



Mitochondrial dysfunction in COPD patients with low body mass index

R.A. Rabinovich^{*,#}, R. Bastos^{*,#}, E. Ardite^{*}, L. Llinàs^{*}, M. Orozco-Levi^{†,‡,+}, J. Gea^{†,‡,+}, J. Vilaró[§], J.A. Barberà^{*}, R. Rodríguez-Roisin^{*}, J.C. Fernández-Checa[†] and J. Roca^{*}

ABSTRACT: Patients with chronic obstructive pulmonary disease (COPD) show abnormal adaptations of skeletal muscle redox status after exercise training. Increased skeletal muscle oxidative stress in COPD patients may prompt mitochondrial dysfunction.

The present study explores the association between body composition and mitochondrial respiration in seven COPD patients with low body mass index (BMI_L), eight COPD patients with normal body mass index (BMI_N) and seven healthy controls. All of them underwent a *vastus lateralis* biopsy in which muscle structure, *in vitro* mitochondrial respiratory function, uncoupling protein 3 (UCP3) mRNA expression and glutathione levels in both isolated mitochondria and the whole muscle were determined.

Mitochondrial respiratory function (assessed by acceptor control ratio (ACR)) was impaired in BMI_L (2.2 ± 0.6) compared with both BMI_N (5.3 ± 1.3) and controls (8.2 ± 1.3). ACR significantly correlated with arterial oxygen tension and with muscle endurance but it showed a negative association with exercise-induced increase in blood lactate levels. UCP3 mRNA expression was reduced in BMI_L patients.

In conclusion, chronic obstructive pulmonary disease patients with low body mass index show electron transport chain dysfunction, which may contribute to low muscle endurance in the current subgroup of patients.

KEYWORDS: Endurance training, glutathione, mitochondrial respiration, muscle dysfunction

Chronic obstructive pulmonary disease (COPD) [1] is associated with several extrapulmonary effects. Skeletal muscle dysfunction, one of the most extensively studied systemic effects, is a prominent contributor to exercise limitation [2]. Limb muscle abnormalities exhibit a multifactorial nature, including physical inactivity, systemic inflammation/oxidative stress and cell hypoxia [3–5]. Loss of muscle mass, which occurs in a subgroup of COPD patients [6], results in a deleterious impact on health status, increased use of healthcare resources and poor disease prognosis, which is partly independent of the degree of forced expiratory volume in one second (FEV₁) impairment [7, 8]. A better knowledge of the interplay among mechanisms determining limb muscle dysfunction and loss of fat-free mass (FFM) in COPD may enhance the understanding of the functional heterogeneities of the disease, which in turn may lead to a better management of patients.

In previous studies [9, 10], the present authors reported that although physical training improves muscle bioenergetics in COPD patients

irrespective of the staging severity, abnormal adaptation to muscle oxidative stress is generated by repeated high-intensity training sessions. While in control subjects reduced glutathione (GSH) concentration is increased in skeletal muscle after 8-week endurance training, the opposite effect is observed in COPD patients, in whom increased oxidised glutathione (GSSG) levels were observed after training. These data suggest that antioxidant buffering did not fully adapt to the higher rate of reactive oxygen species (ROS) production, leaving the trained muscle more susceptible to oxidative stress. Altered adaptations of muscle redox status after 8-week endurance training were particularly evident in patients with low body mass index (BMI_L) [9, 10]. Interestingly, GSH and GSSG concentrations at baseline did not differ between COPD patients and control subjects [9]. Nevertheless, since a fall in mitochondrial glutathione can lead to deleterious consequences for the cell, even in the presence of cytosol GSH [11], and because it represents 10–15% of the total GSH pool size, the regulation of mitochondrial GSH needs to be evaluated in isolated organelles.

AFFILIATIONS

*Pneumology Service (ICT), Hospital Clinic, and
†Liver Unit (IMD)-CSIC, IDIBAPS, Barcelona University,
‡Muscle and Respiratory System Research Unit, IMIM, CEXS, Pompeu Fabra University,
+Respiratory Medicine Department, Hospital del Mar, and
§EUIF Blanquerna, Ramon Llull University, Barcelona, Spain., and
#R.A. Rabinovich and R. Bastos contributed equally to the study.

CORRESPONDENCE

R.A. Rabinovich
ELEGI Laboratory
Centre for Inflammation Research
MRC/University of Edinburgh
47 Little France Crescent
Edinburgh, EH16 4TJ
UK
Fax: 44 1312426582
E-mail: roberto.rabinovich@ed.ac.uk

Received:

June 30 2006

Accepted after revision:

December 10 2006

SUPPORT STATEMENT

The present study was supported by the following grants: La Marató de TV3 - 072(2004); SEPAR (N-2003-500621-D); Comissionat per a Universitats i Recerca de la Generalitat de Catalunya (SGR-00386); and Red Respira - ISCIII - RTIC-03/11.

STATEMENT OF INTEREST

None declared.

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

It has been recently shown that uncoupling protein 3 (UCP3) levels are diminished in the skeletal muscle of patients with COPD [12, 13], which in turn return to normal levels after 8-weeks of endurance training [14]. The exact physiological function of UCP3 is unknown. Although it is primarily linked to enhanced rest energy expenditure through mitochondrial respiratory chain uncoupling [15], it was hypothesised that the physiological role of UCP3 is to protect mitochondria against lipotoxicity in cases where fatty acid influx exceeds the capacity to oxidise them. Interestingly, activity of 3-hydroxyacyl-CoA dehydrogenase (HAD), an important enzyme involved in the oxidation of fatty acids, is reduced in the skeletal muscle of COPD patients [16]. Furthermore, lipid peroxidation has been associated to mitochondrial dysfunction [17, 18]. It is important to emphasise that in two of the aforementioned studies [12, 14] the FFM index (FFMi) was assessed and was found to be significantly lower in COPD patients compared with control subjects. With this in mind, it was of interest to analyse the relationship between UCP3 levels and body composition.

