Skeletal Muscle Morphology in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Our experience about skeletal muscle morphology, capillarity and fibre type composition in patients with chronic obstructive pulmonary disease (COPD) is reported. Sixteen CODP male patients (mean age 64.8) having severe exercise limitation by dyspnoea were recruited. All patients underwent a needle biopsy from the quadriceps femoris muscle under local anesthesia. Results have been compared with data obtained in normal muscle from healthy subjects. Morphological evaluation of muscle samples from COPD patients showed reduction in the fibre diameter and occasional degenerative changes identified by increase of sarcoplasmic acid phosphatase activity and by electron microscopy. Intramuscular capillary distribution and morphology were normal. COPD patients showed plastic reorganisation of skeletal muscle by reduction of type 2B fibres with shifting to type 2 A fibres. We conclude that the pulmonary impairment together with sedentary life and exercise limitation may influence skeletal muscle morphology and composition of COPD patients.

Key words: quadriceps femoris muscle, myosin heavy chains, morphology, capillaries, chronic obstructive pulmonary disease.

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic airflow limitation due to airways obstruction and emphysema. The patients show abnormal expiratory flow tests that do not change over periods of several months of observation and gas exchange disorders at rest and during exercise [1, 4, 20]. The major symptom of COPD is a chronic respiratory impairment with limitations in the exercise capacity caused by dyspnoea [1, 3, 4, 11]. Recent investigations suggest that chronic hypoxemia in COPD patients may play some role in skeletal muscle function [7, 10, 18, 23]. Since the functional respiratory damage and the exercise limitation may be related to a structural and functional impairment of the skeletal muscle, we studied the morphology, fibre type size and composition, and capillarity of quadriceps femoris muscle in COPD patients having evident exercise limitation by dyspnoea.

Patients and Methods

Study population

Sixteen male patients (mean age 64.8 years) with COPD, defined according to criteria of the American Thoracic Society [1], were studied.

Criteria of admission to the study were: a) airways obstruction demonstrated by a forced expiratory flow in one second (FEV 1) less than 70% than predicted and lack of reversibility (less than 10% of increase of FEV 1 after inhaled salbutamol 200 mcg); b) dyspnoea during exercise; c) age < 75 and body weight > 90% of the ideal weight [1, 5].

The following exclusion criteria were applied: a) PaO_2 < 60 mmHg and $PaCO_2$ > 45 mmHg at rest; b) bronchitis exacerbation in the month before the enrollment; c) cardiovascular, metabolic and skeletal muscle disorders; d) subjects taking oral steroids during and in the year before the study; e) regular practice of physical activity. The study

was performed after ammission to the Respiratory Unit Care of the S. Maugeri Foundation Medical Centre of Tradate and was approved by the Ethical Committee of the Foundation. Patients were informed about the study aims and methods and a written consent was obtained.

Respiratory function

Baseline measurements of the respiratory function was performed; vital capacity (VC), FEV1, pneumothacography (Masterlab body, Jaeger, Wurzburg, Germany), carbon monoxide diffusion capacity (TLCO) measured with the single breath method (Masterlabe transfer, Jaeger, Wurzburg, Germany); arterial partial pressure of oxygen (PaO_2) was measured immediately after sampling from a puncture of the radial artery at rest (Gas analyzer ABL 33O, Radiometer, Copenhagen, Denmark).

Exercise testing

Each patient performed an incremental (10 watts/minute) exercise test to the maximal tolerated level (EOS sprint Jaeger, Wurzburg, Germany) on a cycle ergometer (Lode, Groningen, Holland). Maximal oxygen uptake (VO_2 max), minute ventilation (VE max) and heart rate (HR max) were measured each minute. All patients stopped their tests due to dyspnoea.

