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Addition of vitamin B12 to exercise training improves cycle ergometer endurance in advanced COPD patients: A randomized and controlled study

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ABSTRACT

Vitamin B12 is essential in the homocysteine, mitochondrial, muscle and hematopoietic metabolisms, and its effects on exercise tolerance and kinetics adjustments of oxygen consumption ($V'O_{2p}$) in rest-to-exercise transition in COPD patients are unknown. This randomized, double-blind, controlled study aimed to verify a possible interaction between vitamin B12 supplementation and these outcomes. After recruiting 69 patients, 35 subjects with moderate-to-severe COPD were eligible and 32 patients concluded the study, divided into four groups (n = 8 for each group): 1. rehabilitation group; 2. rehabilitation *plus* B12 group; 3. B12 group; and 4. placebo group. The primary endpoint was cycle ergometry endurance before and after 8 weeks and the secondary endpoints were oxygen uptake kinetics parameters (time constant). The prevalence of vitamin B12 deficiency was high (34.4%) and there was a statistically significant interaction ($p < 0.05$), favoring a global effect of supplementation on exercise tolerance in the supplemented groups compared to the non-supplemented groups, even after adjusting for confounding variables ($p < 0.05$). The same was not found for the kinetics adjustment variables ($\tau V'O_{2p}$ and $_{MRT}V'O_{2p}$, $p > 0.05$ for both). Supplementation with vitamin B12 appears to lead to discrete positive effects on exercise tolerance in groups of subjects with more advanced COPD and further studies are needed to establish indications for long-term supplementation.

1. Introduction

Micronutrients are essential for life and many elderly people are at risk of presenting deficiency of several components, including vitamin B12 (cobalamin). Chronic disease increases this risk [1]. COPD patients, whose mortality will reach among the highest levels in 2020, have lower micronutrient and vitamin B12 levels than healthy controls [2,3]. The presence of absolute or relative deficiency of vitamin B12 is associated with hyperhomocysteinemia and COPD patients have higher homocysteine levels compared to aged matched controls [3–5], which is in turn related to a decline in lung function in this population [6]. Whether or not an epiphenomenon, there is evidence of a relationship between hyperhomocysteinemia, low blood levels of vitamin B12 and endothelial dysfunction [7,8], mitochondrial dysfunction and muscular weakness [9] and altered physical and neuromuscular performance [10–12].

As well as pulmonary rehabilitation, nutritional assessment and the role of micronutrients or supplements is a component recommended as a priority in research related to COPD [13]. Thus, there are several studies demonstrating a relationship between physical performance, with or without pulmonary rehabilitation, with vitamin D status [14,15], iron deficiency without anemia [16] or with anemia [17], on the physical capacity of patients with COPD. Vitamin B12 also has an important role as an antioxidant [18] and cobalt, its mineral constituent, plays a role in the regulation of

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tissue hypoxic conditioning through hypoxia-inducible factor (HIF-1α) [19,20], HIF-1α expression being one important molecular way to regulate adaptation responses to high-intensity training [21].

Thus, we hypothesized in this study that supplementation could improve cycle ergometer endurance (primary outcome) and/or could positively affect the kinetics adjustment of oxygen consumption on rest-to-exercise transitions (secondary outcome), due to the pleiotropic actions of vitamin B12 on cardiovascular, neuromuscular and hemorheological axes, all physiologically related to exercise.

2. Material and methods

2.1. Study design and subjects

In this randomized, double-blind, controlled study in a specialist COPD tertiary clinic, 69 patients were initially invited to participate. After exclusions for various reasons (Fig. 1), 35 patients were randomized by simple drawing on an Excel® program to four groups: Ex + S, received pulmonary rehabilitation and vitamin B12 supplementation, Ex + P, received pulmonary rehabilitation and placebo only, S or received oral supplementation with vitamin B12 and conventional medical treatment without pulmonary rehabilitation and P, or placebo, received conventional treatment and oral placebo substance (maltodextrin). The dose of vitamin B12 was 500 mg daily for 8 weeks.

