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Supplementation with Qter® and Creatine improves functional performance in COPD patients on long term oxygen therapy

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ABSTRACT

Background: Skeletal muscle dysfunction and poor functional capacity are important extra-pulmonary manifestations of chronic obstructive pulmonary disease (COPD), especially in COPD patients on long-term O_2 therapy (LTOT). Beside the role of pulmonary rehabilitation, the effect of nutritional interventions is still controversial, and there are knowledge gaps on the effective role of nutraceutical supplementation on hard endpoints. The aim of this study was to investigate the effects of nutritional supplementation with Coenzyme Q10 (QTer®) – a powerful antioxidant with the potential to reduce oxidative stress and improve mitochondrial function – and Creatine on functional, nutritional, and metabolomic profile in COPD patients on long-term O_2 therapy.

Methods: One-hundred and eight patients with COPD from 9 Italian hospitals were enrolled in this double-blinded randomized placebo-controlled clinical study. At baseline and after 2 months of therapy, the patients underwent spirometry, 6-minute walk test (6MWT), bioelectrical impedance analysis, and activities of daily living questionnaire (ADL). Also, dyspnea scores and BODE index were calculated. At both time points, plasma concentration of CoQ10 and metabolomic profiling were measured.

Findings: Ninety patients, who randomly received supplementation with QTer® and Creatine or placebo, completed the study. Compared with placebo, supplemented patients showed improvements in 6MWT (51 ± 69 versus 15 ± 91 m, $p < 0.05$), body cell mass and phase angle, sodium/potassium ratio, dyspnea indices and ADL score. The CoQ10 plasma concentration increased in the supplementation group whereas it did not change in the placebo group. The metabolomics profile also differed between groups. Adverse events were similar in both groups.

Interpretation: These results show that in patients with COPD, dietary supplementation with CoQ10 and Creatine improves functional performance, body composition and perception of dyspnea. A systemic increase in some anti-inflammatory metabolites supports a pathobiological mechanism as a reason for these benefits. Further trials should help clarifying the role of QTer® and Creatine supplementation in patients with COPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD), an important cause of morbidity and mortality worldwide [1], is frequently associated with nutritional abnormalities and skeletal muscle dysfunction that contribute to exercise intolerance and poor health status [2]. The

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prevalence of malnutrition and micronutrient abnormalities [3] in COPD varies between 10 and 60% [4–10], being highest in patients with advanced COPD and/or chronic respiratory failure on long-term oxygen therapy (LTOT) [11]. Several mechanisms link malnutrition and COPD. Among these, systemic inflammation [12] seems to play a role in protein turnover [13,14] while the presence of hypoxemia in patients with COPD may increase the generation of inflammatory molecules [15] and worsen skeletal muscle dysfunction by reducing peripheral O_2 use [16]. Indeed, muscle biopsies from COPD patients have shown reduced mitochondrial density, lower oxidative enzyme activity and increased mitochondrial reactive oxygen species [17–19], with a shift in the proportion of muscle fiber type I to those of type II [20]. As a consequence, a lower content of high energetic phosphates has been documented in skeletal and respiratory muscles of these patients compared to healthy individuals [21,22]. Attempts have been made to reverse this state in malnourished COPD patients, using different anabolic medications [23–25] and hormones [26]. Although hormonal supplementation has led to increases in weight and muscle mass [10], there has been little if any improvement in functional capacity or health status [27]. The effect of nutritional supplementation is also controversial, although recent evidence provides evidence for some effect on malnourished patients [28].

Coenzyme Q10 (CoQ10) is a fat-soluble compound present in the mitochondria of cells. It is a member of the electron transport chain that participates in cellular respiration, helping generate energy in the form of adenosine three phosphate (ATP). Due to its limited solubility in water, CoQ10 has poor bioavailability and chemical instability [29]. One multicomposite variant of CoQ10 is the Qter®, which is more soluble in water, while retaining its antioxidant capacity [30]. Creatine is an organic acid that occurs naturally in vertebrates. It facilitates the recycling of ATP by recycling adenosine diphosphate (ADP) to ATP via donation of phosphate groups. Supplementation with QTer®, and Creatine has the potential to reduce oxidative stress and improve mitochondrial function [31], by improving protein turnover and mitochondrial energy production in patients with chronic heart failure [30]. Recently, a pilot randomized study of QTer® and Creatine supplementation in COPD showed that, compared with controls, COPD patients taking supplementation had beneficial effects on lean body mass and exercise tolerance [32].