Unlike peripheral skeletal muscles, respiratory muscles show adaptive changes in response to the chronic mechanical load, *i.e.* increased mitochondrial density, increased oxidative capacity, increased density of interfibrillar capillaries and increased proportion of fibre type I [19, 20]. Equally, mitochondrial electron transport chain function is enhanced in inspiratory muscles of patients with COPD [21]. Therefore, different behaviour of the mitochondrial respiratory chain could be expected in peripheral and respiratory muscles.

It was hypothesised that mitochondrial function may be impaired in the peripheral muscles of COPD patients, especially in the subgroup of patients with BMIL. In the present study, the current authors assessed the *ex vivo* peripheral muscle mitochondrial function and its relationship with arterial oxygen tension (P_{a,O_2}), whole muscle and mitochondrial GSH and GSSG concentrations and UCP3 regulation.

For this purpose, an open biopsy of the *vastus lateralis* was performed in eight COPD patients with normal body mass index (BMIN), seven COPD patients with BMIL ($<21 \text{ kg}\cdot\text{m}^{-2}$) and seven healthy sedentary individuals. Muscle structure, *in vitro* muscle mitochondrial respiration, UCP3 mRNA expression and GSH and GSSG levels in both muscle homogenate and isolated mitochondria were examined [11].

METHODS

Study group

A total of 15 male, clinically stable COPD patients (table 1) [22] took part in the study. All patients were currently receiving bronchodilators and inhaled corticosteroids. They were clinically stable at the time of the study, *i.e.* without episodes of exacerbation or oral steroid treatment in the previous 4 months. None presented significant comorbidities. Seven age-matched healthy sedentary subjects (all male) were included in the study as controls. Eight COPD patients were characterised as presenting BMIN ($\text{BMI} \geq 21 \text{ kg}\cdot\text{m}^{-2}$) and the remaining seven COPD patients were characterised as presenting BMIL ($\text{BMI} < 21 \text{ kg}\cdot\text{m}^{-2}$) [23]. Selection procedures for inclusion in the study were: 1) clinical assessment; 2) pulmonary function testing (Master Screen; Jaeger, Würzburg, Germany) [24, 25]; 3) chest radiograph film; and

4) general blood analysis. Characterisation of the subjects included: 1) body composition analysis by Bioimpedance (Quantum X; RJL Systems Instruments, Clinton Township, MI, USA); 2) incremental cycling exercise protocol (CardiO₂ cycle; Medical Graphics Corporation, St. Paul, MN, USA) [23]; 3) left quadriceps static (isometric) and dynamic (isokinetic) strength and endurance (Cybex 6000; Lumex Inc., Ronkokoma, NY, USA); and 4) muscle morphometry using immunohistochemical procedures [26]. All participants were informed of any potential risks and discomfort associated with the experiment. Written informed consent was obtained in accordance with the Committee on Investigations Involving Human Subjects at the Hospital Clinic, Barcelona University (Barcelona, Spain), which approved the study.

Muscle biopsy

An open muscle biopsy of the *vastus lateralis* (~0.8 g) was performed and processed as follows: 1) ~0.65 g of the muscle

TABLE 1 Characteristics of the study groups

	COPD BMIN	COPD BMIL	Controls
Age yrs	63.1±2.1	61.3±5.6	61.8±2.2
Weight kg	81.0±5.9	55.4±2.3 ^{#, †}	78.9±5.5
BMI kg·m ⁻²	29.0±1.7	19.2±0.6 ^{‡, §}	27.9±1.9
FFM kg	59.6±2.7	49.1±1.9 ^{#, †}	62.8±2.9
FFMi kg·m ⁻²	21.4±0.9	17.0±0.5 ^{‡, §}	22.3±0.9
FEV ₁ L	1.5±0.2 [‡]	1.2±0.2 [‡]	3.2±0.2
FEV ₁ % pred	47.4±6.3 [‡]	32.5±5.7 [‡]	95.3±5.2
FVC % pred	79.5±4.9	54.9±5.0 ^{‡, §}	97.0±4.6
TLC % pred	109.8±3.7	107.9±6.5	
FRC % pred	141.4±9.5	159.3±13.9	
RV % pred	159.3±14.3	190.3±18.8	
DL _{CO} % pred	59.6±5.3	38.8±3.3 [†]	
P_{a,O_2} at rest mmHg	77.3±5.5	72.9±1.6 [#]	94.6±5.6
P_{a,O_2} during exercise mmHg	71.8±9.2 [#]	62.6±4.1 [#]	103.7±2.2
P_{a,CO_2} mmHg	42.5±2.6	41.7±2.3	37.4±1.7
Peak power W	96.9±7.9 [‡]	44.6±9.4 ^{‡, §}	154.3±14.2
$V'O_2$ peak L·min ⁻¹	1.5±0.1	0.6±0.1 ^{‡, §}	2.1±0.2
Isometric strength N·m	184.1±16.4	117.1±19.5 ^{†, †}	204.8±11.7
Isokinetic ^f N·m	117.7±12.1	61.6±11.6 ^{†, †}	129.1±11.2
Endurance J	2097.4±173	790.8±153.5 ^{†, †}	2199.9±225.9
fCSA μm^2	1988.4±217.3	1816.8±212.8	2362.1±181.7
Type I %	31.8±2.2	25.7±6.9	31.3±3.4
Capillary/fibre ratio	0.6±0.1	0.5±0.1	0.5±0.1

