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## Does branched-chain amino acid supplementation improve pulmonary rehabilitation effect in COPD?

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25

26 **Abstract**

27 **Background:** Muscle wasting is frequent in chronic obstructive lung disease (COPD) and  
28 associated with low branched-chain amino acids (BCAA). We hypothesized that BCAA  
29 supplementation could potentiate the effect of a pulmonary rehabilitation program (PRP) by  
30 inducing muscular change.

31 **Materials and methods:** Sixty COPD patients (GOLD 2-3) were involved in an ambulatory  
32 4-week PRP either with BCAA oral daily supplementation or placebo daily supplementation  
33 in a randomized double-blind design. Maximal exercise test including quadriceps oxygenation  
34 measurements, functional exercise test, muscle strength, lung function tests, body  
35 composition, dyspnea and quality of life were assessed before and after PRP.

36 **Results:** Fifty-four patients ( $64.9 \pm 8.3$  years) completed the protocol. In both groups,  
37 maximal exercise capacity, functional and muscle performances, quality of life and dyspnea  
38 were improved after 4-week PRP ( $p \leq 0.01$ ). Changes in muscle oxygenation during the  
39 maximal exercise and recovery period were not modified after 4-week PRP in BCAA group.  
40 Contrarily, in the placebo group the muscle oxygenation kinetic of recovery was slowed down  
41 after PRP.

42 **Conclusion:** This study demonstrated that a 4-week PRP with BCAA supplementation is not  
43 more beneficial than PRP alone for patients. A longer duration of supplementation or a more  
44 precise targeting of patients would need to be investigated to validate an effect on muscle  
45 recovery and to demonstrate other beneficial effects.

46 **Keywords.** COPD, pulmonary rehabilitation, BCAA supplementation, exercise, quadriceps  
47 oxygenation, muscle adaptation.

48

49 **List of abbreviations**

|    |  |  |
|----|--|--|
| 50 | BCAA                                   | Branched-chain amino acids                                     |
| 51 | BMI                                    | Body mass index  |
| 52 | COPD                                   | Chronic obstructive lung disease                               |
| 53 | CPP                                    | Comité de protection des personnes                             |
| 54 | Ctrl                                   | Control  |
| 55 | $\Delta$ [O <sub>2</sub> Hb]           | Concentration changes in oxygenated haemoglobin (in muscles)   |
| 56 | $\Delta$ [HHb]                         | Concentration changes in deoxygenated haemoglobin (in muscles) |
| 57 | $\Delta$ [tHb]                         | Concentration changes in total haemoglobin (in muscles)        |
| 58 | FEV <sub>1</sub>                       | Forced expiratory volume during the first second               |
| 59 | FVC                                    | Forced vital capacity  |
| 60 | GOLD                                   | Global initiative for lung disease                             |
| 61 | HRQoL                                  | Health-related quality of life                                 |
| 62 | IMS Quad                               | Isometric maximal quadriceps strength                          |
| 63 | mMRC                                   | Modified Medical Research Council                              |
| 64 | NIRS                                   | Near-infrared spectroscopy                                     |
| 65 | PaO <sub>2</sub> and PaCO <sub>2</sub> | Arterial oxygen pressure and carbon dioxide pressure           |
| 66 | % pred                                 | Percentage predicted values                                    |
| 67 | PRP                                    | Pulmonary rehabilitation program                               |
| 68 | RER                                    | Respiratory exchange ratio                                     |
| 69 | SaO <sub>2</sub>                       | Arterial haemoglobin oxygen saturation                         |
| 70 | SpO <sub>2</sub>                       | Pulse haemoglobin oxygen saturation                            |
| 71 | 6MWT                                   | Six-minute walk test   |
| 72 | SVC                                    | Slow vital capacity  |
| 73 | TEI                                    | Total energy intake  |

|    |               |                                  |
|----|---------------|----------------------------------|
| 74 | TSI           | Tissue (oxygen) saturation index |
| 75 | $\dot{V}E$    | Ventilatory flow                 |
| 76 | $\dot{V}O_2$  | Oxygen consumption               |
| 77 | $\dot{V}CO_2$ | Carbon dioxide production        |
| 78 |               |                                  |

79

## 80 **1. Introduction**

81           Chronic obstructive lung disease (COPD) has a large panel of deleterious effects. Even  
82 though it is classified as a respiratory disease, COPD also induces extra-bronchial and extra-  
83 pulmonary impairments. Breathlessness likewise is probably not induced by airway damage  
84 alone. Pathophysiological processes progressively involve manifold organs or functions that  
85 play an important role in disease burden and progression. Systemic inflammation, skeletal  
86 muscle dysfunction and nutritional status anomalies have associated deleterious synergic  
87 effects. In addition, COPD is frequently associated with aging and chronic comorbid diseases.

88           Alteration in skeletal muscle function is one of the most widely recognized extra-  
89 pulmonary manifestations of COPD [1]. Atrophy is characterized by a loss of muscle mass and  
90 cross-sectional area [2]. Muscle fiber redistribution has likewise been well-demonstrated in  
91 COPD, with an increased proportion of type II fibers relative to type I fibers [3, 4, 5, 6, 7]. In  
92 association, enzymatic metabolism activity is also altered with increased glycolytic activity and  
93 decreased aerobic metabolism [8, 9, 10] and altered muscle capillarization [11, 12]. Nutritional  
94 depletion that is also frequent in COPD additively contributes to sarcopenia [13]. Skeletal  
95 dysfunction translates into a loss of muscle mass, strength and endurance and has a significant  
96 negative impact on functional capacity and exercise tolerance [14, 15, 16, 17, 18, 19, 20, 21].

97           Multidisciplinary pulmonary rehabilitation has demonstrated its effectiveness in cases  
98 of COPD by counteracting the devastating non-pulmonary consequences of the disease [22,  
99 23, 24, 25]. Since malnutrition and muscle wasting are associated with low branched-chain  
100 amino acids (BCAA) levels probably due to acidosis-induced degradation [26, 27, 28] some  
101 authors have proposed oral supplementation [29, 30]. Nutritional strategies associated with  
102 exercise rehabilitation are interesting insofar as they aim to stimulate muscle protein synthesis  
103 and to limit the negative impact of disease on muscle [31, 32, 33, 34, 35, 29]. However, these

104 strategies do not appear sufficiently proven (in theory) and effective (in practice) to provide  
105 concrete, accurate and specific supplementation for COPD patients [36, 37].

106           The aim of this study was to investigate the effect of a 4-week PR including complete  
107 exercise training associated with daily BCAA supplementation, in a double-blind experiment.  
108 A large panel of respiratory and physiological measurements including quadriceps  
109 oxygenation were performed, before and after PR, during a maximal exercise test. Functional  
110 performance and strength were also tested. Quality of life, exertion and dyspnea perception  
111 were recorded. We hypothesized that BCAA supplementation could potentiate the effect of  
112 PR and improve exercise and recovery capacity by stimulating muscle function.

