REVISED VERSION

ABNORMAL MITOCHONDRIAL FUNCTION IN LOCOMOTOR AND RESPIRATORY MUSCLES OF COPD PATIENTS

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Running head: Muscle mitochondrial function in COPD

Word count (excluding abstract, references and legends): 2809
ABSTRACT

Rationale: Several cellular and molecular alterations have been described in skeletal and respiratory muscles of patients with chronic obstructive lung disease (COPD), but information on potential abnormalities of mitochondrial function is scarce.

Objective: To study mitochondrial function in the Vastus Lateralis (VL) and External Intercostalis (EI) of COPD patients.

Methods: Biopsies from VL and EI were obtained during surgery for lung cancer in 13 patients with mild to moderate COPD (68±6yrs; FEV₁ 66±15%ref.) and 19 control subjects (67±9yrs; FEV₁ 95±18%ref.). State 3 and 4 mitochondrial oxygen consumption (V’O₂m), ATP synthesis, citrate-synthase (CS), cytochrome-oxidase (COX), and complex I-III activities as well as reactive oxygen species (ROS) production were determined.

Results: In COPD patients, in both muscles COX activity (VL: COPD 3.0±0.8 vs control 2.0±0.8; EI: 3.7±1.6 vs 2.4±0.9 µmol·min⁻¹·mg⁻¹) and ROS production (VL:1643±290 vs 1285±468; EI:1033±210 vs 848±288 arbitrary units) were increased (p<0.05) whereas state 3 V’O₂m reduced (p<0.05) (VL:2.9±0.3 vs 3.6±0.4, EI:3.6±0.3 vs 4.1±0.4 mmol·min⁻¹·kg⁻¹)

Conclusions: Skeletal muscle mitochondria of patients with COPD show electron transport chain blockade and excessive production of ROS. The concurrent involvement of both VL and EI suggests a systemic (rather than a local) mechanism(s) already occurring in relatively early stages (GOLD II) of the disease.

Abstract word count: 199
INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is frequently associated with several extra-pulmonary manifestations that influence significantly the course and prognosis of the disease [1]. Skeletal muscle dysfunction (SMD), one of the first systemic effects of COPD identified, contributes to limit exercise capacity and to jeopardize health status in these patients [2]. Further, it heralds poor prognosis, independently of the degree of airflow limitation [3]. Thus, a better understanding of the mechanisms leading to SMD in COPD is of great clinical interest.

Previous studies have described a number of cellular and molecular abnormalities in the skeletal muscle of patients with COPD that can contribute to SMD. These include, among others, a shift in the fibre type distribution, the presence of fibre atrophy, evidence of oxidative and nitrosative stress, protein nytrosilation, and enhanced apoptosis [2;4-6]. Despite the fact that mitochondria are involved in energy production and oxidative metabolism, as well as in the control of apoptosis, direct measurements of mitochondrial function in COPD muscles have seldom been obtained. Sauleda et al were the first to report that the activity of Cytochrome oxidase (COX), the terminal enzyme of the mitochondrial electron transport chain, was up-regulated in skeletal muscle of COPD patients in direct proportion to arterial hypoxemia [7]. More recently, Gosker et al found that the content of uncoupling protein 3 (a protein channel that facilitates proton leak, thus uncoupling oxidative phosphorylation from ATP synthesis, with energy dissipated as heat) was decreased in skeletal muscle of patients with COPD [8]. Finally Ribera et al reported increased mitochondrial electron transport in saponine-skinned isolated fibres of respiratory muscles of severe emphysematous patients [9]. Collectively, thus, these observations suggest that mitochondrial function in skeletal muscle of COPD patients may be altered.

This study sought to extend these previous and partial observations by characterizing mitochondrial function in skeletal muscles of patients with COPD comprehensively. To this end, we determined state 3 and 4 respiration, ATP production, and the activities of COX, respiratory chain complex I+III, citrate-synthase (CS) and the production of reactive oxygen species (ROS) in mitochondria isolated from the Vastus Lateralis (VL) of patients with moderate COPD and control subjects with normal lung function, matched for age and degree of regular physical activity. Further, because respiratory muscles seem to behave differently from skeletal muscle in patients with COPD [7], we also studied mitochondria isolated from the External Intercostalis (EI) in these same patients.
METHODS

Study design
Biopsies from VL and EI were obtained during thoracic surgery for lung cancer (stage I or II) in 13 patients with moderate COPD [1] and 19 patients with normal lung function and no significant co-morbidities or locomotor dysfunction. None of them had received chemotherapy before biopsies were obtained. All participants signed an informed consent after being fully aware of the objectives and nature of the study. The Institutional Committee for Ethics in Human Research of our hospital had approved the study. All the aspects of this study comply with the declaration of Helsinki.

