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**TITLE:**

**BLUNTED MUSCLE ANGIOGENIC TRAINING-RESPONSE IN COPD PATIENTS  
VERSUS SEDENTARY CONTROLS**

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**RUNNING HEAD: BLUNTED MUSCLE ANGIOGENIC RESPONSE IN COPD**

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## **ABSTRACT**

The impaired skeletal muscle of chronic obstructive pulmonary disease (COPD) patients reduces exercise capacity. Similar to the oxidative muscle fibers, the angio-adaptation of muscle to training may be blunted in these patients, as in other chronic conditions. We therefore compared muscle functional responses and angio-adaptations after training in COPD patients and sedentary healthy subjects (SHS).

Twenty-four COPD patients [ $FEV_1$  (in %pred.):  $45.6 \pm 17.5$ ] and 23 SHS (<150 min/week of moderate-to-vigorous exercise) completed a 6-week rehabilitation program based on individualized moderate-intensity endurance training. Histomorphological muscle analysis and measurements of pro-angiogenic vascular endothelial factor-A (VEGF-A) and anti-angiogenic thrombospondin-1 (TSP-1) were conducted before and after training.

COPD patients and SHS showed improved symptom-limited oxygen consumption and muscle endurance, although improvements were lower in COPD patients ( $+0.96 \pm 2.4$  vs.  $+2.9 \pm 2.6$  mL/kg/min,  $p < 0.05$  and  $+65\%$  vs.  $+108\%$ ,  $p = 0.06$ , respectively). The capillary-to-fiber (C/F) ratio increased less in COPD patients than SHS ( $16 \pm 10\%$  vs.  $37 \pm 20\%$ ,  $p < 0.05$ ) and no fiber type switch occurred in patients. The VEGF-A/TSP-1 ratio increased in COPD patients and SHS ( $+65\%$  vs.  $+35\%$ ,  $p < 0.05$ ). Changes in C/F and  $VO_{2SL}$  were correlated ( $r = 0.51$ ,  $p < 0.05$ ). In addition to a lack of fiber switch, COPD patients displayed a blunted angiogenic response to training.

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**KEY-WORDS:** exercise testing, chronic diseases, muscle dysfunction in COPD (myopathy), vascular remodeling, rehabilitation programs, remodeling

## **INTRODUCTION**

Skeletal muscle dysfunction has substantial implications in many chronic diseases [chronic heart failure (CHF), diabetes, peripheral arterial disease [1], obesity, etc.]. In chronic obstructive pulmonary disease (COPD), the skeletal muscle dysfunction affects the patient's prognosis, exercise tolerance, and health-related quality of life [2]. This muscle impairment is characterized by cellular changes, with decreases in the proportion of type I oxidative fibers, oxidative enzymes [3], and capillaries [4]. In particular, the alteration in the capillary supply [4] is well-correlated with the impaired muscle function and aerobic exercise capacity of these patients.

The impaired skeletal muscle of COPD patients has been associated with physical inactivity and, although exercise training is the most efficient therapy for the skeletal muscle dysfunction in these patients [5], it has never been able to fully reverse this dysfunction [6]. Systemic factors may explain this incomplete recovery [2], but a lack of muscle adaptation at the cellular level has also been incriminated. For example, several studies have shown no increase in the type I fiber proportion after exercise training in the quadriceps of COPD patients [7-10], in contrast to the observations in healthy subjects [11].

Consistent with the role of capillarization in muscle function, it is possible that impaired training-induced angiogenesis would also explain the incomplete muscle function recovery in COPD patients. Because exercise training significantly increases muscle capillarization in these patients [7, 8, 12], it could be hypothesized that the angiogenic response of muscle to training is present but blunted in COPD, as in other chronic conditions with similar skeletal muscle impairment (diabetes [13] and CHF [14]). Unfortunately, no study has ever compared the training-induced capillarization in COPD patients versus sedentary healthy subjects

(SHS). Moreover, the impact of COPD on the training-induced expression of the main molecular factors controlling skeletal muscle angio-adaptation, such as pro-angiogenic vascular endothelial growth factor-A (VEGF-A) or anti-angiogenic thrombospondin-1 (TSP-1), remains unknown.

Therefore, the aim of our study was to determine whether COPD patients experience a specific alteration in their muscle angio-adaptive capacity. To do so, we compared functional responses, myofiber remodeling, capillarization, and the main angiogenic factors after a moderate-intensity training program in COPD patients and sedentary healthy subjects with the same pattern of physical inactivity.

## **MATERIALS AND METHODS**

See additional information and references in the online supplement.