113

## 114 **2. Material and methods**

### 115 **2.1. Patients:**

116 Stable COPD patients were recruited by the University Hospital of Poitiers (France).  
117 They fulfilled the following inclusion criteria: a diagnosis of stage 2 or 3 COPD according to  
118 the Global initiative for lung disease (GOLD) [38] with FEV<sub>1</sub> between 30 and 80%pred and  
119 FEV<sub>1</sub>/FVC <70%; age between 40 and 80 years. Patients committed to stop smoking during  
120 the study and agreed to take part in a pulmonary rehabilitation program (PRP). Patients were  
121 excluded from the study if they met any of the following criteria: exacerbation during the last  
122 three months, long-term oxygen therapy or non-invasive long-term ventilation, other  
123 pulmonary diseases, cardiac disease, renal disorder, physical practice contraindication,  
124 involvement in another clinical trial at the time or during the previous three months.

125 The study was conducted by the University Hospital of Poitiers, and for which the  
126 approval was granted by the ethical board [Comité de Protection des Personnes (CPP) - Ouest  
127 3, protocol number 14.12.48). This trial was registered in the French Clinical Trials Registry  
128 (registration number ID-RCB n° 2014-A01737-40 (HPS)). A written consent form was signed  
129 by the patient prior to the beginning of protocol in accordance with the Declaration of  
130 Helsinki and the current guidelines for Clinical Good Practice (International, ICH-E6 and  
131 French decision of 2006-11-24). PRP was conducted in a readaptation center “ Le Moulin  
132 vert” (Nieuil-l'Espoir , France).

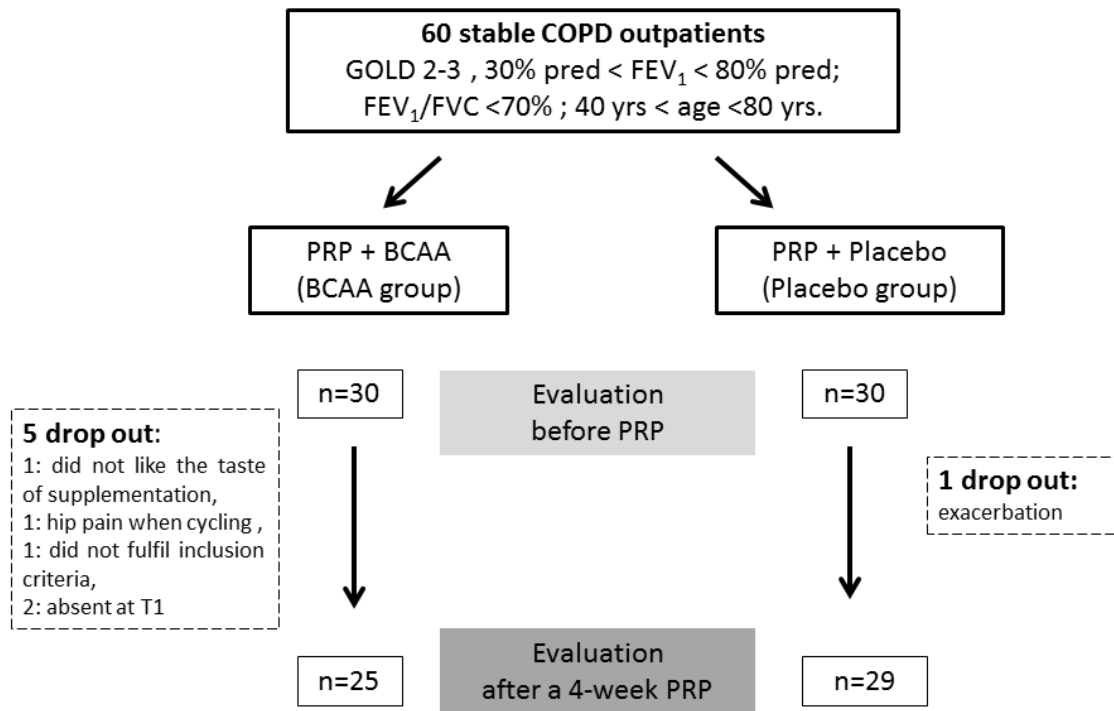
### 133 **2.2. Experimental protocol**

134 Randomized double-blinded patients participated in an ambulatory 4-week PRP either  
135 with BCAA oral daily supplementation or placebo daily supplementation (Fig 1).

136

137





138 Figure 1. Study protocol and patients flow chart. Branched-chain amino acids (BCAA);  
 139 pulmonary rehabilitation program (PRP).  
 140  
 141

142 ***Pulmonary rehabilitation program***

143 Ambulatory PRP took place on 20 days over a 4-week period. It consisted of daily  
 144 exercise training and semi-weekly therapeutic education. Exercise training included (a) a  
 145 daily 30-minute personalized cyclo-ergometer session using intermittent or continuous  
 146 modalities, (b) daily 1-hour adapted physical activity focusing on breathing (c) daily muscle  
 147 strengthening session (45 min) and (d) daily 1-hour outdoor walking. Therapeutic education  
 148 aimed to improve patient knowledge about the disease and its management, medication,  
 149 nutrition, physical practice and also included psychological support.

150 ***BCAA oral supplementation***

151 BCAA supplementation and its placebo had similar appearance and taste. BCAA  
152 supplement was made of partially hydrolyzed whey protein isolates mixed with the other  
153 constituent of the powder and was replaced by polydextrose in the placebo mixture. Both  
154 were administrated in a 25g sweetened chocolate drinking powder diluted in 150 mL water.  
155 Daily supplementation was prepared in individual packages. Patients were instructed to take  
156 one portion per day at the end of their lunch.

157 Active daily dose BCAA supplementation contained 18.44g of proteins (82% total  
158 energy intake (TEI)), 1.59g of glucids (7% TEI), 0.51g of lipids (5% TEI), 2.67g of fiber and  
159 0.3g of salt. Each dose contained 4.3g BCAA of which the proportions were 25.83% valine,  
160 27.05% isoleucin and 47.12% leucin. Matching placebo supplementation consisted of 0.7g of  
161 proteins (5%), 2.05g of glucids (16%), 0.38g of lipids (6%) and 19g of fibers.

### 162 **2.3. Materials and measurements**

163 Clinical, pulmonary and anthropometric data, maximal exercise data, functional  
164 performance, quality of life and dyspnea grade were collected before and at the end of PRP.

#### 165 *Pulmonary function tests*

166 Routine lung function tests were performed before and at the end of PRP. Forced vital  
167 capacity maneuver (FVC) and slow vital capacity (SVC) maneuver were performed following  
168 recommendations [39] to obtained maximal expiratory flows and pulmonary volumes and  
169 capacities (Hyp' Air compact, Medisoft, Be). Predicted normal values were derived from the  
170 reference values by [40].