Lung function and physical activity
Spirometry was performed using a Neumoscreen II (Jaeger, Hochberg, Germany) spirometer following international guidelines [10]. Regular physical activity was evaluated by the International Physical Activity Questionnaire (IPAQ) [11]. To further characterize the exercise capacity of participants, all of them performed an incremental exercise tests on an electromagnetically-braked cycle-ergometer (ER-900, Jaeger, Hochberg, Germany) using a ramp protocol at 20 Watts · min⁻¹ to a symptom-limited maximum. Ventilation and pulmonary gas exchange were measured breath-by-breath by a Quark b² cardiopulmonary exercise system (Cosmed, Roma, Italy).

Investigations on isolated mitochondria
Surgical biopsies of VL and EI were obtained while the patient was anesthetized by performing a small incision about 25 cm proximal to the patella (VL) and from the incised muscles during thorax opening at the level of the 5th or 6th intercostal spaces (EI). Biopsies were transferred within 1 min to ice-cold isolation buffer (300 mM manitol, 1 mM EGTA, 10 mM Trizma (Sigma-Aldrich, Inc, USA). CIH, 1 mM PO₄H₂K, 1,74 mg/ml Phenylmethylsulfonylfluoride, 0.2% bovine serum albumin, amoxicillin 10 mg/l, pH 7.4). Then, muscle samples were placed on a Petri plaque surrounded by ice slurry. Mitochondria were isolated from these biopsies following previously described methodology [12]. Briefly, after dissecting the fat and fibrous tissue, samples were weighted, washed with EGTA chopped up in small pieces with a blade and suspended with isolation buffer (1/2 wt/vol). This was then homogenized with a Potter-Eveljehm homogenizer (B.Braun Medical) avoiding heating of the sample. The homogenate was centrifuged at 4°C in a Sorvall RC-5: rotor SS-34 once for 10 min at 1075 g, followed by three steps of 10 min at 8635 g 4°C. The final mitochondrial pellet was re-suspended in an Eppendorf tube with 150µl of measurement buffer (300 mM Manitol, 10 mM Trizma 1 mM CIH, PO₄H₂K, pH 7.4) and stored on ice.

Mitochondrial oxygen consumption was measured with a Clark-type electrode (YSI Inc., Yellow Springs, Ohio, USA) in a metacrilate chamber at 37º equipped with magnetic stirring in the presence of 10µl of succinate 5 mM with (state 3) or without ADP 5mM (state 4). Mitochondrial oxygen consumption is expressed in mmol · min⁻¹ · kg⁻¹ of muscle tissue. The respiratory control index (RCI) was calculated as the ratio of state 3 to state 4 rates of respiration. ATP synthesis was indirectly assessed by measuring spectrophotometrically (Uvikon 930 spectrofluorometer, Kontron, Milano, Italy) the increase in NADPH concentration coupled to glucose phosphorylation by hexoquinase and later oxidation of glucose-6-phosphate to 6-phosphogluconate by glucose 6 phosphate-dehydrogenase [13]. To account for potential differences in mitochondrial density, measured values of State 3 and 4 mitochondrial oxygen consumption and rate of ATP synthesis were expressed as tissue activities[14] (i.e.
taking into account the dilution factor, the yield of mitochondria - an indirect validated marker of mitochondrial volume density in skeletal muscle - and the biopsy mass as mmol \cdot \text{min}^{-1} \cdot \text{kg}^{-1} of muscle. The activity of COX (E.C. 1.9.3.1, Complex IV), citrate-synthase (E.C. 2.3.3.1), and complex I+III (rotenone-sensitive NADH cytochrome c reductase (EC 1.6.2.1) were determined according to previously described spectrophotometric methods [12;15]. Aliquots of the homogenate and final mitochondrial suspension were assayed for total protein content [16]. Mitochondrial yield was estimated as CS in the mitochondrial suspension relative to that of the actual biopsy. All reagents were obtained from Sigma Chemical (St. Louis, MO).