Data are presented as mean±SEM. COPD: chronic obstructive pulmonary disease; BMIN: normal body mass index (BMI); BMIL: low BMI; FFM: fat-free mass; FFMi: FFM index; FEV₁: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; DL_{CO}: diffusing capacity of the lung for carbon monoxide; P_{a,O_2} : arterial oxygen tension; P_{a,CO_2} : arterial carbon dioxide tension; $V'O_2$: oxygen uptake; fCSA: fibre cross-sectional area. [#]: $p < 0.01$ versus controls; [†]: $p < 0.01$ versus BMIN; [‡]: $p < 0.001$ versus controls; [§]: $p < 0.001$ versus BMIL. *p*-Values were calculated using ANOVA via a Newman-Keuls *post hoc* analysis of contrasts. ^f: isokinetic strength measured at 90°·s⁻¹. 1 mmHg=0.133 KPa.

sample was added to Krebs's buffer (pH 7.40) solution for immediate processing assays (homogenate and mitochondrial glutathione concentrations and mitochondrial respiration assays); 2) ~0.15 g of the muscle sample was embedded in paraffin for immunohistochemical analysis; and 3) ~0.1 g of the muscle sample was added to RNA stabilisation reagent (RNAlater®, Ambion Inc., Austin, TX, USA) and stored at -20°C for RNA extraction.

Lung function at rest, body composition and exercise testing

Lung function

Forced spirometry, lung volumes and the transfer factor of the lung for carbon monoxide ($T_{L,CO}$) were measured (MasterScreen; Jaeger) and the results were expressed as a percentage of the reference values obtained in the present authors' laboratory [24, 25]. P_{a,O_2} , carbon dioxide tension, pH and blood lactate were analysed on a blood gas analyser (Ciba Corning 800, Medfield, MA, USA).

Body composition

Body composition was estimated using single-frequency (50 kHz) bioelectrical impedance analysis (Quantum X; RJL Systems Instruments) while subjects were in a supine position. FFM was calculated from sex-specific regression equations [27]. The FFMi was obtained by dividing FFM (in kg) by height (in m²).

Incremental exercise protocol

After the placement of an arterial catheter (Seldicath; Plastimed, Saint Leu la Forêt, France), subjects were installed on the cycle ergometer with the mouthpiece in place. Online calculations of whole body oxygen uptake ($\dot{V}O_2$), carbon dioxide output, minute ventilation, respiratory exchange ratio and cardiac frequency were obtained. Arterial blood samples were taken every 3 min throughout the test for analysis of blood gases and lactate. Blood samples were kept on ice until analysis (Ciba Corning 800). After 3 min of unloaded pedalling, the work rate was increased by 5 or 10 W·min⁻¹. All studies were performed using an electromagnetically braked cyclo-ergometer (CardiO₂ cycle; Medical Graphics Corporation) with mechanical assistance to overcome the internal frictional resistance.

Muscle strength

Measurements of maximal voluntary contractions of the left quadriceps were performed during static (isometric) and dynamic (isokinetic) contractions against an isokinetic system (Cybex 6000; Lumex Inc.). After a previous muscle warm-up, the peak extension torque was evaluated at 60° of knee flexion and the best of five repetitions was used for the analysis. The isokinetic strength test consisted of five repetitions at the following different angular speeds: 60, 90, 120, 180 and 240°·s⁻¹. The best peak torque and peak power were used for the analysis. An endurance test was also carried out in all patients. The test consisted of a set of 30 knee extensions at a speed of 90°·s⁻¹. The total work realised during the repetitions was used to analyse quadriceps endurance. To ensure the maximal muscle strength in each test, rest periods were introduced between them.

Muscle structure

The muscle was embedded in paraffin and 10-µm thick sections were cut, varying the inclination of the holder by 5° increments until the minimum fibre cross-sectional area (fCSA) was obtained, which was defined as truly transverse [28]. Consecutive cross-sections were processed by immunohistochemical techniques using monoclonal antibodies directed against myosin heavy chain isoforms type I and type II (MHCs and MHCf clones; Biogenesis, Poole, UK). The fCSA, *i.e.* mean least diameter, and proportions of type I and II fibres were assessed using a light microscope (OLYMPUS Series BX50F3; Olympus Optical Co., Hamburg, Germany) coupled with an image-digitising camera (Pixera Studio, version 1.2; Pixera Corporation, Los Gatos, CA, USA) and a morphometry program [29]. At least 100 fibres were measured from each biopsy [28]. Fibre diameters 40–80 µm were considered normal [26, 30].

Mitochondrial respiration

Mitochondria were obtained by centrifugation through a Percoll density gradient [31]. Enrichment and recovery of mitochondria were ascertained by the specific activity of succinic dehydrogenase. The rate of oxygen consumption was measured using a Clark-type oxygen electrode (Hansatech Instruments Ltd, King's Lynn, UK) within 1 h of muscle biopsy [32]. The respiratory function was analysed in a medium containing 225 mM sucrose, 5 mM MgCl₂, 10 mM KH₂PO₄, 20 mM KCl, 10 mM Tris and 5 mM hydroxyethyl piperazine ethane sulphonic acid, which was maintained at 25°C and continuously stirred using an electromagnetic stirrer and a bar flea. The mitochondrial suspension was added to the reaction medium and adenosine diphosphate (ADP)-limited respiration (state 4) was initiated by the addition of succinic acid (13.5 mM) in the absence of ADP. State 3 respiration was determined upon the addition of ADP (0.63 mM), a phosphate acceptor. Oxidative phosphorylation efficiency was assessed by acceptor control ratio (ACR), dividing the slope of state 3 by the slope of state 4 [11].

