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MITOCHONDRIAL DYSFUNCTION IN COPD PATIENTS WITH LOW BODY MASS INDEX

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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. This study explores the association between body composition and mitochondrial respiration in seven patients with low body mass index (BMI_L), eight COPD patients with normal BMI (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, isolated mitochondria and whole muscle glutathione levels were determined. Mitochondrial respiratory function (ACR, acceptor control ratio) was impaired in BMI_L (2.2$\pm$0.6) compared to both BMI_N (5.3$\pm$1.3) and controls (8.2$\pm$1.3) (ANOVA, $p<0.01$). ACR significantly correlated with PaO_2 ($r=0.70$, $p<0.01$) and with muscle endurance ($r=0.44$, $p<0.05$); but, it showed a negative association with exercise-induced increase in blood lactate levels ($r=-0.60$, $p=0.01$). UCP3 mRNA expression was reduced in BMI_L patients ($p=0.07$). We conclude that COPD patients with low BMI show electron transport chain dysfunction that may contribute to low muscle endurance in this subgroup of patients.

Keywords: Mitochondrial respiration; Glutathione; Muscle dysfunction; Endurance training

INTRODUCTION

Chronic obstructive pulmonary disease (COPD)[1] is associated with several extra-pulmonary effects. Skeletal muscle dysfunction, one of the most extensively studied systemic effects, is a prominent contributor to exercise limitation[2]. Limb muscle abnormalities have a multi-factorial nature, including physical inactivity, systemic inflammation/oxidative stress and cell hypoxia[3-5]. Loss of muscle mass, present in a subgroup of COPD patients[6] has a deleterious impact on health status, increased use of health care resources and poor disease prognosis, which is partly independent of the degree of FEV_1 impairment[7;8]. A better knowledge of the interplay among mechanisms determining limb muscle dysfunction and loss of fat-free mass in COPD may enhance our understanding of the functional heterogeneities of the disease which, in turn, may lead to a better management of the patients.

In previous studies[9;10] we 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 control subjects increase skeletal muscle reduced (GSH) glutathione concentration after 8-week endurance training, the opposite effect occurs in COPD patients, which indeed showed increased oxidized (GSSG) glutathione levels 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[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 a 10-15% of the total GSH pool size, the regulation of mitochondrial GSH needs to be evaluated in isolated organelles.

It has been recently shown that uncoupling protein-3 (UCP3) levels are diminished in 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 not known. Although primarily linked to enhanced rest energy expenditure through mitochondrial respiratory chain uncoupling[15], it is hypothesized that the physiological role of UCP3 is to protect mitochondria against lipotoxicity in cases where fatty acid influx exceeds the capacity to oxidize them. Interestingly, 3-hydroxyacyl-CoA dehydrogenase (HAD) activity, an important enzyme involved in the oxidation of fatty acids, is reduced in skeletal muscle of COPD patients[16]. Moreover, lipid peroxidation has been associated to mitochondrial dysfunction[17;18].It is important to underscore that in two of the aforementioned studies[12;14], fat free mass index (FFMi) was assessed and was found to be significantly lower in COPD patients compared to control subjects. With this in mind, it is of interest to analyze the relationship between UCP3 levels and body composition.

Unlike peripheral skeletal muscles, respiratory muscles show adaptive changes in response to the chronic mechanical load namely, increased mitochondrial density, increased oxidative capacity, increase in density of interfibrillary capillaries and increased percentage of fiber type I[19;20]. Likewise, mitochondrial electron transport chain function is enhanced in inspiratory muscles of patients

with COPD[21]. Therefore, a different behavior of the mitochondrial respiratory chain could be expected between peripheral and respiratory muscles.

We hypothesized that mitochondrial function might be impaired in COPD patient's peripheral muscles, especially in the subgroup of patients with low body mass index. In this study, we assessed "ex vivo" peripheral muscle mitochondrial function and its relationship with arterial PO_2, whole muscle and mitochondrial GSH and GSSG concentrations; and, UCP3 regulation.