### **Study population**

From March 2008 to February 2009, we recruited 24 SHS (age from 50 to 75 years) with no disease and less than 150 minutes of moderate-to-vigorous physical activity per week (corresponding to the recommended threshold for health improvement [15]) and 24 patients with COPD defined by dyspnea, chronic cough or sputum production and/or a history of exposure to risk factors for the disease, and post-bronchodilator FEV1/FVC <70% [16]. Exclusion criteria included other respiratory diagnosis, decompensated comorbidity, exacerbation in the last 3 months, and previous participation in an exercise training program. All subjects and patients performed the tests at INSERM U-1046, CHRU Montpellier, France, or “La Solane” and “La Vallonie” Pulmonary Rehabilitation Centers in Osseja and Lodève, France, respectively. Informed written consent was obtained from all subjects, and the

research protocol was approved by the institutional ethics committee of the Montpellier University Hospitals (n° 2008-EESSS-V2 and n°2009-04-BPCO-V2) and conducted in accordance with the Helsinki declaration and the European guidelines for “good clinical practice.

### **Physical activity**

In order to assess the physical activity (PA) of our sedentary-selected healthy population, we used the Voorrips questionnaire (modified Baecke’s questionnaire), a PA questionnaire validated and used in this indication [17]. “Objective” PA level was assessed in 20 COPD patients and 20 SHS who wore a triaxial accelerometer (Tritrac RT3 Research Tracker, Stayhealthy, Monrovia, CA, USA), validated in COPD [18], for 7 consecutive days.

The QUANTAP interview-administered survey is a computer-assisted tool designed to determine PA over a lifetime in 4 dimensions (sports at school, leisure sports, occupation, and daily activities). This questionnaire is reliable to assess lifetime PA and has been validated for use in elderly French subjects and in the context of various pathologies, recently in COPD [19].

### **Pulmonary function tests**

All subjects underwent whole body plethysmography (Transmural Bodybox 2800; Sensomedics, Yorba Linda, CA, USA). The values were compared with normal values [20] (see online supplement).

### **Exercise testing and muscle function assessment**

The 6-minute walking test (6MWT) was performed in a 30-meter corridor. The distance walked during the test (6MWD) was compared with the reference values [21]. Participants performed an incremental test on an electrically-braked cycle ergometer (Ergoselect 200P,

Ergolyne, Bitz, Germany) to determine symptom-limited oxygen consumption ( $\text{VO}_{2\text{SL}}$ ) and the ventilatory threshold [22] (see online supplement). If the ventilatory threshold could not be detected, we assessed the dyspnea threshold by rating breathlessness every minute of the test, using the patient's own vocabulary (see online supplement) [23] (see online supplement). The quadriceps strength and endurance were measured by isometric maximal voluntary contraction (qMVC) and endurance time (T.lim). Fat-free and muscle mass were estimated with multi-frequency bioelectrical impedancemetry (QuadScan 4000, Bodystat, Isle of Man, UK). Additional information is provided in the online supplement.

### **Exercise training**

The exercise training sessions were part of a multi-component and comprehensive pulmonary rehabilitation course, including an education program [5]. Twenty sessions of endurance exercise were conducted 3 or 4 times per week for 6 weeks. The exercise intensity was set as the heart rate at the ventilatory or dyspnea threshold [5, 22, 23]. Each session lasted 45 minutes, and in 1 out of 2 sessions, was followed by 30 minutes of strength-building exercise performed at 40% of the one-repetition maximum (1-RM). All sessions were supervised by an experienced clinician, and the training intensity was increased during the training protocol.

### **Muscle biopsy analysis**

Muscle biopsies were performed in the vastus lateralis of the quadriceps before the exercise training program and 48 hours after the last training session [24].

### **Muscle immunohistochemistry**

Muscle fiber type and mean cross-sectional area (CSA) were assessed after immunohistochemistry on frozen sections (10- $\mu\text{m}$  thickness) from the muscle biopsies, using



antibodies specific to each myosin heavy chain (MHC) isotype (University of Iowa, IO, USA) [4, 25]. Capillaries were visualized by immunochemistry with the monoclonal antibody against CD31 (BD Bioscience, #550389, Franklin Lakes, NJ, USA). CSA, capillary density and the capillary-to-fiber ratio were determined after counting capillaries and myofibers on 1 to 2 cryosections from each muscle biopsy (see online supplement).

### **Western blotting**

Blots were probed for vascular endothelial growth factor-A (VEGF-A, clone VG-1, Millipore #05-1117, Etobicoke, ON, Canada) and thrombospondin-1 (TSP-1, clone A6.1, Invitrogen #399300, Burlington, ON, Canada).  $\beta$ -actin protein was also measured as our loading control (see online supplement).

