




# BMJ Open Beta-alanine supplementation in patients with COPD receiving non-linear periodised exercise training or neuromuscular electrical stimulation: protocol of two randomised, double-blind, placebo-controlled trials

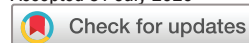
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## ABSTRACT

**Introduction** Exercise intolerance is common in patients with chronic obstructive pulmonary disease (COPD) and, although multifactorial, it is largely caused by lower-limb muscle dysfunction. Research has shown that patients with severe to very severe COPD have significantly lower levels of muscle carnosine, which acts as a pH buffer and antioxidant. Beta-alanine (BA) supplementation has been shown to consistently elevate muscle carnosine in a variety of populations and may therefore improve exercise tolerance and lower-limb muscle function. The primary objective of the current studies is to assess the beneficial effects of BA supplementation in enhancing exercise tolerance on top of two types of exercise training (non-linear periodised exercise (NLPE) training or neuromuscular electrical stimulation (NMES)) in patients with COPD.

**Methods and analysis** Two randomised, double-blind, placebo-controlled trials have been designed. Patients will routinely receive either NLPE (BASE-TRAIN trial) or NMES (BASE-ELECTRIC trial) as part of standard exercise-based care during their 8-to-10 week pulmonary rehabilitation (PR) programme. A total of 222 patients with COPD (2×77 = 154 patients in the BASE-TRAIN trial and 2×34 = 68 patients in the BASE-ELECTRIC trial) will be recruited from two specialised PR centres in The Netherlands. For study purposes, patients will receive 3.2 g of oral BA supplementation or placebo per day. Exercise tolerance is the primary outcome, which will be assessed using the endurance shuttle walk test (BASE-TRAIN) or the constant work rate cycle test (BASE-ELECTRIC). Furthermore, quadriceps muscle strength and endurance, cognitive function, carnosine levels (in muscle), BA levels (in blood and muscle), markers of oxidative stress and inflammation (in blood, muscles and lungs), physical activity and quality of life will be measured.

## Strengths and limitations of this study

- Two prospective, randomised, double-blind, placebo-controlled trials are designed and will be performed in two specialised pulmonary rehabilitation centres to assess the effects of oral beta-alanine (BA) supplementation on exercise tolerance and muscle carnosine in patients with chronic obstructive pulmonary disease (COPD).
- These are the first studies in which BA supplementation will be combined with a supervised exercise intervention (non-linear periodised exercise or neuromuscular electrical stimulation as part of pulmonary rehabilitation) in patients with COPD.
- In-depth assessment of outcomes enables to identify relevant factors that explain variances in muscle carnosine loading and to analyse carnosine's role in skeletal muscle dysfunction in COPD.
- Long-term benefits will not be investigated as limited funding makes it difficult to set up a comprehensive follow-up period in these trials.

**Ethics and dissemination** Both trials were approved by CMO Regio Arnhem-Nijmegen, The Netherlands (NL70781.091.19. and NL68757.091.19).

**Trial registration number** NTR8427 (BASE-TRAIN) and NTR8419 (BASE-ELECTRIC).

## INTRODUCTION

Exercise intolerance is one of the key disabling factors in patients with chronic obstructive pulmonary disease (COPD). Although complex and multifactorial, exercise intolerance is largely caused by lower-limb



muscle dysfunction.<sup>1</sup> Structural and metabolic muscular alterations in patients with COPD lead to a decreased muscle oxidative capacity, which results in early lactate acidosis and contractile fatigue.<sup>2</sup> To date, non-linear periodised exercise (NLPE) training and neuromuscular electrical stimulation (NMES; for the most disabled and dyspnoeic patients) are among the best available strategies to improve exercise tolerance and lower-limb muscle function in COPD.<sup>3</sup> Indeed, muscle strength and endurance improved following 8–12 weeks of exercise training. Improved muscle function was accompanied by increased exercise tolerance, a better quality of life and less symptoms, without a change in lung function.<sup>4–6</sup> Even though exercise-based interventions, such as NLPE and NMES, significantly improve exercise tolerance in COPD, targeted nutritional modulation should also be considered as a potential ergogenic aid to further enhance their efficacy.<sup>7</sup>

Beta-alanine (BA), a naturally occurring beta amino acid, is a specific nutritional supplement that is mostly used by athletes to increase exercise tolerance.<sup>8</sup> Once ingested, BA combines with histidine to form carnosine, one of the most abundant metabolites in human muscle cells.<sup>9</sup> Carnosine's main physiological roles in skeletal muscle include its function as a pH buffer and calcium regulator. High concentrations of muscle carnosine appear to delay the onset of contractile fatigue, as carnosine is able to bind muscle hydrogen ions during intense exercise, thereby moderating the decline in intracellular pH<sup>10</sup> and to increase calcium sensitivity in muscle fibres.<sup>11</sup> Moreover, carnosine can serve as an antioxidant by directly interacting with reactive oxygen species (ROS).<sup>12 13</sup>

The muscle homeostasis and contractile function of especially fast-twitch fibres seem to depend on the presence of carnosine, since they contain twice as much carnosine as slow-twitch fibres.<sup>14</sup> It is well known that the proportion of fast-twitch fibres in the quadriceps muscle of patients with COPD is increased in those with more advanced airflow limitation compared with patients with less severe airflow limitation.<sup>15</sup> Therefore, patients with severe COPD intuitively are expected to have the highest muscle carnosine concentrations. Interestingly, patients with a severe degree of airflow limitation had lower muscle carnosine levels compared with healthy controls.<sup>16</sup> This carnosine deficiency may be closely related to exercise-induced oxidative stress in the lower-limb muscles of patients with COPD<sup>1</sup> or with the fact that in these muscle-wasted COPD patients fast-twitch fibre size is significantly decreased.<sup>17</sup>

Oral supplementation of BA, the rate-limiting factor for carnosine synthesis in skeletal muscle, is reported to be safe<sup>18</sup> and can increase muscle carnosine levels by 60%–80% in both healthy young adults<sup>19</sup> and elderly,<sup>20 21</sup> without exercise training. However, research has shown that trained muscles load carnosine even more efficiently than untrained muscles.<sup>22</sup> Hence, it is very plausible to hypothesise that BA supplementation in patients with

COPD receiving either NLPE or NMES training (as part of pulmonary rehabilitation (PR)) will increase muscle carnosine levels, which, in turn, will result in a positive effect on exercise tolerance and lower-limb muscle function, by buffering pH,<sup>23 24</sup> and scavenging ROS.<sup>12 13</sup> These adaptations may translate into improved functional capacity during activities of daily living and improved quality of life.

The primary targets of both exercise training and BA supplementation are the muscles of ambulation. Nevertheless, it seems reasonable to hypothesise that an enhanced bioavailability of carnosine in the body, by means of BA supplementation, may have an antioxidative effect in both the lungs and the brain. Interestingly, on oral supplementation, carnosine is taken up by the PEPT2 transporter at the lung membrane, resulting in enhanced carnosine levels in lung tissue.<sup>25 26</sup> Likewise, elevations in carnosine in all compartments of the brain were measured after BA supplementation in rats.<sup>27</sup> These elevations in carnosine increased the resiliency to stress and were inversely associated with anxiety index.

