Subcellular adaptation of the human diaphragm in chronic obstructive pulmonary disease

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ABSTRACT: Pulmonary hyperinflation impairs the function of the diaphragm in patients with chronic obstructive pulmonary disease (COPD). However, it has been recently demonstrated that the muscle can counterbalance this deleterious effect, remodelling its structure (*i.e.* changing the proportion of different types of fibres). The aim of this study was to investigate whether the functional impairment present in COPD patients can be associated with structural subcellular changes of the diaphragm.

Twenty individuals (60±9 yrs, 11 COPD patients and 9 subjects with normal spirometry) undergoing thoracotomy were included. Nutritional status and respiratory function were evaluated prior to surgery. Then, small samples of the costal diaphragm were obtained and processed for electron microscopy analysis.

COPD patients showed a mean forced expiratory volume in one second (FEV1) of 60±9% predicted, a higher concentration of mitochondria (n_{mit}) in their diaphragm than controls (0.62±0.16 *versus* 0.46±0.16 mitochondrial transections (mt)·μm^{-2}, p<0.05). On the other hand, subjects with air trapping (residual volume (RV)/total lung capacity (TLC) >37%) disclosed not only a higher n_{mit} (0.63±0.17 *versus* 0.43±0.07 mt·μm^{-2}, p<0.05) but shorter sarcomeres (L_{sar}) than subjects without this functional abnormality (2.08±0.16 to 2.27±0.15 μm, p<0.05). Glycogen stores were similar in COPD and controls. The severity of airways obstruction (*i.e.* FEV1) was associated with n_{mit} (r=-0.555, p=0.01), while the amount of air trapping (*i.e.* RV/TLC) was found to correlate with both n_{mit} (r=0.631, p=0.005) and L_{sar} (r=-0.526, p<0.05). Finally, maximal inspiratory pressure (PI,max) inversely correlated with n_{mit} (r-0.547, p=0.01).

In conclusion, impairment in lung function occurring in patients with chronic obstructive pulmonary disease is associated with subcellular changes in their diaphragm, namely a shortening in the length of sarcomeres and an increase in the concentration of mitochondria. These changes form a part of muscle remodelling, probably contributing to a better functional muscle behaviour.
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Chronic obstructive pulmonary disease (COPD) is a respiratory condition mainly characterized by persistent airflow limitation [1]. It affects >5% of the general population [2], is a cause of permanent disability and constitutes one of the leading and increasing causes of death in developed countries [3].

The function of the diaphragm, the main respiratory muscle, can be impaired in patients with COPD owing to both airways obstruction and pulmonary hyperinflation (air trapping) [4–6]. However, it has been demonstrated that this deleterious effect can be partially counterbalanced by the muscle [7]. TAMAOKI [8] found that fibres were hypertrophic in the diaphragm of guinea-pigs with elastase-induced emphysema. Recently, LEVINE *et al.* [9] have demonstrated that patients with severe COPD show a higher percentage of type I fibres in their diaphragm than subjects with normal lung function or mild COPD. These and other changes could be considered adaptive [10–16], contributing to a better functional behaviour of the muscle against respiratory loads. However, other authors have described structural changes, such as atrophy of respiratory muscles [17–19], that do not appear to be so advantageous. Different factors, such as malnutrition and a persistent increase in the mechanical impedance of the respiratory system have been involved in this atrophy. Surprisingly, there are little data concerning the diaphragm at a subcellular level in patients with respiratory diseases. This study was carried out to investigate whether or not the functional impairment present in COPD can be associated with changes in the contractile and energetic systems of the muscle: specifically, with modifications in the length of sarcomeres and density of mitochondria and glycogen stores.

Methods

Study design and population

This was a cross-sectional study prospectively carried out over a 4 yr period (1991–1995). Subjects were

progressively included from patients undergoing thoracotomy for a localized lung neoplasm. Individuals suffering from bronchial asthma, severe undernourishment, chronic respiratory failure, chronic metabolic diseases, previous abdominal or thoracic surgery, paraneoplastic syndromes, or those receiving chemotherapy, steroids or other drugs known to modify muscle structure were excluded. Twenty male subjects (60±9 (mean±SD) yrs) were finally included. Eleven had COPD (forced expiratory volume in one second ($FEV1$)/forced vital capacity (FVC) <70%) and the rest disclosed normal spirometric values. All were sedentary individuals and their daily physical activities were similar. The study was approved by the Research Committee on Human Investigation at the authors' institution, and informed written consent was obtained after the purposes and potential risks of the investigation were fully explained and understood by each patient.

