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Inspiratory muscle training in patients with COPD: Structural adaptation and physiological outcomes.

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WORD COUNT FOR THE BODY MANUSCRIPT

• Abstract	:	202
• Introduction	:	312
• Methods	:	799
• Results	:	331
• Discussion	:	1400
• TOTAL	:	2842

ABSTRACT. (Word count, n=202).

The present study was aimed at evaluating the effects of a specific inspiratory muscle training protocol on the structure of inspiratory muscles in patients with chronic obstructive pulmonary disease (COPD). Fourteen patients (males, FEV_1, 24±7 %pred) were randomised to either inspiratory muscle or sham training groups. Supervised breathing using a threshold inspiratory device was performed 30 min per day, five times a week during five consecutive weeks. The inspiratory training group was subjected to inspiratory loading equivalent to ≅40-50% of their maximal inspiratory pressure. Biopsies from external intercostal muscles and vastus lateralis (control muscle) were taken prior to and following the training period. Muscle samples were processed for morphometric analyses using monoclonal antibodies against myosin heavy chain isoforms I and II. Increases in both the strength and endurance of the inspiratory muscles were observed in the inspiratory training group. This improvement was associated with increases in the proportion of type I fibers (by ≅38%, $p<0.05$) and size of type II fibers (by ≅21%, $p<0.05$) in the external intercostal muscles. No changes were observed in the control muscle. The study demonstrates that inspiratory training induces a specific functional improvement of the inspiratory muscles and adaptive changes in the structure of external intercostal muscles.

KEY WORDS.

COPD, respiratory muscles, fiber structure, training.

RUNNINGHEAD.

Structure of trained intercostal muscles in COPD.

1. INTRODUCTION.

Chronic obstructive pulmonary disease (COPD) is characterised by a long natural history and elevated costs for health care services[1]. From an individual perspective, COPD patients disclose diverse degrees of dyspnea and deterioration in exercise capacity in association with impaired pulmonary and cardiovascular functions[2]. However, weakness and deconditioning of respiratory and peripheral muscles are currently recognised in these patients as additional factors implicated in the reduction of exercise capacity as well as quality of life[3]. The function of inspiratory muscles is frequently found to be impaired (decreased strength and/or endurance) in COPD patients[4]. Inspiratory muscle dysfunction appears to be the result of geometric changes of the thorax, systemic factors, and/or potential structural changes of the inspiratory muscles[5,6]. It is probable that inspiratory muscle dysfunction does not limit minimal ventilatory needs at rest but it does appear to contribute to dyspnea, decreased exercise capacity and ventilatory failure during exacerbations[7]. For these reasons, specific inspiratory muscle training could be justified as a strategy with potential clinical benefits in COPD patients who remain symptomatic even despite optimal therapy[8]. Although controversy still exists, several studies in healthy individuals and COPD patients have demonstrated that inspiratory muscle training can increase the strength and

endurance of inspiratory muscles[9,10]. This functional improvement is observed only when specific inspiratory muscle training is performed, but not when general exercise programmes are applied.[8] Based on previous experimental studies,[11,12] the authors hypothesised that inspiratory muscle training could be associated with adaptive changes within the structure of inspiratory muscles. Consequently, the present study was aimed at evaluating the effects of a specific short-term inspiratory muscle training protocol on the structural characteristics of the external intercostal muscles in severe COPD patients. Using an outpatient biopsy model, samples from the external intercostal muscles were taken prior to and after five weeks of a supervised training period.

2. CONDENSED METHODS.

2.1 PATIENTS AND STUDY DESIGN. This was a randomised, placebo-controlled trial conducted in accordance with World Medical Association guidelines for research on humans. Our institutional ethics board approved all protocols and all the patients gave their informed consent prior to participating in the study. Fourteen community-based COPD patients (males, 66±5 yr.) were selected for the study from a hospital respiratory clinic. The COPD diagnosis was determined from a clinical history consistent with chronic bronchitis and/or emphysema, a long history of cigarette smoking, and pulmonary function tests revealing fixed airflow obstruction (FEV_1<75% predicted, and FEV_1/FVC ratio < 65 %)[13]. Subjects were sedentary and were observed over a 4-week period while their regular treatment was maintained, to evaluate functional status and verify clinical stability. Patients showing severe hypoxemia (i.e., PaO_2 lower than 60 Torr breathing room air), asthma, coronary disease, undernourishment (body mass index <20 kg/m^2), chronic metabolic diseases (e.g., diabetes, hypo- or hyperthyroidism), orthopaedic diseases, previous abdominal or thoracic surgery, and/or treatment with steroids, hormones, or cancer chemotherapy were not considered eligible for the study. The patient's

characteristics are summarised in Table 1. All were randomised into two groups, with one group receiving specific inspiratory muscle training and the other group receiving the placebo (sham training).