#### 171 *Body composition*

172 Body composition was assessed by a multifrequency bioelectrical impedance  
173 technique (BioparhΩm, Z Metrix®, Le Bourget du lac, France). Resistance and reactance  
174 were measured between 5 and 325 kHz. The amplitude of the electric current was 70 μA.

175 After calibration of the device and completion and verification of the required data,  
176 patients were placed in supine positions with their arms and legs extended. Two pairs of  
177 electrodes, spaced 10cm apart, were attached on the same body side, to the dorsal surface of  
178 hand and forearm and to the exterior side of the leg above the ankle. Usual tissue indices were  
179 collected (fat mass, thin mass, non-fat mass, bone mineral content and muscular mass [41]).

## 180 ***Maximal exercise tests and functional performance***

### 181 *Maximal exercise test*

182 All subjects performed a maximal exercise test on an electrically braked stationary  
183 cycle ergometer (ERGOLINE 800S, Germany). The test began at 30 watts in males and 20  
184 watts in females, and the workload was increased by 10 W every minute. All patients were  
185 encouraged to exercise to exhaustion or to symptom limitation.

186 *Ventilatory and metabolic measurements.* The subject breathed through a facemask (Hans  
187 Rudolph, Kansas City, Mo, USA). Expired gas flows were measured using a  
188 pneumotachograph (Type 3, Hans Rudolph, Kansas City, MO, USA) and analyzed breath-by-  
189 breath using an automated system (Médisoft, Exp'air 1.26, Belgium, 1999). Before each test,  
190 the pneumotachograph was manually calibrated with a three-liter syringe and the gas  
191 analyzers with a known gas mixture of known composition vs room air composition. The O<sub>2</sub>  
192 consumption ( $\dot{V}O_2$ ), CO<sub>2</sub> production ( $\dot{V}CO_2$ ) and ventilatory flow ( $\dot{V}E$ ), breathing frequency,  
193 respiratory exchange ratio (RER) and other usual respiratory parameters were monitored  
194 continuously breath by breath and averaged every 15 seconds. Peak  $\dot{V}O_2$  was defined as the

195 highest 15 sec averaged O<sub>2</sub> recorded during the maximal incremental exercise test. Heart rate  
196 was continuously recorded using a 12-lead ECG.

197 Pulse oximetry. Pulse haemoglobin oxygen saturation (SpO<sub>2</sub>) was recorded continuously with  
198 a pulse oximeter (Wrist-Ox2 3150, Nonin Medical Inc., Plymouth, MN, USA) placed at the  
199 right index.

200 Arterial blood pressure. A sphygmomanometer (Omron-M3, Health Care CO. Ltd, Kyoto,  
201 Japan) was used to measure systolic and diastolic blood pressure (SBP and DBP) at every  
202 stage of the maximal exercise test.

203 Respiratory and muscle pain. Perception of dyspnea and muscle pain experienced during  
204 maximal exercise was obtained immediately after the end of maximal exercise and was  
205 assessed using the modified 0-10 Borg-scale [42][43].

206 Muscle oxygenation. Near-infrared spectroscopy (NIRS) (Oxymon Mk-III, Artinis Medical  
207 Systems, Zetten, The Netherlands) was used to assess the tissue saturation index (TSI) and the  
208 concentration changes in oxygenated, deoxygenated and total haemoglobin in vastus lateralis  
209 ( $\Delta$  [O<sub>2</sub>Hb],  $\Delta$  [HHb] and  $\Delta$  [tHb] respectively) during the maximal exercise test and 5 min  
210 post-exercise recovery. The optode was placed on the right thigh vastus lateralis, at 1/3 of the  
211 distance between the upper patella and the antero-superior iliac spine /crest. The interoptode  
212 distance was 4 cm given that the penetration depth of the light is approximately half of the  
213 distance between transmitter and receiver [44]. A differential pathlength factor of 4 was  
214 chosen [45]. Wavelengths of 859 and 763 nm were used. Data were recorded at 10 Hz and  
215 exported at 1 Hz after running average filtering. Concentration changes were calculated from  
216 the resting period (1 minute before exercise).

217 *6 min walk test*

218 A six-minute walk test (6MWT) was performed before and at the end of PRP to  
219 evaluate functional capacity [46]. The test was performed in a 30-m long quiet corridor. The  
220 patients were asked to cover as much ground as possible within 6 minutes. SpO<sub>2</sub> and cardiac  
221 frequency were continuously recorded with a pulse oximeter (Wrist-Ox2 3150, Nonin  
222 Medical Inc., Plymouth, MN, USA) placed at the right index. Dyspnea was assessed using the  
223 modified 0-10 Borg-scale, at rest, immediately at the end of the test and after 5 min recovery  
224 [43].

#### 225 *Maximal quadriceps strength and muscle endurance*

226 Isometric maximal quadriceps strength (IMS Quad) was measured on a Koch bank  
227 device. Patients were seated with the knee flexed at 90° and the ankle connected to a force  
228 sensor (Salter mechanical dynamometer, 235 6S, Foundry Lane, Smethwick, West Midlands  
229 B66 2LP, UK). Patients were instructed to perform the strongest extension against a resistance  
230 and to maintain it during 3-5 sec. Three trials were performed for each leg separated by 5 min  
231 rest. The better value among two reproducible trials was selected.

232 Quadriceps endurance was measured by performing repeated extensions of the leg  
233 against a load equivalent to 30% maximal isometric strength while respecting a frequency of  
234 10 movements/min. The number of extensions performed was recorded [47].

#### 235 *Quality of life assessment*

236 Health-related quality of life (HRQoL) was assessed by the validated VQ11  
237 questionnaire to quantify potential short-term change in HRQoL in patients with COPD [48].  
238 This questionnaire includes 11 items (with a 1-to-5 response scale) pertaining to functional,  
239 psychological and relational components. The questionnaire was completed by patients before  
240 and at the end of the PRP. The lower the global score, the better the HRQoL.

#### 241 *Dyspnea and BODE index*

242 BODE index [49] was calculated before and after PRP. Modified Medical Research  
243 Council (mMRC) scale was used to assess dyspnea.

#### 244 **2.4. Sample size estimation and statistical analysis**

245 A total sample size of 60 patients (30 patients per group) was computed to detect with  
246 80% power and one-sided type-one error of 5% a minimum 2 mL/min/kg difference in mean  
247 peak  $\dot{V}O_2$  change between groups, with a standard deviation of 3 mL/min/kg (SAS Proc  
248 Power).

249 Data are expressed as mean  $\pm$  SD. Statistical analyses were performed using SAS  
250 Statistical Package version 9.4 (SAS Institute Inc., Cary, NC, USA). The nonparametric  
251 Mann-Whitney test was applied for comparison of means between groups and the Wilcoxon  
252 test for paired within-group comparison. Fisher's exact test was used for the comparison of  
253 proportions. Correlation analysis used Spearman rank coefficients. After assessment of  
254 distribution normality, two-way repeated-measures mixed ANOVA was performed on NIRS  
255 variables recorded during maximal exercise. Fisher's LSD post-hoc pairwise comparisons  
256 were performed when ANOVA was significant. P-value < 0.05 was considered significant.