Among the inclusion criteria were a diagnosis of COPD through the Global Obstructive Lung Disease Initiative (GOLD) with FEV_1 < 60% predicted for the Brazilian population, being exacerbation-free in the previous 60 days and under respiratory medication optimization, not presenting any known comorbidities except controlled systemic arterial hypertension (SAH) and presenting physical and cognitive ability to perform functional and exercise testing. The exclusion criteria included patients with ACOS (Asthma-COPD overlap syndrome) and left heart failure or cor pulmonale by clinical criteria. The participants were also required to participate in at least 60% of the rehabilitation program. The study was in accordance with the Helsinki's statements and registered with the Brazilian Clinical Trial Registry under number 123456/2014.

2.2. Questionnaires

To quantify the weekly intake intensity of food sources rich in vitamin B12 a recall questionnaire was used, adapted to the Brazilian population, with a score based on the composition of vitamin B12 in each food-source [22]. To describe the general condition of the disease we used the Clinical COPD Questionnaire (CCQ).

2.3. Training protocol

The Ex + S and Ex + P groups performed aerobic and resistance

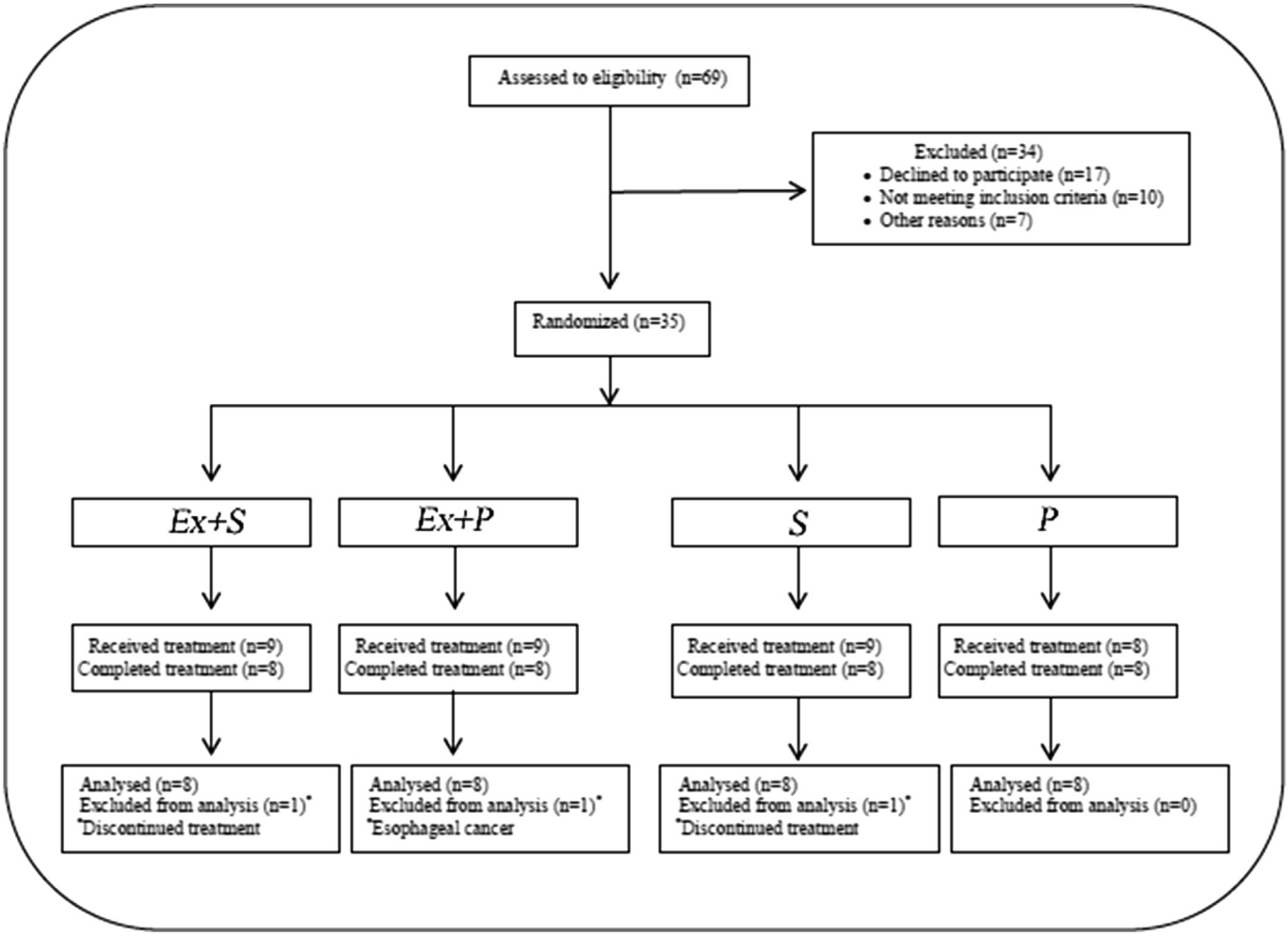

Fig. 1. Flowchart of the study.

training for 8 weeks, generally three times a week for 40 min each session. The initial power on the electromagnetic cycle ergometer was individualized, starting at 70% of the maximum load in the incremental CPET, with an average increase of 5 Watts every 5 days, with monitoring of individual tolerance, always under the close supervision of an experienced professional. Flexion and extension exercises of the large muscle groups with 1–2 kg and dumbbell weights were routinely carried out in both groups, with three sets of 12 repetitions and individual increments.

2.4. Lung function

All patients underwent pre and post bronchodilator spirometry and carbon monoxide diffusing capacity, tested according to the criteria of the ERS/ATS [23] and referenced to predicted values for the Brazilian population [24,25] using the pulmonary function system Vmax229 (Viasys, YorbaLinda, CA, USA, 2011).