Muscle biopsy

Needle biopsy specimens from the lateral portion of the right quadriceps femoris muscle (vastus lateralis) were obtained under local anesthesia. Some specimens were rapidly frozen in isopentane cooled in liquid nitrogen and cryostat sections (8 μm) were stained with histological and histochemical techniques (hematoxylin-eosin, modified Gomori trichrome, PAS, Oil red O, oxidative enzymes and myosin ATP-ase after preincubation at pH 9.6 and 4.6 which gives a best distinction in 3 fiber types (1, 2A and 2B). The immuno-histochemical *in situ* detection of DNA fragments for apoptosis was performed by means of ApopTag method (Oncor) [21]. Some specimens were fixed in formalin and then embedded in paraffin; 10 μm thick sections were stained with hematoxylin and eosin, and with the Gomori' silver impregnation for detection of intramuscular capillaries. Small muscle specimens were fixed in Karnovsky fluid for electron microscopy. Morphometric evaluation was made according to previously described methods [16, 17] and the following parameters were considered: percentage of fibre types, mean fiber diameter, number of intramuscular capillaries per fibre (C/F), and capillary density per square mm of muscle fibre (CD). The determination of the slow isoform of myosin heavy chains (MHC1) was performed by a previously described electrophoretic micromethod [14]. Results have been compared with previously published normal parameters obtained from healthy subjects of the same age [15, 17].

Statistical analysis

Mean and standard deviation (SD) were calculated for each data. Normal and COPD muscle data were compared with the unpaired T-test.

Results

Table 1 provides the functional data of the studied patients. It should be pointed out that they show a severe pulmonary functional damage and some impairment of gas exchange; arterial blood gases are still near the normality. Table 2 shows the results of exercise tests. There is a severe limitation of the maximal exercise capacity, due to the ventilatory and gas exchange damage that induces dyspnoea. Obviously this limitation involves a sedentary life style.

Table 3 report the results of the enzyme-histochemical analysis of the fibre type composition and diameter, and the percentage of the slow isoform of myosin heavy chains (MHC1) performed by an electrophoretic micromethod [14]. COPD patients showed reduction of the fibre size of both type 1 and 2 fibre types. Occasional degenerative alterations, as seen after acid phosphatase reaction (fig. 1a) and an excess of Oil red O positive lipid droplets in scattered fibres were observed. No nuclear changes, inflammatory cellular reaction, necrosis, regeneration or fibrosis were observed. In comparison with normal subjects, COPD patients showed significant reduction of type 2B fibres, with shifting to increased type 2A fibres (fig. 1b). No significant differences have been observed in type 1 fibre and MHC1 myosin isoform percentage between COPD and normal subjects. No immuno-histochemical and ultrastructural patterns of apoptosis was observed in muscle fibres. The overall capillary measures in COPD patients are reported in Table 4. The C/F was within the normal range; the capillary density was higher than normal in the rare cases in which the muscle atrophy was more evident. Electron microscopy showed scattered fibres with

Table 1. Mean values and standard deviation of COPD patients: VC, FEV1, TLCO and KCO are expressed as percent of predicted values. Arterial blood gases in Kpa.

	YRS	VC	FEV1	TLCO	KCO	PaO_2	$PaCO_2$
MEAN	64.6	77.6	51.6	76.5	63.9	10.1	7.4
SD	10	12.8	11.7	26.4	22.7	0.4	0.03

Table 2. Mean values and standard deviations of exercise test of COPD patients: $V0_2$ and VE max are expressed in liters per minute, HR in beats per minute. These data show the exercise impairment of COPD subjects.

	$V0_2$ max	VE max	HR max
MEAN	1.03	41.17	125.3
SD	0.3	9.6	9.8

Table 3. Muscle fibre type diameter, composition and MHC1 content in quadrceps femoris from COPD patients and normal subjects: mean values and standard deviations.