257

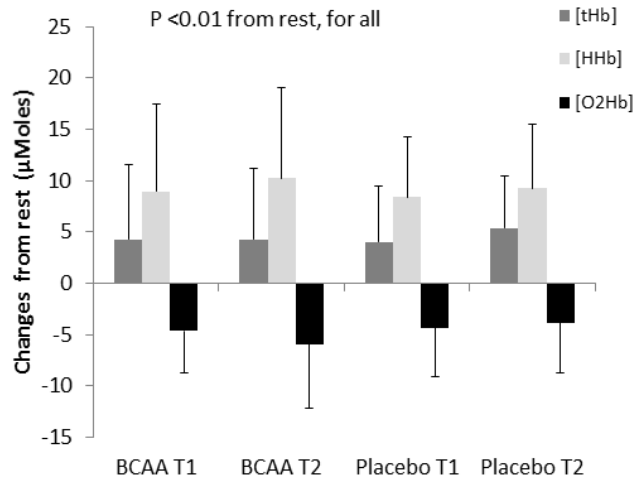
### 258 **3. Results**

259 The study took place between March 2015 and March 2018. Sixty patients were  
260 included, among whom six dropped out as soon as the study started, for different reasons  
261 (exacerbation, pain while cycling, injury, taste of the supplement, exclusion criteria,  
262 unjustified absence) (Fig 1). The characteristics of the remaining 54 patients ( $64.9 \pm 8.3$  years,  
263 18 ♀ and 36 ♂), who completed the protocol, are listed in Table 1. Many of them had  
264 comorbidities (systemic arterial hypertension  $n=29$  (54%), arteritis  $n=7$  (13%), coronary  
265 artery disease  $n=5$  (9%), diabetes  $n=2$  (4%) stroke  $n=1$  (2%).

#### 266 **3.1. Maximal exercise test**

267 Values recorded at maximal exercise before and after PRP are presented in Table 2 for  
268 the two groups. Peak  $\dot{V}O_2$  at T1 were  $16.1 \pm 4.1$  mL/min/kg and  $15.3 \pm 4.6$  mL/min/kg  
269 respectively in BCAA and placebo group and  $1.2 \pm 0.7$  for both groups when expressed in  
270 percentile of the health-related reference values of the American College of Sport Medicine  
271 [50]. Only in the placebo group did the peak of  $\dot{V}O_2$  improve significantly ( $+1.69 \pm 3.35$   
272 mL/min/kg,  $p<0.01$ ). However, peak exercise power was significantly increased in the two  
273 groups without difference between them ( $+8.6 \pm 11.5$ W and  $+11.5 \pm 10.5$ W respectively in  
274 BCAA and placebo group,  $p<0.01$ ).

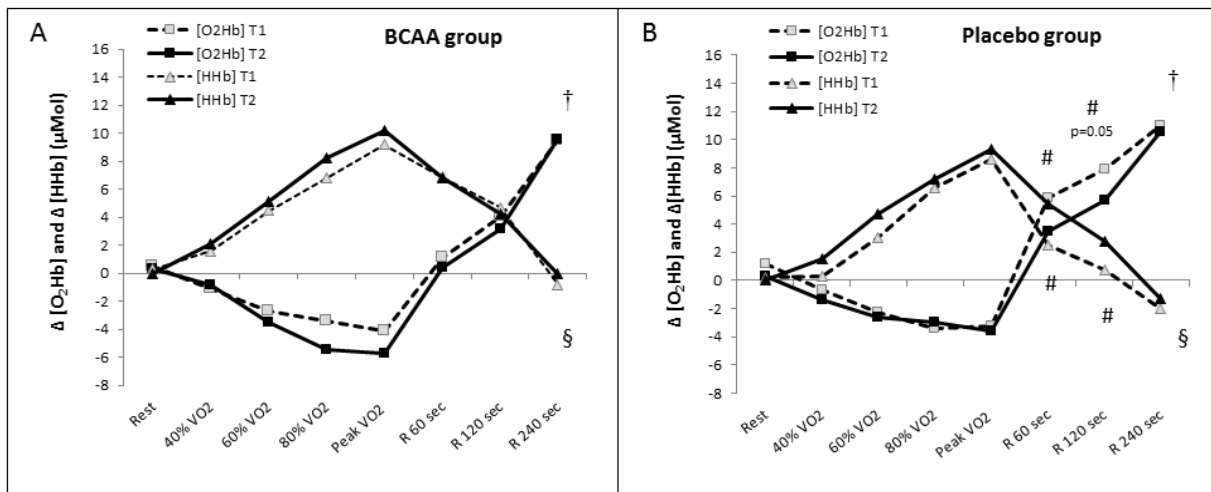
275 All vastus lateralis oxygenation variables were significantly modified at maximal  
276 exercise in the two conditions. TSI is reported in Table 2 and other variables in Figure 2.  
277  $[O_2Hb]$  and TSI were decreased from rest ( $p<0.01$ ) during exercise while they recovered and  
278 exceeded rest values ( $p<0.01$ ). Conversely,  $[HHb]$  and  $[tHb]$  were increased ( $p<0.01$ ) during  
279 exercise.  $[HHb]$  returned progressively to rest during recovery, while  $[tHb]$  stay increased  
280 ( $p<0.01$ ). Kinetics of changes throughout incremental exercise and recovery are presented on  
281 Figure 3 for  $[O_2Hb]$  and  $[HHb]$ . These changes were not modified after 4-week PRP in the  
282 BCAA group, while recovery was slowed down in the placebo groups at T2 ( $p<0.05$ ).



283

284 Figure 2. Concentration changes in quadriceps muscle oxygenation from rest, at maximal  
 285 exercise during incremental exercise test. Total haemoglobin ( $\Delta$ [tHb]); oxyhaemoglobin ( $\Delta$   
 286 [O<sub>2</sub>Hb]); deoxyhaemoglobin ( $\Delta$  [HHb]); p<0.01 from rest.

287



288

289 Figure 3. Concentration changes in oxyhaemoglobin ( $\Delta$ [O<sub>2</sub>Hb]) and deoxyhaemoglobin  
 290 ( $\Delta$ [HHb]) during incremental exercise test and recovery in branched-chain amino acid group  
 291 (BCAA) and placebo groups (panels A and B). T1 and T2: before and after pulmonary  
 292 rehabilitation program respectively; #: p<0.05 T2 vs T1; †:  $\Delta$ [O<sub>2</sub>Hb] was significantly



293 different from rest ( $p < 0.05$ ) at all measurement times, except at 40% (both groups) and R 60  
294 sec (BCAA group); §:  $\Delta$ [HHb] was significantly different from rest ( $p < 0.05$ ) at all  
295 measurement times, except at 40% (both groups), R 120 sec (T1 placebo group) and R 240  
296 sec (BCAA group and T2 placebo group). For clarity they are not more detailed on the figure.