Hereby, we tested the hypothesis that supplementation of QTer® and Creatine in COPD patients with chronic respiratory failure on LTOT would improve functional capacity and dyspnea, as well as body composition and metabolomics profile.

2. Methods

2.1. Participants

One-hundred and eight patients from 9 Italian hospitals were screened for this study between May 2014 and June 2015. A consort table is shown in supplemental Figure S1. Patients of both genders between 60 and 85 year-old, with a modified Medical Research Council (mMRC) score > 2, a forced expiratory volume in 1 s (FEV_1)/forced vital capacity < 0,7, FEV_1 < 70% predicted, and receiving supplemental LTOT were included. The patients were clinically stable, without exacerbations of COPD or hospitalizations in the 4 weeks prior to enrollment. All the patients were receiving pharmacological therapy consisting in the administration of bronchodilators in the different combinations (LABA, LAMA, LABA + LAMA, LABA + ICS, LABA + ICS + LAMA). The therapy was optimized in order to achieve an optimal control of the symptoms. The therapeutic scheme was not changed for the entire duration of the study in any of the study subjects. Patients were excluded if they were on mechanical ventilation, had uncontrolled diabetes mellitus, severe heart, renal, or hepatic failure and current or pre-existing malignant disease within the 3 years. Other exclusion criteria were: persistent infections, chronic oral steroid and/or immunosuppressive therapy, inability to complete the tests included, and use of statins or amino acid supplements.

Table 1
Main clinical variables collected during the study at the baseline.[a]

	Active N = 45	Placebo N = 45
Age (years)	73 ± 7	73 ± 7
Gender (M/F)	34/11	34/11
BMI (Kg/m^2)	32.1 ± 10.2	29.6 ± 8.4
FEV_1 (% pred)	55 ± 21	57 ± 19
FVC (% pred)	68 ± 20	67 ± 22
Fat-free mass (%)	68 ± 15	69 ± 11
Fat mass (%)	32 ± 15	31 ± 11
BCM (%)	24 ± 7	24 ± 8
Na/K	1.32 ± 0.39	1.30 ± 0.36
PhA (degrees)	4.56 ± 1.05	4.55 ± 1.26
6MWD (m)	214 ± 143	213 ± 134
SpO_2-pre test (%)	92 ± 3	92 ± 3
SpO_2-post test (%)	87 ± 5	85 ± 4
mMRC	2.07 ± 0.78	2.20 ± 0.63
BDI1	1.98 ± 0.85	1.82 ± 0.94
BDI2	1.91 ± 0.73	1.84 ± 0.60
BDI3	1.84 ± 0.67	1.98 ± 0.66
Borg scale	3.78 ± 1.76	3.36 ± 1.67
BODE index	4 (3; 6)	5 (3; 6)
ADL	5.36 ± 1.13	5.44 ± 1.16
CoQ10 (ng/mL)	413 ± 188	484 ± 333

Note: Data are presented as mean ± SD, except for BODE, presented as median (25°,75°).
AbbreviationsBMI = body mass index; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; BCM = body cell mass; PhA = phase angle; 6MWD = 6-minute walk test; SpO2 = arterial oxygen saturation; mMRC = modified Medical Research Council; BDI = Baseline Dyspnea Index; ADL = activities of daily living; CoQ10 = Coenzyme Q10.
[a] No significant difference between Active group and Placebo group at the baseline was observed in any of the variables.

2.2. Study design

In this multicenter double-blinded placebo-controlled study (EUFSC-13-001), one-hundred and six patients were randomized to 160 mg Coenzyme QTer® + 170 mg Creatine, or placebo twice daily for 2 months (8 weeks, Table 1 in the supplemental section). No additional nutritional supplementation was prescribed. Randomization schedule was generated using PROC PLAN statistical analysis software. The protocol was approved by the ethical committee of each hospital, and written informed consent was obtained from all patients. The protocol was notified to the Italian Ministry of Health according to guidelines on studies of food supplements [33].

Clinical evaluation and plasma samples were obtained at baseline (V1) and after two months of treatment (V2). At 6 and 12 months, patients were contacted via telephone and information about exacerbations, defined as a rapid change in clinical symptoms of such intensity to require the use of antibiotics and/or systemic steroids [34], or hospitalizations due to due to exacerbation of COPD, during the past year was obtained (shown in supplemental Material).

2.3. Primary endpoint and lung function

A 6-minute walk test (6MWT) with measurement of blood oxygen saturation (SpO2) was performed according to American Thoracic Society (ATS) standards [35]. Predicted values of the 6MWT were calculated using a reference equation [36] and the difference between predicted and measured values were recorded. Post-bronchodilator spirometry was performed following international guidelines [37].

