable condition and had not suffered from respiratory tract infection or exacerbation of their disease at least 4 weeks prior to the study. Medication usage and doses were retrieved from available hospital files and the referring physician. Patients received standard optimized inhalation treatment. Due to the potentially adverse effects of oral glucocorticoids on the behaviour of metabolic enzymes (oxidative and glycolytic) and their relationship to the fibre-type distribution, the hallmark of emphysema [14], was performed by HRCT as described previously [15]. The severity and extent of emphysema in each scan were visually scored by two independent observers according to the direct observational method developed by SAKAI et al. [16]. HRCT scores ranged from 0 (no emphysema) to 120 (severe emphysema). Patients were stratified by HRCT score into two groups: HRCT score <30: non-emphysema subgroup (Emph-); HRCT score ≥30: emphysema subgroup (Emph+).

Assessment of emphysema

Evaluation of the presence of parenchymal destruction, the hallmark of emphysema [14], was performed by HRCT as described previously [15]. The severity and extent of emphysema in each scan were visually scored by two independent observers according to the direct observational method developed by SAKAI et al. [16]. HRCT scores ranged from 0 (no emphysema) to 120 (severe emphysema). Patients were stratified by HRCT score into two groups: HRCT score <30: non-emphysema subgroup (Emph-); HRCT score ≥30: emphysema subgroup (Emph+).

Tissue collection and processing

Arterial oxygen tension (PaO2) was determined (ABL 330; Radiometer, Copenhagen, Denmark) in a blood sample obtained by puncture of the radial artery while breathing room air. Postabsorptive muscle biopsies of the lateral part of the quadriceps femoris were obtained under local anaesthesia by the needle biopsy technique [17]. Biopsies were placed in a drop of Tissue-tek® (OCT compound) on a piece of cork with the fibre orientation perpendicular to the plane of the cork. The specimen was frozen in melting isopentane, precooled in liquid nitrogen and stored at -35°C. Serial cryostat cross-sections (10 μm) were made on a cryostat microtome at -20°C and mounted on slides which were stored at -35°C until analysis. Nine consecutive slides (each carrying two or three cross-sections) per biopsy sample were used for fibre-type characterization and enzyme-activity staining. At least 100 (but up to 200) fibres co-existing on all nine slides were measured and analysed as described previously [18]. Fibres were included if they were part of a cluster of ≥30 fibres and excluded if disturbing
artefacts were present. All staining intensities were analysed by the same person.

**Fibre-type characterization**

The mATPase acidic pre-incubation was performed at pH 4.4 [19] and the mATPase alkali pre-incubation was performed at pH 10.4 [20]. The double pre-incubation method was performed at pH 10.4 and pH 4.6 [21], the only difference being that the fixation of sections was performed prior to the alkaline pre-incubation. After pre-incubation, sections were stained and mounted [22]. Figure 1a–c show examples of mATPase staining.

Immunohistochemistry was performed with a panel of monoclonal antibodies (MAbs); anti-type I MyHC (MAB 219-1D1), anti-type IIA MyHC (MAB 333-7H1), anti-types IIA+IIX MyHC (MAB 332-3D4) [23]. Figure 1d–f show examples of MyHC staining.

Fibres were assigned to categories, first on the basis

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Fig. 1. – Examples of histochemical analysis in nine consecutive cross-sections. Myofibrillar adenosine trisphosphatase (mATPase) staining with: a) acidic, b) alkaline and c) double pre-incubation (positive fibres stain dark); immunohistochemistry with: d) anti-type I, e) anti-type IIA and f) anti-types IIA+IIX (positive fibres stain dark). Fibres numbered 1–5 are assigned to the I, IIA, IIA, IIA/IIX and IIX combination of mATPase and myosin heavy chain fibre type categories, respectively (staining intensities are given in table 2). g) Cytochrome c oxidase activity staining (positive fibres are coloured brown). h) Succinate dehydrogenase activity (positive fibres are coloured blue). i) Glycogen phosphorylase positive fibres are coloured purple and negative fibres yellow. Internal scale bars=150 μm.
of three mATPase staining intensities (-, + or ++) and secondly by immunoreactivity (- or +) (table 2). The following combination of mATPase and MyHC (CMM) fibre-type categories were made: type I, I/IIA, IIA, IIA/IIX, IIX and miscellaneous (misc).