For this purpose, an open biopsy of the *vastus lateralis* was obtained in eight COPD patients with normal BMI (BMI_N), seven BMI_L (< 21 $kg.m^{-2}$), and seven healthy sedentary individuals. We examined muscle structure, *in vitro* muscle mitochondrial respiration, UCP3 mRNA expression and both reduced and oxidized glutathione levels in muscle homogenate and in isolated mitochondria[11].

METHODS

Study Group

Fifteen clinically stable COPD patients (all men) (**Table 1**)[22] were included in the study. All patients were on bronchodilators and inhaled corticosteroids. They were clinically stable at the time of the study, without episodes of exacerbation or oral steroid treatment in the previous four months. None of them presented significant co-morbidities. Seven age-matched healthy sedentary subjects (all male) were included as controls. Eight COPD patients were characterized as BMI_N (BMI ≥ 21 $kg.m^{-2}$) and the remaining seven COPD patients were BMI_L (BMI <21 $kg.m^{-2}$)[23]. Selection procedures for inclusion in the study were: a) Clinical assessment; b) Pulmonary function testing (Jaeger, Master Screen; Würzburg, Germany)[24;25]; c) Chest X-ray film; and, d) General blood analysis. Measurements to characterize the subjects included: i) Body composition analysis by Bio Impedance (Quantum X, RJL Systems instruments, Clinton Twp., USA); ii) Incremental cycling exercise protocol ($CardiO_2$ cycle Medical Graphics Corporation, USA)[23]; iii) Left quadriceps static (isometric) and dynamic (isokinetic) strength and endurance (Cybex 6000. Lumex Inc., USA); and, iv) Muscle morphometry using immunohistochemical procedures[26]. All participants were informed of any risks and discomfort associated with the experiment, and written informed consent was obtained in accordance with the Committee on Investigations Involving Human Subjects at the Hospital Clínic, Universitat de Barcelona, which approved the study.

Muscle Biopsy

An open muscle biopsy of the *"vastus lateralis"* (~0.8 g) was obtained and

processed as follows: a) approximately ~0.65 g of the muscle sample was included in Kreb´s buffer (pH 7.40) solution for immediate processing assays (homogenate and mitochondrial glutathione concentrations and mitochondrial respiration assays), b) approximately ~0.15 g of the muscle sample was embedded in paraffin for immunohistochemical analysis; and, c) ~0.1 g was included in RNA stabilization reagent (RNAlater®, Ambion, Inc., USA) and stored at -20^{o}C for RNA extraction.

Lung function at rest, body composition and exercise testing

Lung function. Forced spirometry, lung volumes, and carbon monoxide transfer capacity (TL_{CO}) were measured (Jaeger, MasterScreen; Wüerzburg, Germany) and the results were expressed as a percentage of the reference values obtained in our own laboratory[24;25]. Arterial oxygen tension (PaO_2), carbon dioxide tension ($PaCO_2$), pH and blood lactate were analyzed on a blood gas analyzer (Ciba Corning 800, USA).

Body composition. Body composition was estimated using single frequency (50 kHz) bio-electrical impedance analysis (Quantum X, RJL Systems instruments, USA) while subjects were in supine position. Fat free mass (FFM) was calculated from gender-specific regression equations[27]. Fat free mass index was obtained by dividing FFM in Kg by height in m^2.

Incremental exercise protocol. After placing an arterial catheter (Seldicath, Plastimed, France), subjects were installed on the cycle ergometer with the mouth piece in place. On-line calculations of whole-body O_2 uptake (VO_2), CO_2 output (VCO_2), minute ventilation (V_E), respiratory exchange ratio (RER), and heart rate (HR) were obtained. Arterial blood samples were taken each three

minutes throughout the test for analysis of blood gases and lactate. Blood samples were kept on ice until analysis (Ciba Corning 800, USA). After three minutes of unloaded pedaling work rate was increased by 5 or 10 Watts per minute. All studies were done using an electromagnetically-braked cyclo-ergometer (CardiO_2 cycle Medical Graphics Corporation, USA) with a mechanical assistance to overcome the internal frictional resistance.

