Eur Respir J 2012; 40: 1130–1136
DOI: 10.1183/09031936.00193411

High-intensity knee extensor training restores skeletal muscle function in COPD patients

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ABSTRACT: Improving reduced skeletal muscle function is important for optimising exercise tolerance and quality of life in chronic obstructive pulmonary disease (COPD) patients. By applying high-intensity training to a small muscle group, we hypothesised a normalisation of muscle function.

Seven patients with COPD performed 6 weeks (3 days·week^{-1}) of high-intensity interval aerobic knee extensor exercise training. Five age-matched healthy individuals served as a reference group. Muscle oxygen uptake and mitochondrial respiration of the vastus lateralis muscle were measured before and after the 6-week training programme.

Initial peak work and maximal mitochondrial respiration were reduced in COPD patients and improved significantly after the training programme. Peak power and maximal mitochondrial respiration in vastus lateralis muscle increased to the level of the control subjects and were mainly mediated *via* improved complex I respiration. Furthermore, when normalised to citrate synthase activity, no difference in maximal respiration was found either after the intervention or compared to controls, suggesting normal functioning mitochondrial complexes.

The present study shows that high-intensity training of a restricted muscle group is highly effective in restoring skeletal muscle function in COPD patients.

KEYWORDS: Exercise, knee extensor, limb, lung, mitochondria, oxygen uptake

Chronic obstructive pulmonary disease (COPD) is now considered to be a multi-organ disease and reduced muscular function is one prominent feature [1]. The principal finding in lower limb skeletal muscle is reduced oxidative capacity with muscle fibre shift, reduced mitochondrial density, reduced mitochondrial biogenesis and impaired mitochondrial respiration [2–5]. Similar changes have been found in sedentary subjects, and it is debated whether these changes are due to the pathogenesis of COPD or a consequence of inactivity [3, 6].

In both sedentary and COPD patients, exercise training is the only intervention shown to partly reverse these changes, mainly by improved muscle oxidative capacity [7, 8]. The effects of exercise on skeletal muscle have mostly been studied with whole body exercise training [8–10] and it has been suggested that the ventilatory limitations prevent the locomotor system from being adequately taxed, thereby reducing the training effects [11]. The existence of a metabolic reserve has been demonstrated in COPD patients when testing in small muscle groups in a model relieved from respiratory constraints [12] and it has also been demonstrated that exercising one leg improves maximal oxygen uptake ($V'_{O_2,max}$) more than whole body training [13]. In healthy individuals, training intensity is one of the key factors determining the training response [14]. Due to the COPD patients' ventilatory limitations, it is often not possible to attain sufficient exercise intensity. By exercising restricted muscle groups, *e.g.* choosing an exclusive lower limb exercise model, the central limitation can be avoided [15, 16]. Furthermore, by performing the training in short high-intensity intervals, maximum exercise effects would be expected [17].

For editorial comments see page 1070.
This article has supplementary material available from www.erj.ersjournals.com

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Received:
Nov 06 2011
Accepted after revision:
Feb 07 2012
First published online:
March 09 2012

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

To further explore whether, to a large extent, reduced muscle function in COPD is caused by specific muscular abnormalities or is reversible by exercise training, we studied the muscle metabolic reserve capacity in a training model unveiled from central constraints to allow the muscle to work at a maximum load. We chose a model of 6-weeks high-intensity knee extensor exercise training to bypass the central limitations. We hypothesised that by interval training a restricted muscle group at high intensity, a normal exercise response in the individual mitochondrial respiration complexes would be found.

METHODS

Study subjects

Eight patients with stable COPD, a smoking history >20 pack-yrs, forced expiratory volume in 1 s (FEV_1) <60% (post-bronchodilator) and age >50 yrs and without resting hypoxaemia were included in the study. Two patients were current smokers. Patients with clinical heart disease or a medical condition limiting exercise training were excluded. None of the patients used systemic steroids and none of the patients changed their medications during the study period. None of the patients had participated in a pulmonary rehabilitation programme during the last 3 months. As controls, five healthy nonsmoking age-matched subjects with normal lung function were included. The control group did not participate in regular sports or leisure activities. These subjects did not participate in the exercise training and served only as controls for the baseline values (knee extensor peak work, muscle biopsy and muscle mass determination by magnetic resonance imaging (MRI)). All subjects underwent pulmonary testing, treadmill aerobic capacity testing and resting echocardiography examination at baseline and after the training period (refer to online supplementary material).