To date, the safety and efficacy of BA supplementation during usual care (not combined with exercise training) in patients with COPD has only been assessed once (NCT02770417). Therefore, we have designed two randomised, double-blind, placebo-controlled trials in which we will assess the beneficial effects of BA supplementation in enhancing exercise tolerance in addition to two types of exercise training (NLPE and NMES) in patients with COPD. This manuscript describes the protocol of the BASE-TRAIN (NLPE) and BASE-ELECTRIC (NMES) study and gives an overview of its strengths, methodological considerations and assumed clinical implications.

## METHODS AND ANALYSIS

The BASE-TRAIN study and BASE-ELECTRIC study are designed by the *BASES consortium*, a multidisciplinary collaboration between CIRO (Horn, The Netherlands), Radboud University Medical Centre Dekkerswald (Nijmegen, The Netherlands), MEREM (Hilversum, The Netherlands), Maastricht University Medical Centre (Maastricht, The Netherlands), Hasselt University (Diepenbeek, Belgium), Ghent University (Ghent, Belgium) and Montpellier University (Montpellier, France). The consortium consists of researchers from organisations with years of experience in various related disciplines. To provide valuable insight from the patient perspective, a patient advisory board, consisting of representatives of both the CIRO client council and Lung Foundation Netherlands, is closely involved by advising and monitoring the *BASES consortium*.

## Study design

Two prospective, randomised, double-blind, placebo-controlled trials enrolling patients with COPD with explicit functional limitations and high symptom burden (GOLD classification B or D) have been designed and

**Table 1** Overview of the inclusion criteria for the two randomised controlled trials

Inclusion criteria	BASE-TRAIN trial	BASE-ELECTRIC trial
Exercise modality	NLPE	NMES
Age	40–80 years	40–80 years
mMRC dyspnoea grade	≥2	≥3
No exacerbation/hospitalisation	<4 weeks	<4 weeks
Endurance time*	Not specified	100–300 s
Quadriceps strength	Not applicable	<80% predicted
PR programme	Inpatient+Outpatient	Inpatient

\*Endurance time is measured as walking endurance time (BASE-TRAIN) or cycle endurance time (BASE-ELECTRIC).

mMRC, modified Medical Research Council; NLPE, non-linear periodised exercise; NMES, neuromuscular electrical stimulation; PR, pulmonary rehabilitation; s, seconds.

will be performed in Radboud University Medical Centre Dekkerswald (Nijmegen, The Netherlands) and CIRO (Horn, The Netherlands). The expected trial duration is 2 years from first patient in (1 September 2020) to last patient out (1 September 2022). In both trials, the intervention (BA supplementation) will be part of a comprehensive, interdisciplinary PR programme consisting of educational sessions, psychosocial and nutritional counselling, supervised physical exercise training, occupational therapy and COPD exacerbation management, as defined in the latest statement by the American Thoracic Society (ATS) and the European Respiratory Society (ERS).<sup>3</sup>

Eligible patients are those who satisfy the criteria listed in table 1. Patients are excluded if they meet at least one of the following criteria: unstable cardiac disease, use of anabolic steroids during PR programme, history of drugs/alcohol abuse, participants treated with NMES (BASE-TRAIN trial) or NLPE (BASE-ELECTRIC trial) during the PR programme, vegetarianism,<sup>28</sup> inability to understand the Dutch language, self-reported BA supplementation in the past 3 months (wash-out period: 9 weeks),<sup>29</sup> participation in a PR programme within the past 12 months, inability to perform an incremental shuttle walk test (BASE-TRAIN trial) or cardiopulmonary exercise test (BASE-ELECTRIC trial). Furthermore, patients with bleeding disorder, a recent trauma of the muscle or an infection in the region of the proposed biopsy will be excluded for the muscle biopsies.<sup>30</sup>

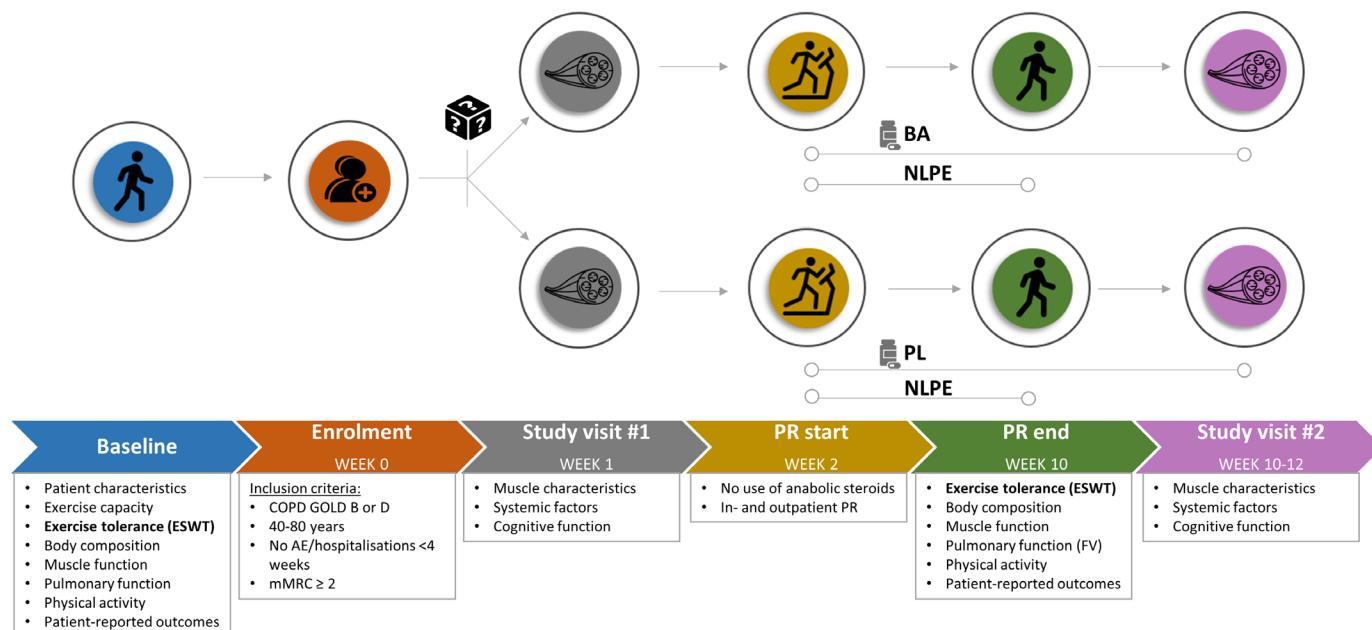
The regular PR programme at the PR centres consists of a baseline assessment, followed by the (BASE-TRAIN: inpatient or outpatient; BASE-ELECTRIC: inpatient) PR programme and is ended with a post-rehabilitation assessment. After completion of the baseline assessment and obtaining informed consent, an additional study-related appointment is scheduled with included patients approximately 1 week prior to the start of the PR programme. The appointment includes fasting venous blood sampling, a vastus lateralis muscle biopsy (optional, not required), two cognitive function tests (modified Wisconsin Card Sorting Test; M-WCST and Stroop Colour-Word Test; SCWT) and, only in the BASE-ELECTRIC trial, two

tests for lung inflammatory biomarkers (volatile organic compounds; VOCs and fractional nitric oxide concentration in exhaled breath; FeNO). This additional testing day will be repeated after the rehabilitation period. The total study duration per subject will be 10–12 weeks. A flow chart is included to give an overview of the study design and the main procedures that subjects will undergo in the course of both trials (figure 1: BASE-TRAIN trial; figure 2: BASE-ELECTRIC trial).