2.5. Cardiopulmonary exercise testing (CPET)

CPET was performed on different days as both incremental or constant-load protocols, the first primarily to characterize the maximum tolerable exercise response and the second in duplicate with 30 min intervals to determine the endurance and kinetics of oxygen consumption. With power increased by 5 Watts ($FEV_1 < 1$ L) or 10 Watts ($FEV_1 \geq 1$ L) in a ramp protocol to maximum tolerance, breath-by-breath oxygen consumption ($V'O_{2p}$), exhaled carbon dioxide ($V'CO_2$), minute volume ($V'E$) and their respiratory rate (f_R) and tidal volume ($V'T$) components were measured by a metabolic system Vmax *Encore* 29 (Viasys, Yorba Linda, CA, USA, 2011), calibrated at two moments with high precision gases (GAMA GASES, São Paulo, Brazil). Heart rate (HR) and arrhythmia were monitored using an ECG system (Cardiosoft®, USA, 2012), integrated into the metabolic system and programmed to control the electromagnetic brake cycle ergometer Vsprint 200 (Viasys, Yorba Linda, CA, USA, 2011). Continuous peripheral digital oximetry monitoring (SpO_2) was performed by a DIXTAL DX2010 system (DIXTAL, Manaus, Brazil, 2010) and blood gas analysis at the beginning and end of the incremental test was analyzed in a COBAS B21® System (Roche, Portugal, 2011).

The constant power tests at 70–80% of maximum power in the incremental test were performed before and after the rehabilitation program. The first was to the maximum limit of tolerance (T_{lim}) and the second for 4 min, both in a calm and temperature controlled (22 ± 1 °C) laboratory atmosphere. Both were preceded by 3 min of rest for collecting gas exchange and hemodynamic parameters (baseline), followed by abrupt pedaling, starting at 50 cycles/min.

2.6. Blood analysis

On the first visit, blood was collected to measure hemoglobin concentration, creatinine, and baseline concentrations of vitamin B12, among others. Vitamin B12 was measured before and after rehabilitation through human plasma analysis using an Elecsys2010 system, Modular Analytics E170 (Roche, Portugal) through Electrochemiluminescence Immunoassay (ECLIA)®.