### 297 **3.2. Other exercise tests**

298 Six-minute walk test data obtained before and after a 4-week PRP are presented in  
299 Table 3 for the two groups. The covered distances were significantly improved ( $40 \pm 53$ m and  
300  $60 \pm 51$ m respectively in the BCAA and the placebo groups,  $p < 0.01$ ). Dyspnea during the test  
301 was significantly decreased in both groups of patients ( $p < 0.01$ ). The 30m minimal important  
302 difference[51, 52] was reached in 12 patients from the BCAA group and in 19 patients from  
303 the placebo group.

304 Quadriceps strength and endurance were significantly increased in the two groups after  
305 a 4-week PRP ( $p < 0.01$ ).

### 306 **3.3. Body composition**

307 Body composition is presented in Table 4 for the two groups. Neither muscular mass  
308 nor fat mass changed after a 4-week PRP in either group. Weight decreased significantly in  
309 the placebo group ( $-0.8 \pm 1.9$ kg;  $p < 0.01$ ).

### 310 **3.4. Quality of life**

311 Quality of life was improved (lower score) in the two groups after PRP ( $\Delta$  score:  $-8.8$   
312  $\pm 8.6$  and  $-6.9 \pm 8.5$  respectively in the BCAA and the placebo groups ( $p < 0.01$ )). The  
313 improvement was significant in the three components evaluated by the VQ11 questionnaire  
314 (functional, psychological, relational).

### 315 **3.5. Comparison as a function of sex**

316 The changes after a 4-week PRP were analyzed as a function of sex in all studied  
317 patients. Differences in change between men and women were observed for the functional  
318 component of quality of life ( $p<0.02$ ) and for muscular endurance ( $p<0.03$ ). Greater  
319 improvements were observed in women.

### 320 **3.6. Correlations**

321 Correlations on the overall patient population showed a positive association between  
322 the positive changes observed with the 6MWT and those in  $VO_2$  peak values (Spearman  
323 coefficient=0.3;  $p=0.03$ ). A negative association was observed between the changes with the  
324 6MWT and those in quadriceps MIS (Spearman coefficient = -0.30;  $P= 0.0506$ ). In other  
325 words, when delta strength increased after PRP, the change in 6MWT distance tended to be  
326 less pronounced.

327 Correlations were also observed between age and change in the psychological  
328 component of the VQ11 (Spearman coefficient 0.28;  $p=0.04$ ) and age and the change in the  
329 total VQ11 score (Spearman coefficient 0.265;  $p=0.054$ ). The decrease in VQ11 scores after  
330 PRP (improvement in quality of life) was more pronounced in younger patients.

331

## 332 **4. Discussion**

333 This study aimed to investigate the effect of a daily BCAA supplementation on the  
334 muscular and exercise performances achieved after a 4-week PRP including a complete  
335 physical training program, as a function of its potential beneficial effects in terms of improved  
336 exercise capacity by induced muscular change. We found that maximal exercise capacity,  
337 functional and muscle performances, as well as quality of life, were increased after the  
338 pulmonary rehabilitation program, but without any supplemental benefit due to BCAA.  
339 However, BCAA supplementation could promote recovery from exercise by comparison to  
340 placebo group.

### 341 **4.1. Benefits of pulmonary rehabilitation**

342 Exercise training performed by the patients in this study was a complete program  
343 including a combination of weekly activities such as walking or cycling, using interval or  
344 continuous training method to improve aerobic capacity; strengthening exercises to increase  
345 muscle strength and endurance; gymnastic sessions to enhance balance, coordination and  
346 flexibility, etc.... The complete approach was chosen because it has been shown to result in  
347 higher physiological response than a more specific one [53].

348 In both groups, functional performance (6MWT) and muscle performance (maximal  
349 quadriceps strength and endurance), as well as peak power reached in maximal incremental  
350 exercise testing were significantly improved after PRP. These encouraging results are not  
351 really surprising insofar as many previous studies have reported large systemic hemodynamic  
352 adaptations and peripheral muscle improvements post-rehabilitation [54, 55, 25]. Cross-  
353 sectional area of oxidative fibers, mitochondrial volume density and oxidative enzyme  
354 activity have been shown to increase after exercise training. Capillary volume and capillary-  
355 to-fiber ratios have also been reported as enhanced, enabling the muscle to be more efficient  
356 during exercise by strongly increasing local blood flow and oxygen supply. By the same

357 token, a reduction in exercise-induced lactic production has also been observed [56]. During  
358 incremental exercise, despite the continued increase in  $\text{VO}_2$ , previous studies have reported a  
359 limited increase in  $\text{O}_2$  delivery and  $\text{O}_2$  extraction in lower limbs in COPD patients [57]. The  
360 limit was attributed to a redistribution of cardiac output and  $\text{O}_2$  to respiratory muscles [58].  
361 The increased proportion of type II fibers observed in COPD could also affect oxygenation  
362 kinetics [59].

363 This study assessed the effect of physical training on peripheral muscle oxygenation as  
364 there is little data currently available on the COPD population. De-oxygenation in the vastus  
365 lateralis was observed for both groups during maximal exercise, while during the recovery  
366 period, immediately after exercise, re-oxygenation was observed. These findings are in  
367 agreement with previous data obtained in healthy adults or patients and in hypoxia [60, 61].  
368 These de-oxygenation kinetics seem to result from exercise requirement while during  
369 recovery  $\text{O}_2\text{Hb}$  kinetics indicate a surplus of oxygen delivery relative to demand [62, 63, 64].

370 After PRP, some differences in the kinetics of muscle oxygenation are highlighted  
371 between the two groups in comparison to their respective initial kinetics. In the placebo  
372 group, recovery appears slowed down in the first few minutes after maximal exercise while  
373 muscle oxygenation values are unchanged during exercise. This delay may be interpreted as a  
374 manifestation fatigue following to a month of heavy and unaccustomed physical activity load  
375 [65, 66]. In the BCAA group, a non-significant trend to lower  $\text{O}_2\text{Hb}$  values was observed at  
376 maximal exercise after PRP followed by unchanged sharp  $\text{O}_2\text{Hb}$  overtake during recovery.  
377 This evolution following PRP may be consistent with lessened fatigue and muscle damage  
378 due to exercise and recovery favored by BCAA supplementation. Similar effects have been  
379 reported in healthy subjects and will be detailed in the next paragraph [67, 68, 69].