### Intervention

Study participants will be randomly assigned to either 3.2 g of sustained release BA (SR CarnoSyn) or identical looking placebo supplementation (both produced by Natural Alternatives International, San Marcos, USA) for a duration of 8–10 weeks. Patients, researchers and all PR staff, except the researchers performing randomisation and distributing the study supplements, will be blinded to treatment allocation.

The intervention will start directly from the onset (day 1) of the regular PR programme and will end after the last measurement of the study. Participants are instructed to coingest the supplements with meals, as this can beneficially influence muscle carnosine loading.<sup>31</sup> As part of standard care, the total protein intake during the rehabilitation period will be set at 1.5–1.9 g of protein per kilogram of fat-free mass per day<sup>32</sup> for all patients and a protein-rich supplement (±20 g of protein) is provided to every patient directly after training sessions. During breakfast and lunch, 2 tablets of BA/placebo (2×0.8 g) will be taken each time, resulting in a total daily intake of 3.2 g of BA/placebo. Furthermore, the participants will receive a personal diary, in which they keep track of intake of tablets and any possible side effects. With subjects' self-reported compliance on tablet intake (diary) and returning of the pill containers, compliance with the intervention will be assessed. In addition, a moment will be scheduled every week in the context of patient safety to discuss possible side effects with the patient. The investigators will report all serious adverse events (SAEs) without undue delay after obtaining knowledge of the events to the sponsor,



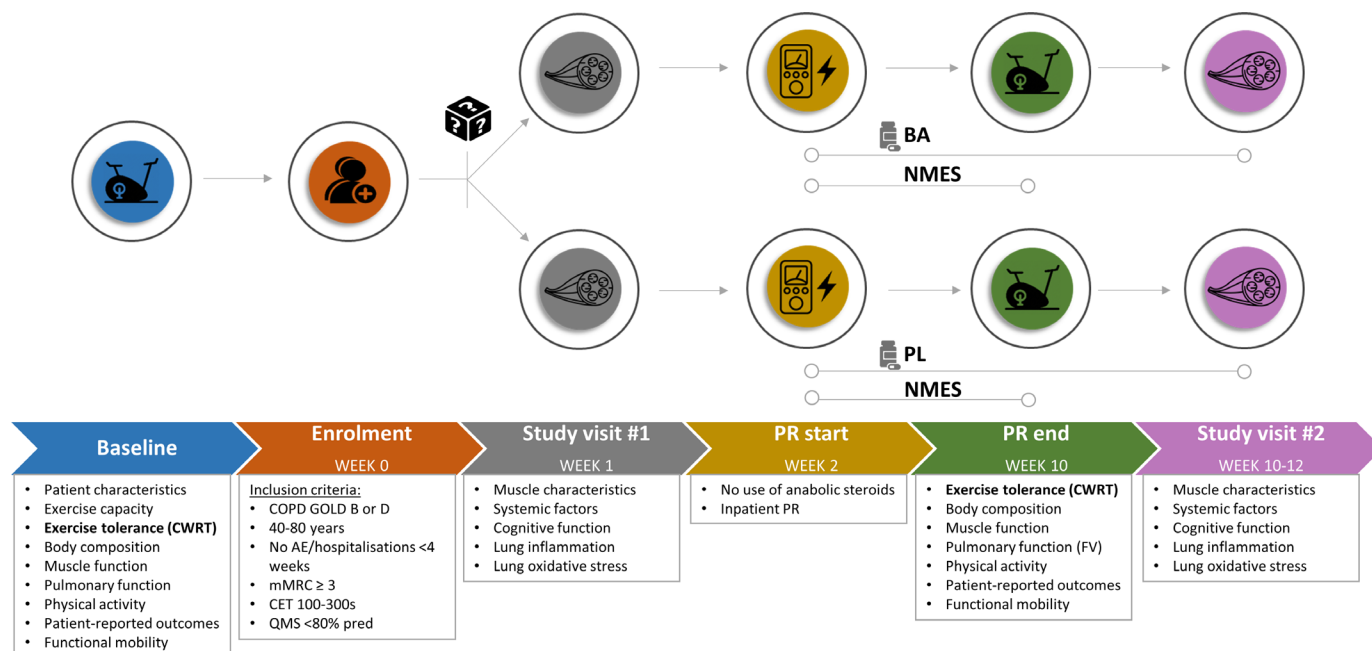
**Figure 1** Timeline for the BASE-TRAIN trial, in which all measurements, inclusion criteria, moment of randomisation and supplementation period are outlined. AE, acute exacerbations; BA, beta-alanine; COPD, chronic obstructive pulmonary disease; ESWT, endurance shuttle walk test; FV, flow volume; GOLD, Global Initiative for Chronic Obstructive Lung Disease; mMRC, modified Medical Research Council; NLPE, non-linear periodised exercise training; PL, placebo; PR, pulmonary rehabilitation.

who will report the SAEs to the accredited METC (CMO Regio Arnhem-Nijmegen).

### Training protocols

In the BASE-TRAIN trial, NLPE training is applied to the patients. Periodisation is an essential organisational strategy and a cardinal principal of exercise training.<sup>5 33 34</sup>

Periodisation involves a division in distinct and smaller, more easy manageable but interrelated phases.<sup>34</sup> The goals of these distinct phases contribute to the overall training plan. NLPE is featured by a frequent manipulation of training intensity, duration and repetition-volume. Exercise protocols are ultimately adapted based



**Figure 2** Timeline for the BASE-ELECTRIC trial, in which all measurements, inclusion criteria, moment of randomisation and supplementation period are outlined. AE, acute exacerbations; BA, beta-alanine; CET, cycle endurance time; COPD, chronic obstructive pulmonary disease; CWRT, constant work rate cycle test; FV, flow volume; GOLD, Global Initiative for Chronic Obstructive Lung Disease; mMRC, modified Medical Research Council; NMES, neuromuscular electrical stimulation; PL, placebo; PR, pulmonary rehabilitation; QMS, quadriceps muscle strength.



on information emerging from the training sessions (eg, dyspnoea, residual fatigue, percutaneous oxygen desaturation, exertion of heaviness, pain).<sup>34</sup> The programme is started with a preplanned NLPE protocol in which the patients receive three training sessions of 1–1.5 hours per week. The training plan and division in phases is shown in the online supplement (online supplemental file 1). As mentioned, individual protocol adaptations are possible, based on patients' responses during training (e.g. cause of exercise intolerance, response to a particular protocol). Whole-body exercise and resistance training are combined in each training session, and training parameters as frequency, intensity, duration and number of repetitions are monitored. The endurance training will be performed on a treadmill and the strength training will primarily be performed on the leg press (lower extremity) and chest press and pull down (upper extremity). Furthermore, category-ratio Borg scores of leg fatigue and dyspnoea are reported immediately following exercise completion to evaluate exercise-induced breathlessness and fatigue.<sup>35</sup>

