



Trabajo Original

Nutrición artificial

Oxidative and inflammatory effects of pulmonary rehabilitation in patients with bronchiectasis. A prospective, randomized study

Efectos oxidativos e inflamatorios de la rehabilitación pulmonar en pacientes con bronquiectasia. Estudio prospectivo aleatorizado

Casilda Oliveira^{1,2}, Eva García-Escobar^{1,3,4}, Esperanza Doña^{1,5}, Francisco J. Palenque⁶, Nuria Porras^{1,4}, Antonio Dorado², Ana M. Godoy⁶, Elehazara Rubio-Martín^{1,3,4}, Francisco J. Bermúdez-Silva^{1,3,4}, Silvana Y. Romero-Zerbo^{1,4}, Gemma Rojo-Martínez^{1,3,4}, Rocío Martín-Valero^{7,8}, José Abuín Fernández⁴, and Gabriel Oliveira^{1,3,4,8}

¹Instituto de Investigación Biomédica de Málaga (IBIMA). Málaga, Spain. ²Pneumology Clinical Management Unit. Hospital Regional Universitario de Málaga. Málaga, Spain. ³CIBER de Diabetes y Enfermedades Metabólicas Relacionadas (CIBERDEM). Málaga, Spain. ⁴Endocrinology and Nutrition Clinical Management Unit. Hospital Regional Universitario de Málaga. Málaga, Spain. ⁵Pneumology Clinical Management Unit. Hospital de Alta Resolución de Benalmádena. Benalmádena, Málaga, Spain. ⁶Rehabilitation Clinical Management Unit. Hospital Regional Universitario de Málaga. Málaga, Spain. ⁷Nursing and Physical Therapy Department. Universidad de Málaga. Málaga, Spain. ⁸Universidad de Málaga. Málaga, Spain

Abstract

Background: systemic inflammation and oxidative stress are important factors in the pathogenesis of bronchiectasis. Pulmonary rehabilitation (PR) is recommended for bronchiectasis, but there is no data about its effect on the inflammatory and REDOX status of these patients.

Aims: to investigate the effect of PR in non-cystic-fibrosis bronchiectasis (NCFB) patients, and to compare it with the effect of PR plus a hyper-proteinic oral nutritional supplement (PRS) enriched with beta-hydroxy-beta-methylbutyrate (HMB) on serum inflammatory and oxidative biomarkers.

Materials and methods: this was an open randomized, controlled trial. Thirty individuals (65 years old or younger with a body mass index over 18.5, older than 65 years with a body mass index over 20) were recruited from September 2013 to September 2014, and randomly assigned to receive PR or PRS. Total neutrophils, and inflammatory and oxidative biomarker levels were measured at baseline, and then at 3 and 6 months.

Results: in the PRS group neutrophil levels were decreased from baseline at 6 months. A significantly different fold change was found between the PR and PRS groups. In the PR group, IL-6 and adiponectin were increased by the end of the study while TNF α levels were decreased from baseline at 6 months. REDOX biomarkers remained stable throughout the study except for 8-isoprostane levels, which were increased from baseline at 6 months in both groups of patients.

Conclusion: a PR program induced a pro-oxidative effect accompanied by changes in circulating inflammatory cytokine levels in NCFB patients. Our results would also suggest a possible beneficial effect of the HMB enriched supplement on neutrophil level regulation in these patients. The information provided in this study could be useful for choosing the right therapeutic approach in the management of bronchiectasis.

Resumen

Introducción: la inflamación sistémica y el estrés oxidativo son factores importantes en la patogénesis de la bronquiectasia. La rehabilitación pulmonar (PR) está recomendada en los sujetos con bronquiectasias, pero no hay datos sobre sus posibles efectos sobre el estado inflamatorio y REDOX de estos pacientes.

Objetivos: investigar el efecto de la PR en pacientes con bronquiectasias no asociadas a fibrosis quística (NCFB) sobre los biomarcadores oxidativos e inflamatorios, y compararlo con los efectos de la PR junto con la suplementación oral de un suplemento hiperproteico (PRS) enriquecido con beta-hidroxi-beta-metilbutirato (HMB).

Material y métodos: ensayo clínico abierto, aleatorizado y controlado. Treinta pacientes (de 65 años o menos con un índice de masa corporal por encima de 18,5, y mayores de 65 años con un índice de masa corporal de más de 20) se aleatorizaron para recibir PR o PRS. Los niveles circulantes de neutrófilos totales y los de biomarcadores de estado inflamatorio y oxidativo se determinaron al inicio del estudio y a los 3 y 6 meses.

Resultados: los niveles de neutrófilos en el grupo de PRS se redujeron desde el inicio a los 6 meses, presentando una tasa de cambio significativamente diferente según el tratamiento. En el grupo de PR, la IL-6 y la adiponectina aumentaron al final del estudio, mientras que los niveles de TNF α disminuyeron desde el inicio a los 6 meses. Los biomarcadores de estrés oxidativo se mantuvieron estables durante todo el estudio excepto por los niveles de 8-isoprostano, que aumentaron desde el inicio a los 6 meses en ambos grupos de pacientes.

Conclusión: el programa de PR indujo un efecto pro-oxidativo acompañado de cambios en los niveles de citoquinas inflamatorias circulantes en pacientes con NCFB. Nuestros resultados también sugieren un posible efecto beneficioso del suplemento nutricional sobre la regulación de los niveles de neutrófilos de estos pacientes.

Keywords:

Bronchiectasis.
Pulmonary rehabilitation. HMB (beta-hydroxy-beta-methylbutyrate).
Oxidative stress.
Cytokines.