Protocol. First day: when informed consent was obtained, patients performed conventional pulmonary function tests and their nutritional status was assessed. Second day: respiratory muscle function was evaluated. Third day: biopsies from costal diaphragm were taken at the beginning of thoracotomy, 24 h after the functional evaluation. Samples were subsequently processed.

Pulmonary function tests

Forced spirometry was performed (Datospir 92; Sibel, Barcelona, Spain) and inspiratory capacity, inspiratory thoracic gas volume (ITGV; or functional residual capacity (FRC)) and the transfer factor of the lung for carbon monoxide (TL,CO, single-breath method) (Masterlab; Jaeger, Würzburg, Germany) were measured in each patient. Reference values were those for a Mediterranean population [20, 21]. Blood samples were obtained from the radial artery and analysed for arterial oxygen tension (Pa,O_2), arterial carbon dioxide tension (Pa,CO_2) and pH, using standard polarographic techniques (ABL 330; Radiometer, Copenhagen, Denmark).

Respiratory muscle function

This was assessed through transdiaphragmatic pressure (Pdi) and inspiratory pressure measured at the mouth. The former was obtained using the oesophageal and gastric balloon technique [22]. The balloon catheters were connected to pressure transducers (Transpac II; Abbott, Chicago, IL, USA) and the signals registered using a multichannel recorder (Sensormedics R-611, Anaheim, CA, USA). Calibrations were performed at the beginning and end of each study. Pdi was determined both during quiet breathing and a maximal inspiratory manoeuvre from FRC (maximal Pdi (Pdi,max)). The sniff technique was chosen for the latter because it appears to be more reproducible and easily performable by C0PD patients [23]. Ventilatory pattern was also recorded, since subjects were breathing through a low-resistance two-way valve (Hans Rudolph; Kansas City, MO, USA), connected to a pneumotachometer (Screenmate; Jaeger). As a result, the tension–time index of the diaphragm (Tdi) was also calculated as:

$$Ttdi = (Pdi/Pdi,max) \times (tI/ttot)$$

where tI represents the inspiratory time and $ttot$ the duration of the total breathing cycle ([24]). The maximal inspiratory pressure (PI,max) measured at the mouth was obtained using a manometer with a mouthpiece that could be occluded (Sibelmed, Sibel). Reference values were those published by WILSON *et al.* [25].

Nutritional assessment

Nutritional status was evaluated using both anthropometric and biochemical parameters. The former included weight (kg), body mass index (BMI= weight/height^2), and the percentage of ideal body weight (%IBW= (actual weight/ideal weight) × 100) [26]. Blood analysis included serum cholesterol, triglycerides, total proteins, albumin, globulins and prothrombin consumption time.

Structural analysis of the diaphragm

Light microscopy. Biopsies from the diaphragm (~0.5 cm × 0.5 cm × 0.5 cm in size) were obtained during thoracic surgery. Samples were taken 2 cm from the costodiaphragmatic angle, below the middle axilar line. Each biopsy was divided into two parts. One was immediately plunged into isopentane (cooled over liquid nitrogen), and stored at -70°C. For histochemical procedures, each block was positioned on a cryostat holder roughly transverse to the muscle fibre axis. Consecutive sections were then obtained inclining the holder by 5°; the section with the smallest cross-sectional area (CSA) was chosen [27, 28]. Fibre diameters and CSA were measured using a semiautomatic system (Videoplan-II, Zeiss, Kontron Electronics, Eching, Germany). At least 100 fibres were measured in each case. Using previous studies as a reference, diameters between 40 and 80 µm were considered normal [18]. Type I and II fibres were identified with adenosine triphosphate (ATPase) stains at different pHs [29].