In the BASE-ELECTRIC trial, NMES, being the primary exercise modality, is applied to the patients. During NMES, quadriceps and calf muscles of both legs will be simultaneously stimulated with a portable battery-operated electrical stimulator, using eight carbon-rubber electrodes (Tensmed S84, Enraf-Nonius, Rotterdam, The Netherlands).<sup>36</sup> The stimulation protocol will consist of a symmetrical biphasic square pulse at 75 Hz (high-frequency NMES), a total duty cycle of 16s and a pulse time of 400  $\mu$ s during a directly supervised session lasting 21 min in total. This high-frequency NMES has shown before to be safe, feasible and efficacious in dyspnoeic and weakened patients with COPD.<sup>6 37</sup> Patients will be specifically encouraged to increase current intensity at their will according to tolerance in each training session. The contraction time will be 6s with 8s relaxation excluding 1s ramp-up and 1s ramp-down. A total of eight carbon-rubber electrodes in moistened sponges are placed on the target muscles (four electrodes on each leg): two pairs of 8×12 cm on the quadriceps muscles and two pairs of 4×6 cm on the calf muscles. The electrodes on the quadriceps muscles are placed transversally 5–10 cm distal to the inguinal fold and 4–8 cm proximal to the patella. The electrodes on the calf muscles are placed longitudinally on the belly of the gastrocnemii muscles (protocol in accordance with study NL30153.068.09/MEC 09-3-072<sup>6</sup>).

### Study procedure

Patients will be informed about the trials by their treating chest physician during the evaluation of their baseline assessment. If patients are eligible and show interest in the study, they will receive the patient information letter. Participation will be verified through a phone call at least 1 week later. At the start of the study-related appointment, written informed consent will be obtained and participants will be randomised to either the experimental or the placebo group using a web-based generated

randomisation programme. Randomisation will be stratified for gender<sup>28 38</sup> in both trials and for one or two baseline endurance shuttle walk tests (ESWT) in the BASE-TRAIN trial. This will be explained in more detail in the Primary outcome section.

Participants will be randomised in a 1:1 ratio to BA (SR Carnosyn) or placebo. Allocation to treatment will be concealed. Randomisation and allocation (sealed opaque envelopes) will be performed by independent researchers not related to the current studies. These persons are neither participating in clinical treatment nor processing the study data. At the start of their PR programme, participants will receive a container with the intervention supplements. The containers will be provided with a blinded label, ensuring that the researchers, the PR staff and the participant will not be able to see which intervention supplement is taken. After inclusion, the participant's general practitioner and chest physician will be informed about study participation.

### Outcomes

#### Primary outcome

The primary outcome in both studies is *exercise tolerance*, a functional outcome that is measured as walking endurance time (BASE-TRAIN trial) or cycle endurance time (BASE-ELECTRIC trial).

Walking endurance will be assessed via the ESWT, a standardised externally controlled constant paced field test at 85% of the predetermined maximal velocity (evaluated by the incremental shuttle walk test (ISWT)) until exhaustion.<sup>39</sup> The preferred ESWT duration in this study is below 10 min, which has multiple reasons. First of all, this allows enough room for an additional improvement due to BA supplementation on pulmonary rehabilitation (MID: 174–279 s).<sup>40</sup> Second, because of the hyperbolic power–duration relationship of the constant-load test, the interindividual preintervention ESWT time should be as much homogeneous as possible to better interpret intervention efficacy.<sup>41 42</sup> At last, BA supplementation is proven to be most effective in exercise durations between 0.5 and 10 min.<sup>43</sup> Therefore, patients with an ESWT >10 min perform the ESWT again at a higher speed.

Cycle endurance time will be determined with the constant work-rate cycle test (CWRT), performed on an ergometer at 75% of the maximal work rate (Wmax; determined by a cardiopulmonary exercise test (CPET) performed on a separate day during assessment) to volitional exhaustion (with a maximum of 20 min).<sup>44</sup>

#### Secondary outcomes

Besides the CPET (both trials) and the ISWT (only in the BASE-TRAIN trial) is the exercise capacity measured with the 6 min walking test (6MWT) in the BASE-ELECTRIC trial and will be performed twice at baseline.<sup>45</sup> Isometric and isokinetic quadriceps strength and endurance will be assessed using a computerised dynamometer (Biodex System 4 Pro, Biodex Medical Systems, New York, USA). Isometric quadriceps strength will be determined by three

maximal unilateral isometric knee extensions for 5 s at a knee angle of 60°, interspersed with 15 s of rest and will be defined as highest peak torque (newton-metre, Nm). Furthermore, participants will perform 30 sequential volitional maximal contractions at an angular velocity of 90° per second, while seated upright and the hip joint in 90° of flexion. Isokinetic quadriceps muscle strength will be defined as the highest peak torque (Nm). The isokinetic quadriceps muscle endurance will be defined as the total amount of delivered work (joules) during the set of 30 repetitions.<sup>46</sup> Maximal dynamic isotonic strength, to determine maximal quadriceps strength (leg press), will be assessed by one-repetition maximum using standard (fitness) equipment.<sup>47</sup>

Body composition is measured by bioelectrical impedance analysis<sup>48</sup> (BASE-TRAIN trial) or dual-energy X-ray absorptiometry<sup>49</sup> (BASE-ELECTRIC trial). Physical activity will be measured using activity monitors (BASE-TRAIN: MoveMonitor; BASE-ELECTRIC: Actigraph GT9X), which provide simple, valid and reliable measures of physical activity in patients with COPD.<sup>50</sup> The Short Physical Performance Battery test is designed to measure functional status and physical performance<sup>51</sup> and will be carried out in the BASE-ELECTRIC trial.

Vastus lateralis muscle biopsies will be performed by an experienced physician during two study-related appointments (pre-rehabilitation, at least 1 week before the start of the PR programme; and post-rehabilitation) using standard aseptic technique and local anaesthesia. A side cutting needle muscle biopsy will be passed through an incision to obtain approximately 100 mg of muscle tissue.<sup>52 53</sup> Muscle components of carnosine metabolism (eg, BA, carnosine, taurine and histidine) will be quantified by means of reserved-phase high-performance liquid chromatography.<sup>54</sup> Muscle oxidative stress and inflammation will be assessed via immunoblotting and specific activity kits.<sup>55 56</sup> Fasting venous blood will be assessed for the analysis of systemic components of carnosine metabolism as well as systematic markers of oxidative stress and inflammation at rest.

