

ORIGINAL ARTICLE

## Acute exercise amplifies inflammation in obese patients with COPD<sup>☆</sup>



F. Rodrigues<sup>a,b,\*</sup>, A.L. Papoila<sup>c,d</sup>, D. Ligeiro<sup>e</sup>, M.J.M. Gomes<sup>c</sup>, H. Trindade<sup>f</sup>

<sup>a</sup> Serviço Pneumologia, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Portugal

<sup>b</sup> Faculdade de Medicina, Universidade de Lisboa, Portugal

<sup>c</sup> NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Portugal

<sup>d</sup> Centro de Estatística e Aplicações da Universidade de Lisboa, Portugal

<sup>e</sup> Centro Luso-Transplante do Sul, Portugal

<sup>f</sup> Instituto Português do Sangue e da Transplantação, Portugal

Received 22 October 2015; accepted 11 May 2016

Available online 16 June 2016

### KEYWORDS

COPD;  
Inflammation;  
Exercise;  
Obesity;  
Reverse transcriptase  
polymerase chain  
reaction

**Abstract** Systemic inflammation has been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD) systemic effects. However, most COPD patients do not suffer from persistent systemic inflammation even after exacerbations and exercise and scientific evidence has provided conflicting results. Our aim is to evaluate inflammatory gene expression at rest and at 1 and 24 h after strenuous exercise in COPD patients and study the patient variables associated with inflammatory expression.

A cross-sectional study was conducted in COPD patients who were recruited on entry to a pulmonary rehabilitation (PR) program. Demographic, clinical and functional data were collected. Blood samples were collected and gene expression was analyzed by reverse transcriptase polymerase chain reaction for IFN $\gamma$ , IL1b, IL6, IL8, TNF $\alpha$ , TGFb1 and iNOS.

The study included 21 patients (15 men, 71.4%), mean age 66.1 years old (SD = 8.27), mean FEV<sub>1</sub> 46.76% (SD 20.90%), 67% belonging to GOLD grade D, mean BODE index of 3.9, 90.5% with smoking history, mean BMI 25.81 (SD = 4.87), median of 1.29 exacerbations in the previous year.

There was no statistical significant difference between inflammatory expression at rest and at 1 h and 24 h after the maximal exercise test for all tested genes.

We found an association between BMI and inflammatory expression at all the points of time checked, a slight inverse association occurs with low BMI for mRNA IL1b, IL6, TNF $\alpha$ , TGFb1 and iNOS, and there was a more pronounced positive association for obese patients for all tested genes.

This preliminary study did not show an enhanced inflammatory gene expression from rest to 1 h and 24 h after short-term exercise, but did show an increased inflammatory gene expression

<sup>☆</sup> Study was carried out at the Pulmonary Rehabilitation Unit, Pneumology Service, CHLN-Hospital Pulido Valente and in South Luso-Transplant Centre.

\* Corresponding author.

E-mail address: [fatima.rodriguesed@gmail.com](mailto:fatima.rodriguesed@gmail.com) (F. Rodrigues).

in both BMI extremes, both at rest and after exercise, suggesting not only malnourishment, but also obesity as potential links between COPD and systemic inflammation. Studies with larger samples and designed to definitely exclude OSA or OHS as confounding factors in obese patients are required.

© 2016 Sociedade Portuguesa de Pneumologia. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Chronic obstructive pulmonary disease (COPD) is associated with important extrapulmonary manifestations, including weight loss, skeletal muscle dysfunction, cardiovascular disease, depression, osteoporosis, reduced exercise tolerance, and poor health status.<sup>1–3</sup> Although the pathobiology of COPD has not been fully determined, persistent systemic inflammation has been implicated in the pathogenesis of the majority of these systemic effects.<sup>3,4</sup> Elevated circulating levels of white blood cells, C-reactive protein (CRP), interleukins 6 (IL-6) and 8 (IL-8), fibrinogen and tumor necrosis factor alpha (TNF $\alpha$ ) have been reported in patients with COPD.<sup>5</sup> However, the ECLIPSE study<sup>5</sup> has demonstrated that a large group of patients with COPD do not suffer from systemic inflammation.

The role of exercise in COPD inflammatory process has also been a matter of debate.<sup>6–15</sup> Patients with COPD are exposed to a systemic inflammation that is amplified by exhaustive exercise. Inflammatory response to exercise is more pronounced in patients with COPD when compared to healthy controls, even at lower levels of exercise intensity.<sup>9,15</sup> However, scientific literature on this subject is also not consensual, as several studies have shown a reduction in the level of TNF $\alpha$  protein expression in COPD subjects.<sup>10</sup> As pointed out by Canavan and colleagues,<sup>11</sup> some of the heterogeneity of these results might be caused by the different methods that were used in the studies (patient characterization, exercise protocols, and assay techniques). Crul and colleagues<sup>12</sup> did not find any evidence of muscle inflammation in patients with COPD, independently of whether they were in a stable or an acute exacerbation state. Conversely, others have suggested an anti-inflammatory effect of regular exercise in some low systemic inflammation chronic diseases, with beneficial outcomes in disease prevention and symptomatic improvement.<sup>6,13,14</sup>

In this study we aim to evaluate the inflammatory and immune regulatory gene expression profiling in peripheral blood determined at rest in patients with COPD, and the possible modification after strenuous exercise, and search for variables and patients' characteristics associated with inflammatory expression.