2.4. Secondary outcomes

2.4.1. Body composition

Body mass index (BMI) was calculated as body weight/height [2]. Fat free mass (FFM) and body cell mass (BCM) were estimated using bioelectrical impedance analysis (BIA) (Akern 101, Italy). Fat mass (FM) was calculated as total body weight minus FFM. Phase angle (PhA) was directly obtained from BIA device at 50 kHz and considered as an indicator of extracellular/intracellular water ratio, BCM, and cellular integrity [38–46].

2.4.2. Dyspnea, BODE index and functional independence degree

Dyspnea was assessed using the mMRC scale [47], the Baseline Dyspnea Index scale (BDI), and the Transition Dyspnea Index scale (TDI) [48]. Exertional dyspnea was assessed using the Borg scale at the end of the 6MWT. The BODE index was calculated on the basis of BMI, mMRC, FEV_1 and 6MWT [49]. The daily activity performance was assessed by the Activities of Daily Living (ADL) questionnaire [50].

2.4.3. Blood samples and metabolomics data

A morning, fasting venous blood sample was collected at V1 and at V2, and plasma was stored at −80 °C until further analysis. Plasma concentration of CoQ10 was measured using Mass Spectrometry [51]. Plasma metabolomic profile was investigated in 88 subjects (43 placebo vs. 45 active) using a targeted quantitative approach combining direct flow injection and liquid chromatography tandem mass spectrometry assay. This strategy allowed simultaneous quantification of 186 metabolites. (See supplemental Table 1 and **Methods** for further details).

2.5. Statistical analysis

A complete case analysis was performed using Matlab 2016. As the trial measured the primary outcome at a single time point, patients who lost visit 2 were excluded from the final analysis.

Data are presented as mean ± standard deviation (SD) unless specified differently in table and figure captions. Clinical values were compared between the two groups by means of Student t-test at both time points V1 and V2. Similarly, the differences within the same treatment group between V2 and V1 time points were compared by using means of Student t-test. For the categorical data we performed the Fisher exact test. For the BODE index we used the Wilcoxon ranksum test and the Wilcoxon signed rank test for the unpaired and paired comparisons, respectively. Finally, for each metabolite, the time-trend variations between the two treatment groups in metabolites concentration (i.e. Δ = V2-V1) were compared by Student t-test. To overcome the problem of the large number of statistical comparisons, we computed also the false discovery rate (FDR).

The multivariate analyses are described in the Supplemental Material.

3. Results

The baseline characteristics of the 90 (45 Active vs. 45 Placebo) patients that completed the study are summarized in Table 1. No significant differences in the demographics or in any of the parameters measured were observed between the two groups.

3.1. Outcomes

3.1.1. Clinical outcomes

After treatment, the Active treatment group showed a statistically significant improvement of 51 m in the 6MWT (primary outcome) from 214 ± 143 to 265 ± 127 m ($P < 0.001$), with a significant increase in SpO_2 of 1.8 ± 3.3% after test. This increase was not observed in the Placebo group. The average increase in the placebo group was from 213 ± 134 to 228 ± 135 m; however, this increase was not significant ($P = 0.280$), (Table 2 and Fig. 1A). The majority of patients (91%) had a 6MWT below the predicted values at baseline. At 2 months, 29% of the patients in the Placebo group worsened their 6MWT compared with 11% in the Active group ($P < 0.05$) as shown in Fig. 2.

Table 2
Variations of the main clinical, nutritional and functional variables from baseline (V1) to the second month of therapy (V2). The asterisks indicate that more variables changed significantly in the Active compared with the Placebo group. There were absolute differences between groups in the 6MWT and the serum concentrations of CoQ10.