Two domains of cognitive function<sup>57</sup> will be evaluated: executive functioning will be assessed using the M-WCST and divided attention will be examined via the SCWT.<sup>58 59</sup> Lung inflammatory and oxidative stress biomarkers will be measured (only in the BASE-ELECTRIC trial) using VOCs<sup>60</sup> and FeNO.<sup>61</sup>

The severity of dyspnoea is measured via the modified Medical Research Council dyspnoea scale.<sup>62</sup> Health-related quality of life will be assessed using the COPD Assessment Test.<sup>63</sup> The Hospital Anxiety and Depression Scale will be used as a screening tool to detect clinical signs of anxiety and depression.<sup>64 65</sup> Fatigue severity will be measured by the subjective fatigue subscale of the Checklist Individual Strength (CIS-Fatigue).<sup>66</sup> Problematic activities of daily life will be measured (only in BASE-ELECTRIC study) using the Canadian Occupational Performance Measure.<sup>67</sup> At last, the subjects' compliance on tablet intake, patient safety and possible side effects

are assessed as secondary outcome measures as described in the Intervention section.

### Other outcomes

Additional outcomes are used to characterise patients at baseline. Pulmonary function will be assessed using post-bronchodilator spirometry, whole-body plethysmography and diffusion capacity according to the ATS/ERS guidelines for pulmonary function testing.<sup>68–70</sup> Furthermore, respiratory muscle strength, using maximal inspiratory pressure and maximal expiratory pressure measurements, will be determined.<sup>71</sup> An arterial blood sample is collected to determine arterial blood gases at rest.<sup>72</sup> Additionally, patient characteristics as age, gender, level of education, marital status, smoking status, exacerbation/hospitalisation frequency and medical history (Charlson Comorbidity Index),<sup>73</sup> medication and/or oxygen use will be assessed at baseline during an intake interview.

Table 2 provides an overview of the measurements performed during the several time points of the studies.

### Sample size calculation

Since no preliminary data are available concerning the effects of BA supplementation combined with exercise training on exercise tolerance (walking or cycle endurance time) in an elderly population (with or without chronic disease), a more conservative approach based on the minimal clinically important differences (MCID) of the walking and cycle endurance time, in COPD patients treated in PR, is used.

In the BASE-TRAIN trial, a total of 154 patients is needed to detect a MCID of 177 s<sup>40 74</sup> in walking endurance time with an estimated drop-out rate of 10%, based on recent local unpublished data ( $\alpha=0.05$ ;  $\beta=0.80$ ).

In the BASE-ELECTRIC trial, a total of 68 patients is needed to detect a MCID of 105 s<sup>75</sup> in cycle endurance time with an estimated dropout rate of 25%, based on the study of Sillen *et al* ( $\alpha=0.05$ ;  $\beta=0.90$ ).<sup>6</sup>

Furthermore, a sample size calculation for the required muscle biopsies is included. Based on the unpublished findings of De Brandt *et al* (NCT02770417; effect size: 1.39), a group size of  $n=15$  is required for each group in each trial, including an estimated dropout rate of 25% ( $\alpha=0.05$ ;  $\beta=0.90$ ). Therefore, the aim will be to perform muscle biopsies in 30 patients with COPD (15 for the BA group and 15 for the placebo group) in each trial.

### Data management and statistical analyses

In order to minimise missing data, only essential information will be collected at each visit, user-friendly case-report forms have been developed, and the majority of the tests and questionnaires will be performed in the presence of a researcher. The study variables will be tested for normality. For all data, point measures and measures of variability will be provided. Demographic variables (such as age, sex, educational level, smoking history) will be compared between patients in the BA and placebo group, using unpaired t-test or Mann-Whitney U

**Table 2** Overview outcome measurements

	Baseline assessment	Additional study appointment 1	PR programme	Post-PR assessment	Additional study appointment 2
<b>Patient characteristics</b>					
Demographics, including age, gender, marital status	X				
Medical history: Charlson Comorbidity Index	X				
COPD history: number of exacerbations and hospital admissions	X				
Smoking history and behaviour	X				
Current medication	X				
Use of long-term oxygen therapy	X				
<b>Safety reporting</b>					
Exacerbations			X		
Hospitalisations			X		
Side effects			X		
<b>Exercise capacity and exercise tolerance</b>					
Cardiopulmonary exercise test	X				
Constant work rate cycle test	X			X	
Incremental shuttle walk test	X*				
Endurance shuttle walk test	X*			X*	
6 min walk test	X†			X†	
<b>Body composition</b>					
Body mass index	X			X	
Bioelectrical impedance analysis	X*			X*	
Dual-energy X-ray absorptiometry scan	X†			X†	
<b>Muscle function</b>					
Quadriceps muscle strength (computerised dynamometer)	X			X	
Quadriceps muscle endurance (computerised dynamometer)	X			X	
One-repetition maximum	X			X	
<b>Muscle characteristics (muscle biopsy)</b>					
Components of carnosine metabolism		X			X
Muscle inflammation		X			X
Muscle oxidative stress		X			X
<b>Pulmonary function</b>					
Spirometry/flow volume	X			X	
Whole-body plethysmography	X				
Diffusion capacity	X				
Maximal inspiratory and expiratory pressure	X			X	
Arterial blood gases, including PaO <sub>2</sub> and PaCO <sub>2</sub>	X				
<b>Systemic factors</b>					
Systemic components of carnosine metabolism		X			X
Systemic markers of oxidative stress		X			X
Systemic markers of inflammation		X			X
<b>Physical activity</b>					
MoveMonitor/Actigraph measurement	X			X	
<b>Questionnaires</b>					
Dyspnoea: Modified Medical Research Council	X			X	
Health-related quality of life: COPD Assessment Test	X			X	

Continued



Table 2 Continued

	Baseline assessment	Additional study appointment 1	PR programme	Post-PR assessment	Additional study appointment 2
Mood status: Hospital Anxiety and Depression scale	X			X	
Fatigue: Checklist Individual Strength (CIS-Fatigue)	X			X	
Evaluation muscle biopsy: feedback form		X			X
Canadian Occupational Performance Measurement (COPM)	X†			X†	
<b>Functional mobility</b>					
Short Physical Performance Battery	X†			X†	
<b>Cognitive function</b>					
Modified Wisconsin Card Sorting Test		X			X
Stroop Colour-Word Test\		X			X
<b>Lung inflammation and oxidative stress</b>					
Volatile organic compounds measurement		X†			X†
Fractional exhaled nitric oxide measurement		X†			X†
<b>Compliance</b>					
Patient diary					X
Counting remaining tablets					X

\*Exclusively performed in BASE-TRAIN trial.

†Exclusively performed in BASE-ELECTRIC trial.

test, as appropriate, for continuous variables and  $\chi^2$  tests for categorical variables.

To evaluate the changes in exercise tolerance, lower-limb muscle function, muscle carnosine, muscle and systemic BA, taurine and histidine, muscle, systemic and pulmonary markers for inflammation and oxidative stress and cognitive function, a 2×2 general linear model repeated measures analysis of variance (ANOVA) will be performed, with ‘supplementation group’ (BA, PL) as between-subjects factor and ‘time’ (PRE vs POST) as a within-subjects factor. If, however, the assumptions of repeated measures ANOVA are not met, POST–PRE deltas ( $\Delta$ ) will be calculated, and exercise tolerance changes between groups will be analysed using an unpaired t-test (non-parametrical alternative: Mann-Whitney U test). Differences in changes ( $\Delta$ ) in quality of life, dyspnoea, symptoms of anxiety and depression, problematic activities of daily life and fatigue between groups will be analysed using an unpaired t-test when the data are normally distributed. For skewed data, the Mann-Whitney U test will be performed. Pearson’s or Spearman’s correlation will be calculated between baseline muscle carnosine content and absolute increase in muscle BA/carnosine. Furthermore, correlations will be conducted between relative changes in muscle BA/carnosine content and performance parameters. To counteract the problem of multiple comparisons, the Bonferroni correction will be used. All statistical analyses will be performed using Statistical Package for the Social Sciences (SPSS) v. 25.0. A priori, a two-tailed p value of  $\leq 0.05$  is considered as significant.

