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Blunted muscle angiogenic training-response in COPD patients *versus* sedentary controls

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ABSTRACT: The impaired skeletal muscle of chronic obstructive pulmonary disease (COPD) patients reduces exercise capacity. Similar to the oxidative muscle fibres, 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).

24 COPD patients (forced expiratory volume in 1 s 45.6±17.5% predicted) and 23 SHS (<150 min·week^{-1} of moderate-to-vigorous exercise) completed a 6-week rehabilitation programme based on individualised moderate-intensity endurance training. Histomorphological muscle analysis and measurements of pro-angiogenic vascular endothelial growth factor (VEGF)-A and anti-angiogenic thrombospondin (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±2.4 *versus* +2.9±2.6 mL·kg^{-1}·min^{-1}, p<0.05, and +65% *versus* +108%, p=0.06, respectively). The capillary-to-fibre (C/F) ratio increased less in COPD patients than SHS (+16±10% *versus* +37±20%, p<0.05) and no fibre type switch occurred in COPD patients. The VEGF-A/TSP-1 ratio increased in COPD patients and SHS (+65% *versus* +35%, p<0.05). Changes in C/F and symptom-limited oxygen consumption were correlated (r=0.51, p<0.05).

In addition to a lack of fibre switch, COPD patients displayed a blunted angiogenic response to training.

KEYWORDS: Chronic diseases, exercise testing, myopathy in COPD, rehabilitation programmes, remodelling, vascular remodelling

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 characterised by cellular changes, with decreases in the proportion of type I oxidative fibres, 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 shown 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 fibre proportion after exercise training in the quadriceps of COPD patients [7–10], in contrast to observations in healthy subjects [11].

Consistent with the role of capillarisation in muscle function, it is possible that impaired training-induced angiogenesis would also explain the incomplete recovery of muscle function in COPD patients. Because exercise training significantly

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increases muscle capillarisation in these patients [7, 8, 12], it could be hypothesised 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 capillarisation 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 (VEGF)-A or anti-angiogenic thrombospondin (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, myofibre remodelling, capillarisation and the main angiogenic factors after a moderate-intensity training programme in COPD patients and SHS with the same pattern of physical inactivity.

MATERIALS AND METHODS

See additional information and references in the online supplementary material.

Study population

From March 2008 to February 2009, we recruited 24 SHS (age 50–75 years) with no disease and <150 min of moderate-to-vigorous physical activity per week (corresponding to the recommended threshold for health improvement [15]) and 24 patients with COPD defined by dyspnoea, chronic cough or sputum production and/or a history of exposure to risk factors for the disease, and post-bronchodilator forced expiratory volume in 1 s/forced ventilator capacity ratio <70% [16]. Exclusion criteria included other respiratory diagnoses, decompensated comorbidity, exacerbation in the last 3 months and previous participation in an exercise training programme. All subjects and patients performed the tests at INSERM U-1046, Centre Hospitalier Regional Universitaire Montpellier (CHRU) Montpellier, France, or ''La Solane'' or ''La Vallonie'' Pulmonary Rehabilitation Centres 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 (2008-EESSS-V2 and 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 of our sedentary-selected healthy population, we used the Voorrips questionnaire (Modified Baecke's questionnaire), a physical activity questionnaire validated and used in this indication [17]. ''Objective'' physical activity 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 Quantification de l'Activité Physique (QUANTAP) interview-administered survey is a computer-assisted tool designed to determine physical activity over a lifetime in four dimensions (sports at school, leisure sports, occupation and daily activities). This questionnaire is reliable in assessing lifetime physical activity and has been validated for use in elderly French subjects and in the context of various pathologies, including 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] (online supplementary material).

Exercise testing and muscle function assessment

The 6-min walking test was performed in a 30-m 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 ($V'O_2SL$) and the ventilatory threshold [22] (online supplementary material). If the ventilatory threshold could not be detected, we assessed the dyspnoea threshold by rating breathlessness every minute of the test, using the patient's own vocabulary [23] (online supplementary material). The quadriceps strength and endurance were measured by isometric maximal voluntary contraction (qMVC) and endurance time (tLIM). Fat-free and muscle mass were estimated with multi-frequency bioelectrical impedancemetry (QuadScan 4000; Bodystat, Douglas, UK). Additional information is provided in the online supplementary material.

