

Structure and function relationships of the respiratory muscles

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ABSTRACT: Potential relationships between the structure of the diaphragm and external intercostals and several indices of respiratory muscle function, lung function and nutrition in 27 patients (61 ± 10 yrs of age) subjected to thoracotomy as a result of a lung neoplasm have been investigated.

Prior to surgery the nutritional status of the patients was assessed and lung function (spirometry, lung volumes, transfer factor of the lungs for carbon monoxide, arterial blood gases) and respiratory muscle function (maximal inspiratory pressure (MIP) and diaphragmatic function were measured). Biopsies of the diaphragm (and external intercostals) were obtained during surgery.

On average, patients showed mild airflow limitation (forced expiratory volume in one second (FEV₁), $70 \pm 14\%$ of predicted value, FEV₁/forced vital capacity (FVC), $70 \pm 9\%$) with some air trapping (residual volume (RV), $139 \pm 50\%$ pred) and normal gas exchange (arterial oxygen tension (P_{a,O_2}), 11.3 ± 1.33 kPa (85 ± 10 mmHg)) and arterial carbon dioxide tension (P_{a,CO_2}) 5.4 ± 0.5 kPa (40.6 ± 4 mmHg). MIP was $77 \pm 25\%$ pred; maximal transdiaphragmatic pressure was 90 ± 27 cmH₂O. Most morphometric measurements of the diaphragm and external intercostals were within the range of values reported previously in other skeletal muscles. The size of the fibres of these two respiratory muscles was positively related ($p < 0.05$) to MIP (% pred). There were no significant relationships between the structure of both muscles and nutritional status or any index of lung function.

In conclusion, in the population studied, the fibre size of the diaphragm and external intercostals appears to relate to their ability to generate force.
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Respiratory muscle dysfunction is a well recognized cause of respiratory failure [1–3]. In clinical practice, the function of the respiratory muscles are usually assessed by measuring maximal inspiratory and expiratory pressures (MIP and MEP, respectively) [3] and sometimes, by evaluating mean transdiaphragmatic pressure (P_{di}) [4]. Yet, the structural basis of these measurements (*i.e.*, the relationship between these functional indices and the structure of the respiratory muscles) has not yet been established, particularly for the diaphragm, which functionally is the most relevant respiratory muscle [1–3]. The main aim of this investigation was to evaluate potential relationships between the structure of the diaphragm (and external intercostals) and several indices of respiratory muscle function commonly used in practice. Because there is only one previous study in this field that related the structure of the external intercostals to a limited number of physiological variables [5], the latter group of muscles was also evaluated.

Finally, how the structure of these two respiratory muscles (diaphragm and external intercostals) relate to several indices of lung function and some nutritional variables was also investigated. This topic has been studied previously [6–10], but because of its potential clinical rele-

vance, further investigation complement and extend knowledge based on previously published data was required.

Materials and methods

Study subjects

A total of 27 consecutive eligible patients with localized lung cancer, who required a thoracotomy were studied. To avoid potential confounding effects upon muscle structure, patients with neuromuscular disorders, cardiac failure diabetes mellitus, alcoholism, and/or those who were receiving treatment with steroids [11–15] were excluded. Only males were included in the study because gender has a direct influence upon muscle structure [16]. All patients gave informed consent after full explanation of the nature of the study. The study was approved by the local Ethics Committee of the hospital.

Study design

Patients with a localized lung neoplasm requiring a thoracotomy were recruited prospectively. In these patients

lung function and respiratory muscle function were measured and nutritional status assessed prior to surgery. Biopsies (1.5×1.0×0.5 cm) of the costal diaphragm and the fifth external intercostal at the level of posterior axillary line were obtained during surgery. Biopsies were immediately frozen in isopentane cooled with liquid nitrogen, and stored at -70°C until analysis (see below).