Muscle Strength

Measurements of maximal voluntary contractions of the left quadriceps were made during static (isometric) and dynamic (isokinetic) contractions against an isokinetic system (Cybex 6000. Lumex Inc., USA). After a previous muscle warm up, peak extension torque was evaluated at 60 degrees of knee flexion, and the best of five repetitions was taken for the analysis. Isokinetic strength test consisted in five repetitions at the following different angular speeds (60, 90, 120, 180 and 240 degrees s^{-1}). The best peak torque and peak power were taken for the analysis. Also, an endurance test was carried out in all patients. This test consisted in a set of 30 knee extensions at a speed of 90 deg s^{-1}: The total work done during the repetitions was used to analyze the 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 ten-micrometer thick sections were cut, varying the inclination of the holder by 5° increments until the minimum fiber cross-sectional area 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 (MyHC) isoforms type I and type II (MHCs and MHCf clones; Biogenesis, New Fields, Poole, UK). The fiber cross-sectional area (fCSA), mean least diameter, and proportions of type I and II fibers were assessed using a light microscope (OLYMPUS, Series BX50F3; Olympus Optical Co., Germany) coupled with an image-digitizing camera (Pixera Studio, Version 1.2; Pixera Corporation, USA) and a morphometry program (NIH IMAGE, Version 1.60). At least 100 fibers were measured from each biopsy[28]. Fiber diameters between 40 and 80 µm were considered normal[26;29].

Mitochondrial respiration

Mitochondria were obtained by centrifugation through a percoll density gradient[30]. Enrichment and recovery of mitochondria were ascertained by the specific activity of succinic dehydrogenase (SDH). The rate of oxygen consumption was measured using Clark-type oxygen electrode (Hansatech Instruments Limited, UK) within one hour after muscle biopsy was obtained[31]. The respiratory function was analyzed in medium containing 225 mM sucrose, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 20 Mm KCl, 10 mM Tris, and, 5mM HEPES at 25^{o}C continuously stirred, using an electromagnetic stirrer and bar flea. The mitochondrial suspension was added to the reaction medium and ADP-limited respiration (state 4) was initiated by the addition of (13.5 mM) succinic acid in absence of ADP. State 3 respiration was determined upon the addition of (0.63 mM) ADP, a phosphate acceptor. Oxidative phosphorylation efficiency was assessed by acceptor control ratio (ACR) by dividing the slope of state 3 to the

slope of state 4[11].

RNA isolation and UCP3 mRNA expression

Total RNA was extracted from muscle tissue samples treated with RNA*later* (Ambion, USA) by using the TRIzol Reagent (Life Technologies, USA) according to the manufacturer's protocol. The quality of RNA samples was evaluated by capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, 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, Germany).

UCP3 mRNA expression was measured by real-time PCR (qPCR) using specific Assay-on-Demand Taqman Gene expression probes from Applied Biosystems. The probe used for UCP3 spans the 1-2 exon junction amplifying both, the long and short variants of the gen. 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 non template control reactions were performed in the ABI Prism 7900 Sequence Detection System (Applied Biosystems, USA) in duplicate**.** The ΔΔCt method[32] was used to calculate relative changes in mRNA abundance. The Ct values were obtained for each target probe and normalized with the corresponding Ct values for the internal control housekeeping gene beta 2-microglobulin (B2M). mRNA quantity was expressed as arbitrary units.

Assessment of muscle redox status

Fresh muscle samples were homogenized in a buffer containing 20 mM Tris, 0.25 M sucrose, 40 mM KCl, 2 mM EGTA and 1mg/ml bovine serum albumin (BSA). 500 µl were separated for glutathione concentration assessment, and the rest of the sample was used for mitochondrial isolation by percoll centrifugation gradient[30].

Glutathione concentration assessment. The two molecular forms, reduced (GSH) and oxidized (GSSG) gluthatione, were obtained by high-performance liquid chromatography (HPLC) in both, homogenate and mitochondrial fractions, as described previously in detail[33]. An HPLC equipped with a 3-aminopropyl column and an UV spectrophotometric detector (365 nm) was used.