2.2 NUTRITIONAL AND FUNCTIONAL EVALUATIONS.

Nutritional assessment, pulmonary function tests, inspiratory and expiratory muscle strength, and transdiaphragmatic pressure[14] during both quiet breathing (PIdi) and maximal *sniff* ($PIdi_{max}$) manoeuvres, were measured in each patient and compared to reference values[15,16,17,18,19] as described elsewhere[20]. The inspiratory muscle pressure-time index (PT_I) while the patient was seated during tidal breathing was calculated according to the equation:

$$PT_I = (Pes / Pes_{max}) * (T_I/T_{TOT})$$

where Pes is oesophageal pressure, Pes_{max} is oesophageal pressure during *sniff* manoeuvre, and T_I/T_{TOT} is duty cycle.

Exercise capacity was evaluated using two tests. Firstly, a submaximal exercise test was performed to assess the distance the patient was able to walk in 6 minutes along a measured flat corridor as described by McGavin et al.[21]. Secondly, maximal exercise

capacity was evaluated during an incremental exercise test to volitional exhaustion on a cyclergometer referenced to values from Jones et al.[22]

Specific inspiratory muscle endurance was assessed during two different threshold inspiratory loading tests performed using a similar device to that described by Nickerson and Keens[23]. In the first loading test, the volunteers breathed against incremental loads (~8 cmH_2O every 2 min) until *maximal sustainable threshold pressure* was reached (Pth_{max}).[24] In the second loading test, subjects breathed against a submaximal constant load (equivalent to 80% of maximal threshold pressure) until exhaustion. The elapsed time was defined as the *inspiratory sustainable threshold endurance time* (Tth_{80}, in min.).

2.3 MUSCLE BIOPSIES AND FIBER ANALYSES. Biopsies from the external intercostal (inspiratory muscle) and *vastus lateralis* (control) muscles were taken prior to and after the inspiratory muscle training period.[25] The pre-training biopsies were taken from the non-dominant or dominant side as previously randomised. Biopsies from the external intercostal muscles were taken along the anterior axillary line at the sixth intercostal space. Biopsies from the *vastus lateralis* were taken from the middle portion of the thigh. The post-training biopsies were obtained from the same anatomical site but

from the contralateral side at the end of the training period. The size of the muscle samples was approximately 5 x 5 x 5 mm. Each biopsy was quick-frozen in isopentane cooled in N_2(l) and stored at -70°C. Ten µm thick sections were cut varying the inclination of the holder by 5° increments until the minimum cross-sectional area was obtained, which was defined as truly transverse.[26,27] Consecutive cross sections were processed by immunohistochemical techniques using monoclonal antibodies directed against myosin heavy chain (MyHC) isoform type I and type II (figure 1) *(MHCs and MHCf clones, Biogenesis, UK).* The cross sectional area, mean least diameter, and proportions of type I and II fibers were assessed using a light microscope *(OLYMPUS, Series BX50F3. Olympus Optical Co., Japan)* coupled to an image-digitising camera *(PIXERA STUDIO, Version 1.2, Pixera Corporation. CA)* and a morphometry program *(NIH IMAGE, Version 1.60).* At least 100 fibers were measured from each biopsy.[26] Fiber diameters between 40 and 80 µm were considered normal.[28,29]

2.4 INSPIRATORY MUSCLE TRAINING. The subjects received either inspiratory muscle training or sham training for 30 minutes breathing through a threshold inspiratory device five days a week for 5 consecutive weeks. Appropriate personnel supervised

training sessions. Loaded breathing was intermittent for 3-min periods, with a 2-min rest period in between. The patients breathed against a load equivalent to 60% of their maximal sustainable inspiratory pressure (which represented approximately 40-50% of the initial PI_{max}). The load could be increased depending on patient tolerance. Patients who received sham training breathed through the same inspiratory muscle training device with no additional load.

2.5 STATISTICAL ANALYSIS. Values throughout the text and tables are expressed as mean±SD. Baseline and post-training data were compared within groups using the non-parametric (Wilcoxon) tests for paired samples. The Mann-Whitney U-test was used to compare data between groups. An alpha (p) value ≤ 0.05 was considered statistically significant.

3. RESULTS.

3.1 General characteristics, lung function and exercise capacity. Sixteen patients were initially recruited for the study. Two of them were excluded due to pulmonary infection at the beginning of the training period in one patient, and the other due to non-compliance with the training dates. Complications derived from either the functional evaluations or muscle sampling were not detected in any patient. The general characteristics of the study population appear in table 1. Changes in conventional pulmonary function tests (table 2) or general exercise capacity were not found after training in any of the study groups (table 3, figure 2) .