The study was approved by the Norwegian (Regional) Ethics Committee, adhered to the Helsinki Convention and registered at ClinicalTrials.gov (NCT01079221). All patients gave their written informed consent. Patients' characteristics are shown in table 1.

Knee extensor peak work testing and exercise training

The muscular work was limited to the quadriceps of one leg only by using a knee extensor exercise model [15, 16]. To determine the quadriceps peak work capacity, an incremental knee extensor protocol was performed at baseline and after the training intervention. After a 5-min warm-up, each subject performed a work protocol with 2-Watt increments every 3 min until reaching exhaustion, with a kicking frequency at 60 kicks·min^{-1}. During the session, oxygen consumption, femoral artery flow and arterial and venous blood gases were sampled. Venous and arterial blood samples were only taken from the COPD group. The femoral artery flow and blood gases were sampled within the last 30 s of each load. All testing was performed on the right leg.

Prior to exercise training each patient adapted to the knee exercise model by undertaking two training sessions. On the last session peak work and oxygen consumption was measured. All COPD patients went through a 6-week exercise programme consisting of three training sessions per week. After a 5-min warm-up without load, four intervals of 4 min at 90% of peak work rate were performed. Each interval was separated by a 2-min active period of unloaded kicking. A kicking frequency at 60 kicks·min^{-1} was pursued. Both legs were exercised separately and the load was increased each week (see Results section) to ensure work at 90% of peak load.

TABLE 1 Study subjects baseline characteristics

	Control group	COPD group	p-value
Subjects n	5	7	
Age yrs	70.0±4.6	67.6±7.2	NS
Height cm	172±6	175±7	NS
Weight kg	75.2±4.9	75.1±6.4	NS
S_{p,O_2} at rest %	ND	96.4±1.2	
BMI kg·m^{-2}	25.9±1.3	24.6±2.1	NS
FEV_1 L·s^{-1}	2.71±0.4	1.50±0.3	0.0027
FEV_1 % predicted	93.3±13.6	45.5±9.8	0.0045
FVC L	3.89±0.31	3.20±0.77	NS
FEV_1/FVC %	73.9±2.6	48.2±8.9	0.0045
$V'_{O_2,peak}$ mL·kg^{-1}·min^{-1}	38.9±5.0	20.4±4.0	0.0045
Pack-yrs	0	38±14	0.000

Data are presented as mean±SD, unless otherwise specified. COPD: chronic obstructive pulmonary disease; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry; BMI: body mass index: FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $V'_{O_2,peak}$: peak oxygen consumption; ND: no data; NS: not significant.

Oxygen uptake in quadriceps muscle

To determinate the oxygen uptake in the working quadriceps muscle, the muscle mass, blood flow and the arterio–venous (AV) difference was measured [11]. Oxygen uptake was calculated by multiplying blood flow with AV oxygen difference (AVO_2). The AVO_2 difference was determined by venous and arterial blood gases sampled from the radial artery and the femoral vein. Samples for blood gas analysis were drawn during the last 30 s of the working loads and analysed (ABL 625 blood gas analyser; Radiometer, Copenhagen, Denmark). Venous and arterial access was gained by placing a catheter in the in the right femoral vein and an artery catheter in the left radial artery. The femoral blood flow was measured with an ultrasound probe (Wingmed, Horten, Norway) placed over the femoral artery [18]. The flow was determined at each load during the last 30 s. Flow data were analysed with EchoPack^{TM} (Buckinghamshire, UK). Muscle mass of the quadriceps was measured by MRI (refer to online supplementary material). The quadriceps muscle volume was calculated by multiplying the surface area of each MRI slice by the slice thickness, and then taking the sum of all the slices [19]. To adjust for the gap between each slide, calculation was performed using the truncated cone method [20]. To calculate the quadriceps mass, muscle volume was multiplied by muscle density [21].

Citrate synthase activity

Biopsies were obtained from the vastus lateralis and performed at baseline and 72 h after the last training session to avoid the acute training effects [22]. The samples were homogenised in CelLytic buffer (Sigma-Aldrich, St Louis, MO, USA) at 6,000 shakes·min^{-1} for 2×8 s in a Precellys24 tissue homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France).

The homogenate was then centrifuged at 10,000 × *g* for 10 min at 4°C and the supernatant tested for citrate synthase (CS) activity. The activity was determined by the method described by SRERE [23] using a Citrate Synthase Assay Kit (Sigma-Aldrich). Absorbance at 412 nm was measured on a FLUOstar Omega spectrometer (BMG Labtech, Ortenburg, Germany). Specific activity was calculated by dividing measured activity on the muscle extract protein concentration.