Data analysis

Results are expressed as mean SEM. Multiple comparisons were performed using ANOVA. Student 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 no normal distributed variables) regression analysis was used to assess univariate correlations. A p value lower than 0.05 was taken as statistically significant.

RESULTS

Anthropometric and functional characteristics of COPD patients (BMI_N n= 8, and BMI_L n= 7) and controls (n= 7) are indicated in **Table 1**. As expected, FFMi was significantly lower in BMI_L (17.0±0.5 $kg.m^{-2}$) than in both BMI_N (21.4±0.9 $kg.m^{-2}$) and controls (22.3±0.5 $kg.m^{-2}$) ($p<0.001$). None of the patients with normal BMI showed abnormally low FFMi (< 16 $kg.m^{-2}$)[34]. Patients with normal BMI showed higher FEV_1 and Dlco than those with reduced muscle mass. The distribution of patients according to the GOLD[1] classification was as follows: 4 in stage II (3 BMI_N and 1 BMI_L); 6 in stage III (4 BMI_N and 2 BMI_L), and 5 in stage IV (1 BMI_N and 4 BMI_L). The two groups of patients showed a similar degree of mild arterial hypoxemia, but exercise-induced hypoxemia was slightly more pronounced in BMI_L. Likewise, BMI_L had lower exercise tolerance (VO_2 peak 11±1 $ml.kg^{-1}.min^{-1}$) than both BMI_N (19±2 $ml.kg^{-1}.min^{-1}$) and controls (26±2 $ml.kg^{-1}.min^{-1}$) (ANOVA, $p<0.0001$).

Quadriceps muscle strength and endurance were also lower in BMI_L compared to the other two groups (**Table 1**). Muscle-fiber CSA (fCSA) showed a trend to be higher (24%) in controls than in COPD patients, with identical results between BMI_N and BMI_L. But no statistically significant differences among groups were detected. Muscle strength normalized by FFM (strength/FFM) showed no differences between all groups (data not shown). It is of note that muscle endurance normalized by FFM (endurance/FFM ratio) was significantly lower in BMI_L (16.7±3.5 J/Kg) than in both BMI_N (36.1±4.1 J/Kg) and C (35.1±3.6 J/Kg), (ANOVA, $p<0.01$).

As shown in **Figure 1**, BMI_L patients presented earlier arterial blood lactate

([La]) release than both BMI_N patients and controls. As expected, exercise-induced increases in [La] showed a strong association with VO_2peak (r= 0.84, p<0.0001) and Watts peak (r= 0.85, p<0.0001).

Mitochondrial respiration

Individual data for ACR are illustrated in **Figure 2**. On average, ACR was significantly lower in BMI_L patients (2.2±0.6) than in both BMI_N patients (5.3±1.3) and in healthy sedentary subjects (8.2±1.3) (ANOVA, p<0.01). State 3 of respiration in energized mitochondria by succinate was lower in BMI_L patients (20.7±7.5 $nmol.mg^{-1}.min^{-1}$; ANOVA, p<0.01) compared to healthy controls (134.1±32.9 $nmol.mg^{-1}.min^{-1}$) but no statistical differences were seen when compared with BMI_N patients (72.1±39.6 $nmol.mg^{-1}.min^{-1}$). No differences in state 4 of respiration were seen among groups (BMI_L 8.8±2.3 $nmol.mg^{-1}.min^{-1}$; BMI_N 10.7±4.1 $nmol.mg^{-1}.min^{-1}$; 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).

Arterial PO_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 as a whole). Exercise-induced increase in [La] from rest to 45 watts was negatively correlated with ACR (r=-0.60, p=0.01). Moreover, a positive correlation was shown between ACR and the 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 was lower in BMI_L patients (618.2±198.8

AU) compared to both BMI_N patients (1371±306.4 AU) and healthy Controls (1118.8±326.8 AU) (ANOVA, p=0.07). There was no association between UCP3 mRNA expression with any of the muscle measurements, either function or structure. A significant association was observed, however, between UCP3 mRNA expression and FFMi (r=0.58, p<0.01) (**Figure 4**).