RNA isolation and UCP3 mRNA expression

Total RNA was extracted from muscle tissue samples treated with RNAlater® (Ambion Inc.) by using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. The quality of RNA samples was evaluated by capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). 1 µg of total RNA was reverse-transcribed to cDNA using the First Strand cDNA synthesis kit in a 20 µL reaction (Roche Diagnostics GmbH, Mannheim, Germany).

UCP3 mRNA expression was measured by real-time PCR (qPCR) using specific Assay-on-Demand Taqman Gene expression probes (Applied Biosystems, Foster City, CA, USA). The probe used for UCP3 spans the 1–2 exon junction amplifying both long and short variants of the gene. PCR reactions were performed with 1 µL of cDNA along with the Taqman PCR Universal Master Mix and the corresponding primers and probe. PCR reaction conditions were those recommended by the manufacturer. All sample and nontemplate control reactions were performed in duplicate in the ABI Prism 7900 Sequence Detection System (Applied Biosystems).

The comparative threshold cycle (CT) method for relative quantification of gene expression [33] was used to calculate relative changes in mRNA abundance. The CT values were obtained for each target probe and normalised with the corresponding CT values for the internal control housekeeping gene β_2 -microglobulin. The mRNA quantity was expressed as arbitrary units (Au).

Assessment of muscle redox status

Fresh muscle samples were homogenised in a buffer containing 20 mM Tris, 0.25 M sucrose, 40 mM KCl, 2 mM ethylene glycol tetraacetic acid and 1 mg·mL⁻¹ bovine serum albumin. A total volume of 500 μ L was separated for glutathione concentration assessment and the rest of the sample was used for mitochondrial isolation according to Percoll centrifugation gradient [31].

Glutathione concentration assessment

The two molecular forms, GSH and GSSG, were obtained by high-performance liquid chromatography (HPLC) in both homogenate and mitochondrial fractions, as described previously in detail [34]. HPLC apparatus equipped with a 3-aminopropyl column and an UV spectrophotometric detector (365 nm) was used.

Data analysis

Results are expressed as mean \pm SEM. Multiple comparisons were performed using ANOVA. A Neuman-Keuls test was used as a *post hoc* test for contrast analysis. When normality test failed, a logarithmic transformation was applied to the data. Pearson (or Spearman for abnormally distributed variables) regression analysis was used to assess univariate correlations. A p-value <0.05 was considered statistically significant.

RESULTS

Anthropometric and functional characteristics of COPD patients (BMiN n=8 and BMiL n=7) and controls (n=7) are indicated in table 1. As expected, FFMi was significantly lower in BMiL (17.0 ± 0.5 kg·m⁻²) than in both BMiN (21.4 ± 0.9 kg·m⁻²) and controls (22.3 ± 0.5 kg·m⁻²; p<0.001). None of the patients with BMiN showed abnormally low FFMi (<16 kg·m⁻²) [35]. Patients with BMiN showed higher FEV1 and diffusing capacity of the lung for carbon monoxide than those with reduced muscle mass. The distribution of patients, according to the Global Initiative for Chronic Obstructive Lung Disease [1] classification, was as follows: four in stage II (three BMiN and one BMiL), six in stage III (four BMiN and two BMiL) and five in stage IV (one BMiN and four BMiL). The two groups of patients showed a similar degree of mild arterial hypoxaemia, but exercise-induced hypoxaemia was slightly more pronounced in BMiL. Similarly, BMiL patients had lower exercise tolerance ($V'O_2$ peak 11 ± 1 mL·kg⁻¹·min⁻¹) than both BMiN (19 ± 2 mL·kg⁻¹·min⁻¹) and controls (26 ± 2 mL·kg⁻¹·min⁻¹; p<0.0001).

Quadricep muscle strength and endurance were also lower in the BMiL group compared with the other two groups (table 1). Muscle fCSA was higher (24%) in controls than in COPD patients, with identical results observed between BMiN and BMiL groups. No statistically significant differences among groups were detected. Muscle strength normalised by FFM (strength/FFM ratio) showed no differences between all

groups (data not shown). It is noteworthy that muscle endurance normalised by FFM (endurance/FFM ratio) was significantly lower in BMiL patients (16.7 ± 3.5 J·kg⁻¹) than in both BMiN patients (36.1 ± 4.1 J·kg⁻¹) and controls (35.1 ± 3.6 J·kg⁻¹; p<0.01).

As shown in figure 1, BMiL patients presented earlier arterial blood lactate release than both BMiN patients and controls. As expected, exercise-induced increases in blood lactate levels ([La]) showed a strong association with $V'O_2$ peak (r=0.84, p<0.0001) and peak power output (r=0.85, p<0.0001).

Mitochondrial respiration

Individual data for ACR are illustrated in figure 2. On average, ACR was significantly lower in BMiL patients (2.2 ± 0.6) than in both BMiN patients (5.3 ± 1.3) and in healthy sedentary subjects (8.2 ± 1.3 ; p<0.01). State 3 of respiration using succinate-energised mitochondria was lower in BMiL patients (20.7 ± 7.5 nmol·mg⁻¹·min⁻¹; p<0.01) compared with healthy controls (134.1 ± 32.9 nmol·mg⁻¹·min⁻¹), but no statistical differences were seen when compared with BMiN patients (72.1 ± 39.6 nmol·mg⁻¹·min⁻¹). No differences in state 4 of respiration were detected between the groups (BMiL 8.8 ± 2.3 nmol·mg⁻¹·min⁻¹, BMiN 10.7 ± 4.1 nmol·mg⁻¹·min⁻¹ and controls 17.4 ± 3.4 nmol·mg⁻¹·min⁻¹). Mitochondrial metabolic state 3 significantly correlated with ACR (r=0.68, p<0.001) and FFMi (r=0.46, p<0.05).