Methods

The nutritional status of the patients was assessed using the body mass index (BMI, kg·m⁻²), percentage of ideal body weight (IBW) [17], lean body mass (LBM, kg) calculated from the prediction equation of Eddy [18] (percentage of fat for males = (1.281×BMI) - 10.13) and several biochemical variables, such as total serum protein (g·dL⁻¹) and serum albumin (g·dL⁻¹). Forced spirometry (Datospir92, Sibel, Barcelona, Spain), static lung volumes and airway resistance by body plethysmography (Masterlab, Jaeger, Würzburg, Germany), single breath transfer factor of the lung for carbon monoxide (TLCO) (Jaeger) and arterial blood gases (ABL 330; Radiometer, Copenhagen, Denmark) were measured 48–72 h prior to surgery with the subjects in the sitting position. Spirometric, TLCO and lung volume reference values were obtained from a mediterranean population [19–21]. Respiratory muscle function was assessed by measuring: 1) MIP at the mouth against an occluded airway (Sibelmed-163; Sibel, Barcelona, Spain), using the method described originally by BLACK and HYATT [22]. MIP was analysed in absolute values and as percentage of predicted values [22]; 2) mean P_{di} : oesophageal (P_{oes}) and gastric (P_{ga}) pressures were obtained using balloon catheters (Jaeger) coupled to pressure transducers (Transpac II; Abbot, Chicago, IL, USA) and connected to a polygraph (Beckman R-6; Sensors-medics, Anaheim, CA, USA). The balloons were calibrated over appropriate pressure ranges at the beginning and end of each study. The oesophageal balloon containing 0.5 mL of air was positioned in the mid oesophagus. The gastric balloon contained 1 mL of air. P_{di} was taken as the difference between mean P_{ga} and mean P_{oes} , while the patient was seated during tidal breathing [23]; and, 3) maximal transdiaphragmatic pressure ($P_{di,max}$) was assessed during a sniff manoeuvre ($P_{di,sniff}$) [24]. The highest $P_{di,sniff}$ value of five different manoeuvres was chosen for analysis.

Sections of the diaphragm and external intercostals were cut 6 µm thick in a cryostat (-20°C). Type I ("slow twitch") and type II ("fast twitch") fibres were identified by the standard adenosine triphosphatase (ATPase) (at 9.4 and 4.6 pH pre-incubation) (fig. 1), and reduced nicotinamide adenine dinucleotide tetrazolium (NADH-TR) stains [25, 26]. Morphometric evaluation was done using a semiautomatic system (Videoplan 2, Zeiss, Kontron electronics, Bremen, Germany). Measurements, performed independently by two different observers, included quantitation of: 1) percentage of type I and II fibres; 2) least fibre diameter for each fibre type (this diameter is minimally influenced by the cutting angle during sample process) [25]; and 3) atrophy and hypertrophy index (AI and HI, respectively), calculated using the method of BROOKE and ENGEL [26]. These two indices evaluate, respectively, the two tails of the least diameter histogram of at least 100

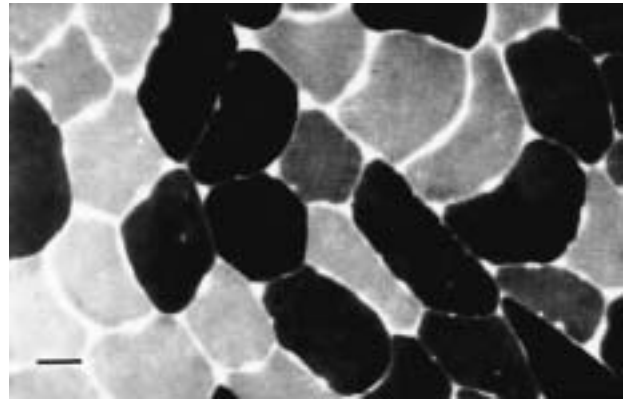


Fig. 1. – Adenosine triphosphatase (ATPase) stain (pH 9.4) from a representative diaphragmatic sample. Type I fibres are stained clear and type II fibres are stained dark. Internal scale bar = 100 µm.

muscle fibres. To calculate the AI a category is assigned to each measured fibre, such that normal fibres (40–80 µm in diameter) are rated 0, intermediate fibres rated 1 and 2 and the smallest fibres rated 3. The AI is derived by summing the values in each category, divided by the total number of fibres in the histogram, and multiplied by 1,000. The larger this index, the greater the degree of atrophy. A separate HI was calculated for each specimen, using a similar scale for fibres larger than 80 µm. Values >250 were considered abnormal [6, 8].