3.2 Changes in the respiratory muscle function. The overall function of the diaphragm and accessory inspiratory muscles showed significant changes after training in the inspiratory muscle training group as expressed by an increase, firstly, in the inspiratory muscle strength (PI_{max}, Pes_{max}, and Pdi_{max}), and secondly, in the inspiratory muscle endurance (table 3). Neither the breathing pattern nor strength of the expiratory muscles (PE_{max}) showed differences after training (table 3).

3.3 Structure of external intercostal muscles. Prior to training, the size of external intercostal fibers were found to be 47 ± 8 μm and a total of 46 ± 18 % of the fibers expressed type I MyHC. The inspiratory muscle training clearly associated with structural changes in the muscle as assessed by both fiber type distributions and fiber size. Specifically, both the proportion of type I fibers ($p<0.05$) and the size of type II fibers ($p<0.05$) increased following training (table 4, figure 2). In contrast, no structural changes were observed in the sham training group (table 4).

3.4 Structure of the *vastus lateralis* muscles. Prior to training, the mean size of *vastus lateralis* fibers was found to be 57±10 μm. A total of 30±11 % of the fibres expressed type I MyHC. The inspiratory muscle training did not promote structural changes within the *vastus lateralis* as assessed by fiber size or fiber type distributions (table 5).

4. DISCUSSION.

The present study is the first to evaluate structural changes in the respiratory muscles of COPD patients after a specific program of respiratory muscle training. Significant increases were observed in both the proportion of type I fibers (by≅38%) and the size of type II fibers (by≅21%) of the external intercostal muscles following the training period. These findings demonstrate that the external intercostal muscles of severe COPD patients have the capacity to express structural remodelling. Functional improvement induced by the inspiratory muscle training (in terms of both inspiratory muscle strength and endurance) could be explained in part by structural adaptation within the inspiratory muscles.

Justification of the study. The present study was aimed at evaluating the effects of a specific inspiratory muscle training on the structure of inspiratory muscles in community-based COPD patients. This could be controversial regarding the current state of respiratory muscle adaptations. In fact, the diaphragm of non-trained COPD exhibit structural changes which presumably represent "adaptive effects". Studies from Levine et al.[30], Mercadier et al.[31] and one study from our group[32] demonstrate that the diaphragm shows an increase of type I fibers, MyHC-I and mitochondria (supporting a fiber type transformation), whereas the length of the

sarcomere decreases (supporting adaptation to chronic diaphragm flattening).[32] A reasonable presumption is that other primary respiratory muscles (such as the external intercostals) could exhibit similar adaptations. From a clinical point of view, however, such structural adaptations only appear to partially restore their functional capacity. In fact, inspiratory muscles of COPD patients show a lower capacity to generate maximal pressures (strength) or tolerate submaximal inspiratory loads (endurance).[4] This muscle dysfunction could be explained by the coexistence of other factors[33,34,35] with deleterious effects such as 1) geometric changes of the thorax due to increased lung volume and shortening of the diaphragm fibers, 2) intrinsic changes within the muscles due to malnutrition, ionic or arterial gas disorders, or 3) the effect of drugs or concomitant diseases or conditions (e.g., aging). Altogether, these arguments have prompted several investigators to hypothesise that inspiratory muscle training could offer clinical benefits to severe COPD patients. However, the results of previous studies have been controversial.

Functional changes following the inspiratory training. *General exercise.* Some authors have demonstrated that inspiratory muscle training may have a more general impact when tolerance is evaluated in terms of exercise capacity, endurance time on a treadmill, or dyspnea[10,36,37,38, 39]. However, other studies[40,41,42,43] including the present one, were unable to

show any changes in either walking distance or maximal oxygen uptake. *Respiratory muscle function*. Whereas some studies have reported that inspiratory muscle strength and endurance can improve with specific training[10,43], others have not found significant changes in inspiratory muscle function[44]. The present results are consistent with the former, since inspiratory muscle strength as well as inspiratory endurance was significantly increased after inspiratory muscle training. The controversy between previous studies regarding the effects of inspiratory training could be related to the differences in either the magnitude or duration of inspiratory muscle loading[45]. Taking this into consideration, specific inspiratory muscle training has been found to be capable of improving inspiratory muscle function when intensity is monitored and exceeds 20% of PI_{max}[46,47]. In addition, some studies have simultaneously included multidimensional intervention as part of COPD patient rehabilitation. From a methodological point of view, such assessment could make it difficult to independently analyse the specific effect of inspiratory muscle training.