ROS production and SOD (EC 1.15.1.1) activity were measured in 9 patients with COPD and 10 control subjects. Fixed volumes (10 µl) of re-suspended mitochondria were incubated with 50µM dichloro-dihydrofluorescein-diacetate (H2DCFDA) at 37°C for 60 min. ROS production is directly proportional to fluorescence emission (between 480 and 520 nm) measured using a multi-detection microplate reader (SBL/AMINCO, Rochester, NY). To assess ROS production during states 3 and 4 respiration, 70 mM of ADP and 10 mM of glutamate were added immediately before the addition of H2DCFDA. The activity of the SOD was measured by blue tetrazolium reduction method (Sigma-Aldrich, USA) [17].

**Statistical analysis**

Results are shown as mean ± standard deviation, unless otherwise specified. Comparisons between groups were performed by t tests. Correlations between variables of interest were explored with the Pearson correlation test. A “p” value lower than 0.05 was considered significant. Analyses were performed with the statistical package SPSS 11.0 (Hispanoportuguesa SPSS SL, Madrid, Spain).

**RESULTS**

**Clinical data**

Patients and control subjects were well matched in terms of age, weight, body mass index (BMI), and the level of usual physical activity, which was relatively low in both groups (Table 1). Patients with COPD had moderate airflow obstruction, whereas spirometry was normal in control subjects (Table 1). Exercise tolerance was preserved in both groups, albeit a tendency to lower peak oxygen uptake was seen in the COPD. At peak exercise patients with COPD had less ventilatory reserve (p=0.02). While dyspnea scores were not different between groups, leg fatigue scores tended to be higher in COPD patients (Table 1). Specifically questioned all the patients declared to have stopped smoking at the time of the admission to the hospital.

**Mitochondrial function**

Table 2 shows mitochondrial function measurements in VL and EI in the two groups of subjects studied. Biopsy weight, mitochondrial yield and mitochondrial protein content were similar in both groups and both muscles, supporting the reproducibility of the methodology used. Compared with control subjects, patients with COPD showed slightly but significantly reduced \( V^\prime O_2 \text{m} \) (state 3) values and ATP production, both in VL and EI (Table 2). The RCI was significantly reduced in the VL of patients with COPD, whereas in the EI, differences just failed to reach statistical significance (Table 2). The P:O ratio (i.e. the efficiency of ATP synthesis coupled with cell respiration) was similar (around 1.5) in both groups and both muscles (Table 2) Compared with control subjects CS activity (a mitochondrial matrix enzyme) was also reduced in the VL of
subjects with COPD (Table 2). In contrast, the activity of mitochondrial membrane enzymes (COX and complex I-III) was significantly higher in COPD patients than in control subjects in both muscles (Table 2). There were a modest, but significant \( p<0.01 \) correlations between the peak \( V^*O_2 \) of the incremental exercise test and CS (\( r=0.58 \)) and state 3 respiration (\( r=0.65 \)). Interestingly, COX activity was negatively related to arterial PaO2, at rest and during exercise (Figure 1). A similar relationship (data not shown) was observed with the activities of the other respiratory chain enzymes.

**ROS production and Superoxide dismutase (SOD) activity**

During state 3 respiration (ADP stimulated) (Figure 2, panel A) the production of ROS was significantly higher (\( p<0.01 \)) in patients with COPD than in control subjects, both in VL (2.4 fold increase) and EI (1.7 fold increase). Similar results were observed during state 4 (glutamate stimulated) respiration (VL 2.9 fold increase; EI 1.8 fold increase; \( p<0.01 \)) (Figure 2, panel B). Figure 2 also corroborates the expectation that ROS production is highest in the absence of ADP (state 4 respiration), when the mitochondrial membrane potential is highest [18]. ROS production was significantly (\( p<0.001 \)) related to CI+III activity in both state 4 and state 3 as we can see in Figure 3, indicating, as anticipated, that the mitochondrial electron transport chain is a major source of ROS production in skeletal muscle [19].

The activity of the antioxidant enzyme superoxide dismutase (SOD) was increased in mitochondria isolated from the VL in patients with COPD (\( p<0.05 \)), but differences failed to reach statistical significance in EI samples (Figure 2, panel C).
DISCUSSION

Our study shows that the mitochondria isolated from the vastus lateralis and external intercostalis of COPD patients present increased ROS production and a set of abnormalities consistent with mitochondrial membrane blockade [20;21] (Table 2 and figures 1 and 2).