Electron microscopy. Samples of the diaphragm were also processed for electron microscopy (EM) according to standard methods (Tissue Processor E9200, BioRad, Boston, MA, USA). In short, each second half of the muscle was immediately fixed with a glutaraldehyde solution (6.25% glutaraldehyde, 0.1 M sodium cacodylate buffer adjusted to 430 mosmol·L^{-1} (16320, EMS, Penn, PA, USA) and 1% osmium tetroxide (0-5500, Sigma, St. Louis, MO, USA)). Stabilization of the fixatives was performed using 125 mM sodium cacodylate buffer (C-0250, Sigma). Samples were then embedded in an epoxy resin mixture (Polarbed 812 Resin, A22161/6, Bio Rad) and subsequently polymerized at 60°C. Several slices were obtained by systematically inclining the holder by 2°, and stained with 0.1% toluidine blue. As suggested by POOLE *et al.* [28], optimal longitudinal sections were identified when a change of the sectioning angle in either direction resulted in a higher sarcomere length. Subsequently, 150-nm thin sections were obtained using a diamond-edged knife (Diatome, Biel, Switzerland) in an ultramicrotome (Ultracut E, Reichert-Jung, Vienna,

Austria). These sections were stained using uranyl acetate–lead citrate. Micrographs were taken from 32 randomly selected fields at constant calibrated magnifications of $1,900 \times$ and $7,100 \times$ (16 pictures each), in a transmission electron microscope (Philips 301, Eindhoven, The Netherlands) with an accelerating voltage of 60 kV.

The structural analysis was performed on the micrographs using the above-mentioned semiautomatic system. The length (L_{sar}) and contraction ratio (C_R) of the sarcomeres, as well as the number (n_{mit}) and size of mitochondria, and the glycogen stores were evaluated in a double-blind manner. L_{sar} was defined as the distance between two consecutive Z discs, and was obtained from the mean value of all sarcomeres present in 16 fields at $1,900 \times$. An index (C_R) for assessing the degree of the contraction artifact was defined. Since L_{sar} decreases during muscle contraction whereas the length of the A band remains constant [30], C_R was calculated from the ratio between these two variables (C_R = A band/L_{sar}). This value was calculated for each sarcomere included in the analysis (3,000 in each individual). The n_{mit} was defined as the number of mitochondrial transections (mt) present in the $7,100 \times$ fields, and was normalized to the micrographed area. The size of these organelles was assessed through their least diameter (LD_{mit}, perimeter (P_{mit}) and surface (A_{mit}). To make pictures representative of any potential increase in size and/or number of mitochondrial clusters and to also take into account the more dispersed interfibrillary mitochondrial compartment, whole muscle fibre fields occupied the micrographs. This included both the subsarcolemmal space and wide areas of intermyofibrillar sarcoplasm. Finally, to assess glycogen stores, the cell area corresponding to aggregates of glycogen particles (A_{gly}) was measured and normalized to the whole area micrographed at $7,100 \times$.

Statistical analysis

Data are presented as mean±SD. Each variable was tested for normal distribution using the Kolmogorov–Smirnov test. Comparisons between groups were performed using the Mann–Whitney U-tests. Spearman's coefficient was obtained to assess correlation, and a linear regression analysis was used where appropriate. A p-value ≤0.05 was considered significant.

Results

Pulmonary and respiratory muscle functions

Individual and mean functional values for the two groups are shown in table 1. Eleven subjects showed mild to severe COPD, and twelve had air trapping (RV/TLC >37%, this included three subjects with normal spirometry). Arterial blood gases were normal except in four COPD patients who displayed light to moderate hypoxaemia. Variables expressing the strength of respiratory muscles (*i.e.* maximal inspiratory pressure (MIP) 106± 40% reference; and $P_{di,max}$ 95±31 cmH$_2$O) were normal, and those related to their functional reserve ($P_{di}/P_{di,max}$ 0.13±0.07; and T_{tdi} 0.05±0.03) were far from the fatigue limits.