Palabras clave:

Bronquiectasias.
Rehabilitación pulmonar. HMB (beta-hidroxi-beta-metilbutirato). Estrés oxidativo. Citoquinas.

Received: 02/07/2019 • Accepted: 22/11/2019

Oliveira C, García-Escobar E, Doña E, Palenque FJ, Porras N, Dorado A, Godoy AM, Rubio-Martín E, Bermúdez-Silva FJ, Romero-Zerbo SY, Rojo-Martínez G, Martín-Valero R, Abuín Fernández J, Oliveira G. Oxidative and inflammatory effects of pulmonary rehabilitation in patients with bronchiectasis. A prospective, randomized study. *Nutr Hosp* 2020;37(1):6-13

DOI: <http://dx.doi.org/10.20960/nh.02763>

Correspondence:

Eva García-Escobar. Laboratorio de Investigación. Edificio 5. Hospital Regional Universitario de Málaga. Plaza del Hospital Civil, s/n. Málaga, Spain
e-mail: eyring@gmail.com

INTRODUCTION

Non-cystic fibrosis bronchiectasis (NCFB) is a chronic disease characterized by permanently dilated airways due to bronchial wall structural component destruction as a result of a vicious cycle involving persistent bacterial colonization and chronic neutrophilic infiltration of the bronchial mucosa (1). Bronchiectasis causes pulmonary infections and loss of lung function, which results in chronic morbidity contributing to premature mortality (1).

Systemic inflammation as demonstrated by increased blood neutrophil levels, elevation of C-reactive protein (CRP), and plasma cytokines has been reported in patients with NCFB, correlating also to disease severity and bacterial colonization (2,3). This systemic inflammation has also been suggested as a cornerstone of reduced exercise capacity and peripheral muscle strength in patients with bronchiectasis (4). Increased blood neutrophil levels have been associated with NCFB disease severity and bacterial colonization in NCFB patients, being considered a possible marker of systemic inflammation (3). Intense oxidant and anti-oxidant activity is also present in the airways and circulating blood of patients with bronchiectasis (5). Overproduction of reactive oxygen species (ROS) results in oxidative stress that can lead to inflammation. This is thought to be an important factor in the pathogenesis and progression of bronchiectasis (5,6).

Enhancing effective expectoration of stagnated bronchopulmonary secretions is essential for the clinical management of bronchiectasis. Exercise training or a pulmonary rehabilitation program (PR) has beneficial effects on NCFB patients (7), and is recommended in current NCFB treatment guidelines (8,9). These effects are related to the respiratory and peripheral skeletal muscle manifestations of bronchiectasis and are commonly associated with improvements in respiratory symptoms, including mucus removal (7,10). Despite the relevant role that inflammation is believed to have in the progression of bronchiectasis (3,4), and its relationship with oxidative stress status (5,6), to the best of our knowledge there are no studies evaluating whether exercise is able to modify the inflammatory and oxidative response of patients with NCFB; only unclear results have been reported in chronic obstructive pulmonary disease (COPD) patients (11-13).

Beta-hydroxy-beta-methylbutyrate (HMB) is a derivative, *in vivo*, of the essential amino acid leucine (14) with a well-documented anti-catabolic effect on skeletal muscle in both healthy and pathological conditions (15-17). Our group has already published the positive effects of the addition of a hyperproteic oral nutritional supplement enriched with HMB together with a PR program on body composition, BMD, muscle strength, health-related quality of life, and breathing parameters in patients with bronchiectasis (16-18). But HMB supplementation has been also suggested as exerting positive anti-inflammatory effects (19), which might be associated with improved pulmonary function in COPD patients (20). It has been suggested that some pro-inflammatory factors, such as interleukin 1 beta and tumor necrosis factor alpha, increase proteolysis and may modulate protein turnover (21). Because HMB is associated with less proteolysis (22), to date, several studies have suggested

that HMB supplementation could affect inflammatory responses even after exercise (19).

The aims of this study were to evaluate in untrained NCFB patients the effects of a PR program, alone or combined with the intake of a hyperproteic oral nutritional supplement enriched with HMB, on oxide-reduction (REDOX) and inflammatory biomarkers.

MATERIALS AND METHODS

Study design, inclusion/exclusion criteria, and screening process are as previously described (16-18). Briefly, a total of 30 stable (without respiratory exacerbations) patients were recruited from the bronchiectasis unit at the Regional University Hospital of Málaga from 2013 to 2014, and randomized in a 1:1 ratio to undergo either PR alone or PR with nutritional support (PRS). Inclusion criteria were as follows: a diagnosis of NCFB, age 18 to 80 years, normo-nourished, and without any acute disease.

INTERVENTION

The exercise program consisted of 60 minutes of exercise bi-weekly at the hospital, and one unsupervised session per week for twelve weeks (16). Hospital and home exercise sessions consisted of a total of 45 min of exercises, coupled with 15 min of breathing retraining with the Orygen-Dual Valve[®], and 7-10 minutes of stretching and relaxation exercises. Attendance of the sessions at hospital was recorded and at-home sessions were reminded by telephone every two weeks. Physical activity was evaluated using the wGT3X (ActiGraph) accelerometer and the IPAQ (International Physical Activity Questionnaire) (23), with no differences in physical activity level according to supplement intake status (18).