### Patient and public involvement

Members of the CIRO client council and patient representatives from Lung Foundation Netherlands are active members of the *BASES consortium* and have been involved in setting up the grant proposal, in reviewing both study designs (particularly the patient informed consent) before submission for ethical approval and in discussing the outcomes assessment. Moreover, this patient advisory board will provide unprecedented insight into improving study feasibility, recruitment and retention, and in understanding patient perceptions and receptivity to current approaches, new practices and possible future (combinations of) therapies. The patient advisory board will also be included in the development of post-trial communication.

### Ethics and dissemination

The studies will be conducted according to the guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013)<sup>76</sup> and in accordance with the Medical Research Involving Human Subjects Act (WMO). Written informed consent will be obtained from all participants before participation. Both trials were approved by CMO Regio Arnhem-Nijmegen, The Netherlands (NL70781.091.19. and NL68757.091.19) and are registered at <https://www.trialregister.nl/>. The results will be submitted for publication in peer-reviewed journals and will be presented at (inter)national conferences. If desired, participants will be informed about the results of the studies.



## DISCUSSION

### Strengths

To date, most studies that examined the effects of BA supplementation have focused on improving exercise tolerance in athletes.<sup>14 24 77</sup> However, results of BA supplementation in the elderly population suggest that there is rationale for further research in this area.<sup>20 21 78</sup>

To the best of our knowledge, our planned studies will be the first in which BA supplementation is combined with a supervised exercise intervention in patients with COPD. A major strength of the present studies is the fact that these are methodologically rigorous randomised controlled trials using a computer-generated randomisation scheme and strict allocation concealment with accurate sample size calculation. This will provide a valid indication of the effectiveness of BA supplementation in patients with COPD receiving PR. In addition, the use of a responsive outcome measure for exercise tolerance will increase the likelihood of being able to assess whether there are any benefits in adding BA supplementation to PR in patients with COPD. In the current studies, we specifically focus on patients with COPD with explicit functional limitations and high symptom burden attending PR. The strict inclusion criteria are another strength of both trials, as patients with severe COPD are expected to benefit the most from BA supplementation.

Furthermore, we optimised both the dosage and the duration of supplementation in order to increase the muscle carnosine response to BA supplementation.<sup>79</sup> In addition, 3.2g of BA per day for a duration of 8–10 weeks will result in a cumulative dose of 179–224g of BA, which will lead to significantly augmented muscle carnosine concentrations (>40%).<sup>80</sup> The protocols also embody other factors that can lead to greater muscle carnosine gains, such as supplement formulation (slow-release formula), ingestion timing in relation to meals (coingestion with breakfast and lunch) and exercise (NLPE or NMES training programme as part of PR).<sup>79</sup> Moreover, the trials examine the mechanism of carnosine loading in a diseased population by analysing levels of carnosine and related dipeptides in blood samples and biopsy samples of the vastus lateralis muscle. This way, we aim to identify relevant factors that explain variances in muscle carnosine loading and try to elucidate carnosine's role as a potential antioxidant. And last, the structure of the *BASES consortium*, including a patient advisory board, a core project team and a steering committee, allows us to enhance the research capacity, to share (academic) resources, to disseminate study results and to accelerate future research.

### Methodological considerations

First, limited funding makes it difficult to set up long-term follow-up, although analysing the sustainability of outcomes would certainly be worthwhile. As such, long-term benefits will not be investigated. Second, despite our efforts to increase compliance through inpatient

follow-up during the PR programme, adherence to the prescribed supplementation protocol is only monitored by asking participants about their intake using a diary and by documenting the remaining tablets at the end of the supplementation period. Therefore, it is possible that the actual compliance is different and forgotten tablets are discarded. However, this is comparable with daily practice as patients may behave in the same manner. Furthermore, the actual efficacy of BA supplementation is monitored by means of quantifying muscle carnosine levels through muscle biopsies of the vastus lateralis muscle.

Third, all patients start with a preplanned NLPE protocol in the BASE-TRAIN trial. However, individual protocol adaptations are possible based on patients' responses during training as it is part of usual care. This might result in (minor) differences in exercise training programme between the BA and placebo group. As we deliver patient-tailored care with the purpose to generate an optimal result for each patient, which depends on the patient's abilities, needs and goals and is therefore patient-specific, we will not be able to apply one single protocol to all patients. Moreover, training parameters as frequency, intensity, duration and number of repetitions will be closely monitored during the study. This will allow additional post hoc analyses to evaluate the effect of potential differences in exercise training programme that arise throughout the study. Furthermore, we acknowledge the fact that the protein-rich supplement, given after each training session as part of standard care, could potentially reduce the between-group difference regarding the primary outcome (exercise tolerance), since additional protein intake could enhance training benefits in the control group. In this way, however, the potential risk of a BA-induced decline in circulating and/or muscle histidine levels<sup>81</sup> is minimised by additional intake of protein (post-training) in these patients with COPD.

### Clinical implications

The BASE-TRAIN and BASE-ELECTRIC studies will examine whether and to what extent BA supplementation can augment the exercise tolerance outcomes of PR. Improving exercise tolerance is a major therapeutic goal in COPD and there are theoretical reasons for presuming that specific oral nutritional supplements may further enhance the outcomes of exercise training, an intervention that is of proven clinical benefit in patients with COPD.<sup>1 3</sup> Furthermore, the present studies will gain insight in the characteristics of patients that do and do not benefit from BA supplementation and whether these benefits directly mirror changes in intracellular carnosine.

## CONCLUSION

In conclusion, both exercise training and BA supplementation effectively increase exercise tolerance in several populations. The BASE-TRAIN and BASE-ELECTRIC trials will be the first to evaluate the effects of BA

supplementation in addition to exercise training (either NLPE or NMES) in patients with COPD. In this article, the study rationale and protocols of both trials are described, and a preliminary analysis of the possible strengths and limitations is outlined.