Exercise training

The exercise training sessions were part of a multicomponent and comprehensive pulmonary rehabilitation course, including an education programme [5]. 20 sessions of endurance exercise were conducted three or four times per week for 6 weeks. The exercise intensity was set as the heart rate at the ventilatory or dyspnoea threshold [5, 22, 23]. Each session lasted 45 min, and in one out of two sessions, was followed by 30 min of strength-building exercise performed at 40% of the one-repetition maximum. 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 programme and 48 h after the last training session [24].

Muscle immunohistochemistry

Muscle fibre type and mean cross-sectional area (CSA) were assessed after immunohistochemistry on frozen sections (10-μm thickness) from the muscle biopsies, using antibodies specific to each myosin heavy chain isotype (University of Iowa, Iowa City, IO, USA) [4, 25]. Capillaries were visualised by immunochemistry with the monoclonal antibody against CD31 (#550389; BD Biosciences, Franklin Lakes, NJ, USA). CSA, capillary density and the capillary-to-fibre ratio were determined after counting capillaries and myofibres on one to two cryosections from each muscle biopsy (online supplementary material).

Western blotting

Blots were probed for VEGF-A (clone VG-1 #05-1117; Millipore, Etobicoke, Canada) and TSP-1 (clone A6.1 #399300; Invitrogen,

Burlington, Canada). β-actin protein was also measured as our loading control (online supplementary material).

Statistical analysis

Data are presented as mean±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 modelling [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 analysed using R.2.13.0 software (www.r-project.org).

RESULTS

Baseline characteristics of the COPD patients and SHS

All the SHS had a Voorrips score of <9.4, indicating that they were all sedentary. The COPD patients and SHS were well matched according to age and physical activity level (table 1). Moreover, there was no significant difference between the COPD patients and SHS for their physical activity level over the past 15 years (in metabolic equivalents 14 949±9470 *versus* 12 341±5201, p=0.26). At the morphological level (online supplementary figure E1), COPD muscle presented a significantly lower proportion of type I fibres compared with that of SHS (table 2). Additional details are provided in the online supplementary material.

Exercise training-induced functional and histomorphological adaptations

Exercise training was performed at the intensity of the ventilatory or dyspnoea (6 out of 24 COPD patients) threshold in COPD patients and SHS (12.7±1.9 mL·kg^{-1}·min^{-1} 48.7±11.4% predicted and 15.4±3.5 mL·kg^{-1}·min^{-1}, 63.5±10.1% pred, respectively, p<0.001). Expressed as % maximal heart rate, the relative intensity was not significantly different in COPD patients and SHS (66±6.8% and 69±6.3%, respectively, p=0.27). Expressed as % of V'_{O_2}max, the relative intensity was slightly higher in COPD patients *versus* SHS (66±6% *versus* 60±5%, respectively, p=0.05). Relative improvements for all functional parameters are shown in figure 1. The training significantly improved 6MWD in both groups (COPD: +37±35 m and SHS: +31±35 m, p<0.001; fig. 1). The V'_{O_2}max showed significant interaction between group and time (p<0.05). Lastly, a significant increase in *t*LIM occurred in both groups (COPD: +121±169 s and SHS +334±274 s, p<0.001), with an interaction between group and time that almost reached significance (p=0.06).

Representative changes in type I fibre proportions in COPD patients and SHS are depicted in figure 2a. The type I fibre proportion was significantly increased in the SHS group (+8±11.6%, p<0.01; fig. 2c) and was mirrored by a decrease in the percentage of type IIx fibres (-8±10.0%, p=0.001).