Mitochondrial respiration

Mitochondrial respiration was studied *in situ* in saponin permeabilised fibres as described by VEKSLER *et al.* [24] and reviewed recently [25]. Briefly, fibres from the biopsies of vastus lateralis were gently separated under a binocular microscope using small forceps (Dumont #5) in separation solution (S) at 4°C, and then permeabilised in the same solution with 50 μg·mL^{-1} saponin for 30 min at 4°C while shaking. After being rinsed for 10 min in solution S at 4°C and then in respiration solution (R) at 22°C under shaking, the skinned fibres were transferred to a water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, UK) equipped with a Clark electrode containing 3 mL of solution R. Solutions R and S contained 2.77 mM CaK_2 ethylene glycol tetra-acetic acid (EGTA), 7.23 mM K_2EGTA (100 nM free Ca^{2+}), 6.56 mM $MgCl_2$ (1 mM free Mg^{2+}), 20 mM taurine, 0.5 mM dithiothreitol, 50 mM potassium-methane sulfonate (160 mM ionic strength), and 20 mM imidazole (pH 7.1 at 22°C). Solution S also contained 5.7 mM Na_2ATP, 15 mM creatine phosphate, while solution R contained 10 mM glutamate, 4 mM malate, 3 mM phosphate and 2 mg·mL^{-1} bovine serum albumin. Basal respiration rate (V'_0) was measured at 22°C under continuous magnet stirring in the oxygraphic cells. Maximal adenosine disphosphate (ADP)-stimulated respiration (V'_{ADP}) above V'_0 was measured by the addition of 2 mM ADP as phosphate acceptor and the maximal respiration rate (V'_{max}) was calculated as (V'_0+V'_{ADP}). The acceptor control ratio was calculated as ratio of V'_{max} to V'_0. Following ADP additions, functioning of various complexes of the electron transport chain function was assessed [26]. Addition of 10 mM succinate, followed by 1 mM amytal, a specific inhibitor of complex I, allowed estimation of the maximal respiration involving complexes II, III, and IV ($V'_{succinate}$). Thereafter, ascorbate (0.5 mM) and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD; 0.5 mM) were added to estimate maximal respiration from complex IV ($V'_{ascorbate+TMPD}$). After the measurements the fibres were dried and respiration rates were expressed as μmoles O_2·min^{-1}·g^{-1} dry weight.

Statistics

Data are presented as mean±SD. The changes in physiologic variables were calculated at baseline and post-training. Control group and COPD baseline differences were analysed by t-test. Assumptions of normality were assessed by normal probability plots. COPD group baseline and follow-up differences were analysed using paired t-tests. The level of significance was set at $p<0.05$.

RESULTS

Knee extensor exercise training and testing

Seven patients completed the exercise programme with a total of 18 sessions each. The average training intensity in the first week was 12.7±3.8 W and in the sixth week 18.4±5.3 W. The change in training load during the training period is shown in figure 1. The peak power at baseline was significantly lower than in the control subjects, and increased from 14.6±4.9 W to 20.0±5.3 W ($p<0.001$) in the COPD patients. We found no change in $V'_{O_2,peak}$ or work economy at the treadmill testing.

Resting echocardiography was performed before and after the exercise training programme and no effects on cardiac output, ejection fraction and stroke volume were observed.

No difference in quadriceps muscle mass was observed either at baseline or at post-test (table 2).

Oxygen consumption during knee extensor exercise

During the knee extensor exercise both muscular and pulmonary $V'_{O_2,peak}$ were measured. After the 6-week exercise programme, muscular $V'_{O_2,peak}$ increased from 200±40 mL·min^{-1}·kg^{-1} to 248±43 mL·min^{-1}·kg^{-1} ($p<0.05$). The difference in AVO_2 did not change, but femoral blood flow increased significantly from 2,127±655 mL to 2,631±348 mL ($p<0.05$). Quadriceps work economy (WE) was measured in the patients at a load of 6 W. Quadriceps WE at baseline was 117±30 mL·min^{-1}·kg^{-1} and at follow-up was 104±29 mL·min^{-1}·kg^{-1}, but failed to reach statistical significance. Likewise the lactate level at WE load was 2.16±1.0 mmol·L^{-1} at baseline and at follow-up 1.67±1.1 mmol·L^{-1} ($p=0.17$). The pulmonary oxygen uptake at peak work during the knee extensor test was not improved (baseline: 10.9±1.8 mL·min^{-1}·kg^{-1}; follow-up: 11.7±1.2 mL·min^{-1}·kg^{-1}; $p=0.30$). However, the pulmonary uptake at submaximal work was reduced by 26%, from 7.8±2.7 mL·min^{-1}·kg^{-1} at baseline to 5.8±1.3 ($p<0.05$) at follow-up (table 2), suggesting improved WE. Moreover, minute ventilation (V'_E) was reduced from 16.7±1.3 L·min^{-1} to 13.3±2.0 L·min^{-1} ($p<0.05$) after the training intervention, suggesting reduced ventilatory demands.