### **Statistical analysis**

Data are presented as mean  $\pm$  SD. Distribution normality was tested by the Kolmogorov-Smirnov test. Pearson coefficients describe the correlations. We used a multiple linear regression model. Comparisons of the training effect between groups were performed by mixed linear regression modeling [26]. Each patient/subject was evaluated twice (repeated measures). Thus, measured variables were adjusted using a linear mixed-effects model for repeated measures to take into account the repeated measures as random variables. Patient group and time of measure were used as fixed effects in the model. The group effect was then tested with the mixed-linear model. A p-value of  $<0.05$  was considered statistically significant. Data were analyzed using R.2.13.0 software.

## **RESULTS**

### *Baseline characteristics of the COPD patients and SHS*

All the SHS had a Voorrips score below 9.4, indicating that they were all sedentary. The COPD patients and SHS were well-matched according to age and physical activity (PA) level (Table 1). Moreover, there was no significant difference between the COPD patients and SHS for their PA level over the past 15 years (in metabolic equivalents:  $14949 \pm 9470$  vs.  $12341 \pm 5201$ ,  $p=0.26$ ). At the morphological level (Figure E1, online supplement), COPD muscle presented a significantly lower proportion of type I fibers compared with that of SHS (Table 2). Additional details are provided in the online supplement.

### *Exercise training-induced functional and histomorphological adaptations*

Exercise training was performed at the intensity of the ventilatory or dyspnea ( $n=6/24$  COPD patients) threshold (mL/kg/min and % of predicted  $VO_{2SL}$ ) in COPD patients and SHS:  $12.7 \pm 1.9$ ,  $48.7 \pm 11.4\%$  and  $15.4 \pm 3.5$ ,  $63.5 \pm 10.1\%$ , respectively;  $p < 0.001$ . Expressed in % of maximal heart rate, the relative intensity was not significantly different in COPD patients and SHS ( $66 \pm 6.8\%$  and  $69 \pm 6.3\%$ , respectively,  $p=0.27$ ). Expressed in % of  $VO_{2SL}$ , the relative intensity was slightly higher in COPD patients vs. SHS ( $66 \pm 6\%$  vs.  $60 \pm 5\%$ , respectively;  $p=0.05$ ). Relative improvements for all functional parameters are shown in Figure 1. The training significantly improved 6MWD (m) in both groups (COPD:  $+37 \pm 35$  and SHS:  $+31 \pm 35$ ,  $p < 0.001$ ; Figure 1). The  $VO_{2SL}$  showed significant interaction between group and time ( $p < 0.05$ ). Last, a significant increase in T.lim (s) occurred in both groups (COPD:  $+121 \pm 169$  and SHS  $+334 \pm 274$ ,  $p < 0.001$ ), with an interaction between group and time that almost reached significance ( $p=0.06$ ).

Representative changes in type I fiber proportions in COPD patients and SHS are depicted in Figure 2A. The type I fiber proportion was significantly increased in the SHS group

(+8±11.6%,  $p<0.01$ ; Figure 2C) and was mirrored by a decrease in the percentage of type IIx fibers (-8±10.0%,  $p=0.001$ ). Conversely, no significant change occurred in the COPD group (Figure 2B). We observed a reduction in fiber CSA in COPD patients (5107±1375 to 4565±1477  $\mu\text{m}^2$ ,  $p<0.05$ ) and no significant change in the SHS group (4409±1679 to 4629±1670,  $p=0.51$ ). Both type I and type IIa fiber CSA showed this reduction in COPD patients (5905±1588 to 5260±1493,  $p<0.05$ , and 5400±2012 to 4657±1460,  $p<0.05$ ).

#### *Exercise training-induced muscle angio-adaptations*

Capillaries were visualized after immunodetection of the endothelial marker CD31 (Figure 3A). In both groups, the exercise training stimulated angiogenesis, as reflected by the increase in the capillary-to-fiber ratio (COPD: 16±10% and SHS: 37±20%,  $p<0.001$ ; Figure 3B.). Yet there was a significant interaction between group and time ( $p<0.01$ ), indicating lower improvement in COPD patients. An improvement in capillary density was observed in both groups (COPD: 29±28% and SHS: 9±17%,  $p<0.01$ ), with no group-time interaction ( $p=0.11$ ).