#### 380 **4.2. BCAA supplementation**

381 BCAA supplement was made of partially hydrolyzed whey protein isolates, which  
382 present the advantage of being assimilated very quickly and thereby providing essential,  
383 immediately available nutrients to muscles [70]. In our study, BCAA supplementation was  
384 ingested for lunch. As patients were involved in physical activities, in both morning and  
385 afternoon, midday supplementation to favor muscle protein synthesis and recovery from the  
386 morning activities seemed more appropriate and relevant than intake in the evening or during  
387 breakfast [70]. Some authors have reported lower muscular gain when protein ingestion is  
388 delayed (2h) after exercise as compared with immediate consumption [71]. Other findings  
389 suggest that increased amino acid availability in close temporal proximity to exercise appears  
390 beneficial, especially in elderly persons [72]. Finally, a midday intake, during the patient's  
391 attendance time in rehabilitation center, helped to ensure that the BCAA supplementation had  
392 been given.

393 BCAA supplementation was comprised of 47% leucine versus 26% and 27% for  
394 valine and isoleucine respectively. Low plasma levels in BCAA, especially in leucine, have  
395 been highlighted in COPD patients presenting muscle atrophy, as compared to age-matched  
396 controls [27, 37]. In BCAA, leucine is able to stimulate myofibrillar protein synthesis [73, 74,  
397 75], which is one reason why the composition of the present supplementation seemed relevant  
398 as a means of counteracting muscle weakness.

399 In most cases, studies performed on healthy subjects demonstrated inconsequential  
400 effect on performance. Thereby, other aspects have been investigated following exercise, such  
401 as recovery and muscle soreness. Various markers have been measured as manifestations of  
402 fatigue, muscle damage, or energy metabolism. Positive effects have been highlighted in  
403 BCAA supplementation group as compared to control. Reduction in tissue damage markers  
404 has been reported [67, 68, 69], and less soreness following muscle exercise [69].

405           Some studies have compared the effect of nutrient supplementation with BCAA  
406 associated with either moderate or intensive exercise on COPD patients [34, 35]. After 3-  
407 month anti-inflammatory supplementation including whey peptide and a low intensity  
408 exercise program, Sugawara et al demonstrated its positive effects on biological inflammatory  
409 and functional markers as well as quality of life compared with an exercise program alone  
410 (Ctrl group). It bears mentioning that supplementation duration was three times longer than in  
411 the present study. This may explain the differences of results between the two studies and  
412 could mean that duration might be a condition for efficiency. Along the same lines, a study  
413 conducted on sarcopenic older adults showed that a single oral 13-week nutritional support  
414 with vitamin D and leucine-enriched whey protein, not alongside exercise, could improve  
415 muscle mass and chair-stand test performance in the supplement group [76]. However, a  
416 recent review [77] of the anabolic response due to BCAA concluded that the consumption of  
417 dietary BCAAs to stimulate muscle protein synthesis was unwarranted in humans.

#### 418 **4.3. Study limitations and perspectives**

419           Pulmonary rehabilitation included 20 sessions, which corresponds to  
420 recommendations. However, according to rehabilitation centers, the sessions are usually  
421 organized over periods ranging from 4 to 12 weeks [23]. In our case, PR duration was the  
422 shortest: 4 weeks. It is possible that too short a duration does not allow patients to fully  
423 benefit from a rehabilitation program, as improvement takes time and because the repetition  
424 of the sessions can generate significant fatigue in previously sedentary patients. In addition,  
425 the duration of BCAA supplementation was relatively short, a factor that may have lessened  
426 its effect.

427           The patients included in the study were stage 2 or 3 COPD patients according to the  
428 GOLD classification; however, as their degree of muscle atrophy was not homogeneous, they  
429 may have responded differently to training, and it might have been advisable to define more

430 specific inclusion criteria [78, 79]. A threshold defining muscle alteration as low body mass  
431 index (BMI), loss of weight or low lean mass might have led to selection of subjects who  
432 could have derived maximum benefit from BCAA supplementation [33].

433 Patients of both sexes were included in the study, and in each of the two groups there  
434 were approximately 1/3 women. Considering that body composition, muscle anabolism or  
435 substrate metabolism during exercise are influenced by hormones, it can be assumed that  
436 exercise rehabilitation effects may be affected by gender [70]. Nutritional adaptations are  
437 likewise impacted by sex-differences [80]. Regarding protein metabolism, given that women  
438 oxydize less leucine than men during endurance exercise [81, 82], sex-based differences  
439 indeed exist. Unfortunately, most studies involve a majority of male subjects and knowledge  
440 on women is lacking. Women like men seem to become less anabolically sensitive with aging  
441 but also appear less anabolically sensitive than their age-matched counterparts [83]. However,  
442 the dose per meal optimally supporting muscle protein synthesis has not been determined for  
443 them and may be closely related to body weight. Therefore, it may be advisable to distinguish  
444 men from women, one objective being to optimize the muscle performance and overall health  
445 of elderly women.

## 446 **5. Conclusions**

447 In conclusion, this study demonstrated that a 4-week complete pulmonary  
448 rehabilitation program improves muscular and functional performance as well as the quality  
449 of life of COPD patients. This study also showed that a month of BCAA supplementation  
450 does not benefit performance more than pulmonary rehabilitation alone on. However, in our  
451 study BCAA supplementation may have promoted better muscle adaptation and recovery  
452 from exercise as compared to the placebo group whose recovery was slowed-down. Longer  
453 duration or more precise targeting of patients liable to benefit from BCAA supplementation

454 could allow to validate our interpretation of findings on its effect on muscle recovery and  
455 demonstrate other beneficial effects.

456



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461

## 462 **CRedit authorship contribution statement**

463 **Claire de Bisschop**: Conceptualization, Methodology, Investigation, Writing - Original Draft,  
464 Visualization. **Fabrice Caron**: Conceptualization, Investigation, Resources, Supervision.  
465 **Pierre Ingrand**: Methodology, Validation, Formal analysis. **Quentin Bretonneau**:  
466 Investigation, Visualization. **Olivier Dupuy**: Investigation, Visualization. **Jean-Claude**  
467 **Meurice**: Conceptualization, Methodology, Visualization, Funding acquisition, Project  
468 administration.

469

## 470 **Declaration of competing interests**

471 The author(s) declared that they have no known competing financial interests or personal  
472 relationships that could have appeared to influence the work reported in this paper.