Table 1. – Main anthropometric, functional and structural characteristics of the patients

Patient No.	Age yrs	BMI kg·m^{-2}	FEV1/FVC %	FEV1 %ref^{-1}	FRC %ref	RV/TLC %	P_{a,O_2} mmHg	$P_{I,max}$ %ref	n_{mit} mt·µm^{-2}	L_{sar} µm
COPD patients										
1	69	25	45	44	170	61	98	38	0.82	2.16
2	47	27	54	46	126	37	88	100	0.91	2.16
3	68	24	43	53	NA	NA	87	67	0.52	2.38
4	53	25	61	55	126	58	75	90	0.70	2.10
5	62	25	68	62	115	39	89	111	0.53	1.90
6	67	27	63	62	106	51	80	121	0.58	2.11
7	46	20	57	64	100	32	100	96	0.43	2.14
8	46	29	67	67	109	39	80	172	0.47	2.05
9	64	29	67	67	130	46	62	153	0.47	2.29
10	53	20	66	67	162	56	68	37	0.64	1.91
11	70	24	68	71	206	65	79	44	0.74	1.84
Mean±SD	59±10	25±4	60±9	60±9	135±34	48±11	82±12	94±45	0.62±0.16	2.09±0.16
Non-COPD patients										
12	46	22	71	80	109	31	87	124	0.33	2.27
13	51	27	73	80	102	37	84	156	0.43	2.37
14	66	28	76	81	NA	NA	NA	156	0.28	1.96
15	69	23	72	85	128	44	95	109	0.83	2.13
16	63	23	76	88	88	38	100	144	0.44	1.94
17	59	24	71	89	102	33	88	95	0.51	2.37
18	64	22	71	96	101	29	95	128	0.49	2.21
19	68	29	79	96	88	28	91	72	0.48	2.50
20	71	23	72	99	87	29	79	107	0.35	2.12
Mean±SD	62±8	25±2	74±3	88±7	100±14	33±6	90±7	121±28	0.46±0.16	2.21±0.19

Nutritional status

This was strictly normal in all patients and no relationships were found between nutritional and structural items.

Structural analysis of the diaphragm

Light microscopy. In all samples, both types of fibres were distributed in the typical mosaic pattern. Type I accounted for 52±3% of the fibres, and the size was similar for type I and type II (diameter, 58±12 *versus* 61±14 μm, respectively).

Electron microscopy. Mitochondria were found to be mainly distributed in the subsarcolemmal compartment. The n_{mit} was 0.55±0.17 mt·μm^{-2}, with an LD_{mit} of 496± 85 nm, P_{mit} of 2.47±0.36 μm and A_{mit} of 7.18±1.85 μm^{-2}. The index of the sample contraction was very homogeneous (72.4±2.8) for the entire population, with L_{sar} showing a value of 2.15±0.18 μm. With respect to glycogen aggregates, these always appeared in the monoparticular form, with A_{gly} representing 8.7±3.6% of the cellular area.