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

	COPD patients	SHS	p-value
Subjects n	24	23	1.00
Age years	60.8±8.0	61.5±5.7	0.756
Sex male/female	18/6	11/12	
FEV_1/VC %	42.3±8.9	75.8±4.3	<0.001
FEV_1 % 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 ratio %	52.6±12.2	37.9±6.0	<0.01
FRC % pred	156.7±22.2	109.7±22.3	<0.001
P_{aO_2} 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 AU	123±44$^{\#}$	137±51¶	0.490
6MWD m	480 (426–568)	615 (580–652)	<0.001
6MWD % pred	70.4±16.2	93.4±10.5	<0.001
V'_{O_2}SL mL·kg^{-1}·min^{-1}	16.8±5.0	25.7±6.0	<0.001
V'_{O_2}SL % pred	64.1±20.2	107.7±13.8	<0.001
BMI kg·m^{-2}	24.7±4.1	25.9±2.8	0.285
FFMI kg·m^{-2}	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

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; VC: slow vital capacity; % pred: % predicted; RV: residual volume; TLC: total lung capacity; FRC: functional residual capacity; P_{aO_2}: arterial oxygen tension; PA: physical activity; AU: activity units; 6MWD: 6-min walking distance; V'_{O_2}SL: symptom-limited oxygen uptake; BMI: body mass index; FFMI: fat-free mass index; qMVC: quadriceps maximal voluntary contraction; *t*LIM: endurance time. $^{\#}$: n=9; ¶: n=19.

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

	COPD patients	SHS	p-value
Subjects n	24	23	
Muscle fibre %			
Type I	37.5±12.6	44.5±11.1	<0.05
Type I/IIa	2.1±4.1	0.8±0.8	0.118
Type IIa	26.4±15.9	22.2±14.0	0.356
Type IIa/IIx	20.0±13.5	19.5±12.1	0.886
Type IIx	13.8±10.8	13.0±8.6	0.784
All fibre cross-sectional area μm^2	5107±1376	4409±1679	0.141
Capillary-to-fibre ratio	1.273±0.247$^{\#}$	1.625±0.327¶	<0.05
Capillary density mm^{-2}	256±539$^{\#}$	309±557¶	0.057

Data are presented as mean±SD, unless otherwise stated. $^{\#}$: n=10; ¶: n=8.

Conversely, no significant change occurred in the COPD group (fig. 2b). We observed a reduction in fibre CSA in COPD patients (from 5107±1375 μm^2 to 4565±1477 μm^2, p<0.05) and no significant change in the SHS group (from 4409±1679 μm^2 to 4629±1670 μm^2, p=0.51). This reduction in both type I and type IIa fibre CSA was seen in COPD patients (from 5905±1588 μm^2 to 5260±1493 μm^2, p<0.05, and from 5400±2012 μm^2 to 4657±1460 μm^2, p<0.05).

Exercise training-induced muscle angio-adaptations

Capillaries were visualised after immunodetection of the endothelial marker CD31 (fig. 3a). In both groups, the exercise training stimulated angiogenesis, as reflected by the increase in the capillary-to-fibre ratio (COPD 16±10% and SHS 37±20%, p<0.001) (fig. 3b). Yet there was a significant interaction between group and time (p<0.01), indicating lesser 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).

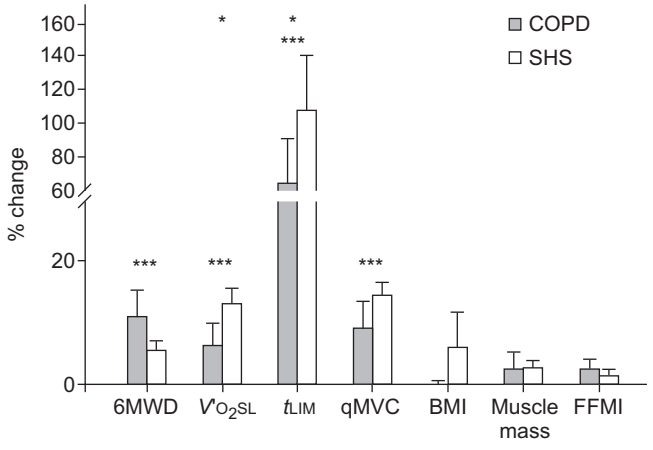

FIGURE 1. Relative changes in functional parameters (%) in chronic obstructive pulmonary disease (COPD) patients and sedentary healthy subjects (SHS) following exercise training. 6MWD: 6-min walking distance; V'_{O_2}SL: symptom-limited oxygen uptake; *t*LIM: endurance time; qMVC: quadriceps maximal voluntary contraction; BMI: body mass index; FFMI: fat-free mass index. Data are presented as mean±SEM. *: group–time interaction, p<0.05; ***: time effect, p<0.001.