473

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478

479 Table 1. Anthropometric, pulmonary function and blood gas characteristics

|                                    | BCAA Grp<br>(N=25) |                 | Placebo Grp (N=29) |                 | Total (N=54) |                 |
|------------------------------------|--------------------|-----------------|--------------------|-----------------|--------------|-----------------|
|                                    | N                  | Mean $\pm$ sd   | N                  | Mean $\pm$ sd   | N            | Mean $\pm$ sd   |
| Sex ( $\text{♂}/\text{♀}$ )        |                    | 18/7            |                    | 18/11           |              | 36/18           |
| Age                                | 25                 | 65.4 $\pm$ 8.8  | 29                 | 64.4 $\pm$ 8.0  | 54           | 64.9 $\pm$ 8.3  |
| Height (cm)                        | 25                 | 168.2 $\pm$ 8.2 | 29                 | 168.7 $\pm$ 9.8 | 54           | 168.4 $\pm$ 9.0 |
| Current smoker                     | 25                 | 8 (32%)         | 29                 | 5 (17%)         | 54           | 13 (24%)        |
| Respiratory variables              |                    |                 |                    |                 |              |                 |
| FVC (L)                            | 24                 | 3.38 $\pm$ 0.94 | 29                 | 3.21 $\pm$ 1.15 | 53           | 3.29 $\pm$ 1.04 |
| FVC (% pred)                       | 24                 | 99.3 $\pm$ 26.3 | 29                 | 94.5 $\pm$ 29.2 | 53           | 96.7 $\pm$ 27.8 |
| FEV <sub>1</sub> (L)               | 24                 | 1.55 $\pm$ 0.52 | 29                 | 1.59 $\pm$ 0.47 | 53           | 1.57 $\pm$ 0.49 |
| FEV <sub>1</sub> (% pred)          | 24                 | 58.2 $\pm$ 17.9 | 29                 | 59.0 $\pm$ 16.3 | 53           | 58.6 $\pm$ 16.9 |
| FEV <sub>1</sub> / FVC (%)         | 24                 | 47.8 $\pm$ 16.8 | 29                 | 51.2 $\pm$ 12.2 | 53           | 49.6 $\pm$ 14.4 |
| FEF 75% FVC (L.sec <sup>-1</sup> ) | 24                 | 2.0 $\pm$ 1.6   | 28                 | 2.3 $\pm$ 1.3   | 52           | 2.1 $\pm$ 1.5   |
| FEF 75% FVC (% pred)               | 24                 | 30.8 $\pm$ 24.1 | 28                 | 36.1 $\pm$ 22.3 | 52           | 33.6 $\pm$ 23.1 |
| Blood gas                          |                    |                 |                    |                 |              |                 |
| pH                                 | 24                 | 7.42 $\pm$ 0.03 | 28                 | 7.42 $\pm$ 0.03 | 52           | 7.42 $\pm$ 0.03 |
| PaO <sub>2</sub> (mm Hg)           | 25                 | 74.9 $\pm$ 9.2  | 28                 | 73.6 $\pm$ 8.3  | 53           | 74.2 $\pm$ 8.7  |
| PaCO <sub>2</sub> (mm Hg)          | 25                 | 38.3 $\pm$ 3.4  | 28                 | 38.4 $\pm$ 4.4  | 53           | 38.4 $\pm$ 3.9  |
| Bicarbonates (mmol/L)              | 24                 | 24.7 $\pm$ 1.4  | 26                 | 24.8 $\pm$ 2.2  | 50           | 24.7 $\pm$ 1.9  |
| SaO <sub>2</sub> (%)               | 25                 | 95.2 $\pm$ 2.1  | 28                 | 95.0 $\pm$ 1.4  | 53           | 95.1 $\pm$ 1.8  |

480 Values are mean  $\pm$  SD. BCAA Grp, branched-chain amino acid group; FVC, forced vital capacity;  
481 FEV<sub>1</sub>, forced expiratory volume during the first second; FEF 75%, forced expiratory flow at 75%  
482 FVC; % pred, percentage predicted values<sup>40</sup>, PaO<sub>2</sub> and PaCO<sub>2</sub>, arterial oxygen and di oxygen pressure;  
483 SaO<sub>2</sub>, arterial haemoglobin oxygen saturation.

484

485

486 Table 2 Maximal values reached during incremental exercise test before and after a 4-week  
 487 pulmonary rehabilitation program either with BCAA supplementation or placebo

|                             | BCAA Grp<br>T1  | BCAA Grp<br>$\Delta$ T2-T1 | P<br>(n)         | Placebo<br>Grp T1 | Placebo Grp<br>$\Delta$ T2-T1 | P<br>(n)                  | P ( $\Delta$ ) |
|-----------------------------|-----------------|----------------------------|------------------|-------------------|-------------------------------|---------------------------|----------------|
|                             | Mean $\pm$ sd   | Mean $\pm$ sd              | Intra<br>Grp     | Mean $\pm$ sd     | Mean $\pm$ sd                 | Intra<br>Grp              | Inter<br>Grp   |
| $\dot{V}O_2$<br>(mL/min/kg) | 16.1 $\pm$ 4.1  | +0.06 $\pm$ 2.54           | NS<br>(n=25)     | 15.3 $\pm$ 4.6    | +1.69 $\pm$ 3.35              | P<0.01<br>(n=29)          | NS             |
| RER                         | 1.11 $\pm$ 0.09 | 0.00 $\pm$ 0.11            | NS<br>(n=25)     | 1.08 $\pm$ 0.10   | +0.01 $\pm$ 0.08              | NS<br>(n=29)              | NS             |
| $\dot{V}E$<br>(L/min)       | 44.4 $\pm$ 8.3  | +1.92 $\pm$ 8.13           | NS<br>(n=25)     | 44.3 $\pm$ 12.2   | +4.42 $\pm$ 9.72              | NS<br>(P=0.057)<br>(n=29) | NS             |
| Dyspnea                     | 6.9 $\pm$ 1.5   | -0.6 $\pm$ 2.4             | NS<br>(n=21)     | 7.2 $\pm$ 1.8     | -0.2 $\pm$ 2.6                | NS<br>(n=22)              | NS             |
| SpO <sub>2</sub> (%)        | 94.3 $\pm$ 4.3  | -1.9 $\pm$ 5.0             | NS<br>(n=22)     | 93.8 $\pm$ 3.7    | +0.5 $\pm$ 3.1                | NS<br>(n=26)              | NS             |
| Peak<br>power (W)           | 74.0 $\pm$ 16.8 | +8.6 $\pm$ 11.5            | P<0.01<br>(n=25) | 75.7 $\pm$ 23.5   | +11.5 $\pm$ 10.5              | <0.01<br>(n=29)           | NS             |
| Leg pain                    | 5.8 $\pm$ 2.4   | -0.5 $\pm$ 1.9             | NS<br>(n=21)     | 4.8 $\pm$ 3.1     | +0.1 $\pm$ 2.8                | NS<br>(n=22)              | NS             |
| Quad TSI<br>(%)             | 45.8 $\pm$ 20.8 | -0.7 $\pm$ 11.6            | NS<br>(n=22)     | 47.4 $\pm$ 13.5   | -2.4 $\pm$ 16.1               | NS<br>(n=24)              | NS             |

488 Values are mean  $\pm$  SD. BCAA Grp, branched-chain amino acid group; T1, before and T2, after a 4-  
 489 week pulmonary rehabilitation program;  $\dot{V}O_2$ , oxygen consumption ; RER, respiratory exchange ratio;  
 490  $\dot{V}E$ , ventilatory flow ; SpO<sub>2</sub>, pulse haemoglobin oxygen saturation; Quad TSI, quadriceps tissue  
 491 oxygen saturation index.