P_{a,O_2} showed a significant correlation with ACR both at rest (r=0.70, p=0.0016) and at peak exercise (r=0.63, p=0.009; all subjects taken as a whole for calculations). Exercise-induced increase in [La] from rest to 45 W was negatively correlated with ACR (r=-0.60, p=0.01). Furthermore, a positive correlation was shown between ACR and muscle endurance (r=0.44, p<0.05).

Muscle UCP3 mRNA expression

Individual figures for qPCR UCP3 mRNA expression are displayed in figure 3. On average, UCP3 mRNA expression

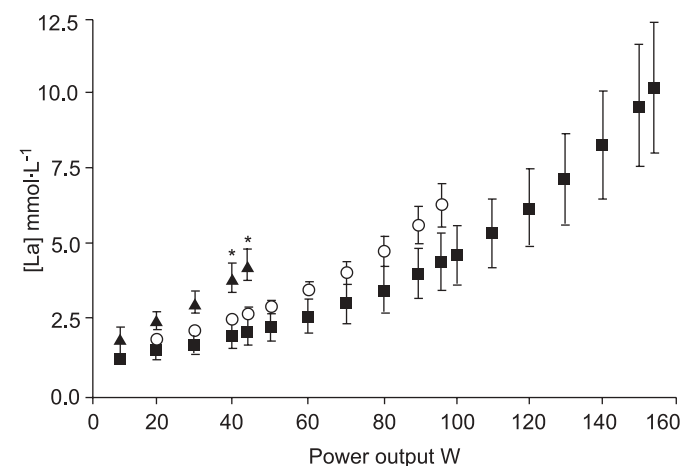


FIGURE 1. Mean arterial blood lactate levels ([La]) during incremental cycling exercise. The graph displays [La] in chronic obstructive pulmonary disease (COPD) patients with low body mass index (BMiL, ▲), COPD patients with normal body mass index (BMiN, ○) and healthy sedentary subjects (controls, ■) during incremental cycling exercise. Early lactate release was seen in BMiL patients compared with both BMiN and controls. *: p<0.05.

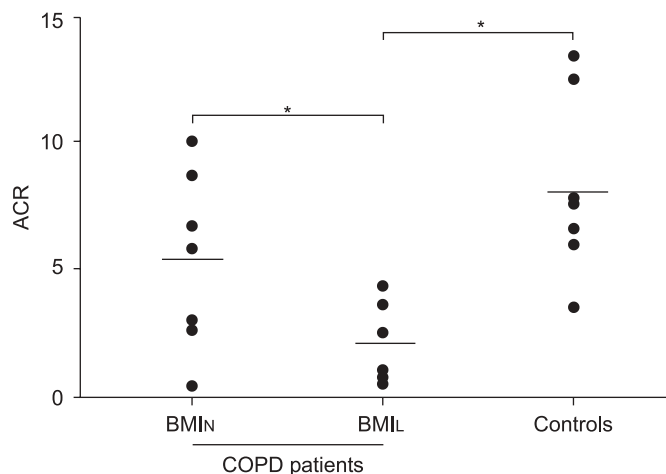


FIGURE 2. *In vitro* mitochondrial respiratory chain function. Individual (●) and mean group (—) data for acceptor control ratio (ACR). Significant differences in ACR were shown between chronic obstructive pulmonary disease (COPD) patients with low body mass index (BMiL) and normal body mass index (BMiN) patients, and between BMiL patients and controls. Data from one COPD patient with BMiN are missing due to technical problems. *: $p < 0.05$.

was lower in BMiL patients (618.2 ± 198.8 AU) compared with both BMiN patients ($1,371 \pm 306.4$ AU) and healthy controls ($1,118.8 \pm 326.8$ AU; $p = 0.07$). There was no association between UCP3 mRNA expression with any of the muscle measurements, either function or structure. However, a significant association was observed between UCP3 mRNA expression and FFMi ($r = 0.58$, $p < 0.01$; fig. 4).

Glutathione assessment

No differences among groups were observed in muscle homogenate concentrations of GSH (BMiL 7.1 ± 0.7 , BMiN 8.9 ± 2.3

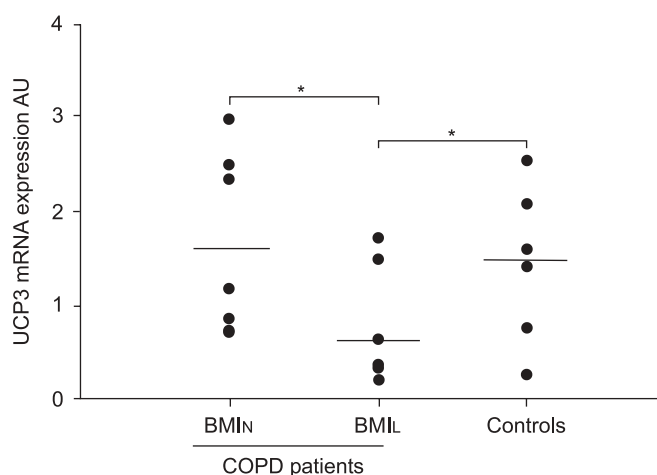


FIGURE 3. Uncoupling protein 3 (UCP3) mRNA expression. Individual (●) and mean group (—) data for UCP3 mRNA expression assessed by real-time PCR (qPCR) are presented. Low body mass index (BMiL) patients show lower values than both normal body mass index (BMiN) patients and controls. Data from one BMiN patient and one healthy subject are missing due to technical problems in qPCR assessment. *: $p = 0.07$.