The supplement intervention (16) was based on the intake or not of the oral nutritional supplement Ensure Plus Advance, which is a 220-mL, oral hyperproteic nutritional supplement providing 330 kcal (1.5 kcal/mL), 18 g of protein, 1.5 g of HMB and 1.7 g of prebiotic fiber (FOS). The participants were advised to take the supplement at least 60 minutes before the rehabilitation sessions. As all were normally nourished, a registered dietitian informed them of how to reduce the intake of natural foods in order to compensate for the increase in calories supplied. Adherence was recorded by patients in a diary and controlled by the investigators in the PR sessions or by telephone. During the intervention, all participants were instructed on general recommendations for a Mediterranean-style healthy diet.

A seven-day, prospective dietary questionnaire was fulfilled by all participants in their homes. The dietary data was registered into their personal computer equipped with the software package Dietstat (Dietstat[®] 1.0, 2010, Málaga, Spain) with up-to-date composition tables for Spanish food (24). Macronutrient intake did not show significant differences according to supplement intake status at any time points (16).

Once the PR finished, participants were instructed and subsequently reminded to maintain a non-sedentary physical activity

level and to use the Orygen-Dual Valve® (25) twice weekly, as well as to maintain a Mediterranean-style healthy diet during the follow-up period.

The study was approved by the Málaga Provincial Research Ethics Committee. All the procedures were conducted according to the Declaration of Helsinki, and all of the participants provided their written informed consent. The study was registered at the clinicaltrials.gov site (<http://clinicaltrials.gov>) NCT02048397.

OUTCOME MEASURES

Outcome assessments were performed at baseline, 3 months and 6 months.

Once no respiratory exacerbations were confirmed, a serum sample was taken and separated: one aliquot was immediately stored at $-80\text{ }^{\circ}\text{C}$ and another aliquot was immediately used to measure the biochemical parameters. The SSPA Biobank has coordinated the $-80\text{ }^{\circ}\text{C}$ storage of the serum samples.

Weight and height were measured and BMI was calculated.

Circulating levels of interleukine 6 (IL-6), tumor necrosis factor alpha (TNF α), and adiponectin were measured by enzyme immunoassay (EIA) (R&D Systems Europe Ltd). Neutrophil and CRP circulating levels were determined by the Central Assistance Laboratory of Hospital Regional de Málaga. Also, serum oxidative markers 8-isoprostane (Cayman Chemical, Michigan, USA), total antioxidant capacity (TAC) (Cayman Chemical, Michigan, USA), and superoxide dismutase activity (SOD) (Cayman Chemical, Michigan, USA) were determined by EIA. The circulating levels of thiobarbituric acid reactive substances (TBARs) were determined by spectrophotometry (26).

Fold change was calculated as the change described by the ratio between the levels of circulating metabolites at 6 months of study and baseline levels. They were expressed as percentages.

STATISTICAL METHODS

Sample size was calculated based on the circulating levels of 8-isoprostane in bronchiectasis patients previously reported by our group (5). For an 80 % probability of detecting a difference in 8-isoprostane levels after months of intervention a total of 30 participants were required, based on the assumption of a difference between groups of 33 pg/mL, with a standard deviation (SD) of 31.9 pg/mL.

Analyses were performed using the SPSS statistical software. Descriptive results are shown as mean and standard deviation. The Shapiro-Wilk test was used to assess whether the variables were normally distributed. Hypothesis contrast for continuous variables between groups used Student's t-test for variables that followed a normal distribution and non-parametric tests for variables that did not conform to normal. Variables tested repeatedly over time following a normal distribution were analyzed using repeated measures multiple analysis of variance. The effects of time on the variables tested repeatedly over time that did not follow a normal

distribution were analyzed by a Wilcoxon test within samples under the same treatment. All analyses were performed using the SPSS statistical software, version 20.0. NY (27).

RESULTS

A total of 30 individuals (15 in each group) were enrolled in the clinical trial. All participants completed the 3-month PR program. Two participants from each arm withdrew from the study during the 6-month follow-up period because of an illness unrelated to bronchiectasis (16-18).

The clinical baseline characteristics, summarized in table I, and the evolution of the dynamometry and quality of life parameters, as well as serum or plasma biomarker levels, of the participants have been previously described (16). The number of exacerbations suffered by the patients during the previous year and one year after commencing the intervention was similar for both groups (17). Additionally, no differences in weight, height were found according to supplement intake status at any time point (data not shown).

NEUTROPHIL LEVELS

There were no differences in white blood cell levels throughout the study period except for neutrophil levels. The PRS group showed significantly lower neutrophil levels at 6 months than at baseline (neutrophils at baseline ($\times 10^9/\text{L}$): 4.24 ± 1.55 vs. neutrophils at 6 months ($\times 10^9/\text{L}$): 3.62 ± 1.09 , $p = 0.01$).

Neutrophil levels were the only blood cells that showed statistically different fold-change percentages according to supplement intake status, being positive in the PR group but negative in the PRS group of patients (Fig. 1).

INFLAMMATORY BIOMARKERS

In table I it is shown the evolution of the evaluated inflammatory biomarkers throughout the study. CRP levels keep stable during the 6 months with a trend to decrease their circulating levels (Table II). The other studied biomarkers showed a different behavior over time; thus, TNF α levels presented an initial decrease at 3 months, significantly different from the baseline level when considering the total group of patients and the PR group, which remained significantly lower by the end of the study in the PR group (Table II), while IL-6 levels tended to rise during all the study period, with IL-6 levels at 6 months being significantly higher than at 3 months in the total group of patients and the PR group (Table II). Alternatively, adiponectin levels were significantly higher from baseline at the end of the study in the total group of patients and the PR group, but not in the PRS group (Table II).

None of the studied biomarkers of inflammatory status or their fold-change percentages were different according to treatment.