	V2-V1				
	Active N = 45		Placebo N = 45		P value
BMI (Kg/m^2)	−0.4 ± 3.0		−0.1 ± 1.2		0.447
FEV_1 (% pred)	1.0 ± 8.9		1.1 ± 8.6		0.976
FVC (% pred)	1.4 ± 8.4		0.9 ± 8.3		0.804
Fat-free mass (%)	1.1 ± 4.4		1.0 ± 4.4		0.927
Fat mass (%)	−1.1 ± 4.4		−1.0 ± 4.4		0.925
Body cell mass (%)	2.3 ± 6.6	*	0.8 ± 5.2		0.231
Na/K	−0.1 ± 0.3	*	−0.1 ± 0.2		0.052
Phase angle (degrees)	0.6 ± 1.6	*	0.0 ± 0.9		0.050
6MWT (m)	**51 ± 69**	*	**15 ± 91**		**0.038**
SpO_2-pre test (%)	0.6 ± 2.5		0.3 ± 2.8		0.633
SpO_2-post test (%)	1.8 ± 3.3	*	2.5 ± 3.4		0.315
mMRC	−0.4 ± 0.8	*	−0.3 ± 0.9	*	0.706
TDI1	0.3 ± 0.8	*	0.2 ± 0.9		0.374
TDI2	0.1 ± 0.8		0.1 ± 0.7		1.000
TDI3	0.1 ± 0.6		−0.1 ± 0.6		0.241
Borg scale	−0.4 ± 1.3	*	0.1 ± 1.3		0.060
BODE index	−1(-2,0)	*	−1(-2,0)	*	0.656
ADL	0.3 ± 0.6	*	0.1 ± 0.6		0.168
CoQ10 (ng/mL)	**164 ± 254**	*	**−31 ± 204**		**< 0.001**

Note: data are presented as mean ± SD, except for BODE index as median (25°,75°).
*significant variation from V1 to V2 ($p < 0.05$).
P values of the difference between active and placebo are reported.
Abbreviations: BMI = body mass index; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; BCM = body cell mass; PhA = phase angle; 6MWD = 6-minute walk test; SpO_2 = arterial oxygen saturation; mMRC = modified Medical Research Council; TDI = Transition Dyspnea Index; ADL = activities of daily living; CoQ10 = Coenzyme Q10.

There was a significant increase in the BCM (+2.27 kg; $P < 0.05$) and a decrease in Na/K (−0.12; $p < 0.01$) in the Active but not in the Placebo group (Table 2 and Fig. 1B and C). Furthermore, phase angle significantly increased by +0.55° in the Active but not in the Placebo group (Fig. 1D).

The mMRC score improved significantly after 2 months in both the Active ($P < 0.01$) and Placebo groups ($P < 0.05$). However, only the Active group showed a significant increase in the TDI scores ($p < 0.01$) and a significant decrease in the exertional dyspnea (lower score in Borg scale at the end of 6MWT, $P < 0.05$) after 2 months. There was a significant improvement in the functional independence index in the Active ($P < 0.01$), but not in the Placebo group.

At V2, the BODE index was significantly decreased in both groups ($P < 0.001$ and $P < 0.05$, respectively) without significant difference between the groups (Table 2).

As additional information, the percentage of patients reaching the minimal clinically important differences (MCID) has been reported in supplemental Table S2 and the differences between groups calculated.

3.1.2. Laboratory outcomes and metabolomics profile

After therapy, the plasma concentration of CoQ10 was significantly increased in the Active ($P < 0.001$), but not in the Placebo group.

The concentrations of all the analyzed metabolites for each subject at V1 and V2 are reported in the supplemental Table S2. These metabolites were mainly glycerophospholipids species, acetylcarnitines and aminoacids. Thirty-four metabolites at V1, and 51 metabolites at V2 were significantly different between the two groups. Fifteen out of 51