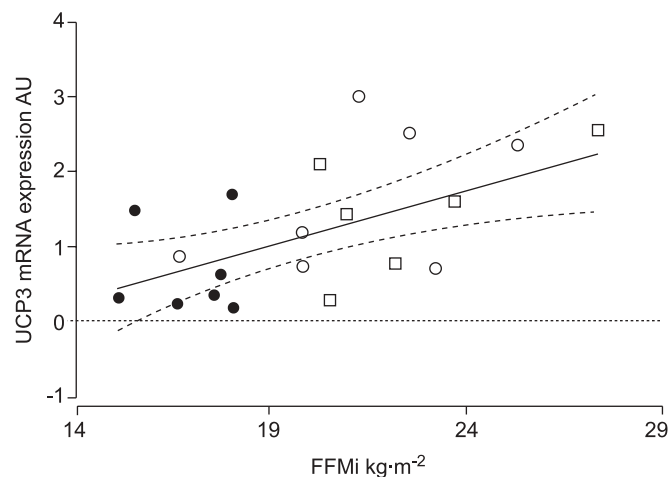


FIGURE 4. Relationship between uncoupling protein 3 (UCP3) mRNA expression and fat-free mass index (FFMi) for low body mass index (BMiL) patients (●), normal body mass index (BMiN) patients (○) and controls (□). A strong correlation is observed ($r = 0.58$, $p < 0.01$), dashed lines represent 95% confidence interval of the regression. Data from one BMiN and one healthy subject are missing due to technical problems in UCP3 mRNA expression assessment by real-time polymerase chain reaction.

and controls 5.3 ± 1.2 nmol·mg⁻¹) and GSSG. Similarly, no differences in mitochondrial GSH (BMiL 1.0 ± 0.5 , BMiN 1.5 ± 0.4 and controls 1.0 ± 0.1 nmol·mg⁻¹) and GSSG were detected.

DISCUSSION

The current study provides original evidence of impaired mitochondrial oxidative phosphorylation, assessed as ACR, in succinate-energised mitochondria in COPD patients with reduced BMI (fig. 2) this has not been observed in BMiN patients or in healthy subjects previously. Since no significant changes were detected in state 4 and state 3 was reduced, altered ACR in BMiL patients seems to be consistent with impaired electron flow from complex II-V and/or impaired adenosine triphosphate synthesis.

Although speculative, it can be hypothesised that altered oxidative phosphorylation in BMiL patients indicates an abnormal mitochondrial adaptation to long-term repeated episodes of cell hypoxia. The association between ACR and P_{a,O_2} seems to support this contention. It is acknowledged, however, that causality cannot be established from the current study.

Relationships between poor arterial oxygenation and systemic manifestations of COPD, *i.e.* low FFMi and high tumour necrosis factor- α plasma levels, have been reported by other authors [36]. Furthermore, abnormal mitochondrial respiratory chain function has been identified as a contributing mechanism to limb muscle dysfunction in patients with tissue hypoxia due to chronic peripheral arterial obstruction [37]. In addition to potential perturbations of the main determinants of systemic oxygen flow, COPD patients are prone to present low muscle oxygen conductance [38] from capillaries to mitochondria and muscle oxygen supply and demand heterogeneities. These two factors may generate cell hypoxia during exercise, even in the absence of overt arterial hypoxaemia.

It is known that tissue hypoxia stabilises hypoxia-inducible factor 1- α [39], which mediates multiple cellular and systemic homeostatic responses to hypoxia [40] by actively down-regulating mitochondrial oxygen consumption [41, 42].

Conversely, the lower UCP3 muscle levels in COPD patients may imply a lower ability to prevent fatty acids oxidation [43], which in turn might prompt mitochondrial abnormalities [17, 18].

Regardless of the potential mechanisms leading to a fall in oxidative phosphorylation in BMIL patients, this has deleterious consequences on adequate muscle performance, probably conditioning an early lactate release and interfering with the sustainability of exercise. The latter seems to be supported by the association between ACR and exercise-induced increase in [La] and muscle endurance. This is the first time, to the present authors' knowledge, that peripheral muscle mitochondrial respiration dysfunction is evidenced in COPD patients with BMIL and, thus outlining a potential association between the latter and the early lactate release during exercise (fig. 1) and the impairment in muscle endurance.

One intriguing finding of the present study was that functional abnormalities (e.g. ACR) were not accompanied by substantial alterations in muscle structure. It is understood that the analysis of muscle structure-function relationships requires further attention due to several confounding factors, *i.e.* physical de-conditioning and geographical and ethnic differences that may partly explain the heterogeneity of the results reported in the literature [44, 45].

Muscle UCP3 mRNA expression

Vastus lateralis of BMIL patients are characterised by reduced levels of UCP3 mRNA expression compared with both BMIN patients and healthy controls (fig. 3). Reduced skeletal muscle UCP3 protein levels [12, 14] and mRNA expression [13] have been previously reported for COPD patients. It should be emphasised that in two of the aforementioned studies in which FFMi was measured, the values of this magnitude were significantly lower in COPD patients compared with controls, a fact that in the present study applies only in the case of patients with BMIL. Similarly, a significant correlation between UCP3 mRNA expression and FFMi has been found in the present study (fig. 4).

Although the exact function of UCP3 is unknown, there is compelling evidence that UCP3 serves as a carrier to move fatty acid anions out of the mitochondrial matrix [46, 47], thereby protecting fatty acids from ROS-induced oxidative damage. Interestingly, the activity of HAD, an enzyme involved in the oxidation of fatty acids, is reduced in the skeletal muscle of patients with COPD [16]. GOSKER *et al.* [14] reported that exercise training restores UCP3 content in limb muscles of patients with COPD, especially in those that fail to increase HAD activity, a factor that supports the hypothesis that UCP3 may protect against fatty acid oxidation. In all, the lower UCP3 muscle levels in COPD patients may account for a reduced ability to prevent fatty acids oxidation [43].