Table I. General clinical characteristics

	Total (n = 28)	PR (n = 14)	PRS (n = 14)	p*
Age	56.1 ± 13	53.7 ± 13.1	58.4 ± 12.9	ns
BMI (kg/m ²)	26.6 ± 4.7	27.3 ± 5.8	25.9 ± 3.4	ns
Men (n/ %)	12/40	4/26.7	8/53.3	ns
FEV1 (%)	66.1 ± 23.6	66.8 ± 28.1	65.3 ± 19	ns
FVC (%)	68.4 ± 18.4	71 ± 21.2	65.8 ± 15.6	ns
Daily bronchorrea (mL)	33.2 ± 43	33.7 ± 54.5	32.7 ± 28	ns

Data are average ± standard deviation. PR: pulmonary rehabilitation; PRS: pulmonary rehabilitation plus oral nutritional supplement; BMI: body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in one second. *Statistical differences comparing PR vs. PRS.

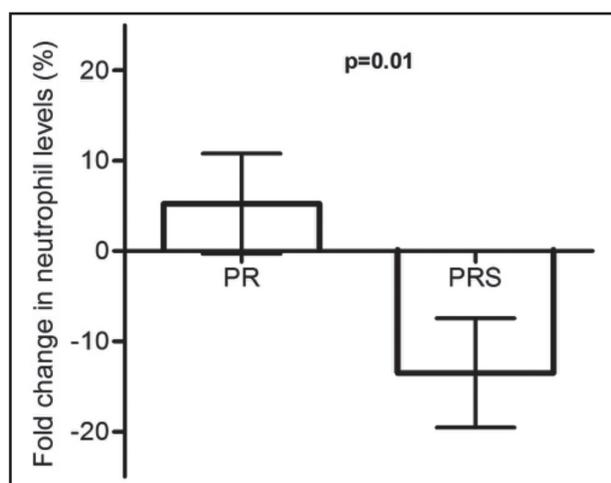


Figure 1.

Fold-change percentage in neutrophil levels. Bars represent the mean and standard deviation values of the fold change percentage (month 6 - baseline) in neutrophil levels of NCFB patients following a pulmonary rehabilitation (PR) program, and NCFB patients following the same pulmonary rehabilitation program supplemented with HMB (PRS).

REDOX BIOMARKERS

The circulating levels of the studied REDOX biomarkers remained stable throughout the study except for 8-isoprostane levels, which were increased to produce significantly higher levels from baseline after 6 months of treatment in all group of patients (Table III). Moreover, in the total and PRS groups, 8-isoprostane levels at 6 months were also significantly higher than at 3 months.

Neither REDOX biomarker levels nor their fold-change percentages were significantly different between the PR and PRS groups.

DISCUSSION

This study revealed that PR could exert a pro-oxidative effect accompanied by changes in circulating inflammatory cytokine levels in normally nourished bronchiectasis patients. Furthermore, the addition of a hyperproteic oral nutritional supplement enriched

with BHT to PR may reduce neutrophil levels when compared to PR alone. These assessments are important because studies suggest that oxidative stress and inflammation are strongly related to and involved in the pathogenesis and progression of bronchiectasis disease (1,5,6). Accordingly, the information provided by this study could be useful for choosing the right therapeutic approach in the management of bronchiectasis disease.

It has been described that inflammatory and oxidative responses in NCFB are mainly caused by chronic neutrophilic activation (1,5,6), this having been considered a potential marker of systemic inflammation (3). The impact of exercise on neutrophil levels is unclear. It has been documented that highly intensive exercise elicits mobilization and functional augmentation of neutrophils in healthy sedentary adults (28,29); however, in adolescents with obesity the number of neutrophils is both reduced and increased after 6 months of high- and low-intensity training, respectively, when compared with baseline (30). To the best of our knowledge, no previous studies have reported information about the regulation of neutrophil levels by exercise in bronchiectasis. In this study, exercise alone did not have any effect on neutrophil levels over time; however, the intake of the nutritional HMB-enriched supplement led to a reduction in neutrophil circulating levels at the end of the study when compared with baseline values.

A different fold-change percentage in neutrophil levels was found between the PR and PRS groups of patients. It is known that HMB is able to modify immune cell function in human (31) and non-human models (32). Similar to the present study, supplementation with HMB in COPD patients was associated with a reduced number of total leukocytes when compared with a control group without supplementation (20). All in all, our results might be suggesting a beneficial effect of HMB on the regulation of systemic inflammation in NCFB patients through a reduction in neutrophil levels; nevertheless, this result should be taken cautiously due to the lack of data about chronic neutrophil activation in the present investigation.

Several investigations of systemic inflammatory markers in COPD patients after an exercise program with and without nutritional supplementation have already been done. However, no information in this regard is available for NCFB patients.