## Material and methods

### Patients

A cross-sectional study was conducted on a sample of 21 patients diagnosed with COPD, according to the Global

Initiative for Obstructive Lung Disease Project (GOLD)<sup>16</sup> as post bronchodilator FEV<sub>1</sub>/FVC < 0.70. Patients were consecutively recruited on entry to a pulmonary rehabilitation (PR) program at our PR Unit, from January to December 2010. Participants were selected if clinically stable in the previous four weeks and able to exercise and to answer health status questionnaires. Patients diagnosed with other significant lung diseases, e.g. asthma, bronchiectasis or other conditions that might cause dyspnea or affect exercise performance, were excluded.

### Data collection

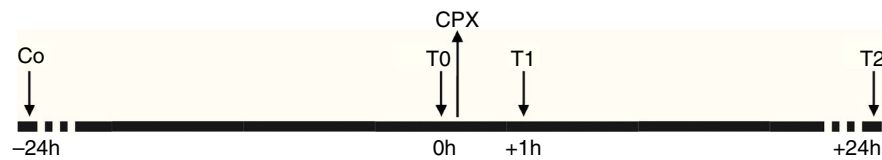
Data collection included age, body mass index (BMI), smoking history, number of exacerbations in the previous year and comorbidities. Clinical data were obtained by interview and from medical records, including the review of concomitant medications. Charlson,<sup>17</sup> Charlson-age<sup>18</sup> and COTE (COPD specific comorbidity test)<sup>19</sup> indexes were calculated based on comorbidities data. Participants completed questionnaires on dyspnea (modified Research Council breathlessness scale and Mahler' baseline dyspnea index),<sup>20</sup> activities of daily living (London Chest Activity of Daily Living scale – LCADL),<sup>21</sup> anxiety and depression (Hospital Anxiety and Depression scale – HADS),<sup>22</sup> and health status (St. George's Respiratory Questionnaire – SGRQ).<sup>23</sup>

Pulmonary function data were obtained using standardized equipment (SensorMedics Corporation, Yorba Linda, CA, USA). Post-bronchodilator spirometric values were obtained. Data were measured as absolute values (L) and as percent predicted of reference values.

### Exercise test and laboratory procedures

Patients were subjected to an incremental exercise test to maximum tolerated on a treadmill or on a cycle ergometer (Fig. 1). Treadmill protocol started with a three minutes warming up at 2.0 km/h and 0° inclination, followed by 0.5 km/h increments per minute and 0° inclination until the patient attained a brisk walking speed without running, and then increments of 2° inclination every minute until exhaustion. Cycle ergometer protocol starts with a three minutes warm up with no added resistance, followed by 10 W of increments each minute until exhaustion. Safety criteria for terminating the exercise test were applied according to ATS/ACCP guidelines.<sup>24</sup>

Whole blood samples were collected from each patient at three different time points: at rest (T0), and at one hour (T1) and 24 h (T2) after the exercise test. Additionally,



**Figure 1** Blood sampling scheme and maximal exercise test.

a blood sample from the first ten patients entering the study was collected the day before (C0), and considered as a calibration sample for relative gene expression analysis to establish the expression pattern of target genes in resting condition. These participants were told to maintain normal low-intensity activities of daily living and avoid exercising above that intensity in the previous 48 h.

RNA integrity from blood cells was immediately preserved at collection with the PAXgene Blood RNA Tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). Each sample tube was kept at room temperature for 2 h, followed by incubation at  $-20^{\circ}\text{C}$  for 24 h and thereafter stored at  $-80^{\circ}\text{C}$ . Thawed tubes were processed with the PAXgene Blood RNA Kit (PreAnalytiX GmbH) for isolation of total RNA according to manufacturers' instructions, including DNase digestion. Yield of purified RNA samples was evaluated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and stored at  $-80^{\circ}\text{C}$ . Gene expression was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR), essentially as previously described.<sup>25</sup> Briefly, a template cDNA was generated by reverse transcription from 1 to 2  $\mu\text{g}$  of total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Measurements of target genes and endogenous control (beta actin) were performed using the Taqman Gene Expressions Assays in combination with TaqMan Fast Advanced Mastermix on a 7900 HT system (Applied Biosystems), according to manufacturers' instructions. The analyzed genes and each primer/probe assay ID were the following: interleukin 1 beta (IL-1 $\beta$ , Hs00174097.m1), interleukin 6 (IL-6, Hs00174131.m1), interleukin 8 (IL-8, Hs00174103.m1), inducible nitric oxide synthase (iNOS, Hs00167248.m1), interferon gamma (IFN- $\gamma$ , Hs00174143.m1), tumor necrosis factor (TNF- $\alpha$ , Hs00174128.m1), transforming growth factor beta 1 (TGF- $\beta$ 1, Hs00998133.m1). The efficiency for each primer/probe assay was above 95% (as determined by the manufacturer).

Endogenous gene expression was used for each assay normalization and gene expression was calculated by the adapted formula  $2^{-\text{DCT}} \times 1000$ , which infers the number of mRNA molecules of the gene of interest per 1000 molecules of the endogenous controls.<sup>26</sup> DCT stands for the difference between the cycle threshold of the target gene and that of the endogenous control genes.