492

493

494 Table 3 Functional capacity, quadriceps strength and quality of life before and after a 4-week  
 495 pulmonary rehabilitation program either with BCAA supplementation or placebo

|                                    | BCAA Grp<br>T1 | BCAA Grp<br>Δ T2-T1 | P<br>(n)        | Placebo Grp<br>T1 | Placebo<br>Grp<br>Δ T2-T1 | P<br>(n)        | P (Δ)        |
|------------------------------------|----------------|---------------------|-----------------|-------------------|---------------------------|-----------------|--------------|
|                                    | Mean ± sd      | Mean ± sd           | Intra<br>Grp    | Mean ± sd         | Mean ± sd                 | Intra<br>Grp    | Inter<br>Grp |
| 6MWT (m)                           | 427 ± 90       | +40 ± 53            | <0.01<br>(n=25) | 421 ± 75          | +60 ± 51                  | <0.01<br>(n=27) | NS           |
| 6MWT<br>(%pred)                    | 67 ± 16        | 6 ± 8               | <0.01<br>(n=25) | 67 ± 12           | 9 ± 8                     | <0.01<br>(n=27) | NS           |
| Delta SpO <sub>2</sub><br>6MWT (%) | -3.1 ± 4.0     | -0.3 ± 3.9          | NS<br>(n=25)    | -4 ± 4.8          | - 0.1 ± 2.3               | NS<br>(n=27)    | NS           |
| Dyspnea<br>mMRC (0-4)              | 1.48 ± 1.1     | -0.6 ± 1.2          | 0.014<br>(n=25) | 1.55 ± 1.1        | -0.6 ± 1.1                | <0.01<br>(n=29) | NS           |
| BODE Index<br>(0-10)               | 2.1 ± 1.8      | -0.3 ± 1.5          | NS<br>(n=25)    | 2.1 ± 1.7         | -0.5 ± 1.2                | 0.047<br>(n=27) | NS           |
| Koch bench                         |                |                     |                 |                   |                           |                 |              |
| IMS right<br>quad (kg)             | 25.5 ± 5.7     | +3.2 ± 3.8          | <0.01<br>(n=24) | 25.8 ± 7.5        | +4.1 ± 5.3                | <0.01<br>(n=29) | NS           |
| IMS left<br>quad(kg)               | 23.0 ± 7.4     | +6.5 ± 4.9          | <0.01<br>(n=24) | 26.6 ± 7.4        | +3.8 ± 4.8                | <0.01<br>(n=29) | NS           |
| Extension<br>number (30%<br>IMS )  | 17.3 ± 9.5     | +6.4 ± 8.0          | <0.01<br>(n=22) | 15.8 ± 8.3        | +6.1 ± 5.5                | <0.01<br>(n=24) | NS           |
| VQ11<br>questionnaire              |                |                     |                 |                   |                           |                 |              |
| Functional                         | 9.6 ± 2.6      | -3.2 ± 3.0          | <0.01<br>(n=24) | 9.1 ± 2.2         | -2.3 ± 2.8                | <0.01<br>(n=29) | NS           |
| Psychological                      | 10.6 ± 3.5     | -3.2 ± 3.7          | <0.01<br>(n=24) | 10.2 ± 2.6        | -2.1 ± 2.9                | <0.01<br>(n=29) | NS           |
| Relational                         | 8.7 ± 3.0      | -2.5 ± 2.9          | <0.01<br>(n=24) | 8.5 ± 3.3         | -2.1 ± 3.5                | <0.01<br>(n=29) | NS           |
| Total                              | 28.7 ± 8.3     | -8.8 ± 8.6          | <0.01<br>(n=24) | 27.8 ± 6.5        | -6.9 ± 8.5                | <0.01<br>(n=29) | NS           |

496 Values are mean ± SD. BCAA Grp, branched-chain amino acid group; T1, before and T2, after a 4-  
 497 week pulmonary rehabilitation program; 6MWT, 6 min walk test; 6MWT (% pred) percentage  
 498 predictive values [84]; Delta SpO<sub>2</sub>, change from rest; IMS Quad, Isometric maximal quadriceps  
 499 strength; mMRC, modified Medical Research Council scale.  
 500

501

502 Table 4. Body composition before and after a 4-week pulmonary rehabilitation program either  
 503 with BCAA supplementation or placebo

|                               | BCAA Grp<br>T1  | BCAA Grp<br>$\Delta$ T2-T1 | P<br>(n)        | Placebo<br>Grp T1 | Placebo Grp<br>$\Delta$ T2-T1 | P<br>(n)         | P ( $\Delta$ ) |
|-------------------------------|-----------------|----------------------------|-----------------|-------------------|-------------------------------|------------------|----------------|
|                               | Mean $\pm$ sd   | Mean $\pm$ sd              | Intra<br>Grp    | Mean $\pm$ sd     | Mean $\pm$ sd                 | Intra<br>Grp     | Inter<br>Grp   |
| Weight<br>(kg)                | 71.2 $\pm$ 15.7 | -0.6 $\pm$ 1.6             | 0.054<br>(n=25) | 78.1 $\pm$ 18.1   | -0.8 $\pm$ 1.9                | P<0.01<br>(n=29) | NS             |
| BMI<br>(Kg/m <sup>2</sup> )   | 25.2 $\pm$ 5.4  | -0.1 $\pm$ 0.7             | NS<br>(n=25)    | 27.5 $\pm$ 6.3    | -0.3 $\pm$ 1.5                | NS<br>(n=29)     | NS             |
| Muscular<br>mass (kg)         | 27.9 $\pm$ 7.1  | +0.07 $\pm$ 1.49           | NS<br>(n=23)    | 27.7 $\pm$ 9.0    | -0.18 $\pm$ 2.24              | NS<br>(n=27)     | NS             |
| Muscular<br>mass<br>(%Weight) | 39.4 $\pm$ 8.7  | -0.1 $\pm$ 2.4             | NS<br>(n=23)    | 35.4 $\pm$ 8.3    | +0.36 $\pm$ 2.71              | NS<br>(n=27)     | NS             |
| Fat mass<br>(kg)              | 19.6 $\pm$ 8.9  | +0.22 $\pm$ 1.04           | NS<br>(n=23)    | 24.2 $\pm$ 12.3   | -0.20 $\pm$ 1.24              | NS<br>(n=27)     | NS             |
| Fat mass<br>(%Weight)         | 26.5 $\pm$ 8.4  | +0.3 $\pm$ 1.3             | NS<br>(n=23)    | 29.9 $\pm$ 10     | +0.01 $\pm$ 1.20              | NS<br>(n=27)     | NS             |

504 Values are mean  $\pm$  SD. BCAA Grp, branched-chain amino acid group; T1, before and T2, after a 4-  
 505 week pulmonary rehabilitation program; BMI, body mass index.

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