Structural changes in the muscles following the inspiratory training. Size of external intercostal fibers was found to be within the normal range prior to specific training.[48] The most important contribution of the present study is that inspiratory muscle training induced structural changes within the trained muscles of stable COPD patients. A few experimental studies

have shown that the diaphragm and other inspiratory muscle show structural changes in animals submitted to inspiratory loads[11]. However, the authors have been unable to find any study evaluating structural changes in human inspiratory muscles after specific training.

The findings of the present study highlights three main concepts. Firstly, **muscle response.** The study demonstrate that external intercostal muscles of COPD patients preserve capacity to be remodelled (conditioned) following a short-term loading period. The muscles exhibited a classical response to training that would be predictable in limb muscles. Similar findings have been described in the peripheral muscles of COPD patients following general muscle training[49]. The novelty of the present report is that this response is demonstrated in an inspiratory muscle group from patients with severe airflow obstruction. Secondly, **functional and structural changes.** The results allow us to hypothesise that the increase in inspiratory muscle endurance and strength after specific training could be related to the observed switching of MyHC isoforms (as assessed by the increase in fibers expressing MyHC-I) and the increase in fiber size (mainly in type II fibers). Other factors such as perceptual adaptation to additional inspiratory loading (e.g., dyspnea desensitisation), learning of specific manoeuvres, or even a placebo effect could have also participated in improving inspiratory muscle strength and

endurance. And thirdly, **specificity.** We found that inspiratory muscle training had specific functional and structural effects only on the trained (inspiratory) muscles. The study included data from an unaffected (limb) muscle as a negative control. No changes were observed in either the fiber size or fiber type proportions of the *vastus lateralis* (control muscle) when pre-training and post-training results were compared. On the same line, a transfer effect to other respiratory muscle groups (e.g., changes in function of the expiratory muscles) was not found. These facts support the conclusion that inspiratory training has a specific effect only on the trained muscles and allow us to hypothesise that structural adaptation occurred only in the inspiratory muscles. However, the design of the study does not permit total confidence in the structural changes within the external intercostals being representative of inspiratory muscles in general. The diaphragm is the most important of the inspiratory muscles but there are obvious ethical and practical difficulties in repeatedly accessing the diaphragm from either healthy subjects or stable COPD patients even in spite of thoracotomy for other reasons (e.g., lung volume reduction, lung cancer or transplant).

The principal limitations of the present study are the relatively small number of subjects studied and the possibility that the results could be related to regional differences in the external intercostal muscles within a given volunteer. The authors felt that a greater number of volunteers

was not essential because the results reached statistical significance from both structural and functional points of view, as well as the study using relatively high invasive procedures. The results do not appear to be related to regional differences within the external intercostal muscles. A previous study demonstrated that biopsies from the external intercostal muscles taken from different anatomic places are comparable in COPD patients even when the samples were taken from either the dominant or non-dominant side[50]. In addition, we feel that anatomical knowledge and experience allow us to ensure that muscle samples came from the external intercostals in all cases. The experience is the result of both anatomical reviews and practical evidence. At least nine arguments should be highlighted: 1) the technique is an "open" biopsy, in contrast to "blind" needle techniques, 2) we have reviewed and we have enough knowledge of the specific body zone, 3) we have practised in cadaver models, 4) we normalised the same body place to obtain the muscle biopsies, 5) we performed a sequential and careful dissection technique with identification of all tissue plans (fascia, muscle), 6) we discarded a potential error by taking biopsies from the pectoralis because the "anatomical window" to access the external intercostals is below and relatively far from the lower insertions of the pectoralis muscles 7) the biopsy was taken from the intercostal space, 8) we identify the orientation of the muscle fibers which are

clearly different for external in comparison to internal intercostals, and 9) due to intimate contact between internal intercostals with the pleura, the absence of yatrogenic pneumo-thorax confirm that such muscles were not sampled.

Clinical relevance. This is the first report demonstrating that structural changes occur in respiratory muscles of COPD patients following specific training in association with improvement of the inspiratory muscle function. The authors feel that this evidence offers additional information, improves the knowledge of the physiological basis of inspiratory muscle training, and reinforces the inclusion of inspiratory muscle training as a part of pulmonary rehabilitation. The results are innovative and could not be directly extrapolated neither from previous animal studies (because many factors present in COPD do not exist in animal models), nor previous studies assessing the effects of peripheral muscle training (because pre-training phenotype is completely different to that reported on the respiratory muscles). Our selection criteria excluded those patients showing malnutrition, respiratory failure, hypercapnia, or systemic steroid treatment, which are features usually found in COPD patients. However, these conditions would introduce confounding factors to the study. Further studies evaluating the potential effect of inspiratory muscle training in patients with these associated conditions, time course of the muscle remodelling process and the minimal

external loading capable of muscle adaptation seems to be warranted.