Glutathione assessment

No differences among groups were observed in muscle homogenate concentrations of GSH (BMI_L 7.1±0.7; BMI_N, 8.9±2.3; and, controls 5.3±1.2 nmol/mg) and GSSG. Likewise, no differences in mitochondrial GSH (BMI_L 1.0±0.5; BMI_N, 1.5±0.4; and, controls 1.0±0.1 nmol/mg) and GSSG were seen.

DISCUSSION

The current study provides original evidence of impaired mitochondrial oxidative phosphorylation assessed as ACR in succinate energized mitochondria in COPD patients with reduced body mass index (**Figure 2**), not seen previously neither in BMI_N patients nor in healthy subjects. Since no significant changes in state 4 were observed and state 3 was reduced, altered ACR in BMI_L patients seems to be consistent with impaired electron flow from complex II-V, and/or impaired ATP synthesis.

Although speculative, it can be hypothesized that altered oxidative phosphorylation in BMI_L patients may indicate an abnormal mitochondrial adaptation to long-term repeated episodes of cell hypoxia. The association between ACR and arterial PaO_2 seems to support this contention. We acknowledge, however, that causality can not be established from the current study.

Relationships between poor arterial oxygenation and systemic manifestations of COPD, namely low FFMi and high TNFα plasma levels, have been reported by other authors[35]. Moreover, 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[36]. In addition to potential perturbations of the main determinants of systemic oxygen flow, COPD patients are prone to present low muscle O_2 conductance[37] from capillaries to mitochondria and muscle O_2 supply-O_2 demand heterogeneities. These two factors may generate cell hypoxia during exercise, even in absence of overt arterial hypoxemia.

It is known that tissue hypoxia stabilizes hypoxia-inducible factor-1α (HIF-1α)[38], which mediates multiple cellular and systemic homeostatic responses to hypoxia[39] by actively downregulating mitochondrial oxygen consumption[40;41].

On the other hand, the lower UCP3 muscle levels in COPD patients might imply a lower ability to prevent fatty acids oxidation[42] (see below) which, in turn, might prompt mitochondrial abnormalities[17;18].

Regardless of the potential mechanisms leading to a fall in oxidative phosphorylation in BMI_L 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 our knowledge, that peripheral muscle mitochondrial respiration dysfunction is evidenced in COPD patients with low BMI; so is the potential association between the latter and the early lactate release during exercise (**Figure 1**) and the impairment in muscle endurance.

One intriguing finding of this study was that functional abnormalities (ACR) were not accompanied by substantial alterations in muscle structure. We understand that the analysis of muscle structure-function relationships requires further attention due to several confounding factors, namely physical de-conditioning and geographical-ethnic differences that may partly explain the heterogeneity of the results reported in the literature[43;44].

Muscle UCP3 mRNA expression

Vastus lateralis of BMI_L patients are characterized by reduced levels of UCP3

mRNA expression compared to both BMI_N patients and healthy controls (**Figure 3**). Reduced skeletal muscle UCP3 protein levels[12;14] and mRNA expression[13] has been previously reported for COPD patients. Although, in the present study this was only the case in patients with low BMI, it should be emphasized that in two of the aforementioned studies in which FFMi was measured, COPD patients FFMi was significantly lower compared to controls. Likewise, a significant correlation between UCP3 mRNA expression and FFMi has been found in the present study (**Figure 4**).

Although the exact function of UCP3 is not known, there is compelling evidence that UCP3 serves as a carrier to move fatty acid anions out of the mitochondrial matrix[45;46] thereby protecting fatty acids from ROS-induced oxidative damage. Interestingly, the activity of the 3-hydroxyacyl-CoA dehydrogenase (HAD), an enzyme involved in the oxidation of fatty acids, is reduced in skeletal muscle of patients with COPD[16]. Gosker et al. reported that exercise training restores UCP3 content in limb muscle of patients with COPD, specially in those that fail to increase HAD activity which complies with the hypothesis that UCP3 may protect against fatty acid oxidation[14]. All in all, the lower UCP3 muscle levels in COPD patients might account for a reduced ability to prevent fatty acids oxidation[42].