It is not clear whether exercise alone may modify inflammatory response in individuals with COPD, with investigations reporting

Table II. Inflammatory biomarker evolution throughout the study period

	Baseline (M ± SD)	3 months (M ± SD)	6 months (M ± SD)	p*	p†	p‡
<i>TNFα (pg/mL)</i>						
Total (n = 28)	4.95 ± 3.45	2.62 ± 2.21	4.07 ± 2.94	< 0.01	< 0.05	ns
PR (n = 14)	6.10 ± 3.43	2.99 ± 2.55	4.20 ± 2.76	0.01	ns	< 0.05
PRS (n = 14)	3.88 ± 3.22	2.28 ± 1.86	3.94 ± 3.20	ns	ns	ns
<i>IL-6 (pg/mL)</i>						
Total (n = 28)	2.79 ± 2.08	3.04 ± 2.69	6.13 ± 5.92	ns	< 0.05	< 0.05
PR (n = 14)	2.32 ± 1.43	2.37 ± 2.18	6.47 ± 7.07	ns	< 0.05	ns
PRS (n = 14)	3.30 ± 2.57	3.76 ± 3.08	5.74 ± 4.50	ns	ns	ns
<i>Adiponectin (mg/L)</i>						
Total (n = 28)	11.48 ± 5.69	12.17 ± 6.59	12.53 ± 5.99	ns	ns	0.001
PR (n = 14)	12.27 ± 4.68	12.08 ± 5.67	14.01 ± 5.31	ns	< 0.05	< 0.01
PRS (n = 14)	10.69 ± 6.61	12.25 ± 7.60	11.06 ± 6.46	0.01	ns	ns
<i>CRP (mg/dL)</i>						
Total (n = 28)	4.88 ± 6.68	3.96 ± 5.50	3.41 ± 4.66	ns	ns	ns
PR (n = 14)	3.64 ± 5.03	3.84 ± 6.68	3.96 ± 5.23	ns	ns	ns
PRS (n = 14)	6.03 ± 7.92	4.07 ± 4.48	2.91 ± 4.19	ns	ns	ns

M ± SD: average ± standard deviation; TNFα: tumor necrosis factor alpha; IL-6: interleukin 6; CRP: C-reactive protein; PR: pulmonary rehabilitation; PRS: pulmonary rehabilitation plus oral nutritional supplement. *Statistical differences comparing baseline vs. 3 months. †Statistical differences comparing 3 months vs. 6 months. ‡Statistical differences comparing baseline vs. 6 months.

Table III. Oxidative biomarker evolution throughout the study period

	Baseline (M ± SD)	3 months (M ± SD)	6 months (M ± SD)	p*	p†	p‡
<i>8-ISOP (pg/mL)</i>						
Total (n = 28)	103.15 ± 32.74	120.54 ± 36.99	166.11 ± 76.17	ns	< 0.01	0.001
PR (n = 14)	105.71 ± 38.04	131.91 ± 46.87	156.94 ± 78.87	ns	ns	< 0.05
PRS (n = 14)	100.59 ± 27.55	109.1 ± 19.01	175.28 ± 75.13	ns	0.01	< 0.01
<i>TAC (nM)</i>						
Total (n = 28)	0.78 ± 0.50	0.62 ± 0.47	0.55 ± 0.47	ns	ns	ns
PR (n = 14)	0.83 ± 0.49	0.51 ± 0.43	0.61 ± 0.48	ns	ns	ns
PRS (n = 14)	0.73 ± 0.53	0.71 ± 0.50	0.48 ± 0.48	ns	ns	ns
<i>TBARS (μM)</i>						
Total (n = 28)	5.4 ± 2.48	6.36 ± 4.1	4.52 ± 2.92	ns	ns	ns
PR (n = 14)	5.32 ± 2.69	6.13 ± 3.57	4.58 ± 2.97	ns	ns	ns
PRS (n = 14)	5.49 ± 2.35	6.59 ± 4.7	4.45 ± 2.98	ns	ns	ns
<i>SOD (U/mL)</i>						
Total (n = 28)	6.45 ± 2.35	6.56 ± 1.90	5.87 ± 1.92	ns	ns	ns
PR (n = 14)	6.04 ± 2.28	5.94 ± 1.35	5.56 ± 2.17	ns	ns	ns
PRS (n = 14)	6.85 ± 2.42	7.18 ± 2.19	6.18 ± 1.65	ns	ns	ns

M ± SD: average ± standard deviation; 8-ISOP: 8-isoprostane; TAC: total antioxidant capacity; TBARS: thiobarbituric acid reactive substances; SOD: superoxide dismutase; PR: pulmonary rehabilitation; PRS: pulmonary rehabilitation plus oral nutritional supplement. *Statistical differences comparing baseline vs. 3 months. †Statistical differences comparing 3 months vs. 6 months. ‡Statistical differences comparing baseline vs. 6 months.

different results (11,13,28,33). In our study, exercise alone was able to reduce TNFα and increase adiponectin levels at the end of the study, and there were also close-to-significant increases in circulating IL-6 levels. The lack of significance in the increased

IL-6 levels found in the PR group was probably due to an insufficient sample size. According to our results, the inflammatory response to a PR program in NFCB patients is unclear, with both an anti-inflammatory effect on TNFα levels and a pro-inflamma-

tory effect on IL-6 and adiponectin levels (34). Contrary to these observations, in the PRS group of patients inflammatory biomarker levels remained stable throughout the study period. All this might be pointing to a balancing effect of HMB on the inflammatory changes caused by exercise in untrained NFCB patients.

There are few works evaluating the effect of HMB on inflammatory biomarkers, and most of them are focused on athletes (35,36); in all of them HMB seemed to attenuate the inflammatory response to intense exercise with a reduction in cytokine production. Studies in COPD patients comparing the effect on inflammatory response of the intake or not of a nutritional supplement added to a PR program returned different results according to supplement composition (37,38). In a study comparing the systemic inflammatory effect of a nutritional supplement based on whey peptide, ω -3 fatty acids and antioxidant vitamins, compared with a control group without the nutritional supplement, in COPD patients, the intake of the supplement decreased CRP, IL-6, and TNF α levels, among others (38). Contrary to this, in our study, the levels of inflammatory biomarkers were no different according to supplement intake status. Two main reasons might be explaining the differences with our study: first, the nutritional composition of each supplement was different. Secondly, the COPD patients of the Sugawara et al. study were malnourished whereas our NFCB patients were normally nourished.