Previous studies

Several studies have previously investigated partial aspects of mitochondrial function in muscle biopsies of patients with COPD. To our knowledge, our study is the first describing mitochondrial function comprehensively in both peripheral and respiratory muscles in these patients. Sauleda et al reported increased COX activity in VL of patients with COPD [7]. Our results are in keeping with this observation (Table 2, Figure 1) and extend it by showing that the activities of other membrane bound respiratory chain enzymes are also up-regulated in patients with COPD (Table 2). Also in keeping with some former studies [22-24], we found decreased CS activity in the VL (Table 2). CS is a citric acid cycle enzyme of the mitochondrial matrix. Thus, the heterogeneous alteration of the mitochondrial oxidative metabolism enzymes found in patients with COPD suggests that the activities of citric acid cycle and the electron transport chain are unmatched in these patients [14:25] and points to different regulatory mechanism for both types of mitochondrial enzymes. In fact, COX and complex III are partially coded by mitochondrial DNA, while citric acid enzymes are regulated by nucleic DNA [26].

Increased ROS production by COPD striated muscle has been previously suspected from indirect findings [27], however we have actually measured it in our study (Figure 2 and Figure 3). Superoxide anions are produced by the electron transport chain on the inner mitochondrial membrane [19] and their generation is strongly depend upon the proton potential across the mitochondrial membrane [18], which was likely increased in the COPD mitochondria as consequence of the increased respiratory enzyme activity together with the mitochondrial respiratory membrane blockade suggested by the lack of translation into more oxygen uptake [20;21]. Finally, Rabinovich et al reported reduced RCI in patients with COPD and low BMI [28]. Our results extend these findings to VL of subjects with normal BMI (Table 1).

Mechanisms

This is a descriptive study that does not address potential mechanisms directly. However, our observations allow some speculation. That mitochondrial dysfunction occurred in COPD both in skeletal and respiratory muscles (Table 2) suggests a systemic, rather than a local factor(s). We can exclude sedentarism, a frequently quoted mechanism in these patients; because both groups had similar activity history and both had a reasonably preserved exercise capacity (Table 1). Smoking may have influenced mitochondrial function [21]; however, as shown in Table 1, there were no apparent differences in cumulative smoking exposure, carboxyhemoglobin levels and/or percentage of current smokers between both groups. Sauleda et al reported increased COX activity in VL of patients with COPD in proportion to arterial hypoxemia [7]. We also found an inverse relationship between respiratory chain activity and PaO2 (Figure 1). This suggests that tissue hypoxia may upregulate respiratory chain enzymes, thus contributing to mitochondrial dysfunction. The absence of resting arterial hypoxemia does not exclude this possibility because it may occur during exercise (Figure 1) or
sleep. An abnormal microcirculatory control that may eventually result in tissue hypoxia cannot be excluded either [29]. Finally, low-grade chronic systemic inflammation in COPD [30] may be another potential mechanism because inflammatory cytokines are related to basal energy metabolism and are known to trigger the production of ROS by striated muscle fibres [27].

**Implications**

Mitochondrial capacity is a well known limiting factor for exercise performance [14]. Our observations of a correlation between State 3 respiration, CS activity and exercise capacity suggests that the functional abnormalities identified here can contribute to limit exercise in patients with COPD. On the other hand, it is worth noting that the enhanced ROS production observed in patients with COPD (Figure 2) can produce further skeletal muscle damage through several, non mutually exclusive pathways. First, oxidative stress can alter the structure of several components of the respiratory chain [31] and cause proton leak and mitochondrial uncoupling [20;32], particularly during exercise [14;33]. As a consequence, the potential for physical activity in these patients and/or their resistance to fatigue under conditions of increased respiratory load, such as episodes of exacerbation of COPD may be impaired. Second, oxidative stress can be involved in muscle atrophy, a well-recognized poor prognostic factor in COPD [3], since it alters the structure of muscle proteins, including myosin, facilitating their degradation by the ubiquitin-proteosome system [6;34]. It can also trigger apoptosis, an event described in the skeletal muscle of COPD [35] patients. Finally, excessive ROS production deteriorates mitochondrial DNA and contributes to perpetuate muscle damage [36].