### Enzyme activity staining

COX staining was performed by incubating sections for 1 h in a 50 mM Tris/HCl buffer (pH 7.6) containing 0.22 M sucrose, 14 mM 3,3'-diaminobenzidine tetrahydrochloride, 80 μM cytochrome c and 1300 U catalase. Sites of COX activity were coloured brown [24]. SDH staining was performed by incubating sections for 1 h at 37°C in a 0.2 M sodium phosphate buffer containing 0.1 M succinic acid and 1.2 mM nitro-blue tetrazolium. Sites of SDH activity were coloured blue [25]. GlyP staining was performed by incubating sections for 5 min in a 43 mM sodium acetate buffer (pH 5.6) containing 7 mM glucose-1-phosphate, 1 mM adenosine monophosphate (AMP), 0.01% glycogen and 15% ethanol [25], after which the newly formed polysaccharide was coloured blue with Lugol's iodine. COX and SDH cross-sections were dehydrated in an ethanol range and mounted in Entallan. GlyP sections were mounted in aqueous glycerin/gelatine that had been coloured with Lugol's iodine. Within each section, three staining intensities were chosen reflecting fibre enzyme activity: positive (+), negative (-) or intermediate (±). For example, a fibre positive for COX, intermediate for SDH and negative for GlyP would be COX+, SDH± and GlyP-. Figure 1g–i show examples of enzyme activity staining.

### Statistical analysis

Values are reported as mean±standard deviation (SD) and error bars in figures reflect standard error of the mean (SEM). Intergroup data were compared using an unpaired t-test (corrected for unequal variances if appropriate) or one-way analysis of variance (ANOVA) (with unpaired t-test as post hoc test).

### Results

Fifteen COPD patients (12 males and three females) and fifteen healthy controls (13 males and two females) participated in this study (table 1). There were no differences in sex or age between the groups. The body mass index (BMI), PaO₂, FEV₁ and the DL,CO were significantly lower, whereas the RV and the ITGV were higher in patients than in controls. Eight patients (with HRCT scores ≥30) were assigned to Emph+ and seven patients (with HRCT scores <30) to the Emph- subgroup. The PaO₂, FEV₁ and the DL,CO were lower and the RV and the ITGV were higher for the Emph+ than for the Emph- COPD subtype.

A typical example of histochemical results is shown in figure 1. First the data was interpreted as if based purely on mATPase staining (fig. 2a). The proportion of mATPase type-I fibres was lower (19% versus...
43%, p<0.001) and the proportion of mATPase type-IIx fibres was higher (46% versus 27%, p=0.004) in the COPD group compared to controls. There was no difference in the percentage of mATPase type-IIa fibres. By combining the three mATPase pre-incubation protocols with immunohistochemistry, five fibre-type categories were defined: CMM type I, IIA, IIx, I/IIa and IIa/IIx fibres, the latter two representing hybrid fibres (table 2). Fibre-type distribution was subsequently re-established with this more accurate method (fig. 2b). Again, the proportion of type-I fibres was lower (16.1% versus 42.4%, p<0.001) and the percentage of type-IIx fibres tended to be higher (23.8% versus 14.7%, p=0.088) in COPD than in controls. In addition, the proportion of hybrid-fibres I/IIa (5.9% versus 1.3%, p=0.044), IIa/IIx (22.9% versus 14.4%, p=0.041), and total (IIa/IIx+I/IIa) (28.8% versus 15.7%, p=0.008) were higher in COPD compared to healthy subjects. Fibres that did not fit into one of the categories discussed earlier were assigned to the miscellaneous group. Although the percentage of these fibres was also higher in COPD than in controls (1.6% versus 0.2%, p=0.017), their overall contribution to fibre-type composition was negligible. There was no difference in the percentage of type-IIa fibres between both groups.