		Fibre type diameter (μm)		Fibre type composition (%)			
		1	2	1	2A	2B	MCH1
	N.						
COPD	16	38.3 ± 4.5	44.9 ± 5.6	40.1 ± 10.3	41.1 ± 9.6	20.6 ± 12.3	37 ± 1.4
NORMAL	10	52.5 ± 1.9	56.0 ± 2.1	40.0 ± 3.7	20.5 ± 3.8	39.3 ± 3.3	40 ± 2.8
p		< 0.001	< 0.001	NS	< 0.001	< 0.001	NS

degenerative changes, as myofibrillar disorganisation and myofilament disruption (fig. 2a), and with presence of autophagic vacuoles and lipofuscin bodies indicating lysosomal activity (fig. 2b). In some fields, an excess of sarcoplasmic vacuoles, formerly filled with lipids, was observed.

Discussion

Airway and pulmonary abnormalities in COPD have distracted attention from the possibility that exercise limitation and the disease "per se" might cause some functional or morphological changes in the skeletal muscle. Studies on COPD and skeletal muscle were usually performed on respiratory muscles [5]; some recent biochemical, spectroscopic and morphological investigations revealed impairment of the main indices of skeletal muscle metabolism and of muscle structure in patients with severe chronic hypoxemia [7, 10, 18, 23]. Most rehabilitation programmes of COPD patients involve muscular exercise reconditioning and the best approach suggested is usually performed by training large skeletal muscles of the lower limbs [3]. It is therefore reasonable to focus the attention on the skeletal muscle of COPD patients. This in the perspective of a better understanding of the disease and of a possible improvement of the rehabilitative process too.

The muscular changes described in the present study in COPD patients, such as muscle fibre hypotrophy associated to focal degeneration and alterations in the fibre type composition as well, may be somewhat related to the same multiple factors which are likely to contribute to respiratory failure in severe COPD; abnormalities of ventilatory mechanics, respiratory muscles, alveolar gas exchange and cardiac function may contribute to enhancing some skeletal muscle alterations, even in less severe stages of the disease. Accordingly, skeletal muscle changes could be observed in patients which have normal blood gases but their gas diffusing capacity is reduced. Furthermore, other important factors such aging and muscle inactivity due to dyspnoea should be taken into account, as demonstrated in humans and in some experimental conditions [15].

The present study of fibre type composition in COPD patients stresses the importance of the reduction of type 2B glycolitic fibres, with shifting to type 2A fibres. In normal

Table 4. Capillary measures in COPD patients and in normal subjects: mean values and standard deviations of C/F (capillary/fibre ratio) and CD (capillary density per square mm of fibre).

	C/F	CD
COPD	1.48 ± 0.04	298 ± 14
NORMAL	1.39 ± 0.02	277 ± 18
p	NS	NS

subjects training and detraining induces adaptation within fibre type population [12, 13]. Type 2B fibres usually perform short, fast, maximal efforts, and their metabolism is anaerobic, depending on glycolysis and lactic acid (LA) production. This involves two consequences: LA has to be reconverted with O_2 consumption and consequent ventilatory demand; its buffering increases CO_2 output and minute ventilation [3]. Type 2A fibres perform a slower and lower contraction, have an oxidative metabolism, but less than type 1 fibres and produce less LA than type 2B fibres.

Does chronic hypoxiemia induce transformation of fibre types? [8]. Our patient population show an arterial blood gas content quite normal, but a reduction in the gas exchange capacity. This might involve a general reduction in the overall oxygen availability to the working muscles, this being a stimulus for type shift. All our patients experienced dyspnoea that is a limitation of their exercise capacity. The observed shift of the fibre type could be explained also with an attempt to reduce the ventilatory demand for a given work load, thus reducing the dyspnoea (type 2A fibres burn less oxygen than type 1 and produce less LA than type 2B). On the other hand dyspnoea itself limits peak working capacities which are supported by type 2B glycolitic fibres (selectively reducing trophic inputs to type 2B fibres).