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#### REFERENCES

- Maltais F, Decramer M, Casaburi R, *et al*. An official American thoracic Society/European respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014;189:e15–62.
- Skeletal muscle dysfunction in chronic obstructive pulmonary disease. A statement of the American thoracic Society and European respiratory Society. *Am J Respir Crit Care Med* 1999;159:S1–40.
- Spruit MA, Singh SJ, Garvey C, *et al*. An official American thoracic Society/European respiratory Society statement: key concepts and advances in pulmonary rehabilitation. *Am J Respir Crit Care Med* 2013;188:e13–64.
- O'Shea SD, Taylor NF, Paratz JD. Progressive resistance exercise improves muscle strength and may improve elements of performance of daily activities for people with COPD: a systematic review. *Chest* 2009;136:1269–83.
- Klijn P, van Keimpema A, Legemaat M, *et al*. Nonlinear exercise training in advanced chronic obstructive pulmonary disease is superior to traditional exercise training. A randomized trial. *Am J Respir Crit Care Med* 2013;188:193–200.
- Sillen MJH, Franssen FME, Delbressine JML, *et al*. Efficacy of lower-limb muscle training modalities in severely dyspnoeic individuals with COPD and quadriceps muscle weakness: results from the DICES trial. *Thorax* 2014;69:525–31.
- De Brandt J, Spruit MA, Hansen D, *et al*. Changes in lower limb muscle function and muscle mass following exercise-based interventions in patients with chronic obstructive pulmonary disease: a review of the English-language literature. *Chron Respir Dis* 2018;15:182–219.
- Derave W, Everaert I, Beeckman S, *et al*. Muscle carnitine metabolism and beta-alanine supplementation in relation to exercise and training. *Sports Med* 2010;40:247–63.
- Harris RC, Tallon MJ, Dunnett M, *et al*. The absorption of orally supplied beta-alanine and its effect on muscle carnitine synthesis in human vastus lateralis. *Amino Acids* 2006;30:279–89.
- Mannion AF, Jakeman PM, Dunnett M, *et al*. Carnitine and anserine concentrations in the quadriceps femoris muscle of healthy humans. *Eur J Appl Physiol Occup Physiol* 1992;64:47–50.
- Dutka TL, Lamboley CR, McKenna MJ, *et al*. Effects of carnitine on contractile apparatus Ca<sup>2+</sup> sensitivity and sarcoplasmic reticulum Ca<sup>2+</sup> release in human skeletal muscle fibers. *J Appl Physiol* 2012;112:728–36.
- Hoetker D, Chung W, Zhang D, *et al*. Exercise alters and β-alanine combined with exercise augments histidyl dipeptide levels and scavenges lipid peroxidation products in human skeletal muscle. *J Appl Physiol* 2018;115:1152/jappphysiol.00007.2018. [Epub ahead of print: 18 Oct 2018].
- Antonini FM, Petrucci E, Pinzani P, *et al*. The meat in the diet of aged subjects and the antioxidant effects of carnitine. *Arch Gerontol Geriatr Suppl* 2002;8:7–14.
- Hill CA, Harris RC, Kim HJ, *et al*. Influence of beta-alanine supplementation on skeletal muscle carnitine concentrations and high intensity cycling capacity. *Amino Acids* 2007;32:225–33.
- Gosker HR, Zeegers MP, Wouters EFM, *et al*. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. *Thorax* 2007;62:944–9.
- De Brandt J *et al*. Late Breaking Abstract - Muscle carnitine in patients with COPD in comparison to age- and gender matched healthy controls: a cross-sectional study. *Eur Respir J* 2019;54:PA1203.