A week after the maximum exercise test, patients performed a 6-minute walking test (6MWT), standardized according to international guidelines.<sup>27</sup> 6MW distance, FEV<sub>1</sub>% predicted after bronchodilator, mMRC dyspnea scale and BMI data were aggregated to calculate BODE index.<sup>28</sup>

Subjects were willing and able to participate in this study and gave written informed consent prior to

baseline measurements. The hospital's Ethics committee and administration board approved the trial (IRB: Study 25/07.CE/027/07), and all data were processed anonymously according to the institution's privacy policy.

### Statistical analysis

Categorical data were presented as frequencies and percentages, and continuous variables as mean or median, SD or interquartile range: 25th percentile (P<sub>25</sub>) to 75th percentile (P<sub>75</sub>). To verify the normality assumption of parametric tests, Shapiro-Wilk goodness-of-fit test and Q-Q plots were used. To compare genetic inflammatory markers between T0 and C0, T0 and T1, and T0 and T2, nonparametric tests were used (exact Wilcoxon signed ranks test or sign test when the differences had no symmetric distributions). To identify associations between patients' characteristics and genetic inflammatory expression in all time points, LOWESS (Locally Weighted Scatterplot Smoother), Spearman's correlation coefficient and Mann-Whitney test were applied. The significance level  $\alpha = 0.05$  was considered. Due to the exploratory nature of the study, no multiple testing procedures were used.

All data were analyzed using the Statistical Package for the Social Sciences for Windows 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

### Results

A total of 21 patients were recruited, agreed to participate and entered the study. There were no dropouts. Participants sample included 15 men (71.4%), with a mean age of 66.1 years (SD = 8.27), 19 (90.5%) with smoking history (16 previous smokers – 76.2% and 3 active smokers – 14.3%), with an average 69 pack-years (range 12–150) and 2 never smokers (9.5%). These latter patients were in fact never smokers with a long-term second-hand smoke exposure. One woman was exposed for years to her husband smoking at home, and the other woman was also exposed for years to her family smoking at home and to her colleagues smoking at work. Fourteen patients (66.6%) had respiratory failure, 54.1% hypoxemic and 9.5% hypercapnic.

According to GOLD categories,<sup>16</sup> 14 (67%) patients belonged to category D. Mean FEV<sub>1</sub> was 46.76% (SD = 20.90%, min 21%, max 97% predicted), median BODE was 3.9 (min 0, max 8) and a median of 1.3 exacerbations (min 0, max 10) had occurred in the previous year.

Mean BMI were 25.81  $\text{kg m}^{-2}$  (SD = 4.9), ranging from 17 to 34  $\text{kg m}^{-2}$ . Three participants (14%) were malnourished, 8 (38%) were overweight, and four patients (19%) were obese

(BMI 30–34 kg m<sup>-2</sup>). Of these latter, one had mild obstructive sleep apnea (OSA) with an Apnea Hypopnoea Index (AHI) of 7.1 events/hour, and the other had moderate obstructive sleep apnea with an Apnea Hypopnoea Index (AHI) of 25.7 events/hour. Neither of these two patients were on CPAP at the time of the study. In the other two obese patients, OSA or hypoventilation were excluded based on the absence of typical night and daytime complaints, on physical examination and on normal arterial blood gases.

In relation to respiratory comorbidities, tuberculous sequelae diagnosis ( $n = 7$ ) were made according to a history of past pulmonary tuberculosis and imagiologic features such as calcified granuloma, focal fibrosis or localized pleural thickening. None of these radiologic features were currently considered as clinically relevant. Similarly, cylindrical bronchiectasis was found in two patients, and interpreted as associated with COPD.

The most prevalent comorbidities in our sample were cardiovascular (CV) disease, arterial hypertension being the most common (52.4%).

Both patients with and without cardiovascular comorbidities exercised on the same ergometers (cycle and treadmill) and used the same protocols: of 15 patients with cardiovascular comorbidity, 5 exercised on cycle ergometer and 10 on treadmill. Of 6 patients without cardiovascular comorbidity, 3 exercised on cycle ergometer and 3 on treadmill.

Patients without cardiovascular comorbidity achieved higher work load on cycle ergometer [mean (SD): 83 (22) W, min–max: 70–108 W versus mean (SD): 58 (25) W, min–max: 40–100 W] but lower workload on the treadmill [mean (SD): 5 (0.76) km/h and 7 (6.93)° inclination, min–max: 4.5 km/h, 2.2° – 6 km/h, 12° versus mean (SD) 5 (0.96) km/h and 10° (7.44), min–max: 4.0 km/h, 0° – 6 km/h, 22°]. All the patients exercised to their maximal tolerance.

Cardiopulmonary exercise tests data are shown in Annex, Table 1a.

Mean 6MWD was 346.1 meters (SD = 86.1), ranging from 185 to 520 meters. Oximetry lowest values at 6-minute walking test were on average 85%, ranging from 68% to 94%. Baseline characteristics are presented in Table 1.