CONCLUSIONS. The present study demonstrates that the external intercostal muscles of COPD patients have the capacity to express structural remodelling following specific inspiratory training. Both the proportion of type I fibers and the size of type II fibers were found to increase following training. These structural adaptations could explain, in part, the functional improvements observed in the trained muscles (increased inspiratory muscle strength and endurance) after specific training.

ACKNOWLEDGEMENTS.

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TABLE 1.

GENERAL CHARACTERISTICS OF STUDY POPULATION

	SHAM TRAINING GROUP	INSPIRATORY TRAINING GROUP
n,	7	7
Age, years	66±6	65±5
Body mass index, kg/m^2	26±4	29±4
Cholesterol, mg%	229±37	235±50
Serum proteins, gr%	7,5±3	7±0,8
Albumin, gr%	4,5±0,2	4,3±0,4
Prothrombin consumption time, s	105±12	104±7

Results appear as mean ± S.D.

TABLE 2

PULMONARY FUNCTION TESTS OF STUDY SUBJECTS

	SHAM TRAINING GROUP		INSPIRATORY TRAINING GROUP	
	Pre	Post	Pre	Post
N,	7	7	7	7
FEV_1, l	836± 184	913±185	974±312	997±341
FEV_1, %pred	27±7	29±7	33±8	34±11
TLC, l	6,8± 7,8	6,7±8,9	6,3±1,5	6,2±1,3
TLC, %pred	115± 7	115±17	112±22	111±19
RV, %pred	190±18	179±32	177±46	179±52
PaO_2, torr	68±5	66±5	68±7	72±10
$PaCO_2$, torr	47±5	46±4	43±7	44±4

Abbreviations: (FEV_1): forced expiratory volume in the first second; (TLC): total lung capacity; (RV): residual volume; (PaO_2, $PaCO_2$): arterial O_2 and CO_2 partial pressure.

TABLE 3

CHANGES IN RESPIRATORY MUSCLE FUNCTION AFTER SPECIFIC INSPIRATORY MUSCLE TRAINING

	SHAM TRAINING GROUP		INSPIRATORY TRAINING GROUP	
	Pre	Post	Pre	Post
INSPIRATORY MUSCLE STRENGTH				
PI_{max}, cmH_2O	77± 9	79± 10	77±22	99±22 *
PI_{max}, %pred	74 ±7	76±7	69±19	90±20 *
Pes_{max}, cmH_2O	-55±17	-58 ±16	-49±16	-74±19 *
$Pdi_{max,}$ cmH_2O	91±24	90±13	74±19	110±23 *
PT_I, index (at rest)	0,06±0,03	0,04±0,04	0,07±0,02	0,04±0,01
INSPIRATORY MUSCLE ENDURANCE				
$Pth_{max,}$ cmH_2O	-39±21	-41±20	-30±19	-39±22 *
Tth_{80}, min	9,3±4	9,2±2	11±6	22± 6 *
EXPIRATORY MUSCLE STRENGTH				
PE_{max}, cmH_2O	147±31	145±28	146±24	144±30
PE_{max}, %pred	77±8	76±12	82±13	81±16
SIX MINUTE WALKING TEST				
Distance, m	429±115	407±114	445±63	433±81
INCREMENTAL CYCLE TEST				
Work rate, $Watt_{max}$	91±25	82±23	79±17	86±23
VO_2max, ml/kg/min	14±2	12±2	16±3	15±5

Abbreviations: (PI_{max}): maximal inspiratory pressure measured at the mouth; (Pes_{max}): oesophageal pressure during maximal (sniff) manoeuvre; (Pdi_{max}): Transdiaphragmatic pressure during maximal (sniff) manoeuvre; (PT_I): pressure-time product for inspiratory muscles during tidal breathing; (Pth_{max}): maximal sustainable inspiratory pressure during progressive inspiratory

threshold loading; (Tth_{80}): elapsed time breathing under constant submaximal inspiratory loads (80% Pth_{max}).
(*): $p<0.05$ when compared with pre-training period.