**Potential limitations**

Several potential limitations of our study deserve comment. First, mitochondrial function was measured “in vitro”. Albeit this was done under strictly controlled experimental conditions following the same standard methodology in both groups [19], our results can not be readily extrapolated to “in vivo” conditions. The fact that we obtained a high yield (~35%) of functionally intact mitochondria [12], and values of RCI, P:O, and mitochondrial oxygen consumptions (Table 2) similar to previously published results [33;37;38] indicate good preservation of the organelle’s membrane and supports the adequacy of our methodology. Moreover, previous papers using similar isolation procedures showed a good relationship between mitochondrial respiration “in vitro” and muscle maximum oxygen consumption “in vivo” [14]. Secondly, using succinate, as we did, will provide electron input starting from complex II that typically renders lower state 3 V’O₂m (about 85% of the maximal state 3 respiration measured with other substrates) [33;38]. However, this fact will not substantially alter our conclusions since we compared COPD results with non-COPD control subjects studied exactly in the same way. Thirdly, smoking may have influenced our results but, declared smoking history and COHb levels were similar between patients and control subjects. Fourthly, because smoking is forbidden in our hospital, it is likely that the majority (if not all) current smokers had not smoked during the 24 hours they were hospitalized before surgery, when muscle samples were obtained. Fourthly subjects studied here had localized lung cancer. This approach has been used in other studies of respiratory muscles in COPD [9] and we do not believe that it had influenced our results because the same happened in both patients and control subjects and previous work has found no differences in the structural characteristics and expression of inflammatory cytokines and growth factors in samples from EI of patients with...
localized lung cancer [39]. Finally, we studied patients with mild-moderate COPD and normal BMI (Table 1) so results may be different in more advanced stages of the disease.

Conclusions
Patients with mild-moderate COPD show evidence of mitochondrial blockade in both skeletal and respiratory muscles, suggesting that functional mitochondrial abnormalities occur in relation to systemic (as opposed to local) factors, and that they are already present at moderate stages of the disease (GOLD II).

Acknowledgements
Authors thank the patients participating in this study for their willingness to contribute to the advancement of science. We are also grateful to Prof. Antoniò L. Andreu and Prof. Helena García-Arumí, from the Centre d'Investigació en Bioquímica i Biologia Molecular (A.L.A.), University Hospital Vall d'Hebron Barcelona, Spain for their help in the determination of the complex I-III of the respiratory chain. CIBERES is an initiative of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación)

Competing interests: All authors declare that the answer to the questions on your competing interest form are all No and therefore have nothing to declare

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Table 1. Demographic and physiologic characteristics of the two groups of subjects studied.

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<tr>
<th></th>
<th>control subjects</th>
<th>COPD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>67±9</td>
<td>68±6</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72±9</td>
<td>71±12</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±3</td>
<td>26±4</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking exposure, pack-yrs</td>
<td>52.1 ±9.3</td>
<td>53.0 ±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Declared current smokers, %</td>
<td>12.5%</td>
<td>15.3%</td>
<td>NS</td>
</tr>
<tr>
<td>Physical activity, IPAQ</td>
<td>1.1±0.2</td>
<td>1.3±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1% ref</td>
<td>95±18</td>
<td>66±15</td>
<td>&lt;0.001</td>
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<tr>
<td>FEV1/FVC, %</td>
<td>77±6</td>
<td>57±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>V’O₂peak, % ref</td>
<td>82±11</td>
<td>73±11</td>
<td>0.053</td>
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<tr>
<td>Vₑ/MVV, %</td>
<td>65±15</td>
<td>81±8</td>
<td>0.02</td>
</tr>
<tr>
<td>Dyspnea, Borg scale</td>
<td>6.4±1.4</td>
<td>5.9±1.4</td>
<td>NS</td>
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<tr>
<td>Leg fatigue, Borg scale</td>
<td>5.0±1.2</td>
<td>6.0±0.7</td>
<td>0.068</td>
</tr>
<tr>
<td>Resting PaO₂ (mmHg)</td>
<td>75.5 ± 6.2</td>
<td>66.6±4.1</td>
<td>&lt;0.001</td>
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<tr>
<td>Exercise PaO₂ (mmHg)</td>
<td>77.5 ± 5.1</td>
<td>63.2±4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>COHb(*)</td>
<td>2.7±1.4%</td>
<td>3.2±1.51</td>
<td>NS</td>
</tr>
</tbody>
</table>

V’O₂peak, % ref: Peak oxygen uptake as percent of predicted Vₑ/MVV: ventilatory reserve usage; NS: non significant
Table 2. Mitochondrial function measurements (mean±SD) in *vastus lateralis* and *external intercostalis* in control subjects and COPD patients.