Glutathione system

Like previous reports[9;10], no differences in glutathione concentration were seen in whole *vastus lateralis* homogenates between BMI_N patients, BMI_L patients and healthy controls at rest. Likewise, no differences in mitochondrial glutathione concentration were seen between groups. This is in contrast to

other disorders[11], in which a decrease in mitochondrial glutathione concentration can occur, even in 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 additional information to the measurements performed in muscle homogenates. This might be a relevant methodological finding 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 may not properly indicate patient's muscle mass. This fact does not seem to alter the interpretation of the current results since none of the COPD patients with normal BMI presented abnormally low FFMi. Moreover, FFM and FFMi (ANOVA, $p<0.01$ and $p<0.001$ respectively) were significantly different when comparing BMI_L with both BMI_N and controls. Patients with BMI_L showed more advanced disease than those with BMI_N. It is well known that the two phenomena (low BMI and severe FEV_1 impairment) are not fully independent and their association is linked to poor disease prognosis.

On the other hand, the UCP3 protein content was not analyzed because of limitations in the availability of tissue sample. Nevertheless, our UCP3 mRNA expression data are in agreement with previously published results[12;13].

In summary, the current investigation clearly identifies mitochondrial dysfunction in COPD 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 UCP3 mRNA is also present in BMI_L patients, which may indicate compromised protection against lipotoxicity. Whether these phenomena occur only in a subset of susceptible advanced COPD patients or it is a general feature of end-stage disease needs to be elucidated. Further research is also required to explore underlying mechanisms that are likely shared by other chronic conditions.

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TABLE 1. CHARACTERISTICS OF THE STUDY GROUPS

		COPD BMI_N		COPD BMI_L		Controls
Age	Years	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
	% 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	‡	±
PaO_2rest	mmHg	77.3 ± 5.5		72.9 ± 1.6	*	94.6 ± 5.6
PaO_2exer	mmHg	71.8 ± 9.2	*	62.6 ± 4.1	*	103.7 ± 2.2
$PaCO_2$	mmHg	42.5 ± 2.6		41.7 ± 2.3		37.4 ± 1.7
Watt peak	Watts	96.9 ± 7.9	†	44.6 ± 9.4	†§	154.3 ± 14.2
VO_2peak	$L.min^{-1}$	1.5 ± 0.1		0.6 ± 0.1	†§	2.1 ± 0.2
Isometric	Nm	184.1 ± 16.4		117.1 ± 19.5	†‡	204.8 ± 11.7
$Isokinetic_{90}$	Nm	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
Cap/Fiber	Ratio	0.6 ± 0.1		0.5 ± 0.1		0.5 ± 0.1

Definition of abbreviations: BMI = body mass index; fCSA = fiber cross sectional area; Cap/Fiber = capillaries to fiber ratio. Quadriceps measurements: Isometric strength in Nm, Isokinetic strength at 90 degrees.s^{-1} in Nm, and Endurance in J.

Comparisons with Controls. * $p<0.01$, † $p<0.001$.
Comparisons between BMI_N and BMI_L. ‡ $p<0.01$, § $p<0.001$
All comparisons done with ANOVA using a SNK post-hoc analysis of contrasts

FIGURE LEGENDS

Figure 1. *Mean arterial blood lactate levels during incremental cycling exercise.* The graph displays arterial blood lactate levels in COPD patients with low BMI (BMI_L, triangles), COPD patients with normal BMI (BMI_N, circles), and healthy sedentary subjects (Controls, squares) during incremental cycling exercise. Early lactate release was seen in BMI_L patients compared to both BMI_N and Controls (* ANOVA, $p<0.05$).