Exercise training had no significant effect on the VEGF-A protein level in either healthy subjects or COPD subjects. TSP-1 protein expression decreased in response to exercise training in both groups ( $p<0.05$ ) with a 44% decrease observed in the COPD population (0.134±0.022 in pre-training vs. 0.075±0.013 in post-training,  $p<0.05$ ; Figure 4D). Interestingly, the VEGF-A/TSP-1 ratio was significantly increased by exercise training in both the COPD and SHS groups (+65% and +35%, respectively,  $p<0.05$ ; Figure 4E), in favor of an angiogenic response.

#### *Angiogenesis-function relationships*

At baseline, the capillary-to-fiber ratio was significantly and positively correlated with  $VO_{2SL}$  and  $qMVC$  in COPD patients and SHS ( $r=0.60$ ,  $p<0.01$  and  $r=0.67$ ,  $p<0.01$ , Figures 5A and 5B). The capillary density was not significantly correlated with the type I fiber proportion ( $p=0.07$ ). The absolute training-induced changes in the capillary-to-fiber ratio was significantly correlated with the changes in  $VO_{2SL}$  in both groups ( $r=0.51$ ,  $p<0.05$ ; Figure 5C).

## **DISCUSSION**

The major finding of our study was that the skeletal muscle in COPD patients showed a blunted response to training in terms of muscle capillarization response, which was linked with the lower functional improvements. We also observed an increase in the VEGF-A/TSP-1 ratio in the two groups, in favor of an angiogenic process. Last, a lack of fiber type switch response to training was observed in the COPD patients compared with the age- and PA-matched healthy subjects.

### *Effect of the training stimulus*

Our program of exercise training at moderate intensity significantly increased the exercise capacity in both COPD patients and SHS. Additional details are provided in the online supplement. Interestingly, there was a significant interaction between group and time effects for  $VO_{2SL}$  and  $T_{lim}$ , indicating that the functional changes were lower in the COPD patients for these parameters. These lower functional improvements in COPD patients compared with SHS are compatible with the lack of exercise-induced muscle fiber type changes [27].

Regarding the exercise training intensity, all participants were trained at their own ventilatory (or dyspnea) threshold, which is a method for targeting the training stimulus to the individual muscle aerobic capacity [28]. The relative intensity was similar in terms of the percentage of

maximal heart rate, which is another classical method to target relative exercise intensity [5, 22]. Studying trained and untrained healthy subjects, it has appeared that the relative but not the absolute intensity was the determinant of the level of transcriptional activation of muscle genes in response to exercise training [29]. Greater muscle responses after continuous exercise performed at similar relative intensity (50 and 65% of the  $VO_{2SL}$ ) have even been observed in COPD patients compared with healthy subjects, especially for PGC-1 $\alpha$ , which is a master regulator of the fiber type switch [30]. Thus, in our study, it is very likely that at the muscle level, the training stimulus was at least similar in COPD patients and SHS.

#### *Muscle histomorphological and functional responses to training in COPD patients*

In agreement with previous works, the baseline level of muscle capillarization was lower in COPD patients [4, 7] and well-correlated with the muscle dysfunction and  $O_2$  consumption before training [4]. We observed an increase in the capillary-to-fiber ratio, which may indicate a training-induced improvement in the capacity to transfer  $O_2$  to myofibers in both COPD patients and SHS. Indeed, recent work has shown that the  $O_2$  transfer to the cell is mostly a function of the capillary-to-fiber interface [31], as reflected by the capillary-to-fiber ratio [32]. This was highlighted by many of our cryosections, which showed an increase in the length of the capillary contact and thus the length of the interface (Figure 3A).

Moreover, the increase in the muscle capillary-to-fiber ratio (i.e., the capillary-to-fiber interface, and thus the  $O_2$  transfer capacity) may have functioned as a master-driver of muscle  $O_2$  uptake and thus improved the endurance after moderate-intensity exercise training in the COPD patients. This would be consistent with evidence showing the critical role of  $O_2$  transfer to cells across capillaries in the maximal  $O_2$  uptake of muscle from healthy subjects [33]. Indeed, in our study, we found no change in the capacity of myofibers to consume  $O_2$ . In

contrast to SHS [34], COPD patients showed no shift toward more oxidative type I fibers [7, 12], and the preliminary data in our COPD patients (n=10) indicated no improvement in the maximal rates of oxygen consumption of permeabilized fibers (see Methods and Results in the online supplement). Last, the significant and positive correlation between the capillary-to-fiber ratio and the  $VO_{2SL}$  changes, as previously observed in healthy subjects [35], strengthens the credibility of a role for capillarization in the improvement of maximal  $O_2$  uptake in our COPD patients.