Statistical analysis

Data are presented as mean±SD and range. The reproducibility of the morphometric measurements between the two observers was tested using the intraclass correlation coefficient [27]. Potential relationships between structure and function of the respiratory muscles, lung function and nutritional status were evaluated using the Pearson's linear correlation coefficient. The results of the morphometric analysis were compared in patients with and without airflow obstruction, the latter being defined as a forced expiratory volume in one second (FEV₁)/forced vital capacity (FVC) ratio <70% and FEV₁ <70% pred, using the Mann-Whitney test. The level of significance was assessed after correction for multiple comparisons (Bonferroni).

Results

The mean age of the 27 patients studied was 61±10 yrs of age. No patient was grossly undernourished. Mean BMI was 24.4±2.76 kg·m⁻², IBW 111±12% pred, and LBM 60±3 kg. Mean serum albumin was 3.9±0.6 g·dL⁻¹ and mean total serum protein was 6.8±0.9 g·dL⁻¹. On average, patients showed mild airflow limitation (with some air trapping) and normal gas exchange (table 1), but some individuals had moderate to severe airflow obstruction and mild hypoxaemia. On average MIP was slightly reduced (77±25% pred). Seven patients showed an MIP value <65% pred (table 2). Mean maximal P_{oes} ($P_{oes,max}$) (64±18 cmH₂O) and $P_{di,max}$ (90±27 cmH₂O) (table 2) were slightly lower than previously published in healthy subjects [28].

Table 1. – Indices of lung function

	Mean±SD	Range
FEV1 % pred	70±14	44–96
FEV1/FVC %	70±9	45–79
RV % pred	139±50	75–254
TLCO % pred	82±16	47–119
P_{a,O_2} kPa	11.3±1.3	8.6–13.6
P_{a,CO_2} kPa	5.5±0.5	4.4–6.3
P_{A-a,O_2} kPa	1.9±1.2	0–4.4

FEV1: forced expiratory volume in one second; % pred: percentage of predicted value; FVC: forced vital capacity; RV: residual volume; TLCO: transfer factor of the lung for carbon monoxide; P_{a,O_2} : arterial oxygen tension; P_{a,CO_2} : arterial carbon dioxide tension; P_{A-a,O_2} : alveolar-arterial pressure difference for oxygen. 1 kPa=7.52 mmHg.

Table 2. – Indices of respiratory muscle function

	Mean±SD	Range
MIP % pred	77±25	26–137
P_{di} cmH ₂ O	6±2.5	2.6–11
$P_{oes,max}$ cmH ₂ O	64±17	27–108
$P_{di,max}$ cmH ₂ O	90±27	45–172
$P_{di}/P_{di,max}$	0.08±0.05	0.03–0.22

MIP: maximal inspiratory pressure; % pred: percentage of predicted value; P_{di} : mean transdiaphragmatic pressure; $P_{oes,max}$: maximal oesophageal pressure; $P_{di,max}$: maximal transdiaphragmatic pressure.

Morphometric measurements were highly concordant between the two observers ($r=0.9$). On average, the different morphometric indices evaluated in both muscles (table 3) were within the range of values previously reported in healthy subjects in other skeletal muscles [29], but some patients showed an HI in the diaphragm >250, which is clearly abnormal [6, 8]. We found no significant differences between the morphometric variables of patients with and without airflow obstruction, as defined above.