Citrate synthase activity

The CS activity in COPD muscle increased after the training programme, from 0.29±0.07 U·mg^{-1} to 0.37±0.11 U·mg^{-1} ($p=0.01$), indicating increased mitochondrial mass and density. We found no difference in baseline CS activity when compared to the healthy controls.

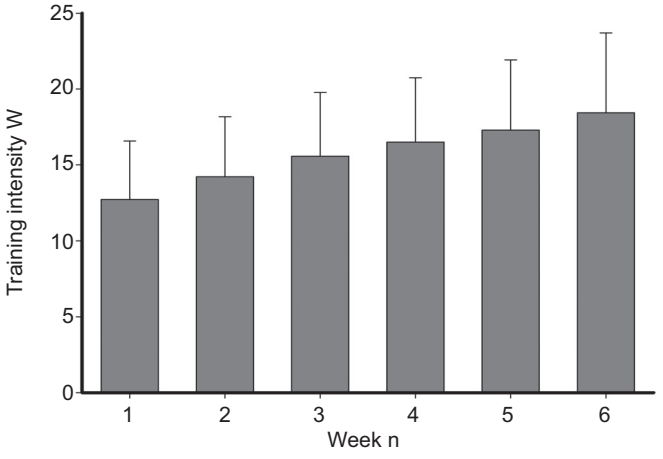

FIGURE 1. Training intensity during the training programme.

TABLE 2 Exercise testing data

	Control group[#]		COPD group[¶]		
	Baseline	p-value[+]	Baseline	Follow-up	p-value[§]
Treadmill testing					
$V'_{O_2,peak}$ mL·kg^{-1}·min^{-1}	38.9±5.0	0.005	20.4±4.0	20.2±3.6**	0.68
WE mL·kg^{-1}·min^{-1}	ND		11.0±0.6	10.6±0.5	0.44
V'_E at peak work L·min^{-1}	87±8	0.001	49±9	49±11***	0.87
Knee-extensor testing					
Peak work W	23.2±6.7	0.03	14.6±4.9	20.0±5.3	0.001
Quadriceps muscle mass kg	1.64±0.20	0.10	1.39±0.27	1.43±0.26	0.45
Femoral blood flow at 6 Watts L·min^{-1}	1185±236	0.85	1213±242	1195±250	0.87
Femoral blood flow at peak work L·min^{-1}	2854±168	0.03	2127±655	2631±348	0.03
Muscle V'_{O_2} at 6 Watts mL·kg^{-1}·min^{-1}	ND		117±30	104±29	0.26
Muscle V'_{O_2} at peak work mL·kg^{-1}·min^{-1}	ND		200±40	248±43	0.048
Pulmonary V'_{O_2} at 6 Watts mL·kg^{-1}·min^{-1}	5.21±1.21	0.07	7.81±2.67	5.78±1.29	0.026
Pulmonary V'_{O_2} at peak work mL·kg^{-1}·min^{-1}	14.6±4.9	0.036	10.9±1.8	11.7±1.2	0.30
Lactate at 6 Watts mmol·L^{-1}	ND		2.16±1.02	1.67±1.13	0.17
Lactate at peak work mmol·L^{-1}	ND		4.4±2.2	6.3±2.3	0.06
V'_E at 6 Watts L·min^{-1}	13.0±1.2	0.02	16.7±2.7	13.3±2.0	0.007
V'_E at peak work L·min^{-1}	33.9±9.9	0.16	27.4±4.3	29.3±2.8	0.18

Data are presented as mean±SD, unless otherwise stated. COPD: chronic obstructive pulmonary disease; $V'_{O_2,peak}$: peak oxygen consumption; WE: work economy; V'_E: minute ventilation; V'_{O_2}: oxygen uptake; ND: no data. [#]: n=5; [¶]: n=7; [+]: significance levels between baseline control and baseline COPD; [§]: significance levels between baseline COPD and follow-up COPD. **: p<0.01; ***: p<0.001 between controls and COPD at follow-up.