Figure 2. *"In vitro" mitochondrial respiratory chain function*. Individual (circles) and mean group (solid lines) data for acceptor control ratio (ACR). Significant differences in ACR were shown between COPD patients with BMI_L and both BMI_N patients and Controls (* ANOVA, $p<0.05$). Data of one COPD patient with normal BMI (BMI_N) is missing because of technical problems.

Figure 3. *UCP3 mRNA expression.* Individual (circles) and mean group (solid lines) data for UCP3 mRNA expression (qPCR). BMI_L patients show lower values than both BMI_N patients and Controls. (*ANOVA, $p=0.07$). One BMI_N patient and one healthy subject are missing due to technical problems in qPCR assessment.

Figure 4. *Relationship between UCP3 mRNA expression and FFMi.* UCP3 mRNA expression (ordinate) and FFMi (abscissa) displayed in the present graphic for BMI_L patients (triangles), BMI_N patients (circles) and Controls (squares) showed a strong correlation ($r=0.58$, $p<0.01$). One BMI_N and one healthy subject are missing due to technical problems in qPCR assessment.

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FIGURE 1

ARTERIAL BLOOD LACTATE LEVELS DURING CYCLING EXERCISE

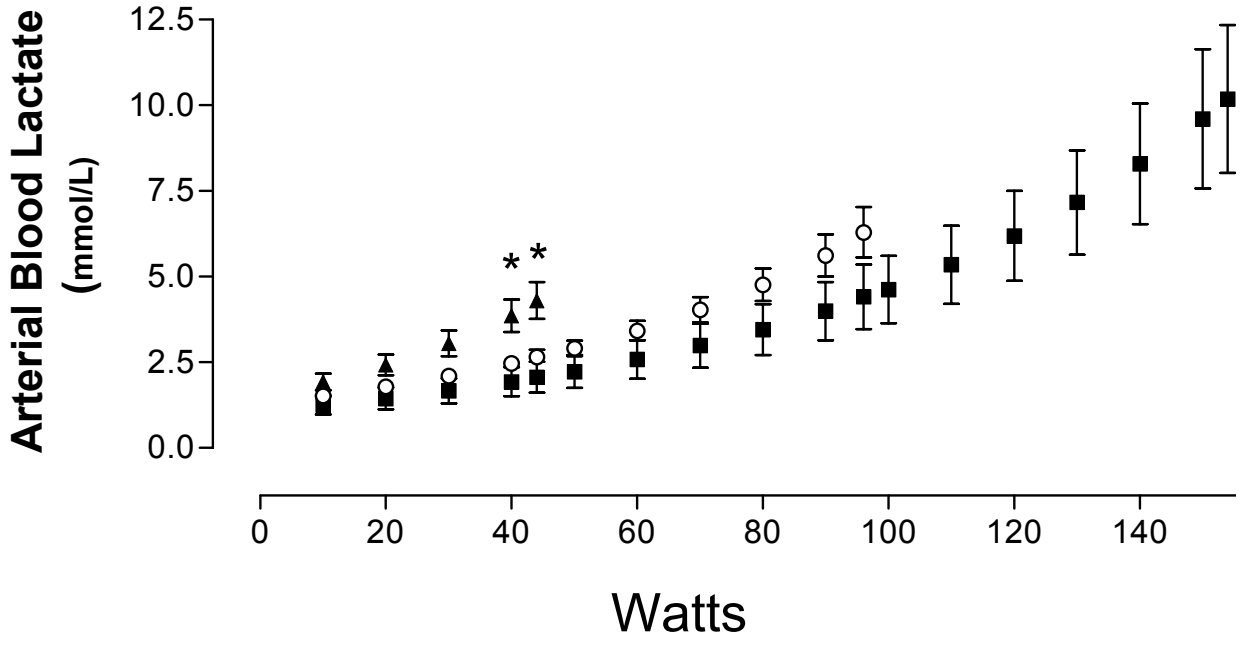

FIGURE 2

MITOCHONDRIAL RESPIRATION

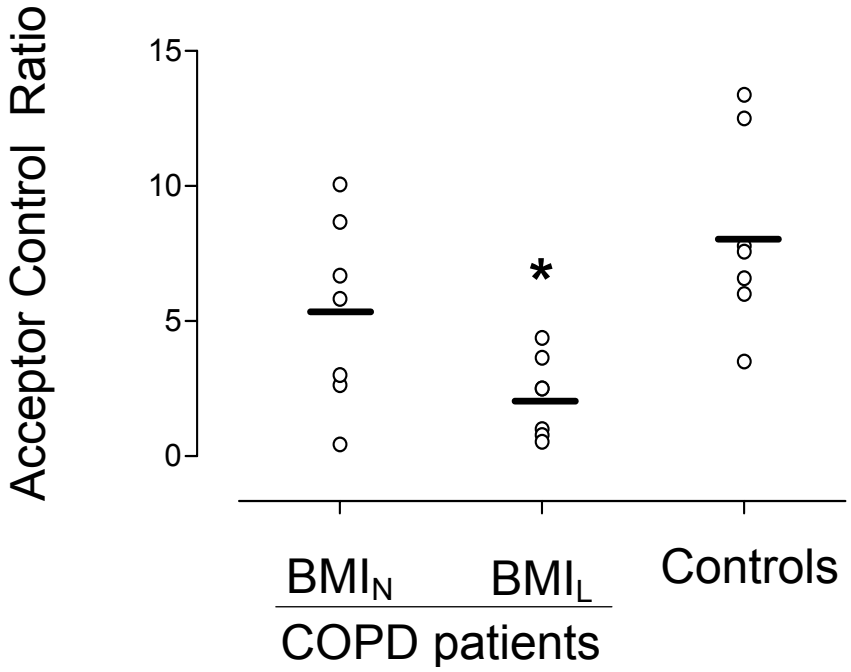

FIGURE 3

UCP3 mRNA EXPRESSION

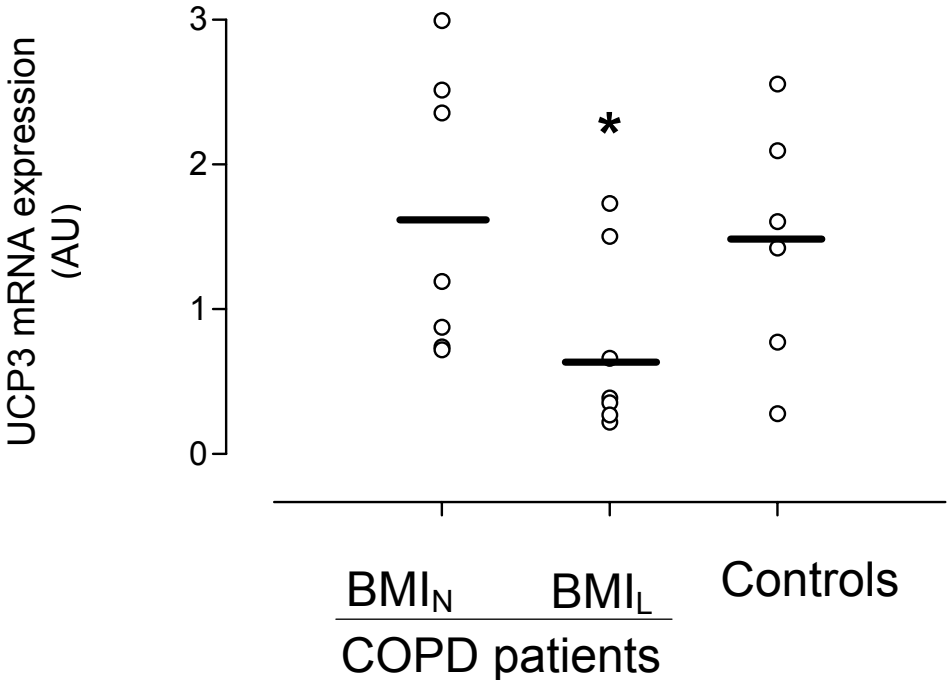

FIGURE 4

RELATIONSHIP BETWEEN UCP3 mRNA EXPRESSION AND FFMi