<table>
<thead>
<tr>
<th></th>
<th><em>Vastus lateralis</em></th>
<th></th>
<th></th>
<th><em>External intercostalis</em></th>
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<th></th>
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<tr>
<td></td>
<td>control subjects</td>
<td>COPD</td>
<td>p value</td>
<td>control subjects</td>
<td>COPD</td>
<td>p value</td>
</tr>
<tr>
<td>Biopsy weight, g</td>
<td>2.2±1.1</td>
<td>1.8±0.6</td>
<td>NS</td>
<td>1.4±0.5</td>
<td>1.4±0.6</td>
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<tr>
<td>Mitochondrial yield, %</td>
<td>35.6±4.2</td>
<td>34.2±3.1</td>
<td>NS</td>
<td>34.1±5.1</td>
<td>34.7±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Mitochondrial protein, g · kg⁻¹</td>
<td>9.5±1.4</td>
<td>9.4±2.7</td>
<td>NS</td>
<td>9.4±2.5</td>
<td>8.9±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Resp. Control Index (RCI)</td>
<td>4.7±1.4</td>
<td>3.7±0.9</td>
<td>0.015</td>
<td>4.6±0.4</td>
<td>3.9±0.9</td>
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<tr>
<td>P:O ratio</td>
<td>1.6±0.5</td>
<td>1.6±0.5</td>
<td>NS</td>
<td>1.5±0.5</td>
<td>1.8±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>V'O₂ m (state 3) mmol·min⁻¹·kg⁻¹(*)</td>
<td>3.6±0.4</td>
<td>2.9±0.3</td>
<td>0.002</td>
<td>4.1±0.4</td>
<td>3.6±0.3</td>
<td>0.03</td>
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<tr>
<td>V'O₂ m (state 4) mmol·min⁻¹·kg⁻¹(*)</td>
<td>0.71±0.21</td>
<td>0.80±0.16</td>
<td>NS</td>
<td>0.71±0.48</td>
<td>0.73±0.32</td>
<td>NS</td>
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<tr>
<td>COX, µmol · min⁻¹ · mg⁻¹</td>
<td>2.0±0.8</td>
<td>3.0±0.7</td>
<td>0.0001</td>
<td>2.4±0.9</td>
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<td>0.02</td>
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<tr>
<td>Complex I-III, µmol · min⁻¹ · mg⁻¹</td>
<td>1.8±0.9</td>
<td>2.3±0.7</td>
<td>NS</td>
<td>2.1±0.9</td>
<td>2.8±1.0</td>
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<tr>
<td>CS, µmol · min⁻¹ · mg⁻¹</td>
<td>18.5±2.7</td>
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<td>0.03</td>
<td>20.5±2.8</td>
<td>19.5±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>ATP production, µmol · min⁻¹ · kg⁻¹(*)</td>
<td>5.4±0.9</td>
<td>4.6±1.1</td>
<td>0.03</td>
<td>6.1±0.8</td>
<td>5.4±0.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

V'O₂m (state 3): maximal oxygen consumption in the presence of succinate and saturating concentration of ADP; P:O ratio: rate of ATP synthesis per rate of respiration. p values refer to comparisons between groups (COPD vs. control subjects) for a given muscle (VL or EI). NS: non significant. (*) Tissue activity (i.e. calculated from the rates of mitochondrial respiration or ATP synthesis, a dilution factor, the yield of mitochondria and the biopsy mass).
FIGURE LEGENDS

Figure 1. Correlation between arterial PO2 at rest (left panels) and during exercise (right panels) and COX activity in VL (upper panels) and EI (bottom panels). For further explanations see text.
Figure 1

- Control
- COPD

Cox activity (iu/ml x mmHg)

Vastus Lateralis

External Intercostal

Exercise PaO2 (mm Hg)

Resting PaO2 (mm Hg)

r = 0.68 (p < 0.01)

r = 0.59 (p < 0.01)

r = 0.60 (p < 0.01)

r = 0.55 (p < 0.01)
Figure 2. Mean (±SD) ROS production (Arbitrary Fluorescence Units in isolated mitochondria during state 3 (ADP stimulated) (panel A) and state 4 (succinate stimulated) respiration (panel B). Panel C shows mean (±SD) Mn$^{2+}$ superoxide dismutase (Mn-SOD) activity. (*) =p<0.05; (**) p<0.01 For further explanations, see text.
Figure 3. Relationship between Complex I+III activity and ROS production. AFU, Arbitrary fluorescents units; Vastus lat., “Vastus lateralis”; Ext. intercostal, External intercostal; CI+III: rotenone-sensitive NADH cytochrome c reductase. Hollow triangles: Control subjects. Solid triangles: COPD patients
References