Mitochondria respiration

V'_{max} per unit of fibre weight was significantly lower in COPD patients compared to healthy controls (3.68±0.73 *versus* 4.52±0.44 μmol O_2·min^{-1}·g^{-1} dry weight; p=0.045). However, 6 weeks of knee extensor exercise training improved V'_{max} of the COPD patients by 40% to 5.15±1.32 μmol O_2·min^{-1}·g^{-1} dry weight (p=0.013) and was no longer different from controls. Basal respiration was similar between groups and did not change after exercise training (fig. 2). We did not find any differences in the maximal activity of complex II ($V'_{succinate}$) and IV ($V'_{ascorbate+TMPD}$) of the respiratory chain between groups, or in COPD patients in the untrained or trained state (fig. 3). When maximal mitochondrial respiration was normalised to CS activity, we found no difference between controls and COPD patients at baseline (fig. 4).

DISCUSSION

To our knowledge, this study is the first to specifically assess the effect of high-intensity interval exercise training on mitochondrial respiration in COPD in an exercise model relieved from respiratory constraints, thereby obtaining maximum training loads on the exercising muscles. Compared to healthy age-matched controls, the COPD group had significantly reduced peak aerobic power, peak quadriceps muscle uptake and maximal mitochondrial respiration at baseline. The 6-week training programme resulted in a significant increase in aerobic power, peak quadriceps muscle oxygen uptake (V'_{O_2}) and maximal mitochondrial respiration. These results demonstrate an improvement of the quadriceps oxidative capacity in COPD patients by exercising a small muscle group. In addition, all respiration differences were attenuated when adjusted for CS activity, which suggests reduced mitochondrial mass rather than specific mitochondrial respiratory impairment in COPD.

Our findings of a reduced maximal mitochondrial respiration to complex I compared to healthy age-matched controls are consistent with previous studies in COPD patients and,

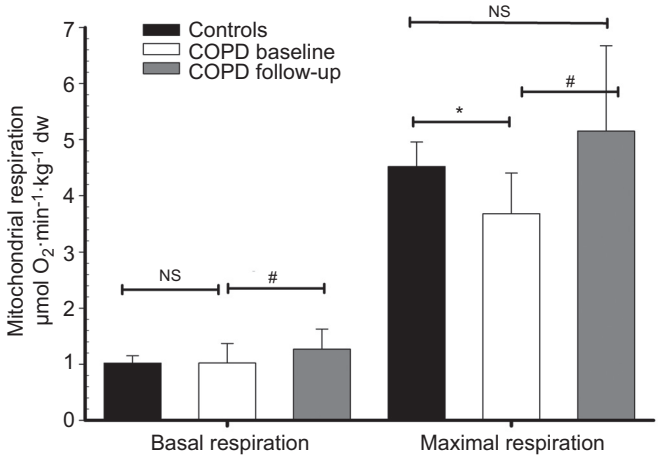

FIGURE 2. Basal and maximal mitochondrial respiration at baseline and after the training period in quadriceps muscle. Basal respiration rate with 10 mM glutamate and 4 mM malate without adenosine diphosphate (ADP) as phosphate acceptor; maximal respiration rate with glutamate and malate after addition of 2 mM ADP. COPD: chronic obstructive pulmonary disease; NS: nonsignificant; dw: dry weight. *: p<0.05; [#]: p<0.02.