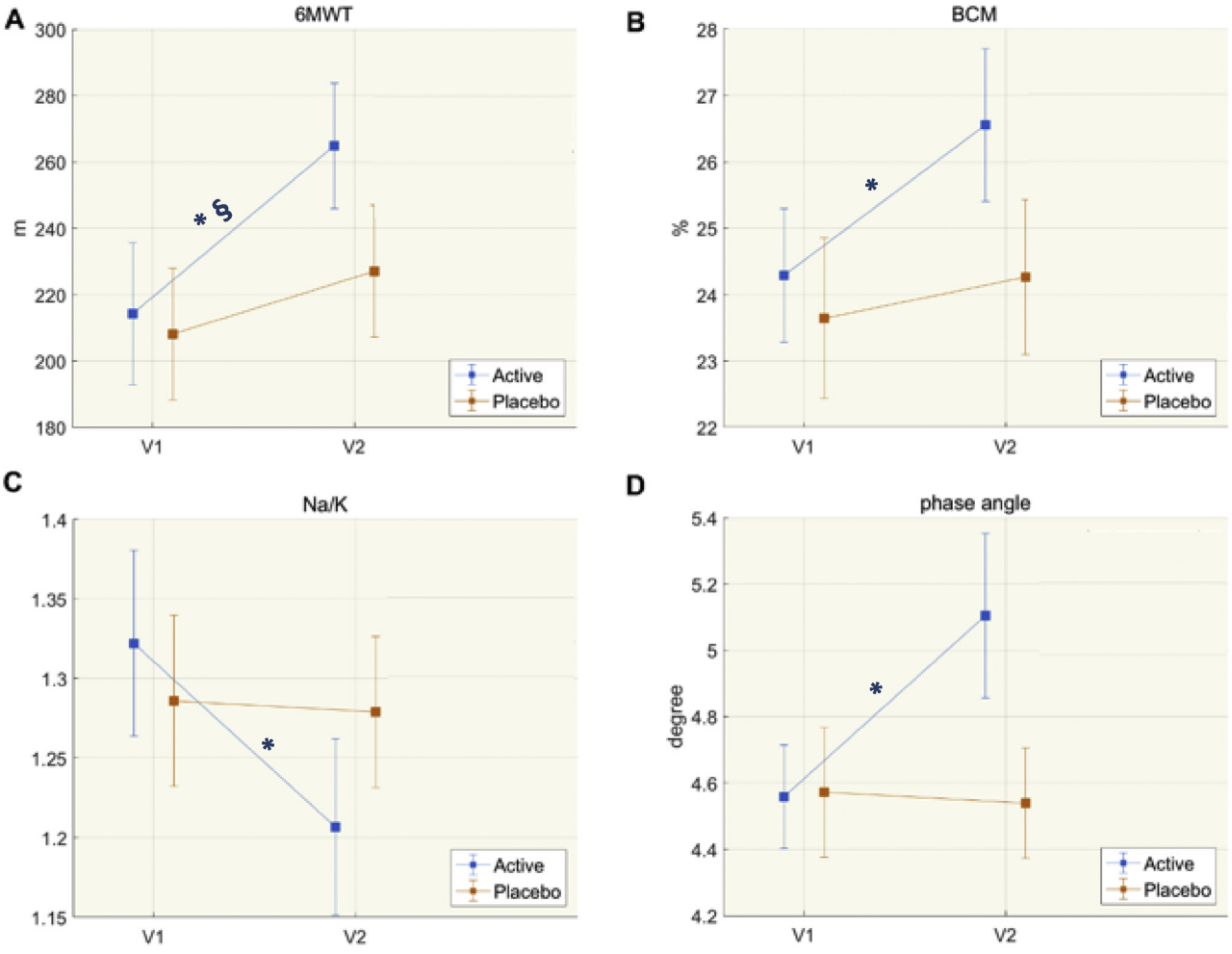

Fig. 1. Distribution of values in the two groups of 6MWT (A), BCM (B), Na/K (C) and phase angle (D) from V1 to V2. The data are expressed as mean ± standard error (SE).
Note: * significant variation from V1 to V2 ($p < 0.05$); § significant difference between active and placebo ($p < 0.05$).

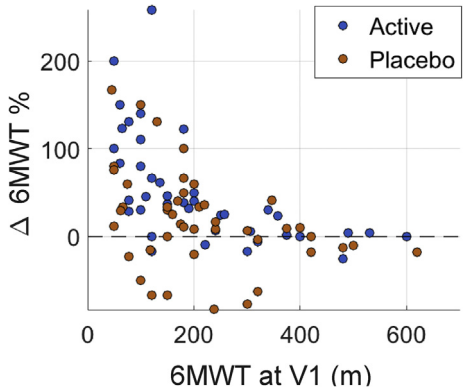

Fig. 2. Scatter plots showing the change (%) of 6MWT after two months treatment in both randomized (Active and Placebo) groups. A larger number of patients in the Placebo compared with the Active treated group decreased their walked distance in their second evaluation.

metabolites were the same species already different at V1 (supplemental table S3) and included one lysophosphatidylcholine species (lysoPC a C26:1), four diacylphosphatidylcholines (PCaa), three acyl-alkyl phosphatidylcholines (PCae), two long-chain sphingomyelins (SM), two short-chain acetylcarnitine (C3:1, C4:1) and three aminoacids such as asparagine, glycine and lysine All the FDRs were < 1.5.

In the Active group, 56 metabolites significantly changed from V1 to V2, whereas 53 metabolites significantly changed in the Placebo group (supplemental table S4). Again, these metabolites comprised mainly glycerophospolipids species (LysoPC, PCaa, PCae, SM), acetylcarnitines, aminoacids, and biogenic amines. Importantly, metabolites of the sphingomyelin class, that are involved in cell membrane damage (SM (OH) C16:1, SM C18:0) decreased in the Active group whereas they increased in the Placebo group. All the FDRs were < 0.1.