2.7. Data exploration and statistics

All data collected in the cardiometabolic system were exported to an Excel spreadsheet® for data processing and analysis. Peak O_2 consumption ($V'O_{2peak}$) was defined as the highest $V'O_2$ near the end of exercise, in the case of the ramp protocol. The OUES (*oxygen uptake efficiency slope*) was calculated as the slope of the linear regression between $V'O_{2p}$ and log ($V'E$), equivalent to the regression coefficient "a" in the equation:

$$V'O_{2P} = a^{*}\log V'E + b$$

In order to calculate $V'O_2$ kinetics, breath-by-breath data were linearly interpolated second-to-second, and the two tests were matched in time for average second-by-second to reduce the noise. After this, the data were analyzed by the nonlinear least-square regression method, with 400 iterations, including time delay, in a mono-exponential model, including the first 180 s of exercise and excluding the first 30 s (cardiopulmonary or phase I), which does not itself represent a muscle $V'O_2$ phase associated with exercise [26,27], or any possible slow component ("excess $V'O_2$") [28], according to the equation below,

$$V'O_{2(t)} = V'O_{2(b)} + A^{*}(1 - \exp^{-(t-\delta)/\tau})$$

where t = time (s), b = baseline $V'O_2$, τ = time constant *tau* and δ = delay time (s), where t is the time required to reach 63% of average $V'O_2$ value reached at the steady state. The baseline (rest) period was considered the average $V'O_2$ of the final 20 s before starting the exercise and the kinetic analysis was performed using a non-linear least square regression in the statistical program GraphPad Prism (GraphPad Software 5.0, San Diego, CA).

Based on a clinically important expected average difference of 105 s and a standard deviation average difference of 262 s for the primary outcome (T_{lim}) [29], the sample size calculation, including two between factors (Rehabilitation and Vitamin B12 supplementation) and one within factor (T_{lim}), for repeated measures ANOVA statistical analysis of 8 individuals in each group (n = 32), resulted in a high power (0.98) for a fixed F Geisser-Greenhouse test at a significance level of 1% (PASS11. NCSS, LLC, Kaysville, Utah, USA). Results are presented as mean ± standard deviation (SD). After the Shapiro-Wilk statistics to determine the sample distribution, some variables were log-transformed for parametric analysis. Mann-Whitney rank sum tests were used for intra-weekly assessment to compare the evolution of the load between the Ex + P and Ex + S groups. Chi-square tests and one-way ANOVA were used for comparison between the four groups, in addition to three way repeated measures ANOVA obtained by the General Linear Model using SPSS 20.0 (SPSS, Chicago, Illinois, USA) and sphericity analysis with the Greenhouse-Geyser correction. In this model we included analysis of covariance for three variables that demonstrated significant between-group differences at baseline or close to significance with a potential confounding role. The level of significance was set at 5%.

3. Results

3.1. Baseline characteristics

Fig. 1 presents the flowchart of the study, with the representative number distributed in each group and a dropout rate of 8.5%, one due to a cancer diagnosis and two patients who dropped out. The main baseline characteristics of the subjects are shown in Table 1. The population consisted of predominantly GOLD III/IV patients and the groups were reasonably homogeneous, with a significant difference between groups for the initial T_{lim} ($p = 0.041$).

3.2. Vitamin B12 status

The prevalence of vitamin B12 serum deficiency ([cobalamin] <300 pg/mL) was 34.4%, with no statistical difference when comparing the groups, with a range of 30–905 pg/mL. There was a significant difference after supplementation in the Ex + S group, $\Delta B12 = 182 \pm 206$ pg/mL ($p < 0.05$). Group S presented

Table 1
Selected baseline clinical and physiological characteristic of the 4 groups of COPD patients.