- 17 Fermoselle C, Rabinovich R, Ausín P, *et al.* Does oxidative stress modulate limb muscle atrophy in severe COPD patients? *Eur Respir J* 2012;40:851–62.
- 18 Dolan E, Swinton PA, Painelli VdeS, *et al.* A systematic risk assessment and meta-analysis on the use of oral  $\beta$ -alanine supplementation. *Adv Nutr* 2019;10:452–63.
- 19 Trexler ET, Smith-Ryan AE, Stout JR, *et al.* International Society of sports nutrition position stand: beta-alanine. *J Int Soc Sports Nutr* 2015;12:30.
- 20 del Favero S, Roschel H, Solis MY, *et al.* Beta-alanine (Carnosyn™) supplementation in elderly subjects (60–80 years): effects on muscle carnosine content and physical capacity. *Amino Acids* 2012;43:49–56.
- 21 Stout JR, Graves BS, Smith AE, *et al.* The effect of beta-alanine supplementation on neuromuscular fatigue in elderly (55–92 years): a double-blind randomized study. *J Int Soc Sports Nutr* 2008;5:21.
- 22 Bex T, Chung W, Baguet A, *et al.* Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles. *J Appl Physiol* 2014;116:204–9.
- 23 Baguet A, Koppo K, Pottier A, *et al.* Beta-Alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. *Eur J Appl Physiol* 2010;108:495–503.
- 24 Derave W, Ozdemir MS, Harris RC, *et al.* Beta-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *J Appl Physiol* 2007;103:1736–43.
- 25 Kamal MA, Jiang H, Hu Y, *et al.* Influence of genetic knockout of PEPT2 on the in vivo disposition of endogenous and exogenous carnosine in wild-type and PEPT2 null mice. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R986–91.
- 26 Yeum K-J, Orioli M, Regazzoni L, *et al.* Profiling histidine dipeptides in plasma and urine after ingesting beef, chicken or chicken broth in humans. *Amino Acids* 2010;38:847–58.
- 27 Hoffman JR, Ostfeld I, Stout JR, *et al.*  $\beta$ -Alanine supplemented diets enhance behavioral resilience to stress exposure in an animal model of PTSD. *Amino Acids* 2015;47:1247–57.
- 28 Everaert I, Mooyaart A, Baguet A, *et al.* Vegetarianism, female gender and increasing age, but not CNBP1 genotype, are associated with reduced muscle carnosine levels in humans. *Amino Acids* 2011;40:1221–9.
- 29 Baguet A, Reyngoudt H, Pottier A, *et al.* Carnosine loading and washout in human skeletal muscles. *J Appl Physiol* 2009;106:837–42.
- 30 Pfenninger JL, Fowler GC. *Pfenninger and Fowler's Procedures for Primary Care*. Elsevier Health Sciences, 2010.
- 31 Stegen S, Blanquaert L, Everaert I, *et al.* Meal and beta-alanine coingestion enhances muscle carnosine loading. *Med Sci Sports Exerc* 2013;45:1478–85.
- 32 Stuurgroep. ondervoeding. Richtlijn ondervoeding, 2019. Available: <https://www.stuurgroepondervoeding.nl/wp-content/uploads/2019/02/SoV01-Richtlijn-Ondervoeding-losse-paginas-210x297februari-2019.pdf> [Accessed 16 May 2019].
- 33 American College of Sports, M., American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2009;41:687–708.
- 34 Klijn P. Nonlinear periodized exercise training in Pulmonary rehabilitation: the devil is in the details! 2020 22-07-2020]. Available: <https://www.thoracic.org/members/assemblies/assemblies/pr/quarterly-bite/nonlinear-periodized-exercise-training-in-pulmonary-rehabilitation-the-devil-is-in-the-details.php>
- 35 Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377–81.
- 36 Maffiuletti NA. Physiological and methodological considerations for the use of neuromuscular electrical stimulation. *Eur J Appl Physiol* 2010;110:223–34.
- 37 Sillen MJH, Franssen FME, Vaes AW, *et al.* Metabolic load during strength training or NMES in individuals with COPD: results from the DICES trial. *BMC Pulm Med* 2014;14:146.
- 38 Maury J, Gouzi F, De Rigal P, *et al.* Heterogeneity of systemic oxidative stress profiles in COPD: a potential role of gender. *Oxid Med Cell Longev* 2015;2015:1–11.
- 39 Holland AE, Spruit MA, Singh SJ. How to carry out a field walking test in chronic respiratory disease. *Breathe* 2015;11:128–39.
- 40 Zatloukal J, Ward S, Houchen-Woloff L, *et al.* The minimal important difference for the endurance shuttle walk test in individuals with chronic obstructive pulmonary disease following a course of pulmonary rehabilitation. *Chron Respir Dis* 2019;16:1–7.
- 41 Whipp BJ, Ward SA. Quantifying intervention-related improvements in exercise tolerance. *Eur Respir J* 2009;33:1254–60.
- 42 Vivodtzev I, Gagnon P, Pepin V, *et al.* Physiological correlates of endurance time variability during constant-workrate cycling exercise in patients with COPD. *PLoS One* 2011;6:e17007.
- 43 Saunders B, Elliott-Sale K, Artioli GG, *et al.*  $\beta$ -alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med* 2017;51:658–69.
- 44 van 't Hul A, Gosselink R, Kwakkel G. Constant-Load cycle endurance performance: test-retest reliability and validity in patients with COPD. *J Cardiopulm Rehabil* 2003;23:143–50.
- 45 Holland AE, Spruit MA, Troosters T, *et al.* An official European respiratory Society/American thoracic Society technical standard: field walking tests in chronic respiratory disease. *Eur Respir J* 2014;44:1428–46.
- 46 Mathur S, Makrides L, Hernandez P. Test-Retest reliability of isometric and isokinetic torque in patients with chronic obstructive pulmonary disease. *Physiother Can* 2004;56:94–101.
- 47 Spruit MA, Gosselink R, Troosters T, *et al.* Resistance versus endurance training in patients with COPD and peripheral muscle weakness. *Eur Respir J* 2002;19:1072–8.
- 48 Moon JR, Stout JR, Smith-Ryan AE, *et al.* Tracking fat-free mass changes in elderly men and women using single-frequency bioimpedance and dual-energy X-ray absorptiometry: a four-compartment model comparison. *Eur J Clin Nutr* 2013;67 Suppl 1:S40–6.
- 49 Graat-Verboom L, Smeenk FWJM, van den Borne BEEM, *et al.* Progression of osteoporosis in patients with COPD: a 3-year follow up study. *Respir Med* 2012;106:861–70.
- 50 Gimeno-Santos E, Raste Y, Demeyer H, *et al.* The proactive instruments to measure physical activity in patients with chronic obstructive pulmonary disease. *Eur Respir J* 2015;46:988–1000.
- 51 Guralnik JM, Ferrucci L, Simonsick EM, *et al.* Lower-Extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med* 1995;332:556–62.
- 52 Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 1975;35:609–16.
- 53 Hayot M, Michaud A, Koechlin C, *et al.* Skeletal muscle micro biopsy: a validation study of a minimally invasive technique. *Eur Respir J* 2005;25:431–40.
- 54 Stegen S, Everaert I, Deldicque L, *et al.* Muscle histidine-containing dipeptides are elevated by glucose intolerance in both rodents and men. *PLoS One* 2015;10:e0121062.
- 55 Pomiès P, Rodriguez J, Blaquière M, *et al.* Reduced myotube diameter, atrophic signalling and elevated oxidative stress in cultured satellite cells from COPD patients. *J Cell Mol Med* 2015;19:175–86.
- 56 Pomiès P, Blaquière M, Maury J, *et al.* Involvement of the FoxO1/MuRF1/Atrogin-1 signaling pathway in the oxidative stress-induced atrophy of cultured chronic obstructive pulmonary disease myotubes. *PLoS One* 2016;11:e0160092.
- 57 Sachdev PS, Blacker D, Blazer DG, *et al.* Classifying neurocognitive disorders: the DSM-5 approach. *Nat Rev Neurol* 2014;10:634–42.
- 58 Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol* 1935;18:643–62.
- 59 Van der Elst W, Van Boxtel MPJ, Van Breukelen GJP, *et al.* The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006;13:62–79.
- 60 Schleich FN, Zanella D, Stefanuto P-H, *et al.* Exhaled volatile organic compounds are able to discriminate between neutrophilic and eosinophilic asthma. *Am J Respir Crit Care Med* 2019;200:444–53.
- 61 American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–30.
- 62 Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest* 1988;93:580–6.
- 63 Jones PW, Harding G, Berry P, *et al.* Development and first validation of the COPD assessment test. *Eur Respir J* 2009;34:648–54.
- 64 Snaith RP, Zigmond AS. The hospital anxiety and depression scale. *Br Med J* 1986;292:344.
- 65 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70.
- 66 Peters JB, Heijdra YF, Daudey L, *et al.* Course of normal and abnormal fatigue in patients with chronic obstructive pulmonary disease, and its relationship with domains of health status. *Patient Educ Couns* 2011;85:281–5.





- 67 Spruit MA, Augustin IML, Vanfleteren LE, *et al.* Differential response to pulmonary rehabilitation in COPD: multidimensional profiling. *Eur Respir J* 2015;46:1625–35.
- 68 Macintyre N, Crapo RO, Viegi G, *et al.* Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720–35.
- 69 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
- 70 Wanger J, Clausen JL, Coates A, *et al.* Standardisation of the measurement of lung volumes. *Eur Respir J* 2005;26:511–22.
- 71 American Thoracic Society/European Respiratory Society. ATS/ERS statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002;166:518–624.
- 72 WHO. Who guidelines on drawing blood: best practices in phlebotomy 2010. Available: <https://www.ncbi.nlm.nih.gov/pubmed/23741774>
- 73 Charlson ME, Pompei P, Ales KL, *et al.* A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;40:373–83.
- 74 Zatloukal J *et al.* Clinical significance for ESWT in COPD after a course of pulmonary rehabilitation. *Eur Respir J* 2015;46.
- 75 Laviolette L, Bourbeau J, Bernard S, *et al.* Assessing the impact of pulmonary rehabilitation on functional status in COPD. *Thorax* 2008;63:115–21.
- 76 World Medical Association. World Medical association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–4.
- 77 Hoffman J, Ratamess N, Kang J, *et al.* Effect of creatine and beta-alanine supplementation on performance and endocrine responses in strength/power athletes. *Int J Sport Nutr Exerc Metab* 2006;16:430–46.
- 78 McCormack WP, Stout JR, Emerson NS, *et al.* Oral nutritional supplement fortified with beta-alanine improves physical working capacity in older adults: a randomized, placebo-controlled study. *Exp Gerontol* 2013;48:933–9.
- 79 Perim P, Marticorena FM, Ribeiro F, *et al.* Can the skeletal muscle carnosine response to beta-alanine supplementation be optimized? *Front Nutr* 2019;6:135.
- 80 Stellingwerff T, Decombaz J, Harris RC, *et al.* Optimizing human in vivo dosing and delivery of  $\beta$ -alanine supplements for muscle carnosine synthesis. *Amino Acids* 2012;43:57–65.
- 81 Blancquaert L, Everaert I, Missinne M, *et al.* Effects of histidine and  $\beta$ -alanine supplementation on human muscle carnosine storage. *Med Sci Sports Exerc* 2017;49:602–9.