Pharmacological treatment was as follows: 18 participants were on triple therapy with long-acting muscarinic antagonist (LAMA) Tiotropium, long-acting beta-adrenergic (LABA) Salmeterol or Formoterol and inhaled corticosteroid (ICS) Budesonide, Beclometasone or Fluticasone; 2 participants were on LABA and ICS, one was on LABA, ICS and short acting muscarinic antagonist (SAMA) Ipratropium and one participant was on rescue short acting beta-adrenergic (SABA) Salbutamol, and Acetylcysteine. Eight patients were on Theophylline, 4 were on statins (Simvastatin, Atorvastatin or Rosuvastatin) and 2 were on Amiodarone.

Seven patients were on long-term oxygen therapy (LTOT) and one was on LTOT and non-invasive ventilation (NIV). One normal-weight patient was on CPAP due to obstructive sleep apnea.

The pharmacological therapy used by the four obese patients and the other participants was essentially the same: three were on triple therapy LAMA, LABA and ICS, one was on LABA and ICS. Three were on theophylline and 2 were on statins. None of the patients were on oral corticosteroids or Roflumilast. None of the four patients were on LTOT, NIV or CPAP.

**Table 1** Baseline characteristics of COPD participants. Spirometry values displayed are post-bronchodilator.

<i>Demographics</i>	
Gender $m/w_n$ (%)	15 (71.4%)/6 (28.6%)
Age <sub>years</sub> mean (SD) min–max	66.05 (8.27) 50–82
BMI <sub>kg.m<sup>-2</sup></sub> mean (SD) min–max	25.81 (4.87) 17–34
Pack <sup>s</sup> <sub>mean</sub> (SD) min–max	69.05 (30.85) 12–150
<i>Symptoms/health status</i>	
mMRC <sub>median</sub> (min–max)	1.00 (0–3)
Mahler's BDI <sub>median</sub> (min–max)	7.00 (3–10)
LCADL <sub>median</sub> (min–max)	19.15 (8–36)
HADS-anxiety <sub>median</sub> (min–max)	6.19 (1–14)
HADS-depression <sub>median</sub> (min–max)	5.62 (0–12)
SGRQ <sub>median</sub> (min–max)	45.95 (27–77)
BODE <sub>median</sub> (min–max)	3.90 (0–8)
Exacerbations previous year <sub>median</sub> (min–max)	1.29 (0–10)
GOLD categories A/B/C/D <sub>n</sub> (%)	2 (9.5)/2 (9.5)/3 (14.3)/14 (66.6)
<i>Comorbidities</i>	
COTE <sub>median</sub> (min–max)	1.24 (0–8)
Charlson <sub>median</sub> (min–max)	1.62 (1–3)
Charlson-age <sub>median</sub> (min–max)	3.57 (2–5)
Respiratory failure/hypoxic/ hypercapnic <sub>n</sub> (%)	14 (66.6)/12 (54.1)/2 (9.5)
Cardiovascular comorbidities <sub>n</sub> (%)	15 (71.4)
Arterial hypertension <sub>n</sub> (%)	11 (52.4)
Congestive heart failure/Cor pulmonale <sub>n</sub> (%)	5 (23.8)
Ischemic heart disease <sub>n</sub> (%)	2 (9.5)
Arrhythmias <sub>n</sub> (%)	2 (9.5)
Tuberculous sequelae <sub>n</sub> (%)	7 (33.3)
Bronchiectasis <sub>n</sub> (%)	2 (9.5)
Obesity or overweight <sub>n</sub> (%)	12 (57.1)
Malnourished <sub>n</sub> (%)	3 (14.3)
Diabetes mellitus <sub>n</sub> (%)	1 (4.8)
Dyslipidemia <sub>n</sub> (%)	4 (19.0)
Osteoarticular pathology <sub>n</sub> (%)	4 (19.0)
<i>Physiology</i>	
FVC <sub>L</sub> mean (SD) min–max	2.75 (0.95) 0.84–5.31
FVC% <sub>mean</sub> (SD) min–max	85.52 (23.74) 32–139
FEV <sub>1</sub> <sub>L</sub> median (P25-P75) min–max	1.02 (0.73–1.46) 0.47–3.25
FEV <sub>1</sub> % <sub>mean</sub> (SD) min–max	46.76 (20.90) 21–97
FEV <sub>1</sub> /FVC% <sub>mean</sub> (SD) min–max	43.48 (14.11) 21–69
TLC <sub>L</sub> ( $n = 19$ ) mean (SD) min–max	7.11 (1.37) 4.14–8.71
TLC% ( $n = 19$ ) mean (SD) min–max	121.53 (15.64) 86–155
RV <sub>L</sub> ( $n = 19$ ) mean (SD) min–max	4.26 (1.27) 2.51–6.43
RV% ( $n = 19$ ) mean (SD) min–max	189.79 (49.19) 128–293
DLCO% ( $n = 17$ ) mean (SD) min–max	45.12 (19.07) 16–95
KCO% ( $n = 17$ ) mean (SD) min–max	41.41 (17.49) 13–76
PaO <sub>2</sub> mmHg mean (SD) min–max	65.46 (8.58) 50–77
PaCO <sub>2</sub> mmHg median (P25-P75) min–max	42.10 (37.20–46.05) 35–60
6MWD <sub>m</sub> mean (SD) min–max	346.05 (86.06) 185–520
6MWD% <sub>pred</sub> mean (SD) min–max	61.00 (19.50) 29–102
6MWT SpO <sub>2</sub> % <sub>mean</sub> (SD) min–max	84.57 (6.67) 68–94



**Table 2** Median (P<sub>25</sub>–P<sub>75</sub>) of the mRNA inflammatory genes at rest (T0), and at 1 h (T1) and 24 h (T2) after maximal exercise test. Comparison of T1 versus T0 and T2 versus T0.