#### *Training-induced response of the angiogenic factors in COPD patients*

Angiogenesis is a complex and multi-step biological process tightly orchestrated by a balance between pro- and anti-angiogenic factors. Therefore, we chose to target key pro- and anti-angiogenic factors with a well-characterized clinical impact, i.e., VEGF-A [36] and TSP-1. The deletion of VEGF-A in the myofibers from transgenic mice dramatically reduced muscle capillarization and decreased exercise endurance time by approximately 80% [37]. Gene therapy targeting VEGF gene expression improved muscle capillarization [38]. TSP-1 is a large matrix glycoprotein whose action principally serves to inhibit angiogenesis [36]. Its clinical impact has been highlighted by increased muscle capillarity and 67% greater endurance in mice knocked out for the TSP-1 gene compared with wild-type [39]. Altogether, the VEGF-A/TSP-1 ratio has emerged as the most relevant marker of the muscle angio-adaptive balance [36]. After hindlimb unloading [40], an increase of the VEGF-A/TSP-1 ratio was observed, consistent with the capillarization increase. Thus, assessing the VEGF-A/TSP-1 ratio appears to be a more accurate approach than analyzing the expression of molecular factors individually [36]. In our study, we also observed a significant increase in this ratio in both groups, which indicates that the angiogenic balance in our patients shifted in favor of angiogenesis, consistent with the capillarization increase.

*Which mechanisms for the impaired muscle cellular responses to training in COPD patients?*

The defective muscle adaptation to exercise training in COPD patients was characterized by a lack of fiber type shift and a blunted capillarization response. Thus, we can hypothesize a pleiotropic patho-biological mechanism. In COPD, systemic factors (hypoxemia, oxidative stress or systemic low-grade inflammation – all enhanced during exercise) have already been incriminated in the peripheral muscle dysfunction [2]. Nonetheless, a molecular link between the cellular pathways regulating muscle angiogenesis and the fiber type cannot be eliminated.

*Clinical implications*

A key observation in our study was the link between reduced function (quadriceps T.lim and  $VO_{2SL}$ ) and capillarization. Moreover, our study indicates a probable role of the blunted muscle capillarization response to training in the reduced  $VO_{2SL}$  improvement in the COPD patients, as angiogenesis has also shown an impact on the improvement in  $VO_{2SL}$  in two other chronic diseases: CHF [41] and peripheral arterial disease [42]. Our finding of lower improvement in the capillary-to-fiber ratio thus appears to be clinically significant. The blunted angiogenic capacity in COPD muscle is consistent with observations in other chronic diseases with similar peripheral muscle dysfunction (diabetes [13] and CHF [14]). In diseases associated with lower limb ischemia, muscle angiogenesis may thus be a relevant target for therapeutic interventions designed to improve muscle capillarization [43]. However, although  $O_2$  transfer to muscle appears to be critical, there is no evidence of chronic lower limb ischemia in COPD patients [44]. Nonetheless, patients with hypoxemia and/or co-morbidities (vascular and/or diabetic) can experience chronic ischemia and in this case therapeutic interventions in association with exercise training might be an adequate approach.

In conclusion, exercise training increased the muscle capillarization in COPD patients, which might have driven the functional improvements. Although the most relevant parameter of the angio-adaptative balance (i.e., the VEGF-A/TSP-1 ratio) increased in favor of an angiogenic process in the COPD patients and SHS, the increase in capillarization was nevertheless significantly blunted in the patients. In addition to the lack of fiber type switch, reduced angiogenesis constitutes another feature of the altered muscle response to training in COPD patients.



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## FIGURE LEGENDS

Figure 1. Relative changes in functional parameters (%) in chronic obstructive pulmonary disease (COPD) patients (dark) and sedentary healthy subjects (SHS) (white) following exercise training. 6MWD (m): 6-minute walking distance in meters; 6MWD (%pred.): 6-minute walking distance in percentage of the predicted value; VO<sub>2SL</sub> (mL/kg/min): peak oxygen uptake in milliliters per kilogram and per minute; T.lim (s): endurance time in

seconds; qMVC: quadriceps maximal voluntary contraction in kilograms; BMI: body mass index (kg/m<sup>2</sup>); FFMI: fat-free mass index in kilograms. Data are presented as mean  $\pm$  standard error of the mean. Statistical differences: \* time effect,  $p < 0.001$ ; # group-time interaction,  $p < 0.05$ .

