BMJ Open

The effect of targeted nutrient supplementation on physical activity and health related quality of life in COPD: study protocol for the randomized controlled NUTRECOVER trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-059252
Article Type:	Protocol
Date Submitted by the Author:	16-Nov-2021
Complete List of Authors:	Beijers, Rosanne; Maastricht University Medical Centre+, Department of Respiratory Medicine van Iersel, Lieke E.J.; Maastricht University Medical Centre+, Department of Respiratory Medicine Hageman, Robert J.J.; Danone Nutricia Research van Helvoort, Ardy; Maastricht University Medical Centre+, Department of Respiratory Medicine; Danone Nutricia Research Schuurman, Lisanne; Maastricht University Medical Centre+, Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism Simons, Sami; Maastricht University Medical Centre+, Respiratory Medicine Gosker, Harry; Maastricht University Medical Centre+, Department of Respiratory Medicine Schols, Annemie; Maastricht University Medical Centre+, Department of Respiratory Medicine
Keywords:	RESPIRATORY MEDICINE (see Thoracic Medicine), NUTRITION & DIETETICS, Chronic airways disease < THORACIC MEDICINE

The effect of targeted nutrient supplementation on physical activity and health related quality of life in COPD: study protocol for the randomized controlled NUTRECOVER trial

Rosanne J.H.C.H. Beijers[1], Lieke E.J. van Iersel[1], Lisanne T. Schuurman[1], Robert J.J. Hageman[2], Sami O. Simons[1], Ardy van Helvoort[1,2], Harry R. Gosker[1], Annemie M.W.J. Schols[1]

[1] Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, The Netherlands.

[2] Danone Nutricia Research, Utrecht, The Netherlands.

Corresponding author: Rosanne J.H.C.G. Beijers

P.O. Box 5800

6202 AZ Maastricht, the Netherlands

Tel: 0031 43 3882990

Fax: 0031 43 3875051

E-mail: r.beijers@maastrichtuniversity.nl

Abstract

Introduction: Physical and mental health is often affected in COPD adversely affecting disease course and quality of life. Abnormalities in whole body and cellular energy metabolism, dietary and plasma nutrient status, and intestinal permeability have been well established in these patients as systemic determinants of functional decline and underexplored treatable traits. The aim of this study is to investigate the efficacy of one-year targeted nutrient supplementation on daily physical activity level and health related quality of life in patients with COPD.

Methods and analysis: This study is a randomized, placebo-controlled, double-blind trial in 166 patients with COPD. The intervention group will receive a multi-nutrient supplement, including vitamin D, tryptophan, long-chain polyunsaturated fatty acids, and prebiotic dietary fibers as main components (94 kCal per daily dose). The control group will receive an isocaloric isonitrogenous placebo. Both groups will ingest one portion per day for at least 12 months. In addition, both groups will receive counselling on healthy lifestyle and medical adherence over the course of the study. Co-primary outcomes are physical activity assessed by triaxial accelerometry and health related quality of life measured by the EuroQol-5D questionnaire. Secondary outcomes are cognitive function, psychological wellbeing, physical performance, patient-reported outcomes, and the metabolic profile assessed by body composition, systemic inflammation, plasma nutrient levels, intestinal integrity and microbiome composition. Outcomes will be measured at baseline and after 12 months of supplementation. In case patients are hospitalized for a COPD exacerbation, a subset outcome panel will additionally be measured during a 4-week recovery period after hospitalization in order to investigate the relative effect of targeted nutrient supplementation on the recovery from this hospitalization.

Ethics and dissemination: This study was approved by the local Ethics Committee of Maastricht University (NL66543.068.18/METC18-011). Study outcomes will be disseminated through presentations at national and international conferences and through peer-reviewed journals.

Trial registration number: clinicaltrials.gov: NCT03807310

Word count: 3921

Article summary:

Strengths and limitations of this study:

Strengths

- A double-blind, randomized, placebo-controlled clinical trial investigating the effect of a novel nutritional intervention product relative to placebo on objective and subjective health status in patients with COPD.
- The study design resembles a real-life setting.
- Next to investigating overall efficacy, the study design allows additional insight in the relative efficacy of the nutritional intervention in clinically stable disease as well as in response to acute disease flare-ups.
- The study product is a newly developed nutritional supplement targeting nutrient status and metabolic alterations in COPD.

Limitation

- The study population targets patients with moderate to very severe COPD; inclusion, long-term compliance and outcome assessment may be challenging.

Keywords: COPD, nutritional supplementation, lifestyle, exacerbations

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow limitation resulting from loss and remodeling of the airways (1). During the course of the disease, patients with COPD experience periods of acute worsening of respiratory symptoms called exacerbations. These exacerbations are often triggered by respiratory infections due to bacteria or viruses. During severe COPD exacerbations (requiring hospitalization) disease-related detrimental factors such as systemic inflammation, hypoxia, physical inactivity, malnutrition and treatment with glucocorticoids and antibiotics, converge and intensify (2). Previous exacerbations and deteriorating airflow limitation are associated with an increasing prevalence of new exacerbations (3, 4). Patients with frequent exacerbations constitute a specific disease phenotype with a worse prognosis, specifically a faster decline in lung function and muscle function, a greater worsening of health status and a substantial reduction in daily physical activity (5-8).

Symptoms of dyspnea and fatigue in patients with COPD often affect their lifestyle. Alongside with disease exacerbations and malnutrition, a sedentary lifestyle contributes to extrapulmonary manifestations including depression, cognitive decline, and loss of muscle mass and function (9-12), causing a vicious cycle towards physical inactivity. Overall high percentages of impaired cognitive function (25%) and depression (27%) have been observed in patients with COPD (13-15) and are negatively influenced by chronic stress and systemic inflammation (16, 17). The risk of developing cognitive dysfunction or depression is even further increased in advanced disease stages (18, 19). Skeletal muscle weakness is another well-established extrapulmonary manifestation of COPD resulting from loss of muscle mass and a decreased muscle oxidative phenotype (20). The latter is characterized by a muscle fiber type shift from oxidative type I towards more glycolytic type II fibers and decreased expression of oxidative enzymes and regulatory proteins (e.g. peroxisome proliferator-activated receptors (PPARs) and their coactivator 1α (PGC-1α)) (21), which render the skeletal muscle more susceptible to fatigue (9, 10). Systemic inflammation and physical inactivity have also been identified as determinants of muscle mass

loss in COPD (21-23). During an exacerbation, enhanced systemic inflammation and physical inactivity lead to acute muscle wasting, which may not be fully restored during recovery as there is evidence for an impaired regenerative potential in these patients (8). Disturbances of intestinal integrity manifested by increased gastro-intestinal (GI) permeability and alterations in intestinal fatty acid binding protein (IFABP) have also been reported in COPD (24). These disturbances are not only observed in stable COPD, but also during hospitalization for a COPD exacerbation (25). Moreover, several factors associated with COPD, including age, gender, smoking, use of corticosteroids and antibiotics, BMI and diet, are known to cause dysbiosis in the gut microbiome (26). However, up till now no published studies of the COPD gut microbiome are available.