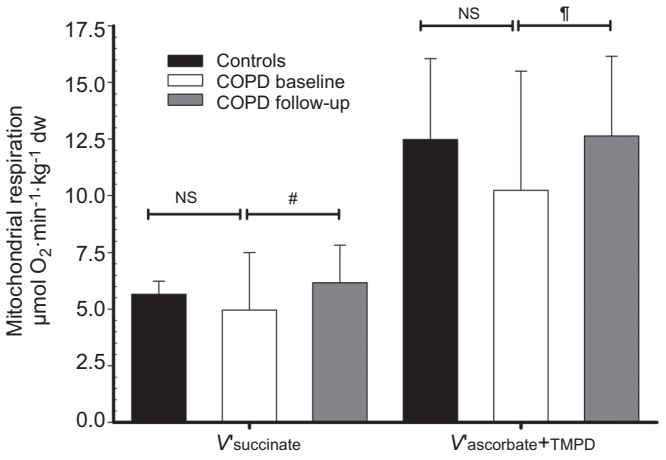

FIGURE 3. After evaluation of maximal respiration with glutamate and malate with adenosine diphosphate as phosphate acceptor, functioning of individual mitochondrial complexes was assessed. Addition of 10 mM succinate followed by 1 mM amytal allowed for estimation of the maximal respiration involving complexes II, III and IV ($V'_{succinate}$). Thereafter 0.5 mM ascorbate and 0.5 mM TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine) were added to estimate maximal respiration from complex IV ($V'_{ascorbate+TMPD}$). COPD: chronic obstructive pulmonary disease; dw: dry weight; NS: nonsignificant. #: p=0.18; ¶: p=0.30.

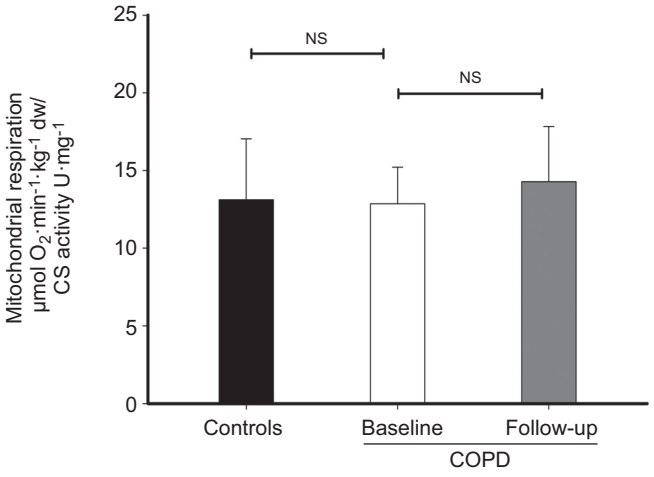

FIGURE 4. Maximal mitochondrial respiration relative to citrate synthase (CS) activity. COPD: chronic obstructive pulmonary disease; dw: dry weight; NS: nonsignificant.

interestingly, also in sedentary individuals' lower limb muscle [3, 27]. Our data are in line with PICARD *et al.* [3], showing a significantly lower maximal respiration per unit of fibre weight involving complex I (V'_{max}) in patients with COPD compared to fibres from healthy subjects. Also, when comparing sedentary persons to individuals participating in regular high-intensity aerobic exercise training, a reduced maximal respiration has been found among sedentary subjects. This difference has been found to be related to a higher complex I activity compared with complex II in more trained subjects [28].

Despite a trend towards lower complex II stimulated respiration ($V'_{succinate}$) in our COPD patients compared to the healthy controls, we were not able to show significantly lower values, in contrast to two earlier studies [3, 27]. A higher fitness level in our COPD patients may explain why we were not able to detect a significant downregulation of complex II respiration or supply of $FADH_2$. Despite a similar FEV1 (% predicted), our COPD patients had a pulmonary $V'_{O_2,max}$ of 20.4 $mL \cdot kg^{-1} \cdot min^{-1}$, which was 40% [3] and 25% [27] higher compared to the two earlier studies. The observation that mitochondrial respiration differences were attenuated after exercise training suggests absence of specific mitochondria respiratory impairment in COPD. This response to exercise training is also consistent with the hypothesis that physical inactivity may cause peripheral muscle respiratory deficiency in COPD patients.

Whole-body exercise in COPD patients has been shown to increase oxidative capacity and reduce lactic acid production during exercise [7–9, 29]. Our findings of an increased maximal mitochondrial respiration and improvement of complex I in COPD are in line with effects found in healthy individuals after endurance training [28]. Compared to athletes, sedentary people have a lower proportion of highly oxidative type I fibres [30], a phenotype also seen in COPD patients [31]. In COPD patients, PICARD *et al.* [3] found a reduced mitochondrial respiration that was normalised when correlated for CS levels. When we normalised our respiration data to CS activity, we observed that the complex I respiration was not different from the healthy controls. Also, the increase in maximal respiration after training was in the same magnitude as the increased CS activity, suggesting improved respiration due to increased mitochondrial mass. More mitochondria, rather than reversal of a dysfunctional respiratory chain, may therefore explain the mechanism behind the increased muscle aerobic capacity. This could point to inactivity, rather than dysfunction, as a possible explanation of the reduced mitochondrial respiration.