Respiratory function and structure of the diaphragm

Interestingly, patients suffering from a COPD showed a higher mitochondrial density than subjects without airways obstruction (0.62±0.16 *versus* 0.46±0.16 mt·μm^{-2}, p<0.05) (fig. 1). Furthermore, an inverse correlation was found between the degree of airways obstruction (represented by FEV1) and nmit (r=-0.555, p=0.01) (fig. 2). In addition, clusters of mitochondria were frequently found in COPD patients (fig. 3) and, in three of them, mitochondrial paracrystalline inclusions were observed (fig. 4). Variables reflecting the size of mitochondrial transections (*i.e.* LD_{mit}, P_{mit} and A_{mit}) did not correlate with any of the functional variables. On the other hand, although the L_{sar} was slightly shorter in COPD (2.09±0.16 *versus* 2.21±0.19), this difference did not reach statistical significance (p=0.1). However, when all individuals with air trapping (RV/TLC >37%) were grouped (most of the COPD patients plus three subjects with normal spirometric values), a higher n_{mit} and shorter sarcomeres were observed, in contrast to subjects without this functional abnormality (0.63±0.17 *versus* 0.43±0.07 mt·μm^{-2}, p< 0.05; and 2.08±0.16 *versus* 2.27±0.15 μm, p<0.05, respectively) (fig. 1). In the same way, L_{sar} was found to inversely correlate with the severity of air trapping (with RV, r=-0.548, p=0.02; with RV/TLC, r=-0.526, p<0.05) and pulmonary hyperinflation (with FRC, r=-0.483, p<0.05) (fig. 5). A similar but direct relationship was obtained between static lung volumes and n_{mit} (with RV, r=0.589, p=0.01; with RV/TLC, r=0.631, p<0.01; with FRC, r=0.657, p<0.01). It is worth noting that CR not only showed a narrow range in this series, but was similar for different groups (COPD *versus* subjects with normal spirometry, 72±3 *versus* 73±2, respectively; individuals with and without air trapping, 73±3 *versus* 72±2, respectively), and did not correlate with n_{mit}. Finally, no differences were observed for glycogen stores or the size
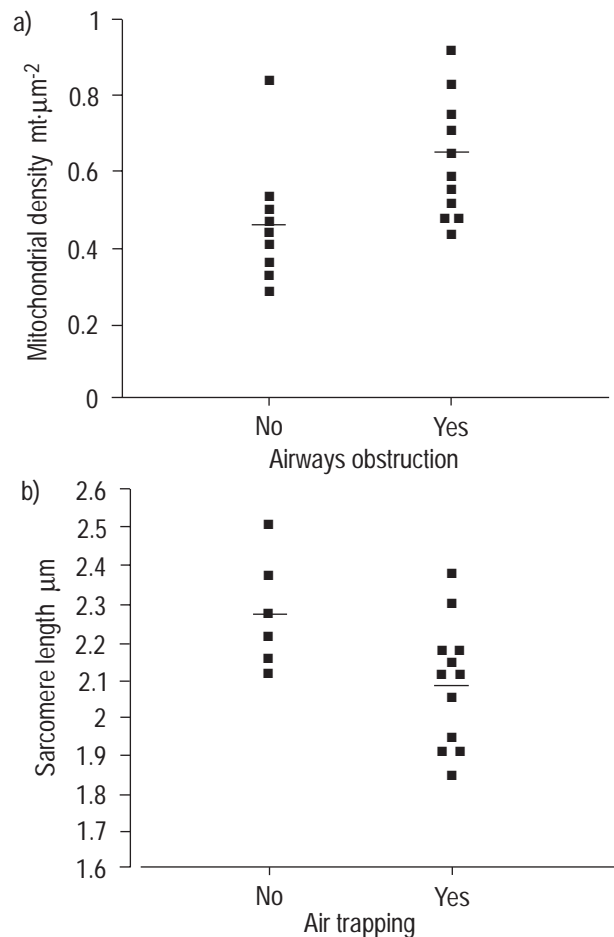

Fig. 1. – Differences in a) diaphragmatic mitochondrial density (n_{mit}) with reference to airways obstruction, and b) in the length of sarcomeres (L_{sar}) with reference to air trapping (p<0.05, both). mt: mitochondrial transections.

of the fibres between COPD and controls (A_{gly} 9.2±3.7 *versus* 8.1±3.6%; diameter of type I fibres, 60±14 *versus* 58±11 μ; diameter of type II, 58±16 *versus* 62±14 μ).

Respiratory muscle function and structure of the diaphragm

An inverse correlation was found between %PI,max and n_{mit} (r=-0.547, p=0.01) (fig. 2). The relationship did not reach statistical significance for Pdi,max. No correlations were observed between L_{sar} and respiratory muscle function.