	T0	T1	T1 vs T0 <i>p</i>	T2	T2 vs T0 <i>p</i>
IFNg	0.16 (0.04–1.57)	0.19 (0.06–0.95)	1.000 <sup>b</sup>	0.15 (0.05–1.17)	0.739 <sup>a</sup>
IL1b	15.46 (6.42–266.69)	19.38 (7.39–268.48)	0.383 <sup>b</sup>	23.15 (5.63–250.58)	0.664 <sup>b</sup>
IL6	0.33 (0.11–2.81)	0.51 (0.23–2.67)	0.189 <sup>b</sup>	0.59 (0.10–2.05)	0.815 <sup>b</sup>
IL8	1.82 (0.97–69.57)	4.70 (1.87–43.26)	0.189 <sup>b</sup>	2.07 (0.94–43.37)	0.664 <sup>b</sup>
TNFa	2.99 (2.55–47.00)	3.36 (2.25–29.61)	1.000 <sup>b</sup>	3.42 (2.51–38.21)	0.383 <sup>b</sup>
TGFb	199.71 (64.86–5462.36)	224.38 (63.91–4456.02)	0.383 <sup>b</sup>	373.45 (75.18–5250.38)	0.078 <sup>b</sup>
iNOS	0.03 (0.01–0.29)	0.04 (0.01–0.60)	0.791 <sup>b</sup>	0.04 (0.01–0.31)	0.398 <sup>a</sup>

<sup>a</sup> Exact Wilcoxon Signed ranks test.

<sup>b</sup> Sign test.

## Laboratory results

In the first 10 patients studied, we did not find a statistically significant difference between C0 and T0, the two time points of baseline resting condition (Annex-Table 2a). The characteristics of these first ten patients entering the study were similar to all group characteristics: 6 men (60%), 4 women (40%), mean age of 63.4 (SD: 6.9) years old (ranging from 56 to 79 years), mean post-bronchodilator FEV<sub>1</sub>%predicted 44.5% (SD: 23.6) (R: 21–85%), mean BMI 24.8 kg m<sup>-2</sup> (SD: 4.3) (R: 19–31 kg m<sup>-2</sup>), mean 69.9 pack-years (SD: 20.9) (R: 45–100 pack-years).

In the entire sample, there was no statistically significant difference between inflammatory expression at rest and at 1 h and 24 h after the maximal exercise test (Table 2).

Looking at associations between patients' characteristics and inflammation, the only evidence of an association was found between BMI and inflammatory expression at all time points. As presented in Fig. 2, a slightly inverse association occurs with low BMI (values under 20 kg m<sup>-2</sup>; *n* = 3) for inflammatory genes mRNA IL1b, IL6, TNFa, TGFb and iNOS. Moreover, a more pronounced positive association was found for obese patients (BMI above 30 kg m<sup>-2</sup>; *n* = 4) for all inflammatory mRNA genes tested, at all time points. No correlation coefficient estimates with corresponding *p* values were reported because only three patients were malnourished and four patients were obese.

We found no associations between inflammatory expression and all other patients' characteristics and variables, including demographics, symptoms/health status, comorbidities and physiologic parameters (data not shown).

Due to the previously reported potential influence of cardiovascular comorbidity in the inflammatory expression of COPD patients,<sup>29</sup> we looked for the association between this variable and inflammatory gene expression. Higher T1-T0 and/or T2-T0 differences were found (sometimes with statistical significance) for patients without cardiovascular comorbidity (Annex-Table 3a).

## Discussion

In our COPD patient sample, real-time polymerase chain reaction of the target mRNA inflammatory genes did not show increased inflammation either at rest or after a