The multivariate analysis (supplemental Figure S3) shows that the two groups differ in their metabolomics profile. Using PLS components, we observed a clear separation between patients in the Active versus the Placebo group (AUC = 0.93, Fig. 3).

The supplemental Table S5 shows the metabolites VIP scores listed according to a descending order of magnitude.

3.1.3. Follow-up

During the 1 year of follow-up, there were no differences in number of subjects with one or more exacerbations or hospitalizations (**supplemental Kaplan Meier** Figures S4 and S5).

No side effects or complications associated with the nutritional supplementation have been reported.

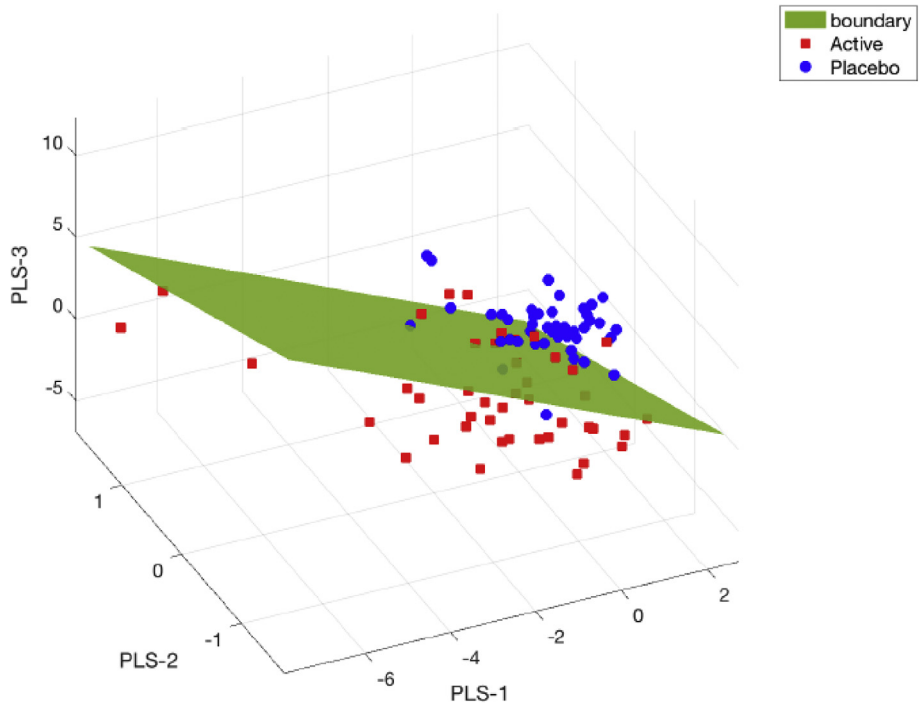

Fig. 3. The 3 partial least square (PLS) components of the metabolomics profile and the hyperplane which separates the two region of the two classes. The profile pattern separated the Active (red squares) and the Placebo group (blue circles).

4. Discussion

This randomized study shows that, in stable COPD patients on LTO_2, a 2-month oral supplementation of Coenzyme Q10 (Qter®) and Creatine resulted in a significant improvement in the exercise capacity as measured by the 6MWT. Supplementation also resulted in improvement in body composition, dyspnea and daily activities, and was associated with positive changes in the plasma metabolomic profile of the treated patients.

Skeletal muscle dysfunction, defined by weakness, reduced endurance, or greater fatigability [52], is a major extrapulmonary manifestation of COPD that results in the impairment of the patients' exercise capacity and quality of life. It reflects both structural and metabolic muscle alterations that are commonly seen in limb muscles in patients with COPD [53]: fiber atrophy, muscle fiber shift from type I to type II and mitochondrial alterations all contribute to loss in muscle oxidative capacity and endurance [54]. More specifically, reduced mitochondrial density, oxidative enzyme activity and increased mitochondrial reactive oxygen species production have been found in peripheral muscles from COPD patients [18,19].

Pulmonary rehabilitation, that includes aerobic exercise training, significantly improves functional capacity of COPD patients, with an average increase in the 6MWT of 44 m [55]. On the other hand, attempts have been made to improve skeletal muscle dysfunction using nutritional and hormonal supplementation with and without simultaneous pulmonary rehabilitation. One trial of testosterone with and without exercise training in COPD men with low testosterone levels, improved muscle mass and strength in the active treatment group compared with controls, but did not improve exercise capacity or health status [23]. These findings were similar to a placebo controlled study which tested human growth hormone supplementation. The growth hormone group had increase in lean body mass without change in overall weight, but showed no difference in the 6MWT [56].