Also, at the whole muscle level, we found increased $V'_{O_2,peak}$ with an increased femoral blood flow at peak exercise after training. In healthy sedentary individuals endurance training results in both improved cellular bioenergetics and muscle oxygen transport [14]. This therefore adds further support to a normal training response in the COPD patients when relieved from ventilatory limitation. Surprisingly, we could not show reduced muscle V'_{O_2} at sub-maximal load, which would reflect an improved WE. Only the pulmonary V'_{O_2} was significantly reduced at sub-maximal levels suggestive of an improved WE in our study. We also observed a reduced V'_E, reflecting the reduced oxygen consumption. This is an important effect of endurance training for COPD patients, as this reflects activity at a moderate level, similar to activities of daily living.

Present data do not suggest that whole-body endurance training is efficient in reversing all aspects of impaired muscle function. Both VOGIATZIS *et al.* [8] and our group (unpublished data) failed to show an improvement in CS activity in thigh muscle even after high-intensity aerobic interval training. DOLMAGE and GOLDSTEIN [13] have shown that one-legged exercise training is superior to whole-body endurance training in improving aerobic capacity in COPD. The present study shows that full reversal of impaired mitochondrial respiration might not be attainable in whole-body endurance training, contributing to the advantages of one-legged exercise training in COPD.

There are some limitations to our study. Due to the invasiveness of this study, the number of participants was low. This may have resulted in loss of statistical significance, especially in some of the sub-analyses of the respiratory chain complexes.

We have suggested inactivity as a possible cause of reduced mitochondrial function, but did not measure the physical activity level of the participants. PITTA *et al.* [32] report that COPD is associated with inactivity, which supports our assumption that COPD patients are more inactive than healthy people. Still, despite it being a possible explanation, we cannot conclude that inactivity is the aetiology of the reduced muscle function in the COPD patients in our study.

Furthermore, we did not train the control subjects as the effects of exercise training on mitochondrial function in healthy individuals are well documented [28, 33, 34]. The measurements of $V'O_2$ in the quadriceps muscle could have been underestimated due to potential mixing of venous blood from calf musculature, and thereby leading to underestimated AVO_2 difference. However, placing a cuff distal to the knee to avoid this was not feasible due to the length of our peak work protocol.

Conclusion

High-intensity aerobic interval training of a limited muscle group restored work performance and oxidative capacity of the quadriceps muscle in COPD patients. The increased mitochondrial respiration was found mainly to be caused by an improvement of mitochondria complex I. Our results are similar to findings in sedentary individuals, and thereby suggest inactivity rather than a dysfunction as a possible aetiology.

SUPPORT STATEMENT

The study was supported by grants from the National Heart and Lung Foundation, the Norwegian Lung Medicine Society, Samarbeidsorganet NTNU and St Olavs Hopsital, K.G. Jebsen Foundation, and the Norwegian Research Council Funding for Outstanding Young Investigators (E. Brønstad, U. Wisløff, S. Steinshamn).

CLINICAL TRIAL

This study is registered at ClinicalTrials.gov with identifier number NCT01079221.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We are grateful to A.S. Fossum, B. Pedersen, I.L. Bjerkan (Lung Dept, St Olavs Hospital Trondheim, Norway), I. Arbo (Institute of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim) and A.E. Meistad (Dept of Heart and Lung Surgery, St Olavs Hospital, Trondheim) for technical assistance.