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 AU pre-training *versus* 0.075±0.013 AU post-training, p<0.05) (fig. 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) (fig. 4e), in favour of an angiogenic response.

Angiogenesis–function relationships

At baseline, the capillary-to-fibre ratio was significantly and positively correlated with V'_{O_2}max and qMVC in COPD patients and SHS (r=0.60, p<0.01 and r=0.67, p<0.01, respectively) (fig. 5a and b). Capillary density was not significantly correlated with type I fibre proportion (p=0.07). The absolute training-induced changes in the capillary-to-fibre ratio were significantly correlated with the changes in V'_{O_2}max in both groups (r=0.51, p<0.05) (fig. 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 capillarisation 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 favour of an angiogenic process. Lastly, a lack of fibre type switch response to training was observed in the COPD patients compared with the age- and physical activity-matched healthy subjects.

Effect of the training stimulus

Our programme of exercise training at moderate intensity significantly increased the exercise capacity in both COPD patients and SHS. Additional details are provided in the online supplementary material. Interestingly, there was a significant interaction between group and time effects for V'_{O_2}max and *t*LIM, indicating that the functional changes were lower in the

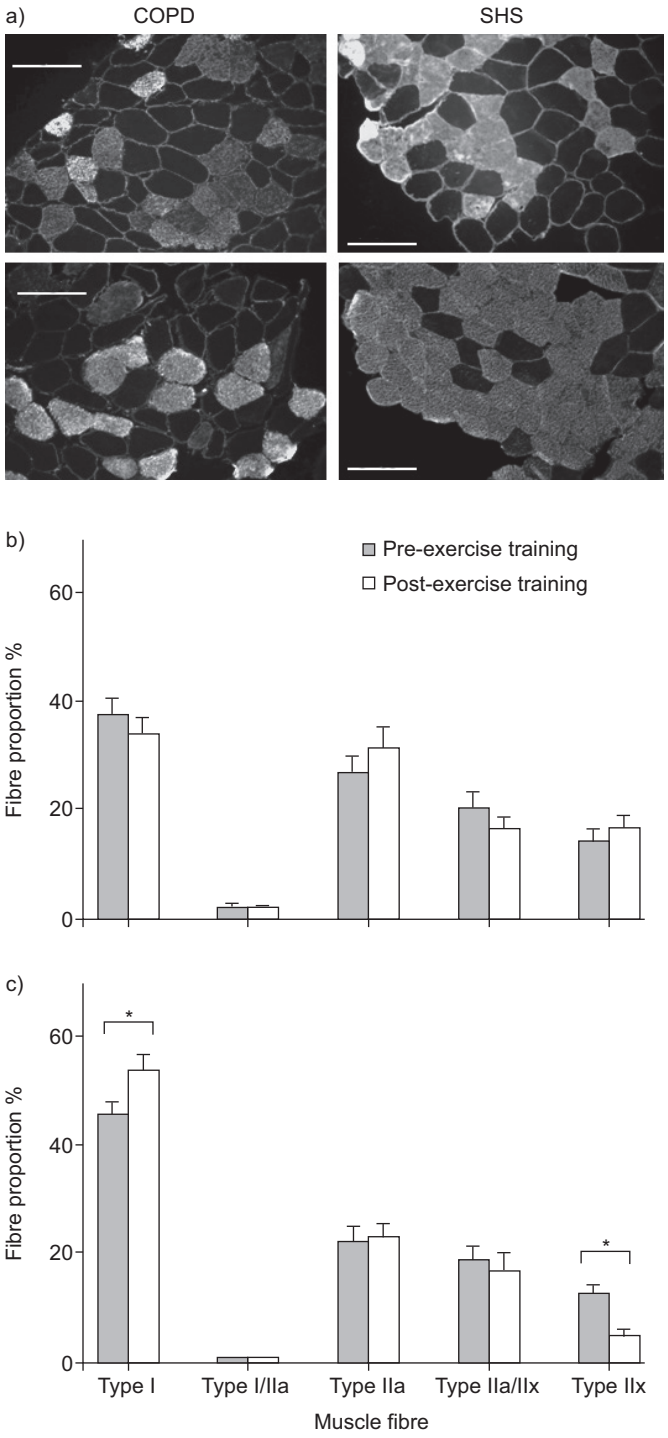