The extrapulmonary manifestations in COPD are linked by the cross-talk between the gut-muscle-brain axis which includes the vagus nerve, gut hormone signaling, the immune system, serotonin and tryptophan metabolism and microbial metabolites such as short chain fatty acids (27). All routes might be disturbed in patients with COPD, but especially serotonin and tryptophan metabolism has recently been shown to be impaired in patients with COPD leading to unfavorable higher kynurenine/tryptophan ratios in the circulation (28, 29). By modulating tryptophan availability, the microbiota can regulate the kynurenine pathway decreasing plasma kynurenine levels. In muscle, kynurenine can also be metabolized and cleared by kynurenine aminotransferases that are regulated by PGC-1α. Interestingly, a recent study of our group showed reduced expression levels of these aminotransferases in skeletal muscle of patients with COPD, which might explain the elevated plasma kynurenine levels (29). Impairments in the kynurenine pathway have been linked to anxiety, depression and cognitive decline (30).

Impairments in the cross-talk between these extra-pulmonary organs could adversely affect daily physical activity level and quality of life (31), accelerating the vicious cycle towards physical inactivity and deterioration of physical and mental status. Interventions facilitating a more physically active lifestyle in patients with COPD are therefore needed. Current pulmonary rehabilitation strategies mainly focus on

improving physical performance by exercise training and counselling targeting respiratory and skeletal muscle function (32), but behavioral translation of improved physical performance to a more active lifestyle in COPD has been disappointing (33-36). Therefore, additional interventions need to be investigated.

Nutritional supplementation targeting impairments of skeletal muscle and gut, by preventing or restoring disease-specific deficiencies in COPD patients and modulation of the function of the gut-brain axis, might be beneficial in patients with COPD. As systematically summarized in **Table 1**, omega-3 polyunsaturated fatty acids (N-3 PUFAs), tryptophan, vitamin D, and nutritional fibers are particularly interesting nutritional components to explore. Intake of these components is generally low in patients with COPD, and we hypothesize that supplementation of these nutrients has positive effects on different pathways of the gut-muscle-brain axis which may lead to increased physical activity levels and improved health status. To minimize risk of other nutritional deficiencies and to ensure presence of essential co-factors, supportive micronutrients are provided together with these core ingredients in the supplement.

We hypothesize that this newly developed multi-nutrient product facilitates maintenance of a more active lifestyle in patients with COPD and improves objective and subjective physical and mental health status relative to placebo. Therefore, the primary aim of this study is to investigate the effect of one-year targeted nutrient supplementation compared to placebo supplementation on daily physical activity level as well as general health status in patients with COPD. Secondary aims are to investigate the effect of targeted nutrient supplementation on potential mediators; cognitive function, psychological wellbeing, physical performance, patient-reported outcomes and the metabolic profile assessed by body composition, systemic inflammation, plasma nutrient levels, intestinal integrity and microbiome composition in patients with COPD.

Table 1. Rationale for the core components to be included in the multimodal nutritional intervention

Component	Proposed effects and underlying mechanisms	Intake, status and supplementation in COPD
N-3 PUFAs	*Proposed effect:* Lowers the risk on depression, improves cognitive function, muscle function and muscle mass as well as mitochondrial function (37-40). *Possible underlying mechanisms:* - Substrate inhibition of pro-inflammatory prostaglandin E2 (41) and 4 series leukotrienes EPA and DHA give rise to resolvins and lipid related signaling molecules such as protectins (42). - N-3 PUFAs are natural ligands for PPAR gamma that attenuates NF-kB activation and inflammatory gene expression and NF-kB activation reducing systematic inflammation (43, 44). - Altering membrane lipid composition (45) and increasing membrane fluidity which facilitate the activation of receptors e.g. adenosine A2A and dopamine D2 receptors in the brain (46). - Decreasing Indoleamine-2,3-Dioxigenase expression and increasing hippocampal serotonin, a neurotransmitter in the central and enteric nervous system (47); - Stimulating skeletal muscle anabolism via the Akt-mTOR-p70S6K pathway (48); - Stimulating muscle oxidative metabolism and boosting mitochondrial function (40).	*Intake and status in COPD:* • Dietary intake of PUFAs is generally low (49-51); • High intake is positively associated with lung function (52). • Blood levels decrease in tune with disease progression in COPD patients (53). *Supplementation in COPD:* • As an adjunct to exercise training, PUFAs significantly enhanced improvement in exercise performance and physical activity level in COPD in an 8-week and 4-months placebo-controlled RCT respectively (36, 54).
Tryptophan	*Proposed effect*: Lowers the risk for depression, improves mood, behavior, and cognitive function (55-57). *Possible underlying mechanisms:* - Being a precursor of serotonin (55-57); - Being a precursor for melatonin which may improve sleep quality which can improve functional capacity, skeletal muscle strength, cognitive function and general QoL in COPD (58, 59); - Being a precursor for niacin (vitamin B3) which for example is essential for mitochondrial function (60).	*Intake and status in COPD:* • Plasma tryptophan levels are decreased during acute exacerbations (61); • Circulating KYN and KYN/tryptophan ratios are elevated compared to controls and is associated with disease severity (28), KYN clearance might be reduced in skeletal muscle tissue of patients with COPD due to reduced PPAR/PGC-1α-mediated KAT expression (29).