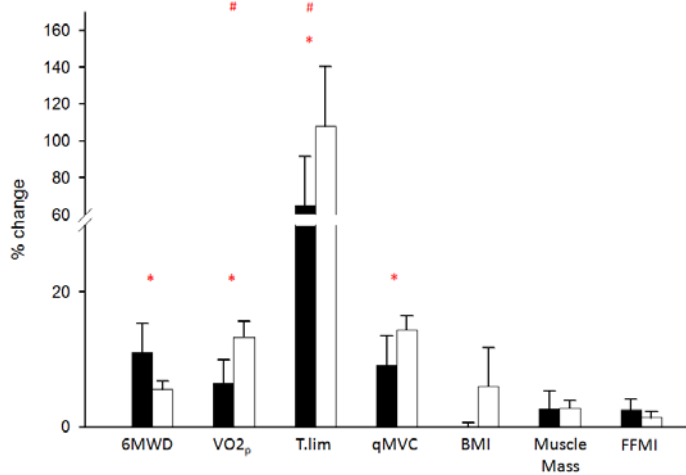


Figure 2. A) Representative transverse cryosections of the *vastus lateralis* stained with the type I myosin heavy chain isotype antibody at a magnification of  $\times 10$ , in a COPD patient and an SHS before and after exercise. Note the increase in the proportion of type I MHC fibers in the SHS. B) Proportion of the muscle fiber type (%) before (dark) and after (white) the exercise training program in COPD patients (n=21) and C) SHS (n=23). Data are presented as mean  $\pm$  standard error of the mean. \*  $p < 0.05$ , after exercise training.



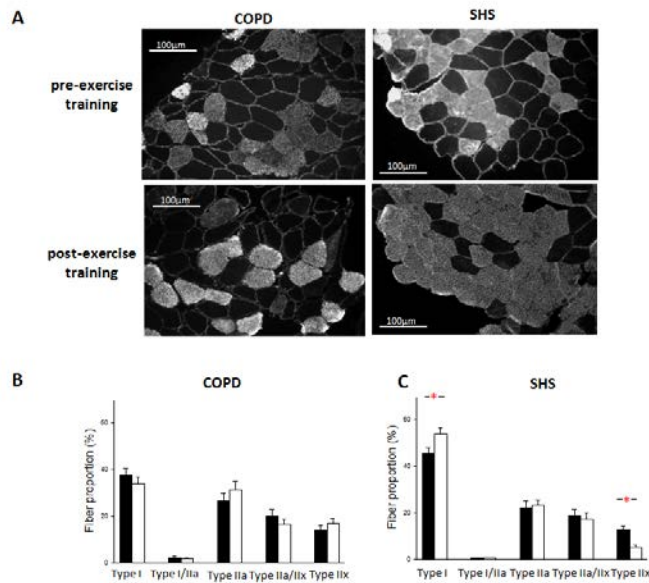


Figure 3. A) Representative pictures of *vastus lateralis* capillarization after staining for the endothelial marker CD31 at a magnification of  $\times 10$ , in a COPD patient and an SHS before and after exercise. Note the transverse capillaries surrounding the myofiber (black arrows) in pre-exercise training and the increased size of the capillary-to-fiber interface post-exercise training (dotted arrows). B) Mean values and standard error of the mean in COPD patients and SHS before (dark) and after (white) training for the capillary-to-fiber ratio. \*  $p < 0.05$ , after exercise training. #  $p < 0.05$ , between groups.