FIGURE 2. a) Representative transverse cryosections of the vastus lateralis stained with the type I myosin heavy chain (MHC) isotype antibody, in a chronic obstructive pulmonary disease (COPD) patient and a sedentary healthy subject (SHS) before (upper row) and after (lower row) exercise. Note the increase in the proportion of type I MHC fibres in the SHS. Proportion of the muscle fibre type (%) before and after the exercise training programme in b) COPD patients (n=21) and c) SHS (n=23). Data are presented as mean±SEM. Scale bars=100 μm. *: $p<0.05$, after exercise training.

COPD patients for these parameters. These lower functional improvements in COPD patients compared with SHS are compatible with the lack of exercise-induced muscle fibre type changes [27].

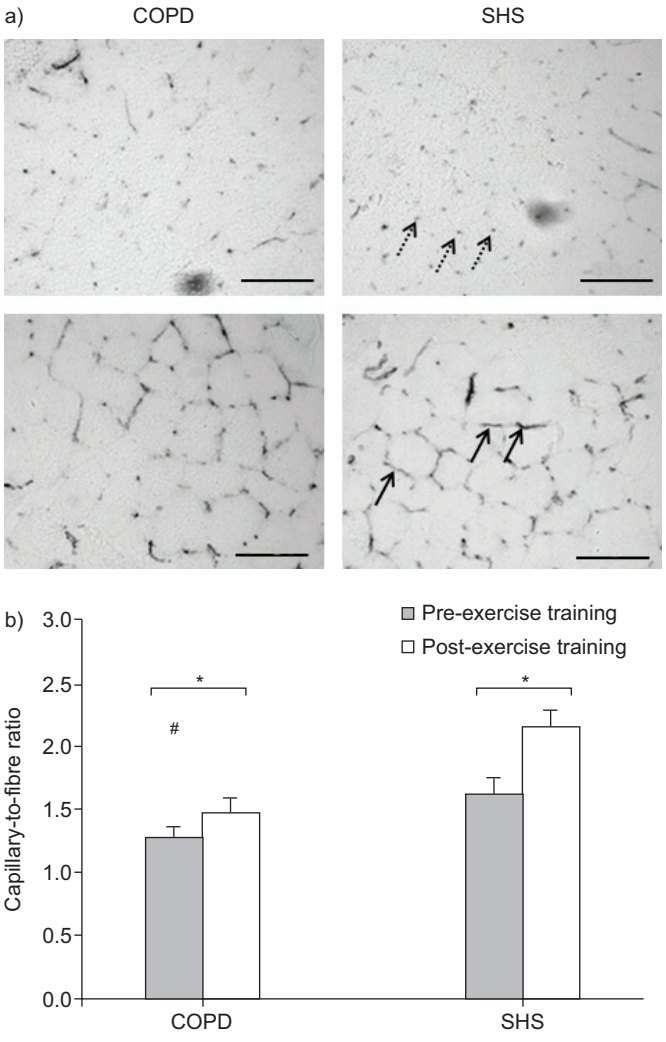

FIGURE 3. a) Representative pictures of vastus lateralis capillarisation after staining for the endothelial marker CD31, in a chronic obstructive pulmonary disease (COPD) patient and a sedentary healthy subject (SHS) before (upper row) and after (lower row) exercise. Note the transverse capillaries surrounding the myofibre (dotted arrows) in pre-exercise training and the increased size of the capillary-to-fibre interface post-exercise training (black arrows). b) Mean values and standard error of the mean in COPD patients and SHS before and after training for the capillary-to-fibre ratio. Scale bars=100 μm. *: $p<0.05$, after exercise training. #: $p<0.05$, between groups.