Glutathione system

As described in previous reports [9, 10], no differences in glutathione concentration were observed in whole *vastus lateralis* homogenates between BMIN patients, BMIL patients

and healthy controls at rest. Similarly, no differences in mitochondrial glutathione concentration were detected between groups. This is in contrast to other disorders [11], in which a decrease in mitochondrial glutathione concentration may occur even in the absence of differences in muscle homogenate, with deleterious consequences for the tissue. Thus, in the current study, analysis of the GSH system in isolated mitochondria did not generate information additional to the measurements performed in muscle homogenates. This may be a relevant methodological finding with the potential to simplify further studies in the field.

Limitations of the study

The rather invasive approach of the study accounts for the small sample size, which may constitute a limitation for some of the areas explored. Patients with COPD may present abnormal fat to lean body mass ratio, such that BMI did not properly indicate patients' muscle mass. This fact does not seem to alter the interpretation of the current results, since none of the COPD patients with BMIN presented abnormally low FFMi. Furthermore, FFM and FFMi ($p < 0.01$ and $p < 0.001$, respectively) were significantly different when comparing BMIL with both BMIN and controls. Patients with BMIL showed a more advanced stage of the disease than those with BMIN. It is well known that BMIL and severe FEV₁ impairment are not fully independent, and that their association is linked to poor disease prognosis.

Conversely, the UCP3 protein content was not analysed due to limitations in the availability of tissue sample. Nevertheless, the present UCP3 mRNA expression data are in agreement with previously published results [12, 13].

Conclusion

The current investigation clearly identifies mitochondrial dysfunction in chronic obstructive pulmonary disease patients with low muscle mass. This abnormality is associated with altered muscle performance in terms of early lactate release and reduced muscle endurance. A reduced expression of uncoupling protein 3 mRNA is also present in low body mass index patients, which may indicate compromised protection against lipotoxicity. Whether these phenomena occur only in a subset of susceptible advanced chronic obstructive pulmonary disease patients or if it is a general feature of the final stages of the disease needs to be elucidated. Further research is required in order to explore the underlying mechanisms that are likely to be shared by other chronic conditions.

ACKNOWLEDGEMENTS

The authors would like to thank F. Burgos, C. Gistau, J.L. Valera (servei de Pneumologia (ICT), Barcelona, Spain) and all the technical staff of the Lung Function Laboratory for their skillful support during the study. E. Gimeno, N. Sánchez, C. Hernando and I. Díaz from EUIF Blanquerna (Ramon Llull University, Barcelona, Spain) are acknowledged for their outstanding work supervising the training sessions. The authors would also like to thank A. Russell (Clinique Romande de Réadaptation SUVA Care, Sion, Switzerland) for his advice on the UCP3 expression analysis, M.C. Carmona (University of Barcelona, Barcelona, Spain) for critical and helpful discussion and B. González for her excellent technical assistance.