Discussion

The main finding of this study is the close association observed between lung function and the subcellular structure of the human diaphragm. Specifically, structural differences in the length of sarcomeres and mitochondrial content were observed in COPD patients, when these were compared with the control group. Furthermore, some of these changes appeared to be proportional to the degree of either airways obstruction, pulmonary hyperinflation or air
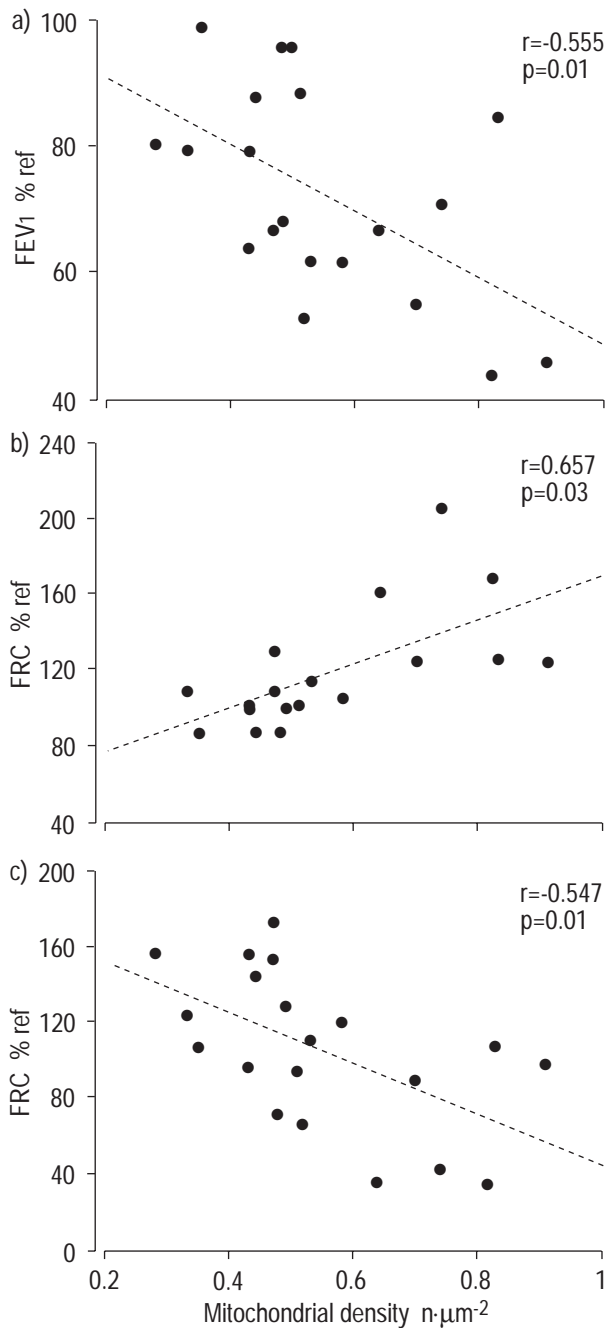
Fig. 2. – Plot of mitochondrial density (n_{mit}) with the severity of airways obstruction in a) forced expiratory volume in one second (FEV1), b) functional residual capacity (FRC) and c) maximal inspiratory pressure ($P_{I,max}$). ref: reference. - - - - - : regression line.
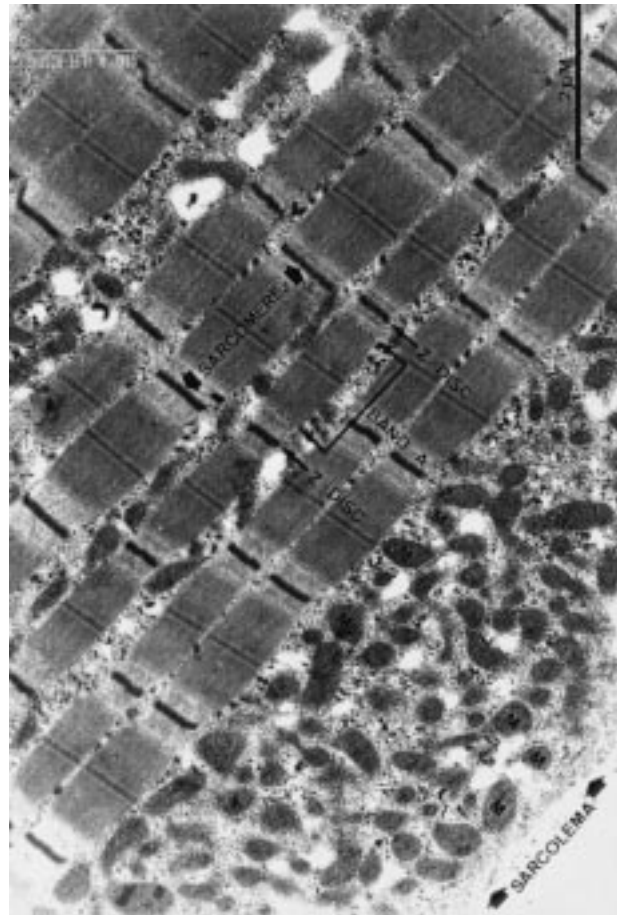