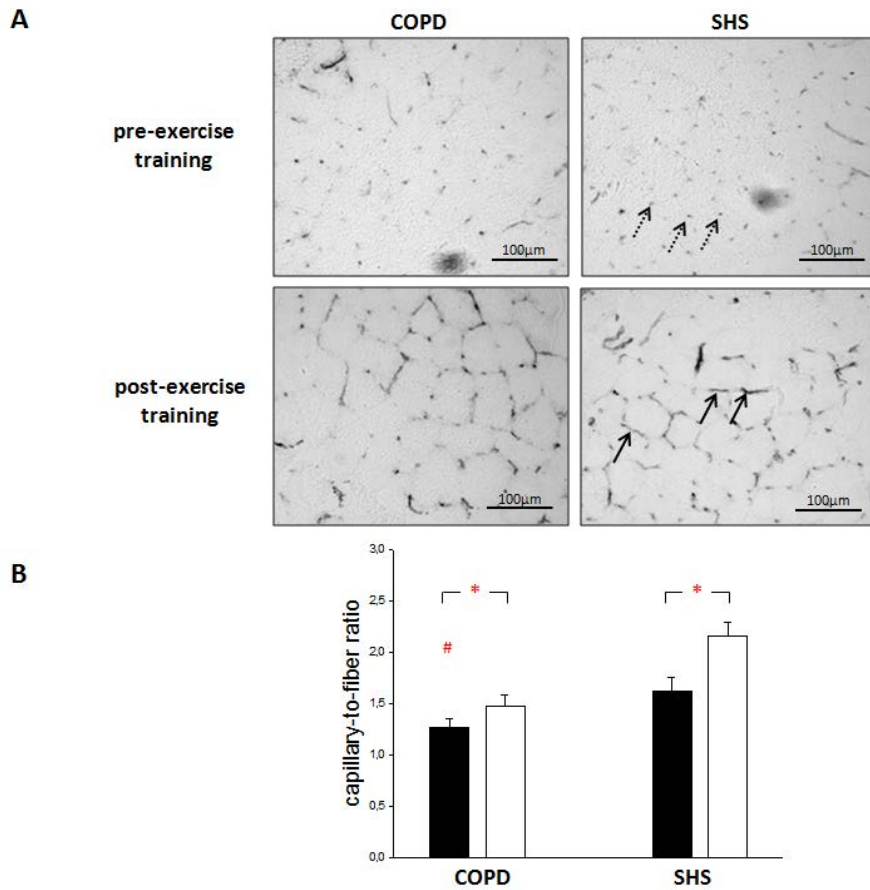


Figure 4. Representative Western blot of vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (TSP-1) (respectively, panels A and B) protein expression in the *vastus lateralis* of COPD patients and SHS before (dark) and after (white) training.  $\beta$ -actin is used as a loading control. Densitometry analysis of VEGF-A (C) and TSP-1 (D) protein and the VEGF-A/TSP-1 ratio (E) in the vastus lateralis of COPD patients and SHS before (dark) and after (white) training. Mean  $\pm$  standard error of the mean. Statistical difference after training: \*  $p < 0.05$ ; between groups (post-training): \*\*  $p < 0.05$ .

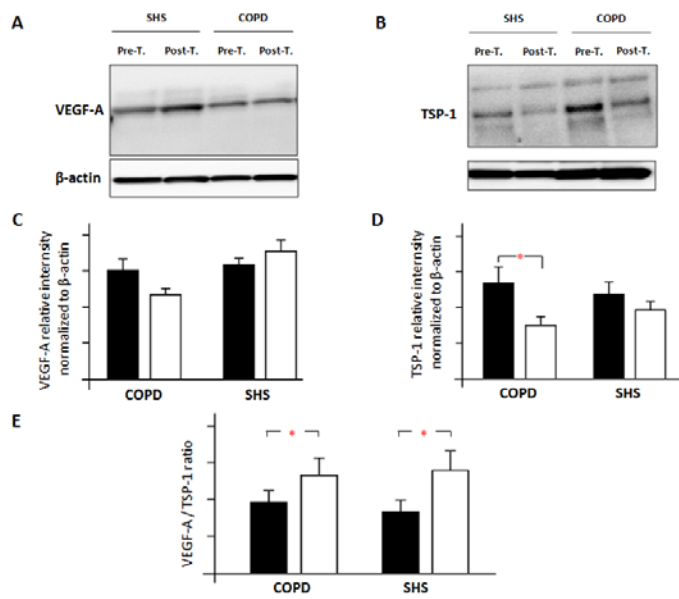
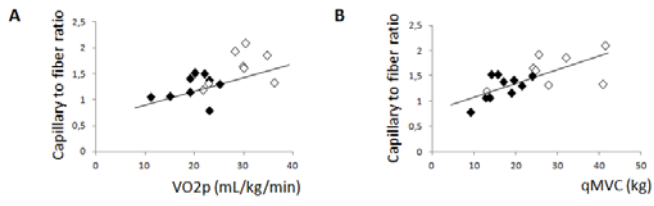
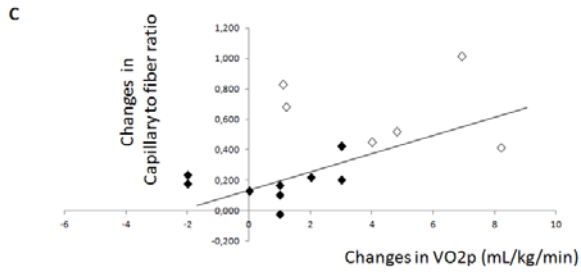


Figure 5. A) The capillary-to-fiber ratio was positively correlated with the peak oxygen uptake ( $VO_{2SL}$ ), expressed in milliliters per kilogram and per minute ( $r=0.60$ ,  $p<0.01$ ), in COPD patients (dark) and SHS (white), and B) with the quadriceps maximal voluntary contraction (qMVC) expressed in kilograms ( $r=0.67$ ,  $p<0.01$ ). C) The capillary-to-fiber ratio changes were positively correlated with the changes in peak oxygen uptake ( $VO_{2SL}$ ), expressed in milliliters per kilogram and per minute ( $r=0.51$ ,  $p<0.05$ ), in COPD patients (dark) and SHS (white).