The results from different forms of nutritional support are still controversial, but are more encouraging than those with hormones: a recent systematic review concluded that nutritional support could improve BMI, muscle mass, 6MWT and health status in malnourished COPD patients, with less positive, but still significant results in normally nourished patients [28]. Furthermore, specific nutritional mixture have been found to effectively bypass electron transport chain defects (providing alternative energy sources), reduce oxidative stress and prevent mitochondrial functional decline [57]. Among these specific compounds, CoQ10 and Creatine have been found to have synergic positive effects on oxidative stress and mitochondrial function [31].

CoQ10 or ubiquinone, is an essential cofactor in mitochondrial oxidative phosphorylation, acting as a mobile electron carrier in the electron transport chain, and participates in the synthesis of ATP. It exerts three main biological roles in humans: contributes to mitochondrial energy production, stabilizes the cell membrane and, in its reduced ubiquinol form, has an antioxidant effect [58]. Protective effects of CoQ10 against oxidative stress and mitochondrial dysfunction have been demonstrated in vitro and in vivo models [59,60]. CoQ10 is normally synthesized endogenously, but it is frequently reduced in the elderly and in chronic diseases, such as COPD [61]. However, its use in humans as a therapeutic agent is not satisfactory, most likely because of its poor solubility in water resulting in poor oral bioavailability. A mechano-physical procedure called terclatration was developed in order to render CoQ10 highly water soluble without chemical modification of the moiety. The resultant multicomposite QTer®, compared with native CoQ10, is about 200 fold more soluble in water, while retaining its antioxidant capacity [62]. As a proof of the great bioavailability and chemical stability of QTer®, the present study demonstrates that patients receiving this compound had increased levels of plasma concentration of CoQ10 over time compared to the placebo patients. Creatine is a guanidine compound whose main role is to maintain high levels of intracellular adenosine triphosphate (ATP) via donation of phosphate groups [63]. It has also been shown that Creatine acts also as antioxidant against reactive oxygen species (e.g. superoxide anions and peroxynitrite) [64], enhances the expression of myogenic regulatory factors that regulate myosin heavy chain expression [65], stimulates protein synthesis and reduces protein degradation [66]. Previous papers demonstrated a clinically protective effect of the association of CoQ10 and Creatine in chronic heart failure [30] and in a small sample of COPD patients (pilot study) [32]. However, the plasma concentration

of CoQ10 nor metabolomics profile were not measured in those studies.

4.1. Effects on the primary end-point

After two months of treatment with QTer® and Creatine, a significant improvement of 6MWD was observed only in the Active treatment group. The 51 m increase in the 6MWT in the Active therapy group compared with placebo group was not only statistically significant, but was also higher than the 25 m, which is also considered to be clinically significant [67]. Nonetheless, the significant increase compared with the 15 m observed in the Placebo group (Fig. 1, Panel A) supports a beneficial effect of the therapy. Importantly, the most significant improvement occurred in patients who had very low baseline performance (i.e. 6MWD < 200 m or values 20% below predicted values) as shown in Fig. 2.

4.2. Effects on the secondary clinical end-points

Significant improvements in the baseline dyspnea index and the Borg scores were observed in the Active, but not in the Placebo group. A statistically significant improvement in the ADL score was seen after 2 months of active treatment. Since the ADL index reflects people's daily self-care activities, which is directly linked to their ability to live independently, a numerically small improvement could be important for the quality of life of the patient. The Active treatment group also had improvement in the markers of body composition. The body cell mass, which represents the total mass of all the cellular elements of the metabolically active components of the body, increased significantly by 2.27%, while it did not change in the placebo group (Fig. 1, Panel B). BCM provides a good estimate of skeletal muscle mass [68,69] or FFM [70]. Actually, repeated measurements of BCM are more informative than those of FFM [71], and represents a biomarker of the nutritional status in COPD and acute respiratory failure [72]. The improvement in phase angle is also noteworthy as PhA is thought to be an indicator of water distribution (extracellular/intracellular water ratio), BCM and cellular integrity [38,42], and has been shown to be independently associated with measures of physical function and disease severity in COPD [40,43,73]. Taken together, the clinical results are in line with previous studies, that reported a clinically protective effect of the association of Qter® and Creatine in chronic heart failure [30] and in a pilot study of COPD patients [32].