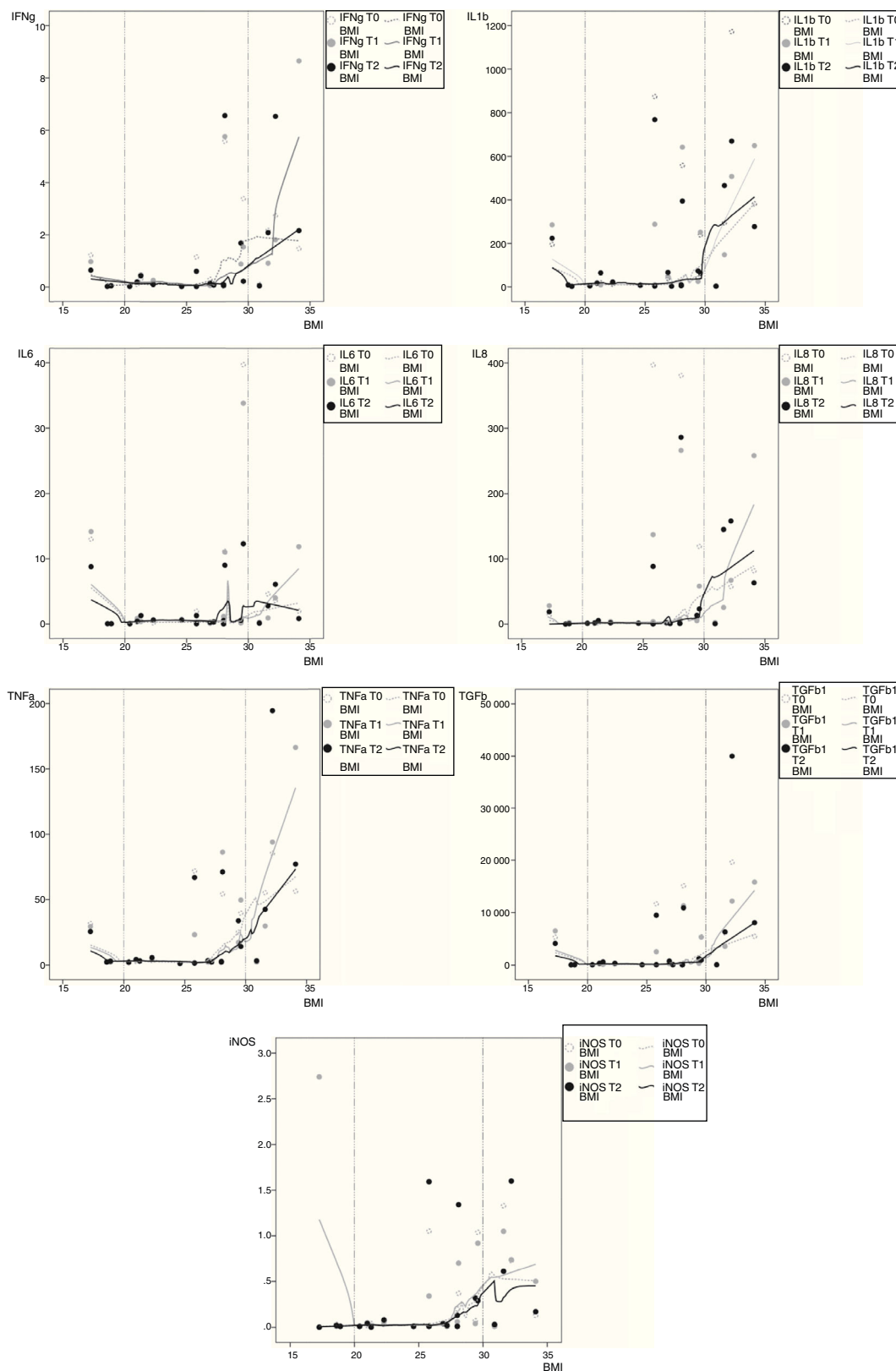
maximum exercise test. When looking at the inflammatory expression measured in resting samples on two consecutive days (control group), there were also no statistical differences between the two time points, which might reflect stability at rest mRNA parameters.

Persistent systemic inflammation is not a universal finding in patients with COPD. ECLIPSE study recently showed that in 1755 COPD patients, about 30% do not have systemic inflammation, and only a minority (16%) have persistent inflammation during 1 year follow up.<sup>5</sup>

Although we expected a significantly different mRNA inflammatory expression at 1 and at 24 h after maximal exercise, in line with other authors, such as Van Helvoort et al.,<sup>15</sup> who demonstrated that COPD patients, when compared to healthy subjects, are exposed to systemic inflammation that is intensified by exhaustive exercise, we did not find a significantly different mRNA inflammatory expression after short-term exercise.

However, in the subgroups of malnourished and obese COPD patients, we found opposite associations with mRNA inflammatory expression. When considering malnourished patients, mRNA levels of iNOS, IL6, IL1b, TNFa, and TGFb1 showed a tendency to decrease with increasing BMI values toward normal. Conversely, overweight and obese patients showed higher mRNA levels of TNFa, IFNg, IL1b, IL8, TGFb1, iNOS and IL6 as their BMI increasing above normal values. When considering all sample data, higher mRNA values of the malnourished on one side, and overweight and obese patients on the other, shown graphically in Fig. 2, might be blurred by the lower mRNA values of normal weight patients. This could explain the absence of a significant association of inflammation and exercise when taking into account the median values of all sample data.

ECLIPSE study also evidenced high BMI as one of the independent risk factors for persistent inflammation, both at baseline and at one year of follow-up.<sup>5</sup> This association was not evident for fat free mass index, which suggests an important role for adipose tissue in systemic inflammation. Garcia-Aymerich and colleagues<sup>30</sup> also identified a "systemic" COPD subtype characterized by a higher proportion of obesity in 342 COPD patients with a significant systemic inflammation; this pattern was maintained through a 4 years' follow-up period. In the study of Tamakoshi and colleagues, an acute phase response evidenced by serum



**Figure 2** Association between inflammatory biomarkers at rest (T0) and at 1h (T1) and 24h (T2) after maximal exercise test and body mass index. A Lowess was fitted to the data. Lowess – locally weighted scatterplot smoother.

levels of C-reactive protein increased in obesity and was associated with insulin resistance.<sup>31</sup>

Most of the scientific evidence has previously shown that peripheral muscle atrophy and cachexia are associated with systemic inflammation in patients with COPD, when compared to patients with no muscle wasting.<sup>32,33</sup> In the same way, in our study, malnutrition was also associated with higher expression on mRNA genes iNOS, IL6, IL1b and TGFb1, but to a lesser extent, and in the opposite direction, when compared to what happened in patients with higher BMI.