Considering that higher inflammatory cytokine levels are associated with higher muscle and weight loss (39), together with the fact that COPD undernourished patients show higher levels of inflammatory cytokines (40,41), nourished status might be affecting the anti-inflammatory effect of the above supplements.

To the best of our knowledge there are no previous studies evaluating the effects of a PR program on the oxidative biomarkers of NFCB patients; however, some of these effects have been described in COPD patients with contradictory results according to disease severity (12,42) or exercise intensity (42-44). Some studies have reported an increment in lipid peroxidation and protein oxidation, accompanied by a decrease in antioxidant mechanisms, in muscle biopsy samples in response to high intensive exercise training in both stable (43) and severely unstable (12) COPD patients, whereas some other authors have reported no differences in muscle lipid peroxidation (44) after high-intensity exercise, or even an improvement of muscle oxidative stress in severe COPD patients (42). In line with those studies reporting a pro-oxidative effect of exercise, in our study an increment in 8-isoprostane levels was observed in response to exercise, regardless of supplement intake status. The fact that no other oxidative biomarker showed differences after the exercise program might be explained because we evaluated systemic oxidative biomarker levels instead of changes in muscle tissue, where the production of ROS is higher (45). Nevertheless, it has been described that in biological fluids the levels of 8-isoprostane seem to represent an accurate measure for *in vivo* oxidative status, and they have been considered the gold-standard biomarker for lipid peroxidation (46). Among the possible physiological functions proposed for 8-isoprostane, some authors have suggested that it could be involved in the regulation

of the exercise response (46). Apart from the deleterious effects of muscle ROS increments in response to non-regular intensive exercise, some previous investigations pointed to a possible positive effect of increased ROS production after regular physical training, which would lead to activate the exercise-induced adaptation of the muscle phenotype (47). In all, this led the authors to suggest a systemic pro-oxidative effect of the PR program that might be associated with a possible exercise-induced adaptation.

In our study, the group of NFCB patients nutritionally supplemented showed a similar increment in 8-isoprostane levels as that of the patients not supplemented, without differences in any other oxidative biomarkers. According to these, the nutritional support enriched with HMB during a PR program did not modify the pro-oxidative effect observed after the exercise in untrained NFCB patients.

Some limitations have been identified in this study. Studies of neutrophil function would be interesting in order to interpret our results; however, no fresh samples for neutrophil function or activation studies were taken during the sample collection.

Additionally, airway samples, in which levels of inflammatory markers could be determined as a more sensible system to detect changes, were not possible to obtain for this study; nevertheless, several studies have proposed systemic inflammatory markers as representative of the inflammatory status of NFCB patients, also correlated to disease severity and bacterial colonization (2,3). It has been described in COPD patients that inflammatory and oxidative status are closely related with nourishment state (40,41) and disease severity (13), so the fact that our NFCB patients showed moderate-to-severe breathing involvement without being malnourished could undervalue our results. In the same way, exercise intensity (44,45) and supplement dose (48) have been associated with differences in the inflammatory or oxidative response of COPD patients. Finally, although the sample size was calculated based on the 8-isoprostane circulating levels of NFCB patients (5), it is possible that this sample size was not enough to detect differences in other markers. All of these factors could be considered limitations that should be solved in future investigations in order to complement our results.

In conclusion, the results of our study provide insights about a pro-oxidative effect of a pulmonary rehabilitation program on untrained NFCB patients, accompanied by changes in circulating inflammatory cytokine levels. Additionally, a possible beneficial effect on the reduction of neutrophil levels, a systemic inflammatory biomarker, is observed after the intake of a hyperproteic HMB-enriched supplement on these patients. However, as neutrophil function or chronic activation has not been determined in this study, and no other inflammatory biomarkers were different between the PR and PRS groups, the possible beneficial effects of HMB on the inflammatory status of these patients should be interpreted cautiously. The authors suggest the necessity of further investigations to confirm the possible benefits of HMB on neutrophil activation and function, and also to evaluate the effects of nutritional supplements in NFCB patients with different grades of malnutrition.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Richard Carlsson for his assistance with the English language.

DISCLOSURES

GO has had occasional interventions of consultancy or speaker fees and grants for non-conditioned investigation from ABBOTT, Nutricia, Vegemat, Fresenius, Adventia and Nestlé. Nonetheless, the study has been carried out independently by the investigators, no clinical nutrition company has participated in the design, execution, evaluation or distribution of the results.

SUPPORT

This study was supported by the Consejería de Salud de la Junta de Andalucía (PI-0239-2013 to GO); SEPAR 016/2013 and Neumosur 3/2013. Costa del Sol Health Agency. Clinical Trial Registration: NCT02048397 (clinicaltrials.gov). F-JB-S and GR-M belong to the regional "Nicolás Monardes" research program of the Consejería de Salud, Junta de Andalucía (C-0070-2012 and RC-0006-2016, respectively). CIBERDEM is an initiative of the Instituto de Salud Carlos III.

AUTHOR'S CONTRIBUTION

CO and GO were the principal investigators and oversaw all aspects of the study (intellectual, practical, and dissemination). EG-E participated in the data analysis and interpretation, and in writing/editing this manuscript. ER-M and S-YR-Z contributed to data acquisition and performed the experiments. CO, ED, F-JP, NP, AD, A-MG, RM-V and GO contributed to data acquisition and patient recruitment. F-JB-S participated in the writing of the manuscript. All the authors contributed to the critical revision of the manuscript.