Regarding the intensity of exercise training, all participants were trained at their own ventilatory (or dyspnoea) 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 $V'O_2max$) have even been observed in COPD patients compared with healthy subjects, especially for peroxisome proliferator-activated receptor-γ coactivator-1α,

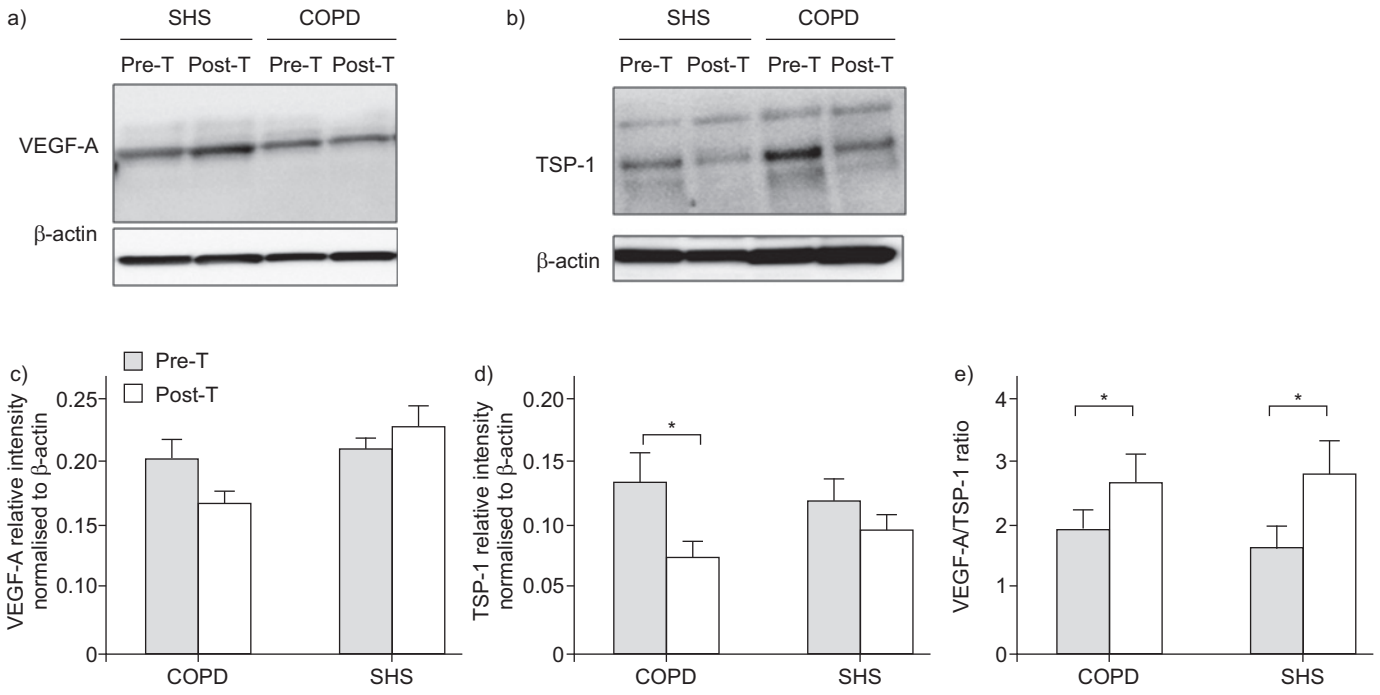

FIGURE 4. Representative Western blot of a) vascular endothelial growth factor (VEGF)-A and b) thrombospondin (TSP)-1 protein expression in the vastus lateralis of chronic obstructive pulmonary disease (COPD) patients and sedentary healthy subjects (SHS) before (Pre-T) and after (Post-T) training. β-actin is used as a loading control. Densitometry analysis of c) VEGF-A and d) TSP-1 protein; e) VEGF-A/TSP-1 ratio in the vastus lateralis of COPD patients and SHS before and after training. Data are presented as mean±SEM. *: $p<0.05$ between groups (post-training).