The relationships between the structure and function of the two respiratory muscles evaluated in this study are shown in figures 2 and 3. MIP (% pred) was significantly related to the diameter of: 1) type I and type II diaphragmatic fibres (fig. 2); and 2) type II fibres of the external intercostals (fig. 3). $P_{di,max}$ showed no correlation with muscle

Table 3. – The structural parameters of the different muscles

Fibres	Diaphragm	External intercostals
Type I %	56±9 (41–77)	62±10 (40–76)
Type II %	44±9 (23–59)	38±10 (24–60)
Type I+ μ m	64±9 (45–81)	60±8 (47–79)
Type II+ μ m	61±12 (45–84)	57±8 (44–74)
Type I AI	52±70 (0–300)	69±81 (0–240)
Type II AI	120±134 (0–500)	157±200 (0–700)
Type I HI	345±404 (0–1560)	170±278 (0–1000)
Type II HI	364±465 (0–1530)	78±150 (0–520)

Values are presented as mean±SD with range in parenthesis. +: least diameter. AI: atrophy index; HI: hypertrophy index.

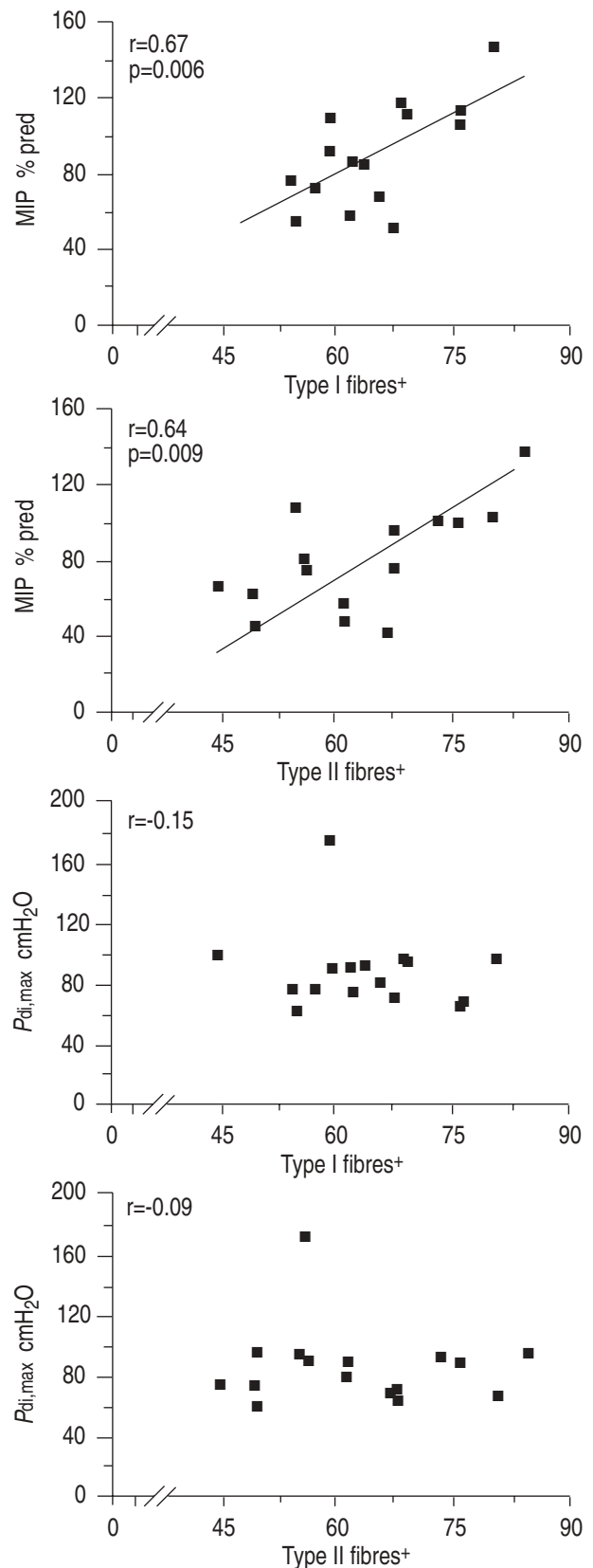


Fig. 2. – The relationships between morphometric parameters of the diaphragm and indices of respiratory muscle function using Bonferroni multiple comparisons. MIP: maximal inspiratory pressure; % pred: percentage of predicted value; $P_{di,max}$: maximal transdiaphragmatic pressure. +: least diameter.