REFERENCES

1 Rabe KF, Hurd S, Anzueto A, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176: 532–555.
2 Rabinovich RA, Bastos R, Ardite E, *et al.* Mitochondrial dysfunction in COPD patients with low body mass index. *Eur Respir J* 2007; 29: 643–650.
3 Picard M, Godin R, Sinnreich M, *et al.* The mitochondrial phenotype of peripheral muscle in chronic obstructive pulmonary disease: disuse or dysfunction? *Am J Respir Crit Care Med* 2008; 178: 1040–1047.
4 Puente-Maestu L, Pérez-Parra J, Godoy R, *et al.* Abnormal mitochondrial function in locomotor and respiratory muscles of COPD patients. *Eur Respir J* 2009; 33: 1045–1052.
5 Gosker HR, van Mameren H, van Dijk PJ, *et al.* Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. *Eur Respir J* 2002; 19: 617–625.
6 Wagner PD. Skeletal muscles in chronic obstructive pulmonary disease: deconditioning, or myopathy? *Respirology* 2006; 11: 681–686.
7 Puente-Maestu L, Tena T, Trascasa C, *et al.* Training improves muscle oxidative capacity and oxygenation recovery kinetics in patients with chronic obstructive pulmonary disease. *Eur J Appl Physiol* 2003; 88: 580–587.
8 Vogiatzis I, Terzis G, Nanas S, *et al.* Skeletal muscle adaptations to interval training in patients with advanced COPD. *Chest* 2005; 128: 3838–3845.
9 Maltais F, LeBlanc P, Simard C, *et al.* Skeletal muscle adaptation to endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996; 154: 442–447.
10 Sala E, Roca J, Marrades RM, et al. Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 1726–1734.
11 Richardson RS, Leek BT, Gavin TP, *et al.* Reduced mechanical efficiency in chronic obstructive pulmonary disease but normal peak VO_2 with small muscle mass exercise. *Am J Respir Crit Care Med* 2004; 169: 89–96.
12 Richardson RS, Sheldon J, Poole DC, *et al.* Evidence of skeletal muscle metabolic reserve during whole body exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 881–885.
13 Dolmage TE, Goldstein RS. Effects of one-legged exercise training of patients with COPD. *Chest* 2008; 133: 370–376.
14 Astrand PO, Rodahl K, Dahl HA, *et al*. Textbook of Work Physiology: Physiological Bases of Exercise. 4th Edn. Champaign, IL, Human Kinetics, 2003.
15 Andersen P, Adams RP, Sjøgaard G, *et al*. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol* 1985; 59: 1647–1653.
16 Richardson RS, Poole DC, Knight DR, *et al.* High muscle blood flow in man: is maximal O_2 extraction compromised? *J Appl Physiol* 1993; 75: 1911–1916.
17 Krustrup P, Hellsten Y, Bangsbo J. Intense interval training enhances human skeletal muscle oxygen uptake in the initial phase of dynamic exercise at high but not at low intensities. *J Physiol* 2004; 559: 335–345.
18 Radegran G. Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol* 1997; 83: 1383–1388.
19 Mathur S, Takai KP, Macintyre DL, *et al.* Estimation of thigh muscle mass with magnetic resonance imaging in older adults and people with chronic obstructive pulmonary disease. *Phys Ther* 2008; 88: 219–230.
20 Ross R, Rissanen J, Pedwell H, *et al.* Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *J Appl Physiol* 1996; 81: 2445–2455.
21 Mendez J. Density and composition of mammalian muscle. *Metabolism* 1960; 9: 184–188.
22 Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 1975; 35: 609–616.
23 Srere PA. Citrate synthase. *Methods Enzymol* 1969; 13: 3–11.
24 Veksler VI, Kuznetsov AV, Sharov VG, *et al.* Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by

using saponin-skinned fibers. *Biochim Biophys Acta* 1987; 892: 191–196.
25 Kuznetsov AV, Veksler V, Gellerich FN, *et al.* Analysis of mitochondrial function *in situ* in permeabilized muscle fibers, tissues and cells. *Nat Protoc* 2008; 3: 965–976.
26 N'Guessan B, Zoll J, Ribera F, *et al.* Evaluation of quantitative and qualitative aspects of mitochondrial function in human skeletal and cardiac muscles. *Mol Cell Biochem* 2004; 256–257.
27 Naimi AI, Bourbeau J, Perrault H, *et al.* Altered mitochondrial regulation in quadriceps muscles of patients with COPD. *Clin Physiol Funct Imaging* 2011; 31: 124–131.
28 Daussin FN, Zoll J, Ponsot E, *et al.* Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle. *J Appl Physiol* 2008; 104: 1436–1441.
29 Casaburi R, Patessio A, Ioli F, *et al.* Reductions in exercise lactic acidosis and ventilation as a result of exercise training in patients with obstructive lung disease. *Am Rev Respir Dis* 1991; 143: 9–18.
30 Zoll J, Sanchez H, N'Guessan B, *et al.* Physical activity changes the regulation of mitochondrial respiration in human skeletal muscle. *J Physiol* 2002; 543: 191–200.
31 Jobin J, Maltais F, Doyon JF, *et al.* Chronic obstructive pulmonary disease: capillarity and fiber-type characteristics of skeletal muscle. *J Cardiopulm Rehabil* 1998; 18: 432–437.
32 Pitta F, Troosters T, Spruit MA, *et al.* Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005; 171: 972–977.
33 Daussin FN, Zoll J, Dufour SP, *et al.* Effect of interval *versus* continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects. *Am J Physiol Regul Integr Comp Physiol* 2008; 295: R264–R272.
34 Norrbom J, Sundberg CJ, Ameln H, *et al.* PGC-1α mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J Appl Physiol* 2004; 96: 189–194.