TABLE 4

HISTOMORPHOMETRY OF THE EXTERNAL INTERCOSTAL MUSCLES PRIOR TO AND FOLLOWING SPECIFIC INSPIRATORY MUSCLE TRAINING

	SHAM TRAINING GROUP		INSPIRATORY TRAINING GROUP	
	Pre	Post	Pre	Post
GLOBAL FIBER SIZE				
Least diameter, µm	47±9	51±10	47±8	55±6 p=0,09
CSA, μm^2	3,08±1,25	3,27±1,28	2,73±0,81	3,88±0,48 *
TYPE I FIBERS				
Proportion, %	50±14	47±16	42±20	58±14 *
CSA, $\mu m^2 \times 10^3$	2,60±0,94	2,94±1,24	2,92±1,39	3,72±0,68 p=0,06
Least diameter, µm	44±7	49±9	49±10	55±10
S.D., µm	6±1	7±1	8±1	10±3
TYPE II FIBERS				
Proportion, %	50±14	53±16	57±20	42±14 *
CSA, $\mu m^2 \times 10^3$	3,67±1,47	3,58±1,32	2,82±0,91	4,06±0,86*
Least diameter, µm	50±12	53±11	47±10	57±8 *
S.D., µm	7±2	8±1	9±2	11±2

Abbreviations: (CSA): cross sectional area; (S.D.): standard deviation of mean least fiber diameters; (*): p value < 0.05 when compared with pre-training evaluation.

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TABLE 5

HISTOMORPHOMETRY OF THE *VASTUS LATERALIS* (CONTROL) MUSCLE PRIOR TO AND FOLLOWING SPECIFIC INSPIRATORY MUSCLE TRAINING

	SHAM TRAINING GROUP		INSPIRATORY TRAINING GROUP	
	Pre	Post	Pre	Post
GLOBAL FIBER SIZE				
Least diameter, µm	58±11	56±14	56±8	60±16
CSA, $\mu m^2 \times 10^3$	2,67±1,06	2,56±1,24	2,44±0,74	2,46±0,65
TYPE I FIBERS				
Proportion, %	33± 10	29 ±18	27±11	21±10
CSA, $\mu m^2 \times 10^3$	2,52 ±1,35	2,47±1,12	2,45±0,49	3,09±1,51
Least diameter, µm	55± 14	54 ±13	56±6	61±14
S.D., µm	8 ±2	9 ±2	11±5	10±4
TYPE II FIBERS				
Proportion, %	67±8	72±16	74±10	79±9
CSA, µm2 × 103	2,72±1,04	2,50±1,13	2,37±0,87	2,36±0,80
Least diameter, µm	58±11	55±13	54±10	60±17
S.D., µm	10±1	11±3	10±4	16±2

Abbreviations: (CSA): cross sectional area; (S.D.): standard deviation of mean least fiber diameters. Difference between pre- and post-training period did not reach significance in any of the analysed variables.

FIGURE LEGENDS.

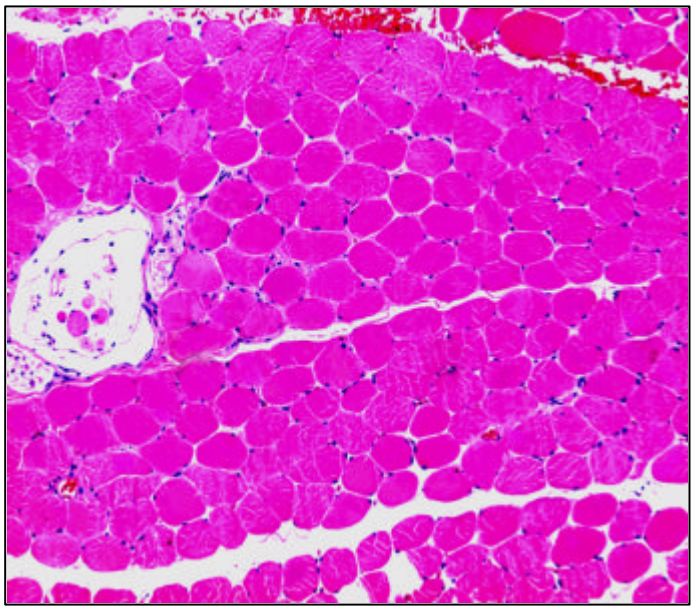
FIGURE 1. Microphotographs of consecutive cross sections of external intercostal muscle processed by conventional hematoxilin (figure a) and immunohistochemical techniques. For the latter, monoclonal antibodies directed against myosin heavy chain (MyHC) isoform type I and type II were used (figure b and c, respectively). For details, see text.