which is a master regulator of the fibre 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 capillarisation was lower in COPD patients [4, 7] and well correlated with muscle dysfunction and O_2 consumption before training [4]. We observed an increase in the capillary-to-fibre ratio, which may indicate a training-induced improvement in the capacity to transfer O_2 to myofibres 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-fibre interface [31], as reflected by the capillary-to-fibre 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 (fig. 3a).

Moreover, the increase in the muscle capillary-to-fibre ratio (*i.e.* the capillary-to-fibre interface, and thus the O_2 transfer capacity) may have functioned as a master driver of muscle O_2 uptake and thus improved 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 myofibres to consume O_2. In contrast to SHS [34], COPD patients showed no shift toward more oxidative type I fibres [7, 12], and the preliminary data in our COPD patients (n=10) indicated no improvement in the maximal rates of oxygen consumption of permeabilised fibres (see Methods and Results sections in the online supplementary material). Lastly, the significant and positive correlation between the capillary-to-fibre ratio and the V'_{O_2SL} changes, as previously observed in healthy subjects [35], strengthens the credibility of a role for capillarisation 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-characterised clinical impact, *i.e.* VEGF-A [36] and TSP-1. The deletion of VEGF-A in the myofibres of transgenic mice dramatically reduced muscle capillarisation and decreased exercise endurance time by ~80% [37]. Gene therapy targeting VEGF gene expression improved muscle capillarisation [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 capillarisation increase. Thus, assessing the VEGF-A/TSP-1 ratio appears to be a more accurate approach than analysing the expression of molecular factors individually [36]. In our study, we also observed a significant increase in this ratio in both groups, which indicates