		Supplementation in COPD: Not available
Vitamin D	*Proposed effect:* Low plasma vitamin D concentrations are associated with depressive symptoms and low muscle function (62-64). *Possible underlying mechanisms:* - An increased region-specific expression of vitamin D receptors in brain areas play a key role in mood regulation (65); - Has anti-inflammatory effects leading to neuroprotective properties (66, 67); - Maintaining a normal calcium and phosphorus balance in skeletal muscle; low vitamin D levels reduce calcium reuptake into sarcoplasmic reticulum, impairing muscle function (62). - Improving mitochondrial function, dynamics and enzyme function in skeletal muscle (63).	*Intake and status in COPD:* • Vitamin D levels are low and deficiency is highly prevalent (68, 69). • Deficiency is associated with COPD severity, osteoporosis, depression and lower muscle strength (70-74). *Supplementation in COPD:* • Significantly improves inspiratory muscle strength, maximal oxygen uptake and QoL in patients with COPD in a 3-months and 8-weeks placebo-controlled RCT (75, 76); • Supplementation reduces number of acute exacerbations in COPD patients with low vitamin D plasma levels as shown in two meta-analyses (77, 78).
Prebiotic fibers	*Proposed effect*: Improve gut function (79-81). *Possible underlying mechanisms:* - Fibers are substrate for intestinal microbes and support their growth (80); - Can be converted into SCFA that support gastro-intestinal integrity and fuel coloncytes (81); - Change gut microbial composition towards a less inflammatory profile (79). - A mixture of prebiotic fiber may prevent alveolar wall destruction, right ventricle hypertrophy and neutrophil infiltration into the lungs after LPS instillation in mice (82).	*Intake and status in COPD:* • Dietary intake is generally low (49); • Low fiber intake is associated with reduced measures of lung function (83) and high intake with a 30% lower risk of COPD (84); • Dietary intake has an inverse relationship with poor lung function and COPD risk (85). *Supplementation in COPD:* Not available

Abbreviations: COPD, chronic obstructive pulmonary disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; KAT, kynurenine aminotransferase; KYN, kynurenine; LPS, lipopolysaccharide; NF-kB, nuclear factor kappa-light chain enhancer of activated B cells; PPARs, peroxisome proliferator-activated receptors; PUFA, polyunsaturated fatty acids; QoL, quality of life; RCT, randomized controlled trial; SCFA, short-chain fatty acids.

METHODS AND ANALYSIS

Study design

The study design was developed according to the SPIRIT 2013 recommendations (85). In a randomized, placebo-controlled, double-blind clinical trial the active nutritional supplement will be compared to a placebo supplement in patients with COPD. Before, after three months and after 12 months (extended in case of hospitalization in the previous 4 weeks) measurements will be performed (**Figure 1**). A one-year follow-up period is chosen to investigate the long-term effect of nutritional supplementation in a real-life setting during which COPD exacerbations might occur. We will investigate in a post-hoc analysis the relative effect of targeted nutrient supplementation on clinical outcome during a 4-week recovery phase after hospitalization for a COPD exacerbation. First of all, because disturbances in the gut-muscle-brain axis as well as specific nutrient deficiencies might be affected by severe COPD exacerbations. Secondly, because the recovery period after severe COPD exacerbations is a completely neglected phase for nutritional interventions thus far. For this, we will perform a subset panel of outcome measures within one week after discharge and one month later (recovery phase) each time a patient is hospitalized for a COPD exacerbation during the intervention period. In case the last measurement day will take place within the post-exacerbation recovery phase, the intervention period will be lengthened until the end of the recovery phase, with a maximum of 2 months. See figure 1 for these different scenarios. The study has been started in October 2020 and is planned to last 3 years.

Participants

The study population will consist of patients with moderate to very severe, but medically stable, COPD according to the GOLD criteria (1) (see Table 2 for detailed in- and exclusion criteria). Participants will be recruited via respiratory physicians from multiple hospitals in the south-east of The Netherlands or via

advertisements. Potential eligible participants will receive detailed information of the study including the informed consent form. The first participant was enrolled in October 2020.

Table 2. Eligibility criteria

Eligibility criteria	
Inclusion criteria	• Moderate to very severe COPD according to GOLD criteria (i.e. GOLD stage II-IV); • Medically stable (no hospital admission <4 weeks prior to the start of the study and no temporary oral steroid or antibiotics use due to a COPD exacerbation in the last 4 weeks);
Exclusion criteria	• Age <18 years; • Allergy or intolerance to components of the study product; • Other acute or unstable chronic diseases that will compromise the study outcome (e.g. active cancer requiring treatments); • Participation in any other study involving investigational or marketed products concomitantly or within four weeks prior to entry into the study; • Terminal illness; • Lung malignance in the previous 5 years; • Diagnosis of dementia or neurodegenerative disease (e.g. Alzheimer's disease, Parkinson's disease, Huntington's chorea, frontotemporal dementia) in the medical records; • Recent diagnosis of cerebral conditions (<1 year e.g. cerebral infarction, hemorrhage, brain tumors, transient ischemic attack) in the medical records; • Any medical condition that significantly interferes with digestion and/or gastro-intestinal (GI) function (e.g. short bowel syndrome, inflammatory bowel disease, gastric ulcers, gastritis, (gastro)-enteritis, GI cancer) as judged by the investigator.

Trial interventions

Nutritional intervention

One group will receive the targeted nutrient supplement, and one group will receive an isocaloric isonitrogenous placebo for at least 12 months. Both study products will be provided as a flavored powder in labelled sachets that are blinded for both the participants and investigators. Participants will be randomly assigned to one of the intervention groups (see randomization and blinding). Both supplements will be produced and supplied by Danone Nutricia Research, Utrecht, The Netherlands. The active supplement consists of 4 g proteins/amino acids, 4 g fat, 8 g carbohydrates and 6 g fibers, providing 1 g of N-3 long-chain PUFAs, 200 mg tryptophan, 20 µg vitamin D, and 6 g of prebiotic fibers as core components (Table 1). To minimize risk of other nutritional deficiencies and to ensure presence of essential co-factors, supportive micronutrients are provided together with these core ingredients in the supplement. The active supplement includes a daily dose of 94 kCal. The placebo product is isocaloric and isonitrogenous where protein/amino acid levels are compensated with L-alanine as neutral amino acid that is known to not modulate outcome parameters in COPD patients, fish oil is replaced by vegetable oils, and fiber/energy is compensated by maltodextrin. The micronutrients will not be added to the placebo powder. The dose of components are all within the recommendations and safety levels as provided by the European Food and Safety Authority (EFSA), as published in their report of December 2017 (86). Each day, one sachet will be consumed after suspension in 60 mL water or any other drink. In case patients are hospitalized during the intervention period, patients will have to quit supplement ingestion temporarily until discharge in order to avoid interactions of the supplement with the medical care.

Healthy lifestyle counselling

Both groups will receive counselling on healthy lifestyle (in particular physical activity and smoking cessation), weight management (loss or gain) and medical adherence. Counselling will be operationalized

through motivational interviewing. Briefly, at baseline subjects will be informed about the results of their physical activity level, body composition and dietary intake. Subsequently, subjects will be asked to construct specific learning goals based on their physical activity level, smoking status and weight management. Every month, one of the counselors will contact the subjects and perform a short interview in which the subjects will be asked to recall and name the specific learning goals. Furthermore, the learning goals will be discussed and potentially adjusted using motivational interviewing.

Study procedures and outcomes

Table 3 gives an overview of measurements taking place at each measurement day in order to assess the study outcomes.