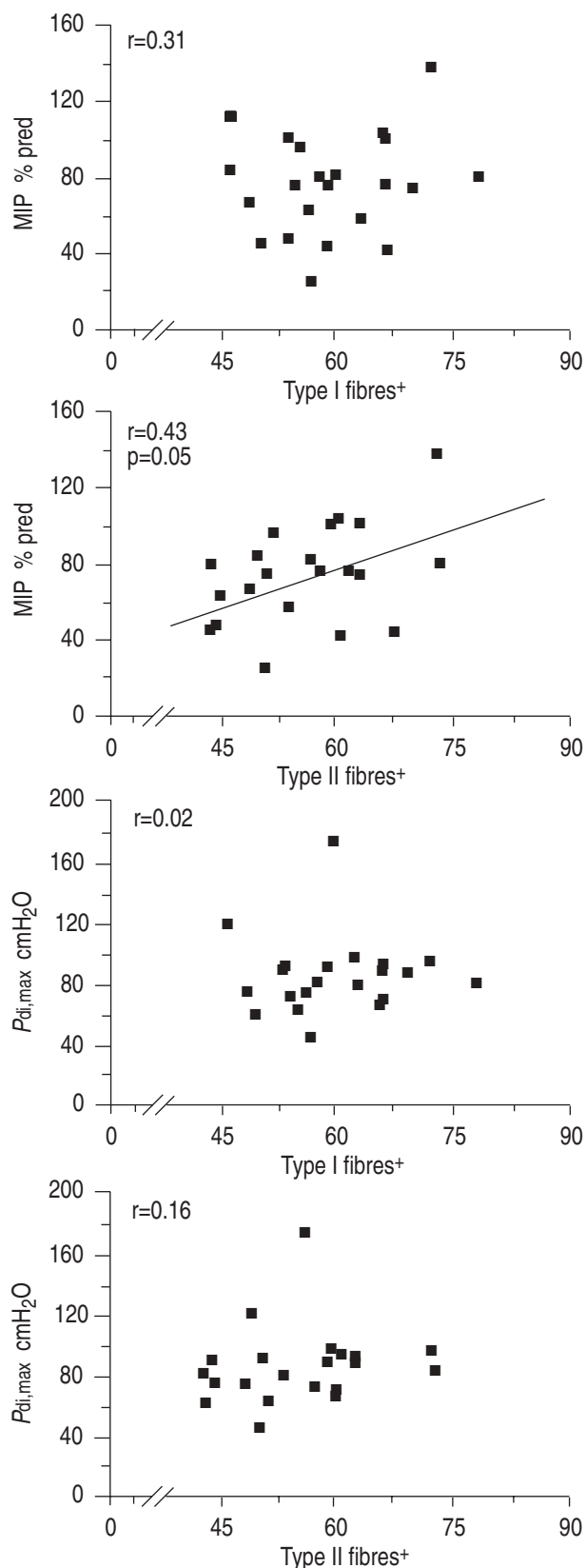


Fig. 3. – The relationships between morphometric parameters of the external intercostals and indices of respiratory muscle function using Bonferroni multiple comparisons. MIP: maximal inspiratory pressure; % pred: percentage of predicted value; $P_{di,max}$: maximal transdiaphragmatic pressure. +: least diameter.

Table 4. – The relationship between nutritional indices and muscle morphometry using Bonferroni multiple comparisons

	BMI kg·m ⁻²	Total serum protein g·dL ⁻¹	IBW %	LBM kg
Diaphragm				
Type I+ μ m	0.36	0.64	0.39	0.12
Type II+ μ m	0.52	0.34	0.53	0.19
Type I AI	-0.28	-0.25	-0.26	-0.02
Type II AI	-0.51	-0.25	-0.50	-0.21
Type I HI	0.28	0.35	0.25	0.49
Type II HI	0.30	0.15	0.26	0.28
External intercostals				
Type I+ μ m	0.17	0.44	0.19	0.07
Type II+ μ m	0.31	0.41	0.30	0.13
Type I AI	-0.09	-0.53	-0.13	-0.01
Type II AI	-0.08	-0.28	-0.10	0.01
Type I HI	0.28	0.33	0.27	0.22
Type II HI	0.28	0.24	0.25	0.21

BMI: body mass index; IBW: ideal body weight; LBM: lean body mass; +: least diameter; AI: atrophy index; HI: hypertrophy index.

morphometry (figs. 2 and 3). Other measurements of diaphragmatic function P_{di} , $P_{oes,max}$ and $P_{di}/P_{di,max}$ showed no correlation either. No correlations between any indices of muscle structure and lung function were found. By contrast, several nutritional variables appeared to be related to muscle structure (table 4), although after correcting for multiple comparisons (Bonferroni), the p-values did not reach the required level of statistical significance ($p < 0.001$).