4.3. Changes in metabolomics profile

The study of metabolomics has received increasing interest, as it represents the result of gene and protein function and activity. As most drugs affect components of metabolism [74], the study of metabolomics provides a sensitive readout of drug response phenotypes. In this study, we used a targeted mass spectrometry-based quantitative metabolomic approach, focusing on glycerophospholipids, aminoacids, biogenic amines and acylcarnitines, as some of them have already been identified as part of key biochemical pathways in COPD [75]. The metabolomics profile differed significantly between the two groups. The patients who received QTer® and Creatine showed increased levels of lysophosphatidylcholine (LysoPC) species, together with a decrease in phosphatidylcholine (PC) species compared with the placebo group. LysoPC [76] act as uncompetitive product inhibitors of plasma secretory phospholipases A2 (sPLA2s), thus providing a feedback mechanism for the anti-inflammatory effects of these compounds [77]. The increase in circulating LysoPC may simply reflect their reduced conversion to lysophosphatidic acid (LPA), which is known to induce a multitude of cellular responses through its action on immunological relevant cells [78]. Thus, elevation of plasma LysoPC can actually help relieve inflammatory conditions. Interestingly LysoPC are reported to impair nitric oxide (NO) production and endothelium-dependent vasorelaxation [79] suggesting an important role of these metabolites in endothelial homeostasis [80].

The Qter®/Creatine-treated patients also showed a decrease in sphingomyelins (SM) species as shown by the multivariate classification modeling. Sphingolipids have been implicated in several lung diseases including COPD [81–83], and increases in plasma sphingomyelins have been associated with more rapid progression of emphysema [75]. Although it is difficult to know how much of the circulating sphingomyelins reflects cellular events occurring into the lung, we can hypothesize that in the Qter®/Creatine-treated patients, the lower plasma sphingomyelins reflect a lower burden of circulating shed cellular membranes (e.g. microparticles, apoptotic bodies), which has been linked to enhanced lung destruction [84].

Increased level of short-chain acylcarnitine species and decreased long-chain acylcarnitines have been found in the Qter®/Creatine treated patients, although the trend in variation over time did not significantly changed between the Active and Placebo groups. Nevertheless, since acylcarnitines are markers of the beta oxidation of fatty acids by the mithocondria, we can hypothesize that changes in circulating acylcarnitine species found in the Qter®/Creatine-treated patients indicate a metabolic switch involving a different rate of mitochondrial beta-oxidation that positively interfere with energy production by ameliorating the mitochondrial functions.

4.4. Strengths and limitations of the study

This is the first study that proves an evident clinical effect of nutraceutical supplementation in terms of hard endpoint, i.e. 6MWT. Furthermore, the multicenter, prospective, randomized, double-blind design of the study ensures impartiality and avoids biases. The study used hard patient-centered outcomes, the improvement of which are supportive not only of a statistical, but importantly of a clinical effect of the supplementation. However, there were several limitations. First, two months of supplementation may be too short to have a significant impact on a chronic disease such as COPD. This may explain the lack of change in the BODE index, exacerbations rates or hospitalizations over the one year follow-up. However, the positive results in PRO's obtained in the 2 month-time frame were important. Secondly, only patients on chronic oxygen therapy were included, and therefore the results cannot be extrapolated to patients who are not on this therapy. Thirdly, no significant relation between 6MWT and plasma concentration of CoQ10 was observed, likely because the plasma levels of CoQ10 are known to poorly reflect the CoQ10 tissue levels and primarily reflect the actual intake of the medication [85]. Finally, we used a targeted metabolomics strategy focused on specific classes of metabolites, thus not representing the full picture of the metabolic changes potentially induced by the CoQ10-Creatine supplementation, thereby limiting its practical implications.

4.5. Conclusions

This study supports the hypothesis that supplementation with Coenzyme Q10 terclatrate (Qter®) and Creatine can improve functional capacity, body composition, dyspnea and daily activities in COPD patients receiving supplemental LTOT. The increases in some anti-inflammatory metabolites after treatment suggest a beneficial effect induced by the active supplementation. Overall, these findings point towards an improvement in functional performances, body composition and perception of well-being and a plausible decrease in cell injury driven by the supplementation with CoQ10 and Creatine. Further studies should be conducted to confirm these findings.

Author contributions

Conception and design of the work: FDB and SM. Data collection: *AIMAR Study Group*, RP, LB. Data analysis and interpretation: FDB, RP, MF, LB, EFMW, BRC. Drafting the article: FdB, RP, MF, FDB, BRC, FP.

Critical revision of the article: EFMW, FP, BRC. Final approval of the version to be published: all the listed authors.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.rmed.2018.08.002.

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