	Ex + S	Ex + P	S	P	*p*-value
Subjects, n	8	8	8	8	1.000
Antropometry					
Age (years)	56.5 ± 5.0	65.2 ± 6,0	63.4 ± 5.2	58.1 ± 10.3	0.156
Gender (M/F)	3/5	3/5	5/3	5/3	0.289
Weigth (kg)	63.7 ± 13.0	64.8 ± 21.0	58.6 ± 7.0	74.0 ± 16.2	0.268
BMI (kg/m^2)	24.5 ± 2.9	25.1 ± 6.0	24.5 ± 4.0	28.3 ± 5.5	0.332
Clinical Rating					
CCQ, total	2.0 ± 0.7	2.5 ± 0.8	2.4 ± 1.1	1.9 ± 1.0	0.505
Smoking, p-y	71.0 ± 44.0	71.2 ± 46.7	50.8 ± 9.0	42.5 ± 11.0	0.218
GOLD stages					
II (n)	1	0	0	2	0.570
III (n)	4	7	6	5	
IV (n)	3	1	2	1	
Lung function					
FVC, % pred	65.2 ± 16.0	72.2 ± 10.0	66.0 ± 13.0	73.0 ± 13.1	0.534
FEV_1, % pred	34.0 ± 11.0	39.2 ± 6.8	32.8 ± 7.6	41.7 ± 12.3	0.235
FEV_1/FVC, %	40.7 ± 10.4	43.5 ± 9.2	41.2 ± 11.4	44.9 ± 6.8	0.695
DLco, % pred	54.4 ± 22.1	48.5 ± 24.2	54.9 ± 16.2	58.6 ± 18.9	0.683
PaO_2, mmHg	66.2 ± 9.3	73.8 ± 9.1	76.4 ± 16.0	75.5 ± 7.6	0.348
CPET data					
$\dot{V}O_{2peak}$, % pred	50.5 ± 16.1	65.1 ± 17.7	68.8 ± 18.2	64.7 ± 18.2	0.191
W_{peak}, % pred	43.6 ± 12.4	46.8 ± 13.1	34.7 ± 12.0	43.1 ± 15.2	0.324
OUES, mL	1823 ± 596	1542 ± 582	1353 ± 367	1936 ± 505	0.141
T_{lim}, s	410 ± 311	314 ± 230	259 ± 110*	436 ± 143	**0.041**
τ, s	65 ± 37	84 ± 39	69 ± 22	69 ± 25	0.617
TD, s	18 ± 10	8 ± 12	20 ± 5	13 ± 9	0.114
MRT, s	83 ± 47	92 ± 31	89 ± 23	82 ± 18	0.929
Medication n(%)					
LABA	8	8	8	8	0.932
LAMA	2	4	2	1	
SABA	1	1	0	1	
IC	8	8	8	8	
Antihipertensive	1	3	2	3	
Blood sample data					
Vitamin B12 (initial), pg/mL	385 ± 207	454 ± 309	451 ± 215	467 ± 114	0.877
Vitamin B12 (final), pg/mL	567 ± 227§	358 ± 177	544 ± 145	442 ± 204	0.148
ΔVitamin B12, pg/mL	182 ± 206	−71 ± 175	93 ± 262	−16 ± 103	0.060
Vitamin B12 < 300 pg/mL, n	3	4	3	1	0.340
Hematocrit, %	47 ± 3	46 ± 4	43 ± 5	43 ± 3	0.118
Haemoglobin, g %	15 ± 1	15 ± 1	14 ± 2	14 ± 1	0.102

Abbreviations: BMI, body mass index; CCQ, clinical COPD questionnaire; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; DLco, carbon monoxide diffusion capacity; PaO2, partial Pressure of oxygen in arterial blood; $\dot{V}O_{2peak}$, peak oxygen consumption; OUES, *oxygen uptake effici-ency slope*; T_{lim}, maximal tolerance exercise time; τ, time constant (*tau*); TD, time delay; MRT, mean res-ponse time; LABA, long action beta-mimetic agonist; LAMA, long action muscarinic antagonist; SABA, short action beta-mimetic agonist; IC, inspiratory capacity.