FIGURE 2. Microphotograph showing immunohistochemical staining using anti-MyHC-I monoclonal antibody for fiber typing and morphometric analyses in cross-sectional sections from the external intercostal muscles of a patient who received specific inspiratory muscle training. In this figures, dark fibers correspond to fibers expressing MyHC type I, whereas light fibers indicate fibers expressing another MyHC phenotype. For details, see text. *Abbreviations: (Pre- and Post-): pre- and post-training biopsy, respectively.*

FIGURE 3. Main changes in both structural and functional variables expressed as the difference (in absolute values) between initial *versus* final values. Dark bars represent the values from inspiratory muscle training group, whereas the light bars represents the values from the sham training group. Abbreviations: *(*): p Value < 0.05 when compared with the intragroup initial value; (n.s.): non-significant difference.*

FIGURE 1

a



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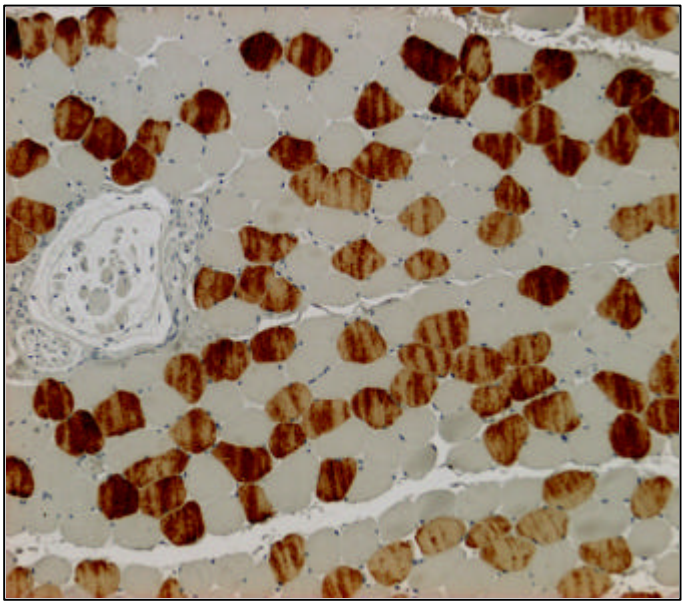