Primary outcomes

In line with the joint American Thoracic Society/European Respiratory Society taskforce on outcome measures in COPD, which recommends a multi-outcome approach in trials with COPD, this study includes both daily physical activity level and general health status as co-primary outcomes (87). Both primary outcomes will be measured on each measurement day (**Table 3**). Physical activity level will be measured using the activPAL™ (PAL Technologies Ltb., Glasgow, Scotland), which is capable of recording step count and physical activity pattern and intensity for a continuous period. The activPAL™ has been validated in patients with COPD (88). The activPAL™ will be attached to the center of the right thigh and subjects have to wear this device for one week. The activPal™ calculates body posture as sitting, lying, standing, and stepping and energy expenditure using static and dynamic acceleration information (89).

General health status will be assessed by the EuroQol five dimensions (EQ-5D), which is a generic classification system used to characterize current health states of patients. The EQ-5D consists of five domains (mobility; self-care; usual activity; pain/discomfort; anxiety/depression) and a Visual Analogue

Scale (EQ-VAS). Subjects will be asked to indicate their level of health by checking one of three levels of functioning for each domain. For the VAS, subjects draw a line from a box to the point on the thermometer like-scale corresponding to their health state, 0-100 (100 is best health state) (90).

Secondary outcomes

Cognitive function

Cognitive function will be measured by the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition Ltb, Cambridge, UK) which has been widely used in a large range of clinical studies, and has been thoroughly documented, replicated and validated (91). The CANTAB will be performed on a tablet computer under supervision of the researchers and will include the following tasks: motor screening task, reaction time task, paired associated learning, delayed matching-to-sample, spatial working memory and stop signal task.

Psychological wellbeing

Multiple questionnaires will be used to assess depression, anxiety and stress. The Depression Anxiety Stress Scale 21 (DASS-21) and the Hospital Anxiety and Depression Scale (HADS) will be used to assess vulnerability to depression. The DASS-21 is a clinical assessment that measures the three related states of depression, anxiety and stress (92). It consists of 21 negative emotional symptoms and subjects will be asked how often they have experienced each symptom over the past week, on a 4-point severity/frequency scale. The HADS is a 14-item instrument designed to detect the presence and severity of mild degrees of mood disorder, anxiety and depression in hospital and community settings and outside (93). It will allow us to measure domain-specific quality of life. The Cohen's Perceived Stress Scale (PSS), which is a measure of the degree to which situations in one's life are appraised as stressful (94), will be used to assess the level of stress. Ten items assess how unpredictable, uncontrollable and overloaded

respondents have found their lives to be over the last month. The scale also includes a number of direct queries about current stress levels.

Acute as well as chronic cognitive stress susceptibility will be measured. To assess acute stress, the socially evaluated cold-pressor test (SECPT) following the protocol described by Schwabe *et al.* will be used (95). Briefly, the subject will immerse his or her right hand including the wrist into ice water (0-4°C) for a maximum of 3 minutes. Before and afterwards, blood pressure, heart rate and cortisol in the saliva will be measured. Furthermore, subjects will be asked to rate how stressful, painful and unpleasant this has been, on a scale from 0 ("not at all") to 100 ("very"). To assess chronic stress cortisol levels in hair samples will be measured, since this reflects the long-term impact of stress (96). Therefore, a small piece of hair will be sampled before the SECPT to determine cortisol level in hair as a marker for long-term stress.

Physical performance

To measure physical performance, the lower extremity performance will be measured by the short physical performance battery (SPPB) and the three individual types of physical maneuvers: the balance test, the gait speed test, and the chair stand test (97). The SPPB is commonly used in older populations, but has also been shown to be a valid and simple assessment tool to measure functional impairment in COPD, independent of the severity of airflow obstruction (98).

Muscle strength will be measured by the handgrip strength test, which has been widely used as a general indicator of frailty with predictive validity for both mortality and functional limitation (99, 100). The handgrip strength will be measured in the dominant hand using a hydraulic grip strength dynamometer.

The six-minute walking test (6MWT) will be used to measure functional exercise performance. First, a practice test will be performed as recommended by the American Thoracic Society whereupon the highest walking distance will be reported (101). Before and after the 6MWT dyspnea and overall fatigue

using the Borg scale will be rated. The 6MWT is commonly used in clinical practice to assess impairment and functional level in patients with pulmonary disease (101).

The MicroRPM monitor™ (Micro Medical/CareFusion, Basingstoke, United Kingdom), will be used to assess the maximum inspiratory and expiratory mouth pressures (MIP/MEP), as a measure for respiratory muscle strength. To measure MIP, the subjects will be asked to exhale to residual volume and then perform a 'Mueller' maneuver, a forced inhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds). To measure MEP subjects will be asked to inhale to total lung capacity and then perform a 'Valsalva' maneuver, a forced exhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds).

Body composition

To assess whole body composition a dual energy x-ray absorptiometry (DEXA)-scan will be performed. Using the DEXA-scan lean mass, fat mass and bone mass can be measured at whole body level and for the extremities. Furthermore, height and weight will be measured using a wall mounted stadiometer and a standard balance beam scale.

Systemic inflammation and nutrient levels

Blood samples will be analyzed on markers of systemic inflammation (such as high-sensitive C-reactive protein, procalcitonin, interleukin-6, interleukin-8 and leucocyte levels), nutrient status (such as vitamin E, vitamin D, PUFAs, amino acids (tryptophan), and homocysteine) and mechanistic markers of gut-muscle-brain crosstalk (such as kynurenine and kynurenic acid).

Patient related outcomes

The checklist individual strength (CIS) will be used to assess fatigue. The CIS is a 20-item self-report questionnaire that measures several aspects of fatigue: fatigue severity, concentration, motivation and physical activity level (102).

The visual analogue scale will be used to assess pain. In case patients experience pain in a specific part of the body, the location of the pain will be reported.

Pittsburgh Sleep Quality Index (PSQI) will be used to assess sleep quality. The PSQI is a self-report questionnaire to assess sleep quality. The questionnaire consists of 19 individual items, creating seven components that produce one global score (103).

Intestinal integrity and gut microbiome

To evaluate the presence and the extent of small intestine injury, intestinal fatty acid binding protein (IFABP) will be determined in rest and after the 6MWT. IFABP is exclusively present in the gut, especially in the mature enterocytes of the small intestine and to a lesser extent in the colon. It rapidly diffuses through the interstitial space into the circulation upon enterocyte membrane integrity loss, making it an early and sensitive marker of small intestine injury (104). One hour after the 6MWT, IFABP will again be determined, since activities of daily living led to enterocyte damage in patients with COPD (24).