Fig. 3. – Micrograph showing subsarcolemmal clustering of mitochondria in a diaphragm cell from a chronic obstructive pulmonary disease patient (forced expiratory volume in one second, 46% predicted). Variables included in the evaluation of the contraction ratio (C_R) of the sarcomeres (A band and length of sarcomeres (L_{sar})) are also shown. (Internal scale bar=2 μM.)

trapping. These findings could be considered representative of an adaptive trend of the muscle against chronic ventilatory loading. The latter is strongly suggested by the association also observed between the structural changes and the strength of respiratory muscles. As far as the authors are aware, this is the first study evaluating the ultrastructure of the human diaphragm and its relationships with both lung and respiratory muscle functions.

Skeletal peripheral muscles have been shown to be very malleable in their adaptive response to different forms of chronic use and disuse [31]. The multitude of observed

phenotypic changes include modifications in mitochondrial content, oxidative capacity, the size and relative frequency of different fibre types, and the length and number of sarcomeres. Descriptions of these adaptive processes in skeletal limb muscles are plentiful. However, relatively few studies have investigated structural remodelling in respiratory muscles. These studies have been focused mainly on their cellular structure (*i.e.* the size and proportion of different fibre types) [9, 17–19]. As a consequence, little is known about the subcellular structure of the diaphragm in experimental models of respiratory diseases, and almost nothing in COPD patients. This lack of data is probably due to technical and ethical limitations in obtaining valid samples of the muscle and at the same time availing of a functional evaluation. Until recently, current models were necropsies or biopsies during a thoracotomy. In the former model, functional assessment is either absent or too far from sampling. In addition, many associated phenomena could interfere with the structural analysis. With reference to sampling during thoracotomy, the procedure employed in the present study, advantages and limitations will be specifically discussed below. Interestingly, in recent years, two new models have been described for obtaining diaphragmatic samples "*in vivo*"

Fig. 4. – Micrograph showing mitochondrial (M) paracrystalline inclusions (arrows) in the diaphragm of the same chronic obstructive pulmonary disease patient as in figure 3. (Internal scale bar=2 μm.)

Fig. 5. – Plots of length of sarcomeres (L_{sar}) with static lung volumes for a) residual volume (RV), b) RV/total lung capacity (TLC), and c) functional residual capacity (FRC). ref: reference. - - - - - : regression.

namely from subjects submitted to upper laparotomy [32] and COPD patients undergoing surgical reduction of lung volume [9].

Sarcomere length

This study shows that air trapping is closely associated with shorter sarcomeres in the diaphragm. Furthermore, the greater the lung volumes, the shorter the length of sarcomeres. These results confirm those observed in previous experimental models. Hamsters with induced emphysema were shown to have a reduction in both the number and length of their diaphragmatic sarcomeres [16]. This structural change was associated with the preservation of the capacity to generate tension, as was the case in the patients of the present study. The hypothesis is that sarcomere shortening probably results in a partial reversion of the displacement of the diaphragmatic length–tension curve due to pulmonary hyperinflation [33]. This would also explain the findings of SIMILOWSKY *et al.* [7] demonstrating that patients with COPD develop even higher diaphragmatic strength than normal subjects at equivalent lung volumes.

Using an inverse train of thought, if the diaphragm preserves its intrinsic properties for generating pressure, a type of structural adaptation should be present: either an increase in the size of the fibres, a change in their relative proportions or a decrease in the length of sarcomeres are the more likely hypotheses. This study demonstrates that the shortening in sarcomeres really does occur in COPD patients. In addition (and as previous authors have described [17, 18]), the data in the present study discard the former hypothesis: fibres were, in fact, similar in COPD and controls. With reference to the second possibility (a change in the proportion of fibres), a recent paper by LEVINE *et al.* [9] has demonstrated a switch to slow type fibres and the homonimous myosin heavy chain isoform in severe COPD patients [7].