REFERENCES

- Mandal P, Hill AT. Bronchiectasis: breaking the cycle of inflammation and infection. *Lancet Respir Med* 2013;1:e5-6.
- Coban H, Gungen AC. Is There a Correlation between New Scoring Systems and Systemic Inflammation in Stable Bronchiectasis? *Can Respir J* 2017;2017.
- Dente FL, Bilotta M, Bartoli ML, Bacci E, Cianchetti S, Latorre M, et al. Neutrophilic Bronchial Inflammation Correlates with Clinical and Functional Findings in Patients with Noncystic Fibrosis Bronchiectasis. *Mediators Inflamm* 2015;2015:642503.
- Alves de Camargo A, Helga Yamane de Oliveira C, Abensur Athanazio R, Zahi Rached S, De Paula Vieira R, Cukier A, et al. Is there a correlation between inflammatory status and exercise capacity in patients with bronchiectasis? Preliminary results, European Respiratory Society (ERS); 2015. p. PA2792.
- Oliveira G, Oliveira C, Dorado A, Garcia-Fuentes E, Rubio E, Tinahones F, et al. Cellular and plasma oxidative stress biomarkers are raised in adults with bronchiectasis. *Clin Nutr* 2013;32:112-7.
- Park HS, Kim SR, Lee YC. Impact of oxidative stress on lung diseases. *Respirology* 2009;14:27-38.
- Lee AL, Gordon CS, Osadnik CR. Exercise training for bronchiectasis. *Cochrane Database Syst Rev*; 2018.
- Smith MP. Diagnosis and management of bronchiectasis. *CMAJ* 2017;189:E828-35.
- Guell Rous MR, Diaz Lobato S, Rodriguez Trigo G, Morante Velez F, San Miguel M, Cejudo P, et al. Pulmonary rehabilitation. Sociedad Espanola de Neumologia y Cirugia Toracica (SEPAR). *Arch Bronconeumol* 2014;50:332-44.
- Dos Santos DO, De Souza HCD, Baddini-Martinez JA, Ramos EMC, Gastaldi AC. Effects of exercise on secretion transport, inflammation, and quality of life in patients with noncystic fibrosis bronchiectasis. *Med (United States)* 2018;97.
- van Helvoort HAC, Heijdra YF, de Boer RCC, Swinkels A, Thijs HMH, Dekhuijzen PNR. Six-minute walking-induced systemic inflammation and oxidative stress in muscle-wasted COPD patients. *Chest* 2007;131:439-45.
- Nemoto K, Oh-Ishi S, Itoh M, Saito T, Ichihata T. Urinary 8-hydroxydeoxyguanosine is a potential indicator for estimating pulmonary rehabilitation-induced oxidative stress in COPD patients. *Tohoku J Exp Med* 2014;233:197-204.
- Rodriguez DA, Kalko S, Puig-Vilanova E, Perez-Olabarria M, Falciani F, Gea J, et al. Muscle and blood redox status after exercise training in severe COPD patients. *Free Radic Biol Med* 2012;52:88-94.
- Cruz-Jentoft AJ. Beta-hydroxy-beta-methyl butyrate (HMB): From experimental data to clinical evidence in sarcopenia. *Curr Protein Pept Sci* 2017.
- Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by β -hydroxy- β -methylbutyrate in cancer-induced muscle loss. *Cancer Res* 2005;65:277-83.
- Oliveira G, Oliveira C, Dona E, Palenque FJ, Porras N, Dorado A, et al. Oral supplement enriched in HMB combined with pulmonary rehabilitation improves body composition and health related quality of life in patients with bronchiectasis (Prospective, Randomised Study). *Clin Nutr* 2016;35:1015-22.
- Doña E, Oliveira C, Palenque FJ, Porras N, Dorado A, Martín-Valero R, et al. Pulmonary rehabilitation only versus with nutritional supplementation in patients with bronchiectasis: A randomized controlled trial. *J Cardiopulm Rehabil Prev* 2018;38:411-8.
- Doña E, Oliveira C, Palenque FJ, Dorado A, Martín-Valero R, Oliveira G. The effect of pulmonary rehabilitation associated with nutritional supplementation on physical activity in patients with bronchiectasis: Randomized trial. *Rev Esp Patol Torac* 2017;29:167-75.
- Arazi H, Taati B, Suzuki K. A review of the effects of leucine metabolite (β -hydroxy- β -methylbutyrate) supplementation and resistance training on inflammatory markers: A new approach to oxidative stress and cardiovascular risk factors. *Antioxidants* 2018;7.
- Hsieh L-C, Chien S-L, Huang M-S, Tseng H-F, Chang C-K. Anti-inflammatory and anticatabolic effects of short-term beta-hydroxy-beta-methylbutyrate supplementation on chronic obstructive pulmonary disease patients in intensive care unit. *Asia Pac J Clin Nutr* 2006;15:544-50.
- Clowes GHA, George BC, Villee CA, Saravis CA. Muscle Proteolysis Induced by a Circulating Peptide in Patients with Sepsis or Trauma. *N Engl J Med* 1983;308:545-52.
- Eley HL, Russell ST, Tisdale MJ. Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin II by β -hydroxy- β -methylbutyrate. *Am J Physiol-Endocrinol Metab* 2008;295.
- Craig CL, Marshal AL, Sjörström M, Bauman AE, Booth ML, Ainsworth BE, et al. International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Med Sci Sport Exerc* 2003;35:1381-95.
- Oliveira G, Oliveira C, Gaspar I, Porras N, Martín-Núñez G, Rubio E, et al. Fat-free mass depletion and inflammation in patients with bronchiectasis. *J Acad Nutr Diet* 2012;112:1999-2006.
- Marco E, Ramírez-Sarmiento AL, Coloma A, Sartor M, Comin-Colet J, Vila J, et al. High-intensity vs. sham inspiratory muscle training in patients with chronic heart failure: a prospective randomized trial. *Eur J Heart Fail* 2013;15:892-901.
- García-Fuentes E, Murri M, Garrido-Sanchez L, García-Serrano S, García-Almeida JM, Moreno-Santos I, et al. PPAR γ expression after a high-fat meal is associated with plasma superoxide dismutase activity in morbidly obese persons. *Obesity (Silver Spring)* 2010;18:952-8.
- Corp IBM. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY; 2011.
- Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. *Cytokine kinetics. Exerc Immunol Rev* 2002;8:6-48.