In addition, composition of the gut microbiome will be measured in selected stool samples. Stool sampling is voluntary for each subject. Preferably, the day before the measurement day subjects will gather stool samples at home in a container and store them in a cool environment. The consistency of the stool samples will be scored using the Bristol stool form scale (105), ranging from 1 (i.e. hard lumpy) to 7 (i.e. watery/liquid stools). Stool samples will be aliquoted and stored at -80°C until microbiome composition and functionality is analyzed.

Other study outcomes

In addition to the secondary outcomes, lung function (post-bronchodilator spirometry), disease impact on wellbeing and daily life, COPD specific health status, breathlessness, medication use, medical history, food intake (3-day food diary), motivation (Self-determination Theory questionnaire), mortality, COPD exacerbations and if applicable characteristics of the exacerbation (viral/bacterial and blood gasses) will be assessed or reported.

Table 3. Overview of study procedures at each measurement day.

Outcome	Measurement	M1	M2	T1	T2	M3
Primary outcomes						
Physical activity level	Accelerometry	X	X	X	X	X
General health status	EQ-5D*	X	X	X	X	X
Secondary outcomes						
Cognitive function						
Cognitive function	CANTAB	X		X	X	X
Psychological wellbeing						
Depression and anxiety	DASS-21*	X		X	X	X
Vulnerability to depression	HADS*	X		X	X	X
Level of stress	PSS*	X		X	X	X
Acute stress	SECPT	X				X
Chronic stress	Hair cortisol	X		X	X	X
Physical performance						
Lower extremity performance	SPPB	X		X	X	X
Handgrip strength	Hydraulic dynamometer	X		X	X	X
Mouth pressure	MicroRPM monitor	X		X	X	X
Exercise performance	6MWT	X				X
Body composition						
Body composition	DEXA-scan	X				X
	Weight	X		X	X	X
	Length	X				X
Blood markers						
Systemic inflammation	Hs-CRP, procalcitonin, IL-6, IL-8, leukocytes	X	X	X	X	X
Nutrient levels	Vitamin E and D, PUFAs, amino acids, homocysteine	X	X	X	X	X
Patient related outcomes						
Fatigue	CIS*	X		X	X	X
Pain	Pain VAS*	X		X	X	X
Sleep quality	PSQI*	X		X	X	X
Intestinal function and gut microbiome						
Intestinal integrity	IFABP rest	X		X	X	X
	IFABP after exercise	X				X
Microbiome composition	Stool sampling (optional)	X	X			X
Other						
Lung function	Spirometry and body plethysmography	X				X
Diffusion capacity	Diffusion capacity	X				X
Impact of COPD	CAT*	X		X	X	X
COPD health status	CCQ*	X		X	X	X
Breathlessness	Modified MRC-scale*	X		X	X	X
Nutritional supplement use#	Self-report	X	X	X	X	X
Medication use	Medical records and self-report	X	X	X	X	X
Medical history	Medical records and self-report	X	X	X	X	X
Food intake	3-day food diary	X				X
Motivation	SDT*	X		X	X	X

*Measurements at baseline, after 3 months and at the end of the study are indicated as M1, M2 and M3 respectively. The recovery period after a hospitalization for a COPD exacerbation is indicated as T1 and T2. * Questionnaire, #Subjects will be requested to refrain from the use of nutritional supplements during the course of the study. In subjects that do not stop due to their medical condition or other arguments, frequency, dose and type of nutritional supplement is recorded. Abbreviations: 6MWT, six minute walking test; CANTAB, Cambridge Neuropsychological Test Automated Battery; CAT, COPD Assessment Test; CCQ, Clinical COPD Questionnaire; CIS, Checklist Individual Strength; DASS-21, Depression Anxiety Stress Scale; DEXA, Dual-Energy X-ray absorptiometry; EQ-5D, EuroQol 5 dimensions; HADS, Hospital Anxiety and Depression Scale; Hs-CRP, High-sensitive C-reactive protein; IFABP, intestinal fatty-acid binding protein; IL; interleukin; MRC, Medical Research Council; PSS, Perceived Stress Scale; PUFAs, Poly unsaturated fatty acids; PSQI, Pittsburgh Sleep Quality Index; SDT, Self-determination theory; SECPT, Socially Evaluated Cold-Pressor Test; SPPB, Short Physical Performance Battery; VAS, Visual Analogue Scale.*

Statistical analysis

Sample size calculation

The recruitment target is calculated to ensure adequate statistical power to detect a difference in the primary outcomes step count and general health status after one year supplementation with the active nutritional supplement compared to the placebo supplement. Both outcomes have been assessed in our previous NUTRAIN-trial (53, 106), so we expect a mean difference between the intervention and placebo group of 900 steps/day with a standard deviation of 1790 steps/day and a mean difference in EQ-5D index score of 0.0775 with a standard deviation of 0.153. Using a power of 80% and a significance level of 0.05, we conclude that 62 patients per group finishing the intervention will be sufficient. Based on our experiences with the NUTRAIN-trial, we estimate the drop-out rate will be 25%. Therefore, 83 patients per group will be included.

Randomisation and blinding

Participants meeting the eligibility criteria will be randomised to the active or placebo supplement by an independent researcher. A block randomisation will be conducted to ensure equal allocation of patients in the intervention and control group. The block size will be 10, including five interventions and five controls. The independent researcher will use the minimisation method to maintain an equal distribution

in age, gender, lung function and history of exacerbations of the subjects (107, 108). The independent researcher will provide the investigator with a number corresponding to a batch of sachets which the investigator will provide to the subjects. The dependent investigators as well as the subjects will be blinded throughout the study.

Data analysis and statistical methods

Data analyses will be conducted using the statistical package IBM SPSS Statistics for Windows (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov normality test will be used to evaluate normal distributions. If necessary, data will be transformed with appropriate functions to achieve normality. In normally distributed data baseline values for the two groups will be compared by the independent sample t-test. If transformation does not result in normally distributed data, non-parametric methods will be used. Chi-square analysis will be performed for categorical variables. Generally, all analyses will be performed according to the intention-to-treat (ITT) principle. Only participants compliant to the intervention will be included in the per protocol analysis, which will be used as a sensitivity analysis for the ITT analyses. A second per protocol analysis will be performed excluding subjects that used nutritional supplements other than the study products. Within the subset of patients that have been hospitalized for a COPD exacerbation, the effect of nutritional supplementation on the recovery phase (T1 and T2) will be investigated as a post-hoc analysis. To determine the effects of the nutritional supplementation on the primary, secondary and other parameters, repeated measures analysis will be used to compare mean changes between M1 and M2, M1 and M3 and between T1 and T2. Two-sided *p*-values <0.05 will be claimed as statistically significant.