Figure 3 shows the results of enzyme activity staining of COX+, SDH+ and GlyP+. Overall, the proportion of COX+ and SDH+ fibres was lower (20.1% versus 45.8%, p<0.001 and 14.2% versus 36.3%, p<0.001), whereas the proportion of COX- and SDH+ fibres was higher (35.3% versus 23.8%, p=0.003 and 47.9% versus 25.0%, p<0.001, data not shown) in COPD than in the control group. The percentage of COX± fibres was also higher in COPD than in controls (44.6% versus 30.3%, p=0.002, data not shown). There were no differences in the proportion of GlyP+, GlyP- and SDH± fibres between patients and controls. The proportion of GlyP+ fibres in patients not treated with glucocorticosteroids (45.8%) was higher than in controls (34.2%, p=0.045) and also higher than in patients who did receive glucocorticosteroids (29.2%, p=0.017). Since enzyme activity staining and fibre-type characterization were performed on serial cross-sections, enzyme activity could be evaluated within fibre-type categories. In the CMM fibre-type categories IIA and IIa COPD patients had a lower proportion of fibres positive for the oxidative enzymes, as shown for COX- and SDH+ (table 3). In CMM fibre-type IIa/IIx the proportion of COX± fibres was higher for patients (data not shown).

Within COPD there was a more pronounced decrease in the percentage of CMM type-I fibres in Emph+ than in Emph- (10.0% versus 23.1%, p=0.045) compared to controls (fig. 4). In each group at least one patient had no CMM type-I fibres at all, was found. No differences between the two subtypes were observed with respect to enzyme activity staining.

Strong relationships were found for COPD between the proportion of CMM type-I fibres and the proportions of COX+ (r=0.77, p=0.001) and SDH+ (r=0.84, p<0.001) fibres (fig. 5). A positive relationship between age and the percentage of type-I fibres in healthy controls (r=0.61; p=0.015) was also observed. This relationship was also seen in the Emph-COPD subtype, although this did not reach statistical

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>COX+ fibres %</th>
<th>SDH+ fibres %</th>
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<tr>
<td>Controls</td>
<td>COPD</td>
<td>Controls</td>
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<tr>
<td>I</td>
<td>87.8</td>
<td>82.7</td>
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<tr>
<td>IIA</td>
<td>73.3</td>
<td>33.3*</td>
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<tr>
<td>IIA</td>
<td>34.1</td>
<td>7.8**</td>
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<tr>
<td>IIa/IIx</td>
<td>2.5</td>
<td>2.4</td>
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<tr>
<td>IIx</td>
<td>0</td>
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</tbody>
</table>

Significance of difference compared to controls. *: p<0.05; **: p<0.01.
correlated with the proportion of COX activity and oxidative capacity in chronic obstructive pulmonary disease patients. Abnormal fibres stain intermediate for mATPase activity after pre-incubation at pH 4.4 and will be identified as IIX fibres.

Fig. 5. – Relationship between the combination of myofibrillar adenosine triphosphatase and myosin heavy chain fibre type-I proportion and oxidative capacity in chronic obstructive pulmonary disease patients. \( \triangle \) and \(-\)-: Succinate dehydrogenase (r=0.84; p<0.001); \( \bigtriangleup \) and \(-\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\·
found in this study. An explanation for the unchanged GlyP+ fibre proportion in the present study is that seven from the 15 COPD patients received low doses of oral glucocorticosteroids. The proportion of GlyP+ fibres in patients not receiving corticosteroids was higher than in controls, suggesting that corticosteroids adversely affect GlyP activity. However, patients frequently suffering from exacerbations are more likely to receive corticosteroids and therefore systemic inflammation may be involved as well. The effect of glucocorticosteroids on glycolytic metabolism needs to be further elucidated.

In COPD patients in this study, the proportion of fibres that stained positive for COX and SDH declined in the same order of magnitude as the percentage of type-I fibres. In fact, the majority of the COX+ and SDH+ fibres were classified as type-II fibres, which is underscored by the strong correlation between their proportions. Thus overall, the relative loss of type-I fibres and the loss of oxidative enzyme activity are linked. It has never been established before that the decline in both percentages of fibre type I and the activity of enzymes involved in oxidative energy metabolism in COPD are linked. Since enzyme activity staining and fibre-type classification in this study were performed on serial cross-sections, it was not only possible to examine the relationship between the fibre-type redistribution and enzyme activity, but also enzyme activities within the fibre-type categories.