c

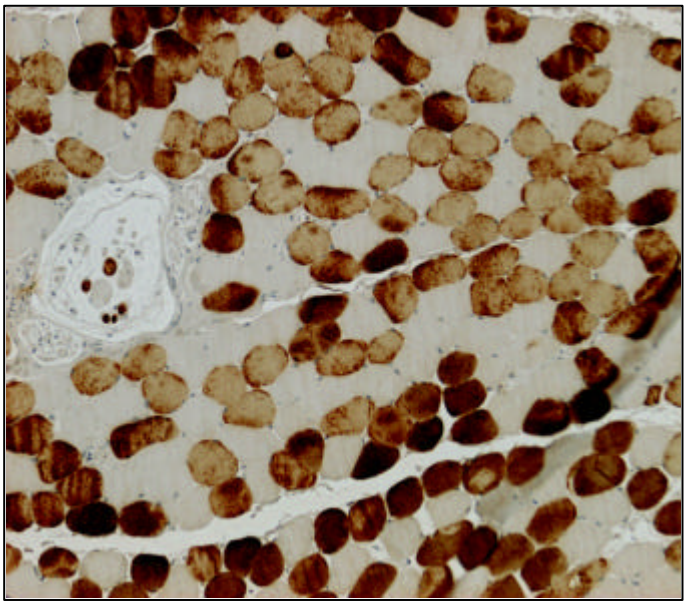


FIGURE 2

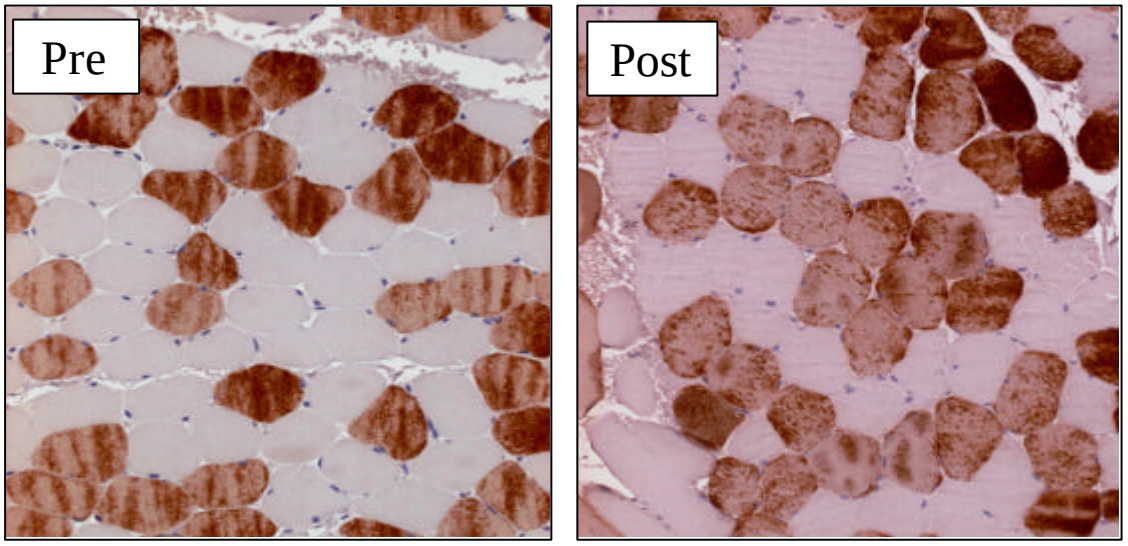
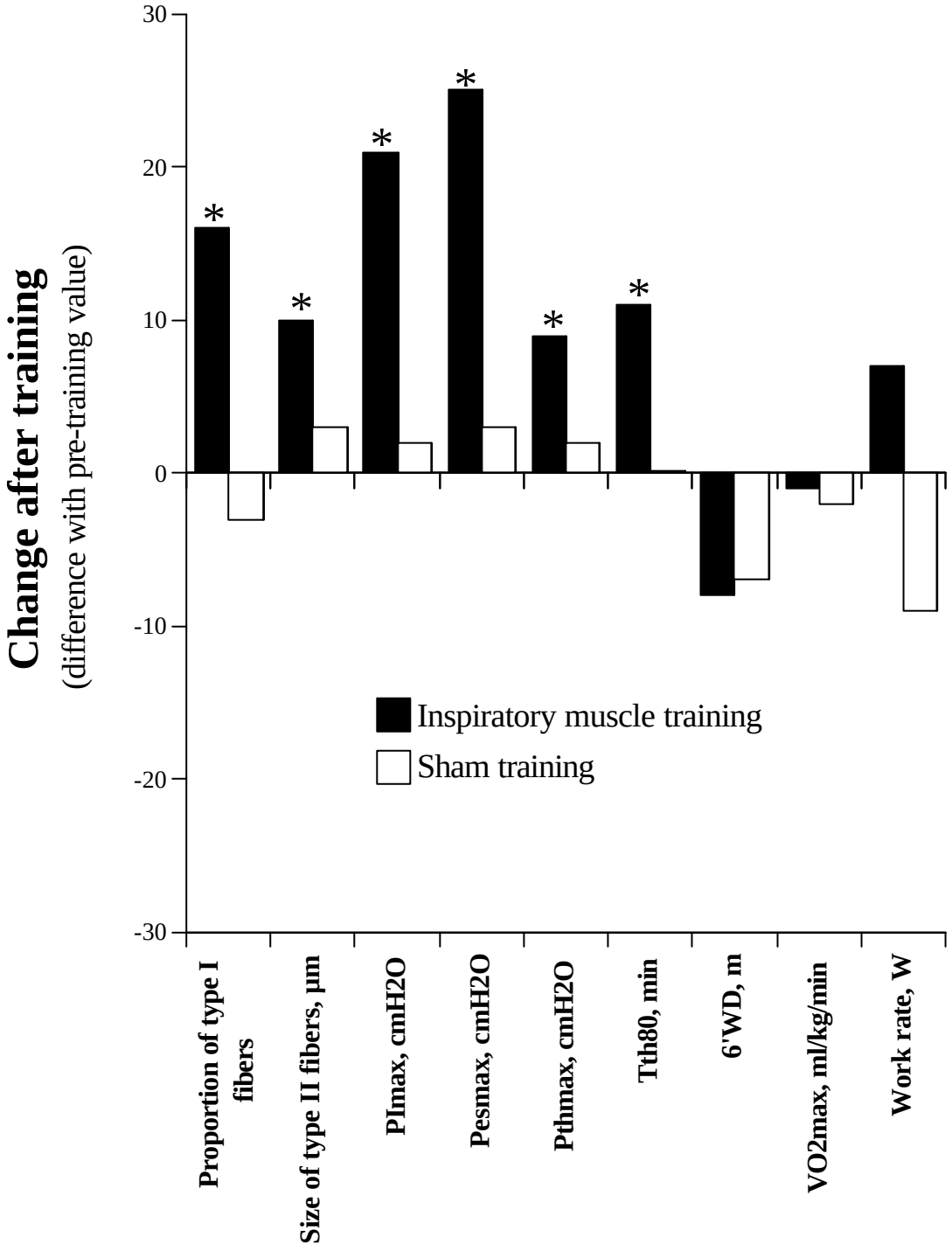


FIGURE 3


Change after training
(difference with pre-training value)
30
20
10
0
-10
-20
-30
*
Inspiratory muscle training
Sham training
Proportion of type I fibers
Size of type II fibers, µm
PImax, cmH2O
Pesmax, cmH2O
Pthmax, cmH2O
Tth80, min
6'WD, m
VO2max, ml/kg/min
Work rate, W

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