29. Bartlett DB, Shepherd SO, Wilson OJ, Adlan AM, Wagenmakers AJM, Shaw CS, et al. Neutrophil and Monocyte Bactericidal Responses to 10 Weeks of Low-Volume High-Intensity Interval or Moderate-Intensity Continuous Training in Sedentary Adults. *Oxid Med Cell Longev* 2017;2017:8148742.
30. Tenorio TRS, Balagopal PB, Andersen LB, Ritti-Dias RM, Hill JO, Lofrano-Prado MC, et al. Effect of Low- Versus High-Intensity Exercise Training on Biomarkers of Inflammation and Endothelial Dysfunction in Adolescents With Obesity: A 6-Month Randomized Exercise Intervention Study. *Pediatr Exerc Sci* 2017;1-10.
31. Nunes EA, Lomax AR, Noakes PS, Miles EA, Fernandes LC, Calder PC. beta-Hydroxy-beta-methylbutyrate modifies human peripheral blood mononuclear cell proliferation and cytokine production in vitro. *Nutrition* 2011;27:92-9.
32. Wojcik R, Malaczewska J, Siwicki AK, Micinski J, Zwierzchowski G. The effect of beta-hydroxy-beta-methylbutyrate (HMB) on the proliferative response of blood lymphocytes and the phagocytic activity of blood monocytes and granulocytes in calves. *Pol J Vet Sci* 2013;16:567-9.
33. van Helvoort HAC, van de Pol MHJ, Heijdra YF, Dekhuijzen PNR. Systemic inflammatory response to exhaustive exercise in patients with chronic obstructive pulmonary disease. *Respir Med* 2005;99:1555-67.
34. Xie J, Yang X-Y, Shi J-D, Deng X-Q, Long W. A new inflammation marker of chronic obstructive pulmonary disease-adiponectin. *World J Emerg Med* 2010;1:190-5.
35. Townsend JR, Fragala MS, Jajtner AR, Gonzalez AM, Wells AJ, Mangine GT, et al. beta-Hydroxy-beta-methylbutyrate (HMB)-free acid attenuates circulating TNF-alpha and TNFR1 expression postresistance exercise. *J Appl Physiol* 2013;115:1173-82.
36. Kraemer WJ, Hooper DR, Szivak TK, Kupchak BR, Dunn-Lewis C, Comstock BA, et al. The addition of beta-hydroxy-beta-methylbutyrate and isomaltulose to whey protein improves recovery from highly demanding resistance exercise. *J Am Coll Nutr* 2015;34:91-9.
37. Laviolette L, Lands LC, Dauletbaev N, Saey D, Milot J, Provencher S, et al. Combined effect of dietary supplementation with pressurized whey and exercise training in chronic obstructive pulmonary disease: a randomized, controlled, double-blind pilot study. *J Med Food* 2010;13:589-98.
38. Sugawara K, Takahashi H, Kashiwagura T, Yamada K, Yanagida S, Homma M, et al. Effect of anti-inflammatory supplementation with whey peptide and exercise therapy in patients with COPD. *Respir Med* 2012;106:1526-34.
39. Sugawara K, Takahashi H, Kasai C, Kiyokawa N, Watanabe T, Fujii S, et al. Effects of nutritional supplementation combined with low-intensity exercise in malnourished patients with COPD. *Respir Med* 2010;104:1883-9.
40. de Godoy I, Donahoe M, Calhoun WJ, Mancino J, Rogers RM. Elevated TNF-alpha production by peripheral blood monocytes of weight-losing COPD patients. *Am J Respir Crit Care Med* 1996;153:633-7.
41. Broekhuizen R, Wouters EFM, Creutzberg EC, Schols AMWJ. Raised CRP levels mark metabolic and functional impairment in advanced COPD. *Thorax* 2006;61:17-22.
42. Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Collins CE, Garg ML. Oxidative stress in cystic fibrosis: dietary and metabolic factors. *J Am Coll Nutr* 2001;20:157-65.
43. Couillard A, Maltais F, Saey D, Debigare R, Michaud A, Koechlin C, et al. Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003;167:1664-9.
44. Rabinovich RA, Ardite E, Troosters T, Carbo N, Alonso J, Gonzalez de Suso JM, et al. Reduced muscle redox capacity after endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164:1114-8.
45. Couillard A, Koechlin C, Cristol JP, Varray A, Prefaut C. Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients. *Eur Respir J* 2002;20:1123-9.
46. Nikolaidis MG, Kyparos A, Vrabas IS. F(2)-isoprostane formation, measurement and interpretation: the role of exercise. *Prog Lipid Res* 2011;50:89-103.
47. Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules* 2015;5:356-77.
48. Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. *Nutr Metab (Lond)* 2008;5:1.