REFERENCES

- 1 Global Initiative for Chronic Obstructive Lung Disease. www.goldcopd.com. Date created: 1997. Date last accessed: November, 2006.
- 2 Saey D, Debigare R, LeBlanc P, *et al*. Contractile leg fatigue after cycle exercise: a factor limiting exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 168: 425–430.
- 3 Skeletal muscle dysfunction in chronic obstructive pulmonary disease, A statement of the American Thoracic Society and European Respiratory Society. *Am J Respir Crit Care Med* 1999; 159: S1–S40.
- 4 Agusti AG, Noguera A, Sauleda J, Sala E, Pons J, Busquets X. Systemic effects of chronic obstructive pulmonary disease. *Eur Respir J* 2003; 21: 347–360.
- 5 Wouters EF, Creutzberg EC, Schols AM. Systemic effects in COPD. *Chest* 2002; 121: Suppl. 5, 127S–130S.
- 6 Schols AM, Soeters PB, Dingemans AM, Mostert R, Frantzen PJ, Wouters EF. Prevalence and characteristics of nutritional depletion in patients with stable COPD eligible for pulmonary rehabilitation. *Am Rev Respir Dis* 1993; 147: 1151–1156.
- 7 Jones PW. Issues concerning health-related quality of life in COPD. *Chest* 1995; 107: Suppl. 5, 187S–193S.
- 8 Connors AF Jr, Dawson NV, Thomas C, *et al*. Outcomes following acute exacerbation of severe chronic obstructive lung disease. *Am J Respir Crit Care Med* 1996; 154: 959–967.
- 9 Rabinovich RA, Ardite E, Troosters T, *et al*. Reduced muscle redox capacity after endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164: 1114–1118.
- 10 Rabinovich RA, Ardite E, Mayer AM, *et al*. Training depletes muscle glutathione in patients with chronic obstructive pulmonary disease and low body mass index. *Respiration* 2006; 73: 757–761.
- 11 García-Ruiz C, Morales A, Ballesta A, Rodes J, Kaplowitz N, Fernández-Checa JC. Effect of chronic ethanol feeding on glutathione and functional integrity of mitochondria in periportal and perivenous rat hepatocytes. *J Clin Invest* 1994; 94: 193–201.
- 12 Gosker HR, Schrauwen P, Hesselink MK, *et al*. Uncoupling protein-3 content is decreased in peripheral skeletal muscle of patients with COPD. *Eur Respir J* 2003; 22: 88–93.
- 13 Russell AP, Somm E, Debigare R, *et al*. COPD results in a reduction in UCP3 long mRNA and UCP3 protein content in types I and IIa skeletal muscle fibers. *J Cardiopulm Rehabil* 2004; 24: 332–339.
- 14 Gosker HR, Schrauwen P, Broekhuizen R, *et al*. Exercise training restores uncoupling protein-3 content in limb muscles of patients with chronic obstructive pulmonary disease. *Am J Physiol Endocrinol Metab* 2006; 290: E976–E981.
- 15 Boss O, Samec S, Paoloni-Giacobino A, *et al*. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 1997; 408: 39–42.
- 16 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.
- 17 Bulteau AL, Szweda LI, Friguet B. Mitochondrial protein oxidation and degradation in response to oxidative stress and aging. *Exp Gerontol* 2006; 41: 653–657.
- 18 Gutiérrez J, Ballinger SW, Darley-USmar VM, Landar A. Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. *Circ Res* 2006; 99: 924–932.
- 19 Orozco-Levi M. Structure and function of the respiratory muscles in patients with COPD: impairment or adaptation? *Eur Respir J* 2003; 22: Suppl. 46, 41S–51S.
- 20 Jimenez-Fuentes MA, Gea J, Aguar MC, *et al*. [Capillary density and respiratory function in the external intercostal muscle]. *Arch Bronconeumol* 1999; 35: 471–476.
- 21 Ribera F, N'Guessan B, Zoll J, *et al*. Mitochondrial electron transport chain function is enhanced in inspiratory muscles of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 167: 873–879.
- 22 Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004; 23: 932–946.
- 23 Schols AM, Wouters EF. Nutritional abnormalities and supplementation in chronic obstructive pulmonary disease. *Clin Chest Med* 2000; 21: 753–762.
- 24 Roca J, Burgos F, Sunyer J, *et al*. Reference values for forced spirometry. Group of the European Community Respiratory Health Survey. *Eur Respir J* 1998; 11: 1354–1362.
- 25 Roca J, Burgos F, Barberà JA, *et al*. Prediction equations for plethysmographic lung volumes. *Respir Med* 1998; 92: 454–460.
- 26 Hards JM, Reid WD, Pardy RL, Pare PD. Respiratory muscle fiber morphometry. Correlation with pulmonary function and nutrition. *Chest* 1990; 97: 1037–1044.
- 27 Kotler DP, Burastero S, Wang J, Pierson RN Jr. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *Am J Clin Nutr* 1996; 64: Suppl. 3, 489S–497S.
- 28 Dubowitz V, Brooke M, eds. Major Problems in Neurology. London, Saunders, 1973; pp. 74–85.
- 29 Research Services Branch (National Institutes of Health). Download Page (NIH Image, version 1.60). <http://rsb.info.nih.gov/nih-image/download.html>. Date last accessed: May 2006.
- 30 Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. 4. Children's biopsies. *Neurology* 1969; 19: 591–605.
- 31 Dunkley PR, Heath JW, Harrison SM, Jarvie PE, Glenfield PJ, Rostas JA. A rapid Percoll gradient procedure for isolation of synaptosomes directly from an S1 fraction: homogeneity and morphology of subcellular fractions. *Brain Res* 1988; 441: 59–71.
- 32 Colell A, García-Ruiz C, Lluís JM, Coll O, Mari M, Fernández-Checa JC. Cholesterol impairs the adenine nucleotide translocator-mediated mitochondrial permeability transition through altered membrane fluidity. *J Biol Chem* 2003; 278: 33928–33935.
- 33 Mahoney DJ, Carey K, Fu MH, *et al*. Real-time RT-PCR analysis of housekeeping genes in human skeletal muscle following acute exercise. *Physiol Genomics* 2004; 18: 226–231.

- 34 Fariss MW, Reed DJ. High-performance liquid chromatography of thiols and disulfides: dinitrophenol derivatives. *Methods Enzymol* 1987; 143: 101–109.
- 35 Baarends EM, Schols AM, Mostert R, Wouters EF. Peak exercise response in relation to tissue depletion in patients with chronic obstructive pulmonary disease. *Eur Respir J* 1997; 10: 2807–2813.
- 36 Takabatake M, Nakamura H, Abe S, *et al.* The relationship between chronic hypoxaemia and activation of the tumor necrosis factor- α system in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 161: 1179–1184.
- 37 Pipinos II, Sharov VG, Shepard AD, *et al.* Abnormal mitochondrial respiration in skeletal muscle in patients with peripheral arterial disease. *J Vasc Surg* 2003; 38: 827–832.
- 38 Simon M, LeBlanc P, Jobin J, Desmeules M, Sullivan MJ, Maltais F. Limitation of lower limb $V'O_2$ during cycling exercise in COPD patients. *J Appl Physiol* 2001; 90: 1013–1019.
- 39 Brunelle JK, Bell EL, Quesada NM, *et al.* Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. *Cell Metab* 2005; 1: 409–414.
- 40 Semenza GL, Agani F, Feldser D, *et al.* Hypoxia, HIF-1, and the pathophysiology of common human diseases. *Adv Exp Med Biol* 2000; 475: 123–130.
- 41 Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006; 3: 177–185.
- 42 Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively down-regulating mitochondrial oxygen consumption. *Cell Metab* 2006; 3: 187–197.
- 43 Barreiro E, Gea J, Corominas JM, Hussain SN. Nitric oxide synthases and protein oxidation in the quadriceps femoris of patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2003; 29: 771–778.
- 44 Coronell C, Orozco-Levi M, Ramírez-Sarmiento A, Martínez-Llorens J, Broquetas J, Gea J. [Low-weight syndrome associated with COPD in our setting]. *Arch Bronconeumol* 2002; 38: 580–584.
- 45 Coronell C, Orozco-Levi M, Gea J. COPD and body weight in a Mediterranean population. *Clin Nutr* 2002; 21: 437–438.
- 46 Schrauwen P, Hesselink MK. The role of uncoupling protein 3 in fatty acid metabolism: protection against lipotoxicity? *Proc Nutr Soc* 2004; 63: 287–292.
- 47 Schrauwen P, Hoeks J, Schaart G, *et al.* Uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *FASEB J* 2003; 17: 2272–2274.