ΔB12 = 93 ± 262 pg/mL ($p > 0.05$). The other groups demonstrated a fall in serum levels after 8 weeks (Table 1). The dietary recall questionnaire for vitamin B12 sources presented scores of 11.8 (Ex + S), 13.1 (Ex + P) 11.0 (S), and 11.9 (P) ($p > 0.05$), showing that there were no significant differences in the average intake of vitamin B12 between the groups. Only one case of mild anemia was found for a range of [Hb] between 11.1 and 17.5 g/dL.

3.3. Main exercise outcomes

As for the primary and secondary outcomes (Table 2), there was an overall trend for improved aerobic performance on the cycle ergometer for T_{lim} in the supplemented groups compared to the non-supplemented groups, with a significant interaction in the ANOVA calculation ($p = 0.044$), which remained significant even after inclusion of confounding variables (baseline T_{lim}, $\dot{V}O_{2p}$ % predicted and [Hb]) in the covariance model studied ($p = 0.045$). The same tendency was not found for the kinetics $\tau\dot{V}O_{2p}$ or $MRT\dot{V}O_{2p}$ adjustment variables with vitamin B12 supplementation, but only a main effect of exercise on $\tau\dot{V}O_{2p}$ ($p = 0.021$). When separating individually the responses to T_{lim} for those who reached or not the minimum clinically important response of 33% for T_{lim} after the intervention [29], we found that globally the supplemented groups performed slightly better (Fig. 2).

3.4. Training consistency

Fig. 3 shows the evolution of training over 8 weeks, with no significant difference in weekly load increment between the two groups ($p > 0.05$ comparing week-to -week between groups).

4. Discussion

This study confirmed the high prevalence of vitamin B12 deficiency among COPD patients and a significant positive overall effect, although slight, on endurance through supplementation with cobalamin. There was no significant effect on the $\dot{V}O_{2p}$ kinetics adjustments in rest-to-exercise transition with supplementation; however we confirmed the positive effects of physical training on acceleration of Phase II oxygen uptake on-kinetics.

Normal cobalamin levels do not preclude functional impairment, which may be present in a large part of populations at risk, especially where there is oxidative stress or associated systemic low-grade inflammation [30,31]. The presence of these factors in

Table 2
Primary and secondary outcomes (Pre and Post rehabilitation) for the groups and adjusted and non-adjusted respective *p*-value.

		Groups				*p*-value
		Ex + S (n = 8)	Ex + P (n = 8)	S (n = 8)	P (n = 8)	(T_{Lim}xSxEx; T_{Lim}xEx; T_{lim}xS;) (τxSxEx; τxEx; τxS) (MRTxSxEx; MRTxEx; MRTxS)
T_{lim}, s	Pre	410 ± 311	314 ± 230	259 ± 110*	436 ± 143	**0.044; <0.001**; 0.642
	Post	690 ± 481	690 ± 495	312 ± 208	353 ± 148	**0.045****; **0.001****; 0.907**
	p	**0.006**	**0.022**	0.234	0.106	
τ, s	Pre	65 ± 37	84 ± 39	69 ± 22	69 ± 25	0.287; **0.021**; 0.950
	Post	55 ± 84	51 ± 97	67 ± 60	85 ± 35	
	p	0.312	0.073	0.905	0.161	
MRT,s	Pre	82 ± 44	92 ± 31	89 ± 23	82 ± 18	0,116; 0,208; 0,343
	Post	83 ± 20	70 ± 11	89 ± 15	95 ± 35	
	p	0.983	0.088	0.942	0.211	

Abbreviatures: T_{lim} = Total time of exercise tolerance; τ = constant time *tau*; MRT = mean response time; Ex = Rehabilitation(+); S = supplementation with Vitamin B12(+); P = Placebo (+).
* = S group significant lower than P group to T_{Lim} (s) in the pre-rehabilitation stage.
** = *p*-values after adjustment of T_{Lim} (s) to potential confounders VO_{2peak} (% predicted), baseline T_{lim} (s) and initial blood [Hb].