The proportion of COX+ and SDH+ fibres within each of the individual CMM fibre types, I/IIA and IIA, were significantly lower for COPD compared to controls (table 3). In addition, the proportion of COX± fibres was higher in CMM type-IIA fibres of patients. It is therefore feasible that COX+ fibres transformed into COX± fibres. It is possible that this extra loss of oxidative capacity is specific for type-IIA fibres. Another possibility is that the loss of oxidative enzyme activity occurs in an early stage of fibre-type transition. It is feasible that as a result of the disease (i.e. hypoxia), oxidative energy metabolism is disturbed and the activities of the enzymes involved are reduced. Type-I and IIA fibres strongly depend on aerobic metabolism, whereas in type-IIX fibres energy conversion is based mainly on anaerobic, glycolytic metabolism. It is therefore tempting to state that in type-I fibres the co-expression of the MyHC-IIA isoform and that in type-IIA fibres the co-expression of the MyHC-IIX isoform is an adaptation to impaired oxidative energy metabolism. Alternatively, it is possible that either the reduced oxidative capacity is secondary to the changes in MyHC-isoform contents or that one or more common factors affect both oxidative capacity and MyHC-isoform contents simultaneously.

The present results indicate that the low fibre type-I proportion in COPD is more pronounced in Emph+ patients (stratified by HRCT) compared to Emph-patients. Like others the authors found a positive relationship between fibre type-I proportion and age in healthy subjects [28–30]. Due to the relative loss of type-I fibres this relationship is disturbed in COPD patients, being totally absent in the Emph+. The proportion of COX+ fibres was also lower in Emph+, but not statistically significant. A positive correlation between the $P_aO_2$ and the percentage of type-I fibres has been reported previously [5, 6]. In addition, a positive relationship between the MyHC-I content, and the DL.CO, and the FEV1, has been shown [10, 11]. However, in the present study these relationships between fibre-type proportions and lung function data were not observed, suggesting that fibre type-I proportion is not related to disease severity per se. COPD patients with a DL.CO <55% predicted are likely to desaturate during exercise [31]. Since the DL.CO is lower in the Emph+ patients, it can be speculated that chronic and especially intermittent hypoxia is directly or indirectly involved in causing the lower fibre type-I proportion compared to Emph-patients. This issue requires further investigation.

At this point, it is not completely understood which factors are causing the muscular abnormalities in COPD. Hypoxia has already been discussed earlier. However, oxidative stress may also play a role in the loss of mitochondrial enzyme activity and relative loss of type-I fibres, since it has been demonstrated that mitochondrial proteins are highly susceptible to oxidative damage [32]. The possible role of malnutrition is demonstrated by animal studies in which nutritional deprivation caused depressed muscular mitochondrial capacity [33–35]. Physical inactivity or disuse of limb muscles are potential contributors to the observed fibre-type shift [36]. However, the authors have not observed previously any difference between the daily physical activity levels of Emph+ and Emph- patients [37], while differences in fibre type-I proportions between these subgroups have now been found. In addition, none of the patients in this study was bedridden and it is therefore unlikely that disuse alone caused the very low fibre type-I proportions that were observed.

To conclude, this study demonstrates the involvement of increased proportions of hybrid I/IIA and IIA/IIX fibre types in the fibre-type shift from I to IIX in the vastus lateralis of chronic obstructive pulmonary disease patients compared to controls. The abnormal fibre type-I proportion is aggravated in emphysema patients. In addition, the percentage of fibres with high oxidative enzyme activities were also reduced in chronic obstructive pulmonary disease patients, being closely linked to the reduced fibre type-I proportion. Moreover, despite comparable IIA fibre-type proportions between patients and controls, oxidative capacity is reduced in this fibre type in patients. Further research should be carried out to identify the factors involved and the mechanisms underlying the muscular alterations in order to evaluate current treatment approaches and to develop new approaches.

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