Discussion

Although the diaphragm is an important component of the respiratory system, there is a surprising paucity of data about the relationship between its structure and function in humans. A significant relationship between its fibre size and MIP was observed (fig. 2). It was surprising though, that more specific indices of diaphragmatic function (P_{di} , $P_{oes,max}$, $P_{di,max}$ and $P_{di}/P_{di,max}$) were not correlated with the morphometric variables assessed. The lack of correlation has several possible explanations. Firstly, the lack of available reference values for $P_{di,max}$ and $P_{oes,max}$ may have jeopardized an ability to disclose potential structure-function relationships. In fact, a relationship with MIP was only found when results were expressed as percentage of predicted value (fig. 2), but not when expressed as absolute values. Secondly, diaphragmatic function is influenced by a number of factors, such as fibre type, number of capillaries and mitochondria and oxygen delivery to tissues, among others [1], that were not directly assessed in our study. It is conceivable that the use of the morphometric indices, such as fibre volume, mitochondrial density, length and/or number of sarcomere, would have given different results, as recently shown in another, as yet preliminary study [30]. Finally, it is thought that methodological errors in the morphometric analyses are unlikely because similar methods to those reported in previous studies [5–11] were used and the morphometric measurements were highly concordant between the two observers.

Only one previous study has related the structure of the external intercostals to MIP [5]. This study failed to show any significant correlation [5]. In contrast, a weak but significant relationship between fibre size of the external intercostals and MIP was observed in the present study, as was also observed in the diaphragm samples of the patients (fig. 3). This apparent discrepancy with previous studies might be explained by the careful selection of subjects in our investigation. In particular, only male subjects (sex has some effects upon muscle structure [16]) were included, and patients with diseases known to influence muscle structure, excluded. This selection and, perhaps also ethnic differences, might also explain the observation of a higher fibre size of the external intercostals than those previously reported [5–8, 10]. An effect of the neoplasm itself upon muscle structure, cannot be completely discounted but this limitation is shared by most previous studies of respiratory muscle structure in humans [5–10].

Some studies have reported significant relationships between muscle structure and nutrition [5, 31] while others failed to show them [6–8]. It was found (table 4) that some nutritional indices appeared to be related to muscle structure, but the correlation coefficients failed to reach statistical significance. This is probably due to the absence of overt malnutrition in any of the patients studied, as shown by the normal values of BMI, IBW and total serum protein. Thus, the results suggest that the effect of nutrition on respiratory muscle structure is small in this population (patients submitted to thoracotomy for a lung neoplasm). Similarly, no significant relationships between the structure of the diaphragm (or the external intercostals) and various indices of lung function were found. Previous studies have reported similar results [5–7, 10]. The relatively narrow range of lung function values in these patients subjected to thoracotomy may also help to explain the lack of relationships observed. However, it is interesting to note that fibre type and fibre size were not different according to the presence or absence of airflow obstruction again in keeping with previous studies [5–7, 10].

In summary, this is the first study assessing possible relationships between the structure of the diaphragm and several indices of respiratory muscle function, lung function and nutrition in humans. A direct correlation between the size of the diaphragmatic fibres and maximal inspiratory pressure was found, but no significant relationships between muscle structure and lung function or several nutritional indices were found. Perhaps, future studies in patients with advanced chronic obstructive pulmonary disease, undergoing lung volume reduction surgery would provide an opportunity to obtain diaphragmatic samples. This would provide a means of assessing, potential remodelling of the diaphragm due to chronic airflow limitation [32].

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