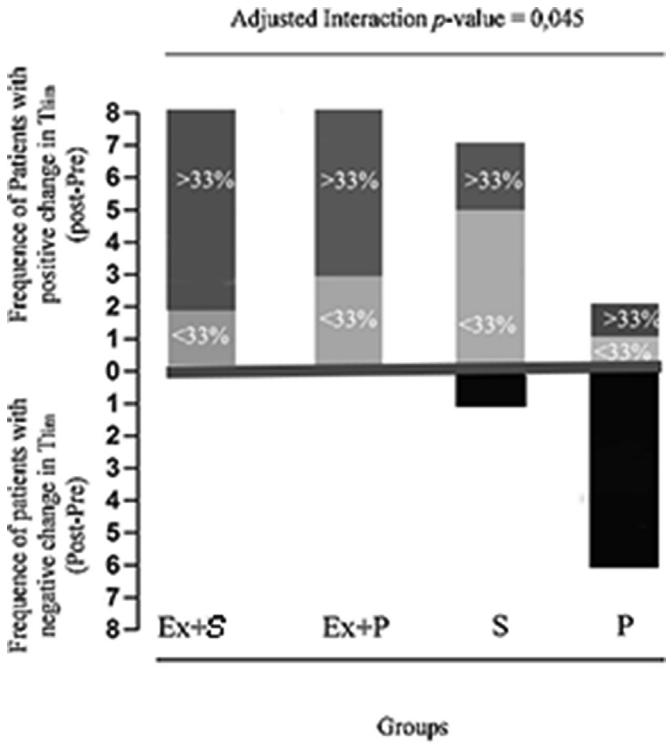

Fig. 2. Number of patients with clinically important ΔT_{lim} and number of patients with negative ΔT_{lim} after 8 weeks by group.

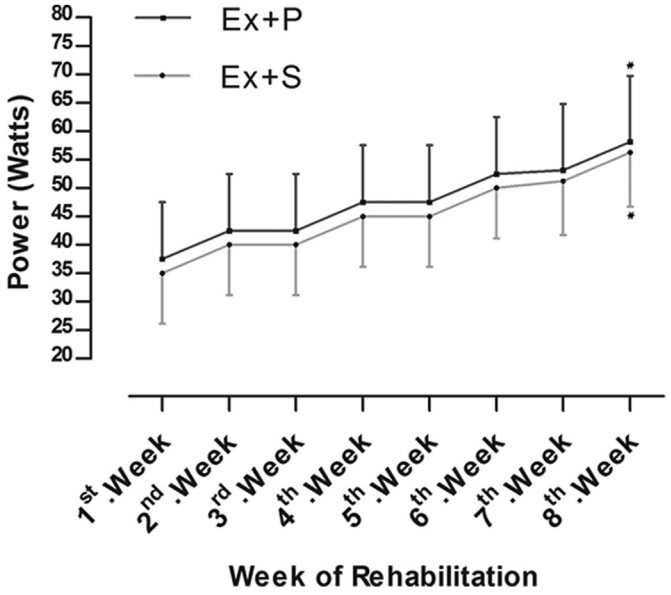

Fig. 3. Evolution of average power (Watts) increase during the rehabilitation program. Figure 3 (footnote): * = $p < 0.001$ comparing 8th. Week to 1st. Week.

COPD and the much higher prevalence of cobalamin deficiency found in this study (34.4%) compared to that in the elderly people in Brazil (18.7%) [32], for the same "low-normal" criteria (<300 pg/mL), suggests a higher risk of other COPD endotypic effects beyond the classic megaloblastic anemia. In spite of the relationship between anemia and reduced aerobic capacity in COPD [17], the finding of only one case of anemia does not indicate that the significant interaction between supplementation and endurance can be explained by anemia correction.

Notwithstanding the many potential neuromuscular, hematological and cognitive molecular targets, real or functional vitamin B12 deficiency, with secondary elevation of harmful byproducts such as homocysteine and methylmalonic acid, there are no studies addressing the effects of cobalamin supplementation on endurance aerobic capacity and VO_{2p} kinetics in patients with COPD. Supplementation with cobalamin and folic acid has discrete effects on physical performance in well-selected populations, such as the elderly [32] or those with ischemic heart disease [7]. However, there are many questions still to clarify, such as the potential association between oxidative stress and systemic inflammation and the real or functional deficiency in cobalamin [30] or association between iron stores and responses to exercise. Recently it was shown that iron deficiency in the absence of anemia reduces the response to pulmonary rehabilitation [16] and, on the other hand, cobalamin deficiency can mask low iron stores in the body, posing a diagnosis of normality where there is iron deficiency. Some authors suggest that a new iron deficiency assessment is necessary after cobalamin replacement [33].