Inflammation in COPD has been associated with a worse prognosis.<sup>34</sup> In the ECLIPSE study, persistent inflamed patients had significantly increased all-cause mortality and exacerbation frequency compared with non-inflamed patients.<sup>5</sup> In our study, 8 patients were deceased after 4 years (38%), of whom, 4 (50%) were obese or overweight, and 3 (37.5%) were malnourished. Only one patient who died had had a normal BMI at baseline (data not shown).

Cardiovascular disease is the most prevalent comorbidity in COPD,<sup>35–37</sup> and it has previously been associated with systemic inflammation (elevated C-reactive protein) in patients with COPD.<sup>29</sup> However, although prevalent (71%), cardiovascular disease was not associated with higher inflammatory expression for all genes studied. These results do not seem to be influenced by different exercise protocols involved, as both patients with and without cardiovascular comorbidities exercised on the same ergometers, used the same protocols and exercised to their maximal tolerance. Patients without cardiovascular comorbidity achieved higher work load in cycle ergometer, but lower workload on the treadmill. However, assuming that the sample studied is too small to be representative, a definite conclusion of cardiovascular comorbidity not being associated with higher levels of inflammation after exercise in COPD patients can only be established with larger samples.

There is still an unmet need to characterize different COPD phenotypes that might benefit from different treatment approaches and respective outcomes. Inhaled corticosteroids, as an example, have limited efficacy in the reduction of systemic inflammation in COPD.<sup>38</sup> On the other hand, a non-pharmacological approach, such as pulmonary rehabilitation with exercise training, has been proven to improve exercise tolerance, while modifying body composition toward a higher fat-free mass, improving muscle function, and, as evidenced by some studies, it might also attenuate systemic inflammation.<sup>6</sup>

The present study has a number of limitations that need to be addressed. A small sample size was not able to rule out the presence of an increased systemic inflammation. Although small, this sample size was enough to show graphically an association between BMI and inflammation. It is an exploratory study and longitudinal studies with larger samples are needed to confirm this association. Studies designed to definitely exclude OSA or OHS as confounding in obese patients are also required.

Another limitation might be the fact that levels of plasma proteins and related mRNA precursors are sometimes not coincident.<sup>39,40</sup> Cytokine protein production is strictly regulated at multiple steps, including transcription (mRNA chain generation) and translation (protein synthesis from RNA). Plasma cytokine expression will depend on the efficiency of translation of mRNA into useful proteins.

Although our study suggested an association between obesity and several inflammatory genes, and other authors also evidenced RT-PCR as an attractive method of studying the gene expression of cytokines in whole blood,<sup>41</sup> an additional study should be carried out to confront mRNA genes and the corresponding plasma proteins.

## Conclusions

This preliminary study evidenced an association between BMI (malnutrition and obesity) and the levels of inflammatory mRNA cytokine precursors, suggesting not only malnourishment, but also obesity as potential links between COPD and systemic inflammation.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

## Conflict of interest statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acquisition of laboratory analysis kits and lab ware were partially funded by a scholarship from Nova Medical School for research projects undertaken in the affiliated hospitals of NOVA Medical School.

## Acknowledgements

We would like to thank Eunice Silva for her help in collecting blood samples and performing laboratory analysis.

## Appendix A. Supplementary data

Supplementary material associated with this article can be found in the online version available at [doi:10.1016/j.rppnen.2016.05.005](https://doi.org/10.1016/j.rppnen.2016.05.005).

## References

1. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J*. 2009;33:1165–85.
2. Garcia-Rio F, Miravittles M, Soriano JB, Munoz L, Duran-Tauleria E, Sanchez G, et al. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. *Respir Res*. 2010;11:63.
3. Agusti AG. Systemic effects of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2005;2:367–70 [discussion 371–2].

4. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax*. 2004;59:574–80.
5. Agusti A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PLoS ONE*. 2012;7:e37483.
6. van der Vlist J, Janssen TW. The potential anti-inflammatory effect of exercise in chronic obstructive pulmonary disease. *Respiration*. 2010;79:160–74.
7. Van Helvoort HA, Heijdra YF, Thijs HM, Vina J, Wanten GJ, Dekhuijzen PN. Exercise-induced systemic effects in muscle-wasted patients with COPD. *Med Sci Sports Exerc*. 2006;38:1543–52.
8. van Helvoort HA, Heijdra YF, Dekhuijzen PN. Systemic immunological response to exercise in patients with chronic obstructive pulmonary disease: what does it mean? *Respiration*. 2006;73:255–64.
9. Rabinovich RA, Figueras M, Ardite E, Carbo N, Troosters T, Filella X, et al. Increased tumour necrosis factor- $\alpha$  plasma levels during moderate-intensity exercise in COPD patients. *Eur Respir J*. 2003;21:789–94.
10. Barreiro E, Schols AM, Polkey MI, Galdiz JB, Gosker HR, Swallow EB, et al. Cytokine profile in quadriceps muscles of patients with severe COPD. *Thorax*. 2008;63:100–7.
11. Canavan J, Linton-Willoughby B, Garrod R. Acute exercise testing of COPD patients: the effect on systemic inflammatory proteins. *Curr Res Med Rev*. 2011;7:464–74.
12. Crul T, Spruit MA, Gayan-Ramirez G, Quarck R, Gosselink R, Troosters T, et al. Markers of inflammation and disuse in vastus lateralis of chronic obstructive pulmonary disease patients. *Eur J Clin Invest*. 2007;37:897–904.
13. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. *J Leukoc Biol*. 2005;78:819–35.
14. Petersen AM, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J Physiol Pharmacol*. 2006;57 Suppl.:43–51.
15. van Helvoort HA, van de Pol MH, Heijdra YF, Dekhuijzen PN. Systemic inflammatory response to exhaustive exercise in patients with chronic obstructive pulmonary disease. *Respir Med*. 2005;99:1555–67.
16. GOLD (Global Initiative for Chronic Obstructive Lung Disease). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease; 2015. Available from <http://www.goldcopd.org>
17. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40:373–83.
18. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *J Clin Epidemiol*. 1994;47:1245–51.
19. Divo M, Cote C, de Torres JP, Casanova C, Marin JM, Pinto-Plata V, et al. Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;186:155–61.
20. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest*. 1988;93:580–6.
21. Garrod R, Bestall JC, Paul EA, Wedzicha JA, Jones PW. Development and validation of a standardized measure of activity of daily living in patients with severe COPD: the London Chest Activity of Daily Living scale (LCADL). *Respir Med*. 2000;94:589–96.
22. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67:361–70.
23. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis*. 1992;145:1321–7.
24. ATS/ACCP. Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med*. 2003:211–77.
25. Videira PA, Amado IF, Crespo HJ, Alguero MC, Dall'Olio F, Cabral MG, et al. Surface alpha 2-3- and alpha 2-6-sialylation of human monocytes and derived dendritic cells and its influence on endocytosis. *Glycoconj J*. 2008;25:259–68.
26. Meijerink J, Mandigers C, van de Locht L, Tonnissen E, Goodsaid F, Raemaekers J. A novel method to compensate for different amplification efficiencies between patient DNA samples in quantitative real-time PCR. *J Mol Diagn*. 2001;3:55–61.
27. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166:111–7.
28. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350:1005–12.
29. Sin DD, Man SF. Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease. *Circulation*. 2003;107:1514–9.
30. Garcia-Aymerich J, Gomez FP, Benet M, Ferrero E, Basagana X, Gayete A, et al. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (COPD) subtypes. *Thorax*. 2011;66:430–7.
31. Tamakoshi K, Yatsuya H, Kondo T, Hori Y, Ishikawa M, Zhang H, et al. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range: a systemic low-grade inflammatory state. *Int J Obes Relat Metab Disord*. 2003;27:443–9.
32. Debigare R, Cote CH, Maltais F. Peripheral muscle wasting in chronic obstructive pulmonary disease. Clinical relevance and mechanisms. *Am J Respir Crit Care Med*. 2001;164:1712–7.
33. Schols AM, Buurman WA, Staal van den Brekel AJ, Dentener MA, Wouters EF. Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. *Thorax*. 1996;51:819–24.
34. Soler Cataluna JJ, Martinez Garcia MA. [Prognostic factors in chronic obstructive pulmonary disease]. *Arch Bronconeumol*. 2007;43:680–91.
35. Soriano JB, Visick GT, Muellerova H, Payvandi N, Hansell AL. Patterns of comorbidities in newly diagnosed COPD and asthma in primary care. *Chest*. 2005;128:2099–107.
36. Carreiro A, Santos J, Rodrigues F. Impact of comorbidities in pulmonary rehabilitation outcomes in patients with chronic obstructive pulmonary disease. *Rev Port Pneumol*. 2013;19:106–13.
37. Areias V, Carreira S, Anciaes M, Pinto P, Barbara C. Co-morbidities in patients with gold stage 4 chronic obstructive pulmonary disease. *Rev Port Pneumol*. 2014;20:5–11.
38. Sin DD, Man SF, Marciniuk DD, Ford G, FitzGerald M, Wong E, et al. The effects of fluticasone with or without salmeterol on systemic biomarkers of inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177:1207–14.
39. Moldoveanu AI, Shephard RJ, Shek PN. Exercise elevates plasma levels but not gene expression of IL-1 $\beta$ : IL-6, and TNF- $\alpha$  in blood mononuclear cells. *J Appl Physiol* (1985). 2000;89:1499–504.
40. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol*. 1998;508 Pt 3:949–53.
41. Netea MG, Drenth JP, De Bont N, Hijmans A, Keuter M, Dharmana E, et al. A semi-quantitative reverse transcriptase polymerase chain reaction method for measurement of MRNA for TNF- $\alpha$  and IL-1  $\beta$  in whole blood cultures: its application in typhoid fever and exentric exercise. *Cytokine*. 1996;8:739–44.