**BASELINE**



**TRAINING-INDUCED CHANGES**



## TABLES

Table 1. Baseline clinical and functional characteristics of the chronic obstructive pulmonary disease (COPD) patients and the sedentary healthy subjects (SHS)

	COPD patients	SHS	<i>p</i>
n	24	23	1.00
Age (y)	60.8 ± 8.0	61.5 ± 5.7	0.756
Gender (M/F)	18/6	11/12	
FEV <sub>1</sub> /VC (%)	42.3 ± 8.9	75.8 ± 4.3	<0.001
FEV <sub>1</sub> (%pred.)	45.6 ± 17.5	106.0 ± 12.9	<0.001
VC (%pred)	90.0 ± 23.7	111.5 ± 14.3	<0.01
RV (%pred.)	183.8 ± 26.3	114.1 ± 34.6	<0.001
RV/TLC (%)	52.6 ± 12.2	37.9 ± 6.0	<0.01
FRC (%pred)	156.7 ± 22.2	109.7 ± 22.3	<0.001
PaO <sub>2</sub> (mmHg)	72.1 ± 10.1	-	
PA level (Voorrips score)	6.3 [2.5-7.9]	4.3 [3.2-5.6]	0.474
PA level (activity counts, AU)	123 ± 44 (n=9)	137 ± 51 (n=19)	0.490
6MWD (m)	480 [426-568]	615 [580-652]	<0.001
6MWD (%pred.)	70.4 ± 16.2	93.4 ± 10.5	<0.001
VO <sub>2SL</sub> (mL/kg/min)	16.8 ± 5.0	25.7 ± 6.0	<0.001
VO <sub>2SL</sub> (%pred.)	64.1 ± 20.2	107.7 ± 13.8	<0.001
BMI (kg/m <sup>2</sup> )	24.7 ± 4.1	25.9 ± 2.8	0.285
FFMI (kg/m <sup>2</sup> )	18.1 ± 2.0	18.9 ± 2.9	0.287
qMVC (kg)	17.6 ± 5.6	21.7 ± 9.0	0.105
Quadriceps T.lim (s)	272 [159-368]	340 [221-656]	0.05

Definition of abbreviations: M: male/F: female; FEV<sub>1</sub> (%pred.): forced expiratory volume in 1 second in percentage of the predicted value; VC (% pred.): slow vital capacity, in percentage of the predicted value; RV (%pred.): residual volume, in percentage of the predicted value; RV/TLC (%): ratio of the residual volume and total lung capacity, in percentage; FRC (% pred.): functional residual capacity, in percentage of the predicted value; PA: physical activity; 6MWD (m): 6-minute walking distance, in meters; 6MWD (%pred.): 6-minute walking distance, in percentage of the predicted value; VO<sub>2SL</sub> (mL/kg/min): peak oxygen uptake, in milliliters per kilogram and per minute; BMI: body mass index (kg/m<sup>2</sup>); FFMI: fat-free mass index (kg/m<sup>2</sup>); qMVC (kg): quadriceps maximal voluntary contraction, in

kilograms; T.lim (s): quadriceps endurance time, in seconds. Results are expressed in mean  $\pm$  SD or median [interquartile range (IQR)].

Table 2. Muscle characteristics of the chronic obstructive pulmonary disease (COPD) patients and the sedentary healthy subjects (SHS)

	<b>COPD patients</b>	<b>SHS</b>	<b><i>p</i></b>
<b>n</b>	24	23	
<b>Muscle fiber %</b>			
<b>Type I</b>	37.5 ± 12.6	44.5 ± 11.1	<0.05
<b>Type I/IIa</b>	2.1 ± 4.1	0.8 ± 0.8	0.118
<b>Type IIa</b>	26.4 ± 15.9	22.2 ± 14.0	0.356
<b>Type IIa/IIx</b>	20.0 ± 13.5	19.5 ± 12.1	0.886
<b>Type IIx</b>	13.8 ± 10.8	13.0 ± 8.6	0.784
<b>All fiber cross-sectional area (µm<sup>2</sup>)</b>	5107 ± 1376	4409 ± 1679	0.141
<b>Capillary-to-fiber ratio</b>	1.273 ± 0.247 (n=10)	1.625 ± 0.327 (n=8)	<0.05
<b>Capillary density (/mm<sup>2</sup>)</b>	256 ± 539 (n=10)	309 ± 557 (n=8)	0.057

Results are expressed in mean ± SD