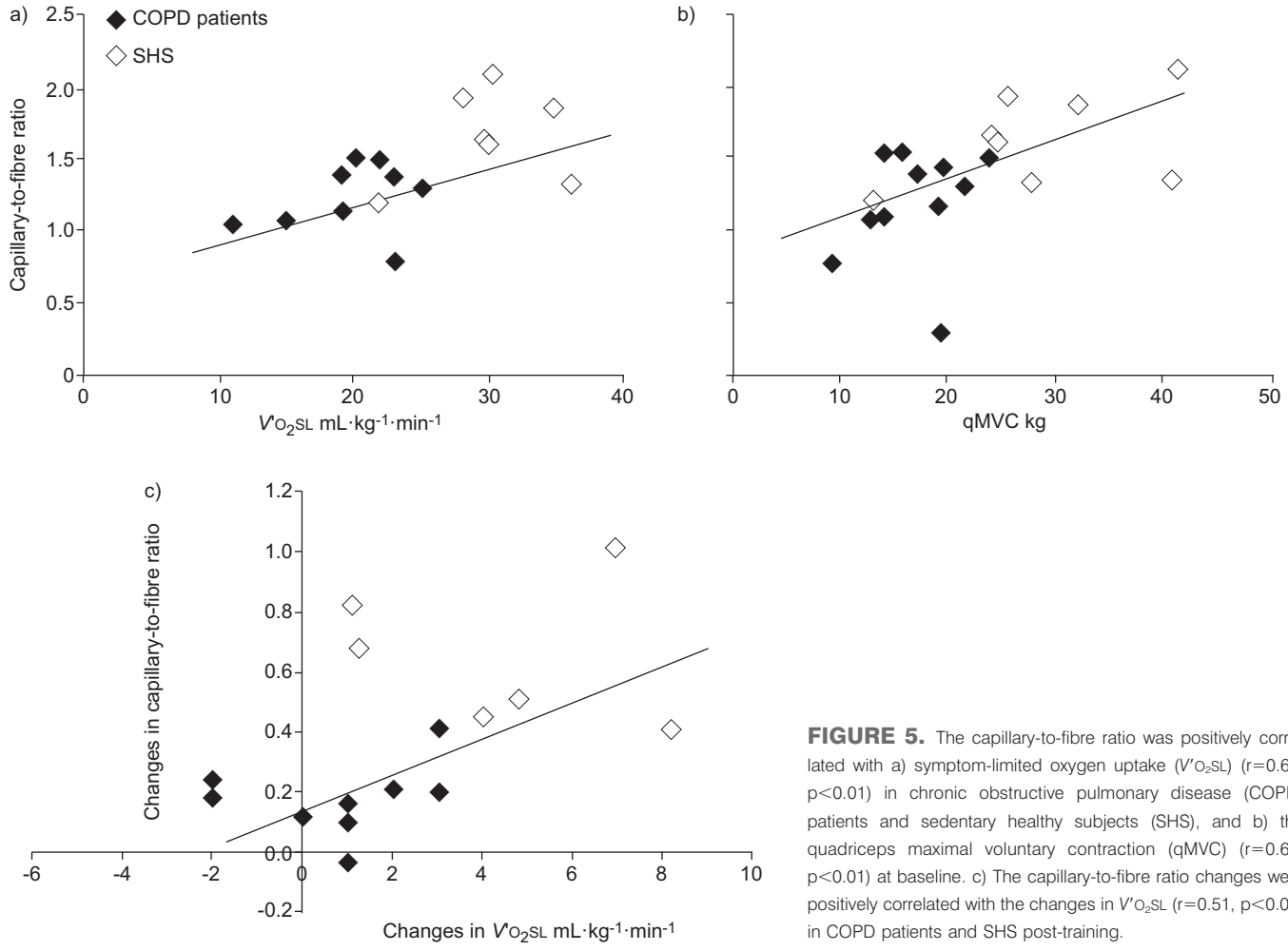

FIGURE 5. The capillary-to-fibre ratio was positively correlated with a) symptom-limited oxygen uptake ($V'O_{2SL}$) ($r=0.60$, $p<0.01$) in chronic obstructive pulmonary disease (COPD) patients and sedentary healthy subjects (SHS), and b) the quadriceps maximal voluntary contraction (qMVC) ($r=0.67$, $p<0.01$) at baseline. c) The capillary-to-fibre ratio changes were positively correlated with the changes in $V'O_{2SL}$ ($r=0.51$, $p<0.05$) in COPD patients and SHS post-training.

that the angiogenic balance in our patients shifted in favour of angiogenesis, consistent with the increase in capillarisation.

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

The defective muscle adaptation to exercise training in COPD patients was characterised by a lack of fibre type shift and a blunted capillarisation response. Thus, we can hypothesise a pleiotropic patho-biological mechanism. In COPD, systemic factors (hypoxaemia, 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 fibre type cannot be eliminated.

Clinical implications

A key observation in our study was the link between reduced function (quadriceps t_{LIM} and $V'O_{2SL}$) and capillarisation. Moreover, our study indicates a probable role of the blunted muscle capillarisation response to training in the reduced $V'O_{2SL}$ improvement in the COPD patients, as angiogenesis has also shown an impact on the improvement in $V'O_{2SL}$ in two other chronic diseases: CHF [41] and peripheral arterial disease [42]. Our finding of lower improvement in the capillary-to-fibre 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 ischaemia, muscle angiogenesis may thus be a relevant target for therapeutic interventions designed to improve muscle capillarisation [43]. However, although O_2 transfer to muscle appears to be critical, there is no evidence of chronic lower limb ischaemia in COPD patients [44]. Nonetheless, patients with hypoxaemia and/or comorbidities (vascular and/or diabetic) can experience chronic ischaemia, and in this case therapeutic interventions in association with exercise training might be an adequate approach.

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

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STATEMENT OF INTEREST

Conflict of interest information can be found alongside the online version of this article at www.erj.ersjournals.com

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