The main condition in which capillary supply to skeletal muscle fibres is modified is endurance training. In this condition both capillary / fibre ratio and capillary density increased; proliferation of capillaries therefore seems to be

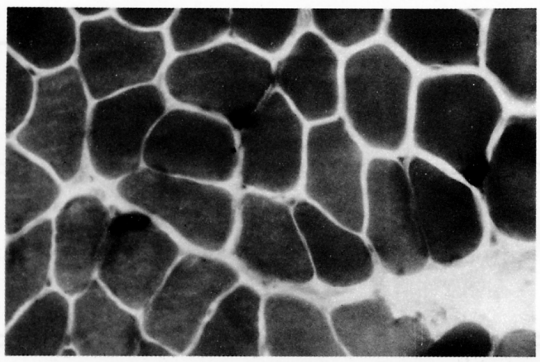

Figure 1a. The reaction for the acid phosphatase is strongly positive in peripheral sarcoplasmic areas of scattered fibres, indicating muscle degeneration. Acid phosphatase reaction. X 100.

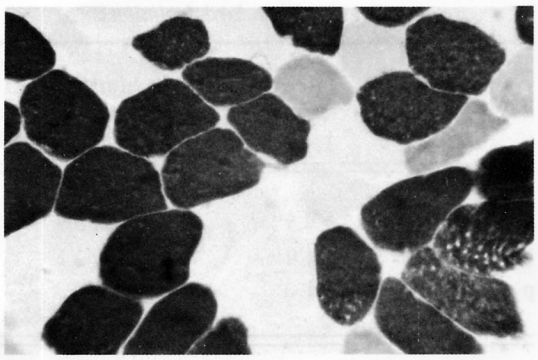

Figure 2a. Myofibrillar disorganisation and myofilament disruption in a fibre perinuclear region. Electron micrograph. X 8,000.

Figure 1b. Numerous type 1 fibres (dark stain) are present. Some of them show sarcoplasmic vacuoles. Rare type 2B fibres (grey stain) are present together with more numerous type 2A fibres (light stain). Myosin ATP-ase pH 4.6. X 250.

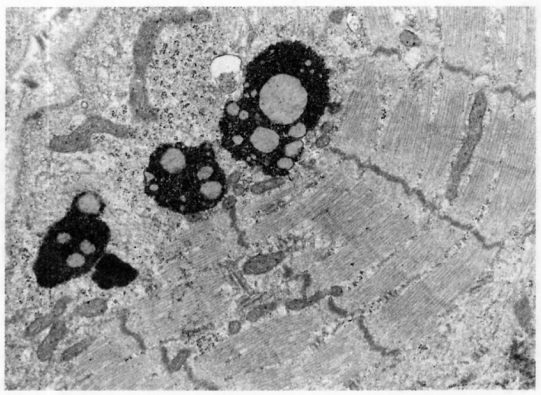

Figure 2b. Dense lipofuscin bodies with vesicular inclusions and myofibrillar disorganisation at the edge of the fibre. Contiguous myofibrils show normal appearance. Electron micrograph. X 9,000.

the most reasonable explanation for this, although it cannot be excluded that altered lengths and patterns of the capillaries may play a role [2]. An interesting observation is that the increase in capillarity per fibre was in the same order of magnitude as the increase in maximal oxygen uptake during the training period [9]. One could expect in COPD patients, which have a low degree of physical fitness, a significant reduction in the intramuscular capillarity. Our findings show no difference in muscle capillaries in respect to normal population, while previous studies demonstrated changes in muscular capillaries in trained subjects [2]. Our subjects are in sedentary condition just like normal subjects; the level of their physical activity may be different, as far as more strenuous efforts are concerned. Muscle capillary density and number might be the basic condition of both groups and could be modified only if muscular training is applied. Muscle fibre hypotrophy in COPD patients could be explained by sedentary life, according with results of other studies on sedentary people [15] and on subjects with chronic heart failure [6, 19, 22]. Nevertheless the rare and focal muscle fibre degeneration could be the consequence of an excessive muscle contraction in relation to the available O_2 supply. In conclusion, many factors such as oxygen availability, physical activity and life style seem to change fibre size and composition of skeletal muscle in COPD patients, while degeneration phoenomena seen at light and electron microscopy, are scanty and focal , and no apoptosis or necrosis of muscle fibres are seen. Further studies are necessary in order to correlate the above factors with muscular damage in COPD subjects.

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