Other authors have failed to find similar relationships between the length of the muscle filaments and functional behaviour of the diaphragm [28], although this is probably owing to the relatively short course of the disease in current experimental models. In other words, the subacute emphysema generated in animals cannot entirely mimic the slow and progressive development of a COPD in humans. This would be specially notable in consequences of chronic mechanical loading on the structural adaptive changes of respiratory muscles and thus further emphasize the relevance of this study.

One possible criticism is a potential artifact in sarcomere length due to muscle contraction. However, this seems unlikely, since muscle bundles were immediately fixed after surgery. In addition, the small dispersion of the variable assessing the degree of contraction reveals that, if present, this factor was similar for all the different samples.

Mitochondria

In this study the number of mitochondrial transections in the diaphragm has been shown to be higher in COPD patients. Moreover, mitochondrial density was associated with the severity of both airway obstruction and pulmonary hyperinflation. Although the design of the study does not make it possible to assign a cause–effect relationship, it is well established that the number of mitochondria increases in other muscles submitted to overloads [31]. Since airflow obstruction imposes a persistent mechanical load on the inspiratory muscles, changes in the concentration of energetic organelles should also be expected here [34, 35].

Either an actual increase in mitochondria or a change in the shape of these organelles could explain the high value of mitochondrial transections observed in these COPD patients. However, the latter seems less probable, since this would implicate a highly convoluted reticulum along an appreciable length of the myocyte. Another possible criticism is the potential bias in the evaluation of mitochondria due to the involuntary selection of a specific fibre type. However, although no standardized method exists which allows us to differentiate the fibre subtypes during electron microscopy analysis, this bias is unlikely: populations of slow and fast-twitch fibres were similar, there were no clusters of a specific subtype, and fields were randomly selected. In addition, as LEWIS *et al.* [11] have demonstrated, the metabolic adaptation of the diaphragm is nonspecific for a determined subtype of fibres.

An interesting point for discussion is whether or not changes in the mitochondrial density have functional relevance. In fact, trained limb muscles increase the content of these organelles, using carbohydrates sparingly and displaying greater levels of β-oxidation enzymes [36]. This results in a smaller disruption of homeostasis during exercise [30]. The present study demonstrated similar structural changes in the diaphragm of COPD patients. If their functional significance is similar, as suggested by the increased oxidative capacity reported by HUGHES *et al.* [37], the muscle would be allowed to work for longer before becoming fatigued [31]. Once again, the strikingly direct relationship observed between the mitochondrial density and the strength of inspiratory muscles in these patients can be viewed as the functional expression of their structural adaptation.

A short comment should be made about potential regulators of the number of mitochondria in the diaphragm of COPD patients. Although no specific studies have been carried out, some of the factors that could be present in this condition (such as muscle hypoxia or depletion of high energy phosphates) can induce changes in the mitochondrial content in limb muscles [31].

Finally, paracrystalline inclusions observed in some of these COPD patients, and previously described by this group [38], probably reflect the metabolic mismatching present in the mitochondria of this population [39].

Limitations of the model

Thoracotomy has been used in this and almost all previous studies concerning the structure of the diaphragm [17–19, 37]. This procedure allows us to obtain samples of the diaphragm *in vivo*, making a close functional and nutritional evaluation possible, and avoiding cellular phenomena related to death. However, it does have its limitations: many severe COPD patients cannot be submitted to this surgery and a possible bias can exist owing to the presence of a malignancy. However, the close selection of these patients reasonably excludes the latter objection: nutritional parameters were normal, the neoplasm was small and limited to the thorax and there were no signs of associated paraneoplastic syndromes.

In conclusion, the present study demonstrates shorter sarcomeres and a higher mitochondrial content in the diaphragm of patients with chronic airways obstruction and/or increased lung volumes. These findings support the notion that respiratory muscles undergo structural remodelling and adaptation in chronic obstructive pulmonary disease patients.

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