The overall positive slight effect on T_{lim} in this small representative sample of patients with COPD was also more important in the group that was supplemented and did not perform exercise (Group S), as there was practically no fall in physical performance after two months, a fact that may be common in patients with advanced COPD and importantly, basal physical inactivity [34,35]. This was despite lower increases in vitamin B12 levels in the blood after

eight weeks of supplementation (ΔVitamin B12). Despite significant baseline T_{lim} group response, the interaction between supplementation and the final effect remained significant after statistical adjustment in the model for the main confounder variables in this study. The observed unbalanced data to some variables, like a 4 kg m^2 average difference in BMI between groups 1 and 4, a predominantly GOLD IV status in group 1 and a 10 years average difference between the groups 1 and 2 could theoretically produce some bias, but age, sex and lung function were considered poor predictors of response to training in a previous large cluster analysis [36].

Since the main components of the acceleration of $\dot{V}O_{2p}$ kinetics are (i) the reduction in metabolic inertia and improvement in the mitochondrial respiratory chain, especially after training [28,37], (ii) associated with cardiovascular adjustment [28], these would theoretically provide indirect reasons for improved $\dot{V}O_{2p}$ kinetics after supplementation with vitamin B12 in patients at risk. A possible reduction in hyperhomocysteinemia, amplified by intrinsic oxidative stress in COPD patients [5] and associated with "low-normal" vitamin B12 levels as defined in this study or possible functional deficiency, has support in the literature to produce improvement in endothelial dysfunction [8], reduction in toxic effects of homocysteine on the mitochondria [9] and/or deleterious effects on heart muscle [38,39]. Overall, this could theoretically lead to better adjustment of the main determinants of $\dot{V}O_{2p}$ kinetics in rest-to-exercise transition. However, these effects would probably be best assessed in a population with increased homocysteine levels over a period long enough to cause these effects.

Important limitations should be considered in this study. First, the low number of participants, despite the power conferred by the design and statistics performed. In studies with a low number of participants in this context, although statistically homogeneous, temporal variations in exercise capacity may depend on the level of baseline physical activity, which was not specifically evaluated. However, this does not appear to be the case, since the scores of functional CCQ between the groups were balanced ($p > 0.05$), an indicator that correlates with the level of physical activity almost as well as the six minute walk test [40]. Another important confounding variable is that supplementation with vitamin B12 can lead to improvement in possible cognitive impairment, one that has not been rigorously evaluated and, as a consequence, produces better exercise performance. We could also not conduct several other tests that could enrich the analysis such as the dosage of serum homocysteine, intrinsic factors, and peripheral muscle and respiratory strength tests, among others. Regarding the relationship between atrophic gastritis and intrinsic factors, a study showed that 500mcg of cobalamin orally was as effective in restoring cobalamin blood levels in people with or without atrophic gastritis [41]. However, further studies are needed with larger numbers of patients to assess the effects of vitamin B12 supplementation alone or in combination with folic acid and other micronutrients to achieve excellence in the treatment of the nutritional risks of these patients.

In conclusion, supplementation of advanced COPD patients with vitamin B12 for eight weeks seems to produce discrete positive effects on exercise tolerance on a cycle ergometer. The effect is uncertain when combined with exercise alone, but the significant interaction observed when assessing globally all patients supplemented compared to controls opens perspectives for further studies in this area.

Ethics

The author and co-authors have contributed substantially to this original work and approved the final submission. This work is not being considered for publication, in whole or in part, in another journal, book or conference proceedings and the author and co-authors have no conflicts of interest. The author and co-authors reviewed the final stages of the manuscript.

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