Safety assessment

Adverse events and serious adverse events will be recorded during the study. An independent data safety monitoring board (DSMB) will be assigned to perform analysis on the serious adverse events data. The

DSMB will meet after 25%, 50% and 75% of the inclusion is completed or when principal investigators do striking observations that need further investigation. The DSMB will check whether serious adverse events are related to the study product and will subsequently give the principal investigators advice on the continuation of the study.

Patient and Public Involvement

A patient advisory panel is included in the current study and gave feedback on the study design and patient information during a face-to-face meeting that was organized by the researchers. During the study, this panel will be informed about the study progression and at the end of the study they will be involved in interpretation and dissemination of the results towards the patient population. Additionally, all individual participants will be informed about their personal results after a measurement day. After the full study has been finished, all participants will be informed about the total study results.

ETHICS AND DISSEMINATION

The study has been approved by the Medical Ethics Committee from Maastricht University Medical Centre+ (MUMC+ [NL66543.068.18/METC18-011]) and is registered at clinicaltrials.gov (NCT03807310). The study will be conducted according to the principles laid down in the Declaration of Helsinki (Brazil, October 2013). Subjects will be provided with at least one week to consider their participation and will be given an opportunity to ask questions before they will sign the informed consent. Subjects will be informed that they can leave the study at any time for any reason. Handling of the personal data in this study will conform to the General Data Protection Regulation. Data will be handled confidentially. To trace data of individual subjects, the study will use a subject identification code list that is linked to the data of the participating subjects. Only the principal investigators have access to this code list. The results of this study

will be disseminated through presentations at national and international respiratory and nutritional conferences and through publications in peer-reviewed journals.

Acknowledgements: The researchers want to thank the patient advisory panel for their involvement thus far in the development of the current study.

Authors contributions: RB, HG, AvH, RH and AS developed the protocol. RB and LvI drafted the manuscript. All authors reviewed the protocol as well as this manuscript.

Funding statement: This work was supported by a LSH-TKI Lung Foundation grant 10.2.16.119, 2017 'Food for thought and active lifestyle in COPD'.

Competing interest statement: AvH is employed by Danone Nutricia Research; RH is retired from Danone Nutricia Research; AvH and RH report that a patent might derive from the research described in this paper; Dr. Simons reports grants and personal fees from GlaxoSmithKline, personal fees from Chiesi, grants from Boehringer Ingelheim, grants from AstraZeneca, all outside the submitted work; HG and AS were granted PPP Allowance made available by Health~Holland, Top Sector Life Sciences & Health to Lung Foundation Netherlands & matching by UM, UU, Nutricia Reasearch; all other authors report no competing interests.

REFERENCES

1. (2020-2021): GSCM, Vogelmeier CF, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease (2021 report). 2021 [Available from: https://goldcopd.org/.
2. Lainscak M, Gosker HR, Schols AM. Chronic obstructive pulmonary disease patient journey: hospitalizations as window of opportunity for extra-pulmonary intervention. Curr Opin Clin Nutr Metab Care. 2013;16(3):278-83.
3. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, Tal-Singer R, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. N Engl J Med. 2010;363(12):1128-38.
4. Mullerova H, Maselli DJ, Locantore N, Vestbo J, Hurst JR, Wedzicha JA, et al. Hospitalized exacerbations of COPD: risk factors and outcomes in the ECLIPSE cohort. Chest. 2015;147(4):999-1007.
5. Demeyer H, Costilla-Frias M, Louvaris Z, Gimeno-Santos E, Tabberer M, Rabinovich RA, et al. Both moderate and severe exacerbations accelerate physical activity decline in COPD patients. Eur Respir J. 2018;51(1).
6. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax. 2002;57(10):847-52.
7. Miravitlles M, Ferrer M, Pont A, Zalacain R, Alvarez-Sala JL, Masa F, et al. Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. Thorax. 2004;59(5):387-95.
8. Gosker HR, Langen RC, Simons SO. Role of acute exacerbations in skeletal muscle impairment in COPD. Expert Rev Respir Med. 2020:1-13.
9. Falck RS, Davis JC, Liu-Ambrose T. What is the association between sedentary behaviour and cognitive function? A systematic review. Br J Sports Med. 2017;51(10):800-11.
10. Teychenne M, Costigan SA, Parker K. The association between sedentary behaviour and risk of anxiety: a systematic review. BMC Public Health. 2015;15:513.
11. Zhai L, Zhang Y, Zhang D. Sedentary behaviour and the risk of depression: a meta-analysis. Br J Sports Med. 2015;49(11):705-9.
12. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. Journal of Thoracic Disease. 2015;7:E418-E38.
13. Matte DL, Pizzichini MM, Hoepers AT, Diaz AP, Karloh M, Dias M, et al. Prevalence of depression in COPD: A systematic review and meta-analysis of controlled studies. Respir Med. 2016;117:154-61.
14. Hung WW, Wisnivesky JP, Siu AL, Ross JS. Cognitive decline among patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2009;180(2):134-7.
15. Yohannes AM, Chen W, Moga AM, Leroi I, Connolly MJ. Cognitive Impairment in Chronic Obstructive Pulmonary Disease and Chronic Heart Failure: A Systematic Review and Meta-analysis of Observational Studies. Journal of the American Medical Directors Association. 2017;18(5):451 e1- e11.
16. van Beers M, Janssen DJA, Gosker HR, Schols A. Cognitive impairment in chronic obstructive pulmonary disease: disease burden, determinants and possible future interventions. Expert Rev Respir Med. 2018;12(12):1061-74.
17. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33(5):1165-85.
18. Poot B, Travers J, Weatherall M, McGinty M. Cognitive function during exacerbations of chronic obstructive pulmonary disease. Intern Med J. 2019;49(10):1307-12.
19. Schneider C, Jick SS, Bothner U, Meier CR. COPD and the risk of depression. Chest. 2010;137(2):341-7.

20. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare 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(9):e15-62.
21. Remels AH, Gosker HR, Langen RC, Schols AM. The mechanisms of cachexia underlying muscle dysfunction in COPD. Journal of Applied Physiology. 2013;114:1253-62.
22. Langen RC, Haegens A, Vernooy JH, Wouters EF, de Winther MP, Carlsen H, et al. NF-kappaB activation is required for the transition of pulmonary inflammation to muscle atrophy. American journal of respiratory cell and molecular biology. 2012;47(3):288-97.
23. Remels AH, Gosker HR, Schrauwen P, Hommelberg PP, Sliwinski P, Polkey MI, et al. TNF-alpha impairs regulation of muscle oxidative phenotype: implications for cachexia? FASEB. 2010;24:5052-62.
24. Rutten EPA, Lenaerts K, Buurman WA, Wouters EFM. Disturbed intestinal integrity in patients with COPD: effects of activities of daily living. Chest. 2014;145(2):245-52.
25. Sprooten RTM, Lenaerts K, Braeken DCW, Grimbergen I, Rutten EP, Wouters EFM, et al. Increased Small Intestinal Permeability during Severe Acute Exacerbations of COPD. Respiration. 2018;95(5):334-42.
26. Vaughan A, Frazer ZA, Hansbro PM, Yang IA. COPD and the gut-lung axis: the therapeutic potential of fibre. J Thorac Dis. 2019;11(Suppl 17):S2173-S80.
27. Foster JA, Rinaman L, Cryan JF. Stress & the gut-brain axis: Regulation by the microbiome. Neurobiol Stress. 2017;7:124-36.
28. Zinellu A, Fois AG, Zinellu E, Sotgiu E, Sotgia S, Arru D, et al. Increased kynurenine plasma concentrations and kynurenine-tryptophan ratio in mild-to-moderate chronic obstructive pulmonary disease patients. Biomark Med. 2018;12(3):229-37.
29. Gosker HR, Clarke G, de Theije CC, Cryan JF, Schols A. Impaired Skeletal Muscle Kynurenine Metabolism in Patients with Chronic Obstructive Pulmonary Disease. J Clin Med. 2019;8(7).
30. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology. 2017;112(Pt B):399-412.
31. Duenas-Espin I, Demeyer H, Gimeno-Santos E, Polkey MI, Hopkinson NS, Rabinovich RA, et al. Depression symptoms reduce physical activity in COPD patients: a prospective multicenter study. Int J Chron Obstruct Pulmon Dis. 2016;11:1287-95.
32. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester 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(8):e13-64.
33. Egan C, Deering BM, Blake C, Fullen BM, McCormack NM, Spruit MA, et al. Short term and long term effects of pulmonary rehabilitation on physical activity in COPD. Respir Med. 2012;106(12):1671-9.
34. Mador MJ, Patel AN, Nadler J. Effects of pulmonary rehabilitation on activity levels in patients with chronic obstructive pulmonary disease. J Cardiopulm Rehabil Prev. 2011;31(1):52-9.
35. Steele BG, Belza B, Cain KC, Coppersmith J, Lakshminarayan S, Howard J, et al. A randomized clinical trial of an activity and exercise adherence intervention in chronic pulmonary disease. Arch Phys Med Rehabil. 2008;89(3):404-12.
36. van de Bool C, Rutten EPA, van Helvoort A, Franssen FME, Wouters EFM, Schols A. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. J Cachexia Sarcopenia Muscle. 2017;8(5):748-58.
37. Nurk E, Drevon CA, Refsum H, Solvoll K, Vollset SE, Nygard O, et al. Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study. Am J Clin Nutr. 2007;86(5):1470-8.
38. Grosso G, Micek A, Marventano S, Castellano S, Mistretta A, Pajak A, et al. Dietary n-3 PUFA, fish consumption and depression: a systematic review and meta-analysis of observational studies. Journal of Affective Disorders. 2016;205:269-81.

39. Ma WJ, Li H, Zhang W, Zhai J, Li J, Liu H, et al. Effect of n-3 polyunsaturated fatty acid supplementation on muscle mass and function with aging: A meta-analysis of randomized controlled trials(). Prostaglandins Leukot Essent Fatty Acids. 2021;165:102249.
40. Remels AH, Langen RC, Schrauwen P, Schaart G, Schols AM, Gosker HR. Regulation of mitochondrial biogenesis during myogenesis. Mol Cell Endocrinol. 2010;315(1-2):113-20.
41. Yaqoob P, Calder P. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. Cell Immunol. 1995;163(1):120-8.
42. Duvall MG, Levy BD. DHA- and EPA-derived resolvins, protectins, and maresins in airway inflammation. Eur J Pharmacol. 2016;785:144-55.
43. Remels AH, Langen RC, Gosker HR, Russell AP, Spaapen F, Voncken JW, et al. PPARgamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. Am J Physiol Endocrinol Metab. 2009;297(1):E174-83.
44. Giudetti AM, Cagnazzo R. Beneficial effects of n-3 PUFA on chronic airway inflammatory diseases. Prostaglandins Other Lipid Mediat. 2012;99(3-4):57-67.
45. Chapkin RS, Akoh CC, Miller CC. Influence of dietary n-3 fatty acids on macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis. J Lipid Res. 1991;32(7):1205-13.
46. Guixa-Gonzalez R, Javanainen M, Gomez-Soler M, Cordobilla B, Domingo JC, Sanz F, et al. Membrane omega-3 fatty acids modulate the oligomerisation kinetics of adenosine A2A and dopamine D2 receptors. Sci Rep. 2016;6:19839.
47. Carabelli B, Delattre AM, Waltrick APF, Araujo G, Suchecki D, Machado RB, et al. Fish-oil supplementation decreases Indoleamine-2,3-Dioxygenase expression and increases hippocampal serotonin levels in the LPS depression model. Behav Brain Res. 2020;390:112675.
48. McGlory C, Calder PC, Nunes EA. The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. Front Nutr. 2019;6:144.
49. Van de Bool C, Mattijssen-Verdonschot C, Van Melick PP, Spruit MA, Franssen FM, Wouters EF, et al. Quality of dietary intake in relation to body composition in patients with chronic obstructive pulmonary disease eligible for pulmonary rehabilitation. European Journal of Clinical Nutrition. 2014;68:159-65.
50. Varraso R, Fung TT, Barr RG, Hu FB, Willett W, Camargo CA, Jr. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US women. Am J Clin Nutr. 2007;86(2):488-95.
51. Varraso R, Fung TT, Hu FB, Willett W, Camargo CA. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US men. Thorax. 2007;62(9):786-91.
52. Hirayama F, Lee AH, Binns CW, Hiramatsu N, Mori M, Nishimura K. Dietary intake of isoflavones and polyunsaturated fatty acids associated with lung function, breathlessness and the prevalence of chronic obstructive pulmonary disease: possible protective effect of traditional Japanese diet. Mol Nutr Food Res. 2010;54(7):909-17.
53. van Beers M, Rutten-van Molken M, van de Bool C, Boland M, Kremers SPJ, Franssen FME, et al. Clinical outcome and cost-effectiveness of a 1-year nutritional intervention programme in COPD patients with low muscle mass: The randomized controlled NUTRAIN trial. Clin Nutr. 2020;39(2):405-13.
54. Broekhuizen R, Wouters EF, Creutzberg EC, Weling-Scheepers CA, Schols AM. Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. Thorax. 2005;60(5):376-82.
55. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 2017;357(6349).

56. Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. International journal of tryptophan research : IJTR. 2009;2:45-60.
57. Young SN. The effect of raising and lowering tryptophan levels on human mood and social behaviour. Philosophical Transactions of the Royal Society of London. 2013;368(1615):20110375.
58. Vardar-Yagli N, Saglam M, Savci S, Inal-Ince D, Calik-Kutukcu E, Arikan H, et al. Impact of sleep quality on functional capacity, peripheral muscle strength and quality of life in patients with chronic obstructive pulmonary disease. Expert Rev Respir Med. 2015;9(2):233-9.
59. Cleutjens FA, Pedone C, Janssen DJ, Wouters EF, Incalzi RA. Sleep quality disturbances and cognitive functioning in elderly patients with COPD. ERJ Open Res. 2016;2(3).
60. Song SB, Park JS, Chung GJ, Lee IH, Hwang ES. Diverse therapeutic efficacies and more diverse mechanisms of nicotinamide. Metabolomics. 2019;15(10):137.
61. Gulcev M, Reilly C, Griffin TJ, Broeckling CD, Sandri BJ, Witthuhn BA, et al. Tryptophan catabolism in acute exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2016;11:2435-46.
62. Dzik KP, Kaczor JJ. Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. Eur J Appl Physiol. 2019;119(4):825-39.
63. Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS, et al. 1alpha,25-Dihydroxyvitamin D3 Regulates Mitochondrial Oxygen Consumption and Dynamics in Human Skeletal Muscle Cells. The Journal of biological chemistry. 2016;291(3):1514-28.
64. Spedding S. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. Nutrients. 2014;6(4):1501-18.
65. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. Journal of chemical neuroanatomy. 2005;29(1):21-30.
66. Buell JS, Dawson-Hughes B. Vitamin D and neurocognitive dysfunction: preventing "D"ecline? Mol Aspects Med. 2008;29(6):415-22.
67. van Etten E, Stoffels K, Gysemans C, Mathieu C, Overbergh L. Regulation of vitamin D homeostasis: implications for the immune system. Nutr Rev. 2008;66(10 Suppl 2):S125-34.
68. Mahlin C, von Sydow H, Osmancevic A, Emtner M, Gronberg AM, Larsson S, et al. Vitamin D status and dietary intake in a Swedish COPD population. Clin Respir J. 2014;8(1):24-32.
69. Zhu M, Wang T, Wang C, Ji Y. The association between vitamin D and COPD risk, severity, and exacerbation: an updated systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis. 2016;11:2597-607.
70. Zhu B, Zhu B, Xiao C, Zheng Z. Vitamin D deficiency is associated with the severity of COPD: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis. 2015;10:1907-16.
71. Romme EA, Smeenk FW, Rutten EP, Wouters EF. Osteoporosis in chronic obstructive pulmonary disease. Expert Rev Respir Med. 2013;7(4):397-410.
72. Persson LJ, Aanerud M, Hiemstra PS, Hardie JA, Bakke PS, Eagan TM. Chronic obstructive pulmonary disease is associated with low levels of vitamin D. PLoS One. 2012;7(6):e38934.
73. Kokturk N, Baha A, Oh YM, Young Ju J, Jones PW. Vitamin D deficiency: What does it mean for chronic obstructive pulmonary disease (COPD)? a compherensive review for pulmonologists. Clin Respir J. 2018;12(2):382-97.
74. Carson EL, Pourshahidi LK, Madigan SM, Baldrick FR, Kelly MG, Laird E, et al. Vitamin D status is associated with muscle strength and quality of life in patients with COPD: a seasonal prospective observation study. Int J Chron Obstruct Pulmon Dis. 2018;13:2613-22.
75. Hornikx M, Van Remoortel H, Lehouck A, Mathieu C, Maes K, Gayan-Ramirez G, et al. Vitamin D supplementation during rehabilitation in COPD: a secondary analysis of a randomized trial. Respiratory Research. 2012;13:84.

76. Alavi Foumani A, Mehrdad M, Jafarinezhad A, Nokani K, Jafari A. Impact of vitamin D on spirometry findings and quality of life in patients with chronic obstructive pulmonary disease: a randomized, double-blinded, placebo-controlled clinical trial. Int J Chron Obstruct Pulmon Dis. 2019;14:1495-501.
77. Jolliffe DA, Greenberg L, Hooper RL, Mathyssen C, Rafiq R, de Jongh RT, et al. Vitamin D to prevent exacerbations of COPD: systematic review and meta-analysis of individual participant data from randomised controlled trials. Thorax. 2019;74(4):337-45.
78. Martineau AR, Jolliffe DA, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. Health Technol Assess. 2019;23(2):1-44.
79. Pelgrim CE, Peterson JD, Gosker HR, Schols A, van Helvoort A, Garssen J, et al. Psychological co-morbidities in COPD: Targeting systemic inflammation, a benefit for both? Eur J Pharmacol. 2018;842:99-110.
80. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125(6):1401-12.
81. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54(9):2325-40.
82. Janbazacyabar H, van Bergenhenegouwen J, Verheijden KAT, Leusink-Muis T, van Helvoort A, Garssen J, et al. Non-digestible oligosaccharides partially prevent the development of LPS-induced lung emphysema in mice. Pharmanutrition. 2019;10.
83. Hanson C, Lyden E, Rennard S, Mannino DM, Rutten EP, Hopkins R, et al. The Relationship between Dietary Fiber Intake and Lung Function in the National Health and Nutrition Examination Surveys. Ann Am Thorac Soc. 2016;13(5):643-50.
84. Szmidt MK, Kaluza J, Harris HR, Linden A, Wolk A. Long-term dietary fiber intake and risk of chronic obstructive pulmonary disease: a prospective cohort study of women. Eur J Nutr. 2020;59(5):1869-79.
85. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krleza-Jeric K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-7.
86. European Food Safety Authority. Dietary reference values for nutrients. Summary report. 2017 [Available from: https://www.efsa.europa.eu/sites/default/files/2017_09_DRVs_summary_report.pdf.
87. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J. 2008;31(2):416-69.
88. Ng LWC, Jenkins S, Hill K. Accuracy and responsiveness of the stepwatch activity monitor and ActivPAL in patients with COPD when walking with and without a rollator. Disability and Rehabilitation. 2012;34(15):1317-22.
89. Lyden K, Keadle SK, Staudenmayer J, Freedson PS. The activPALTM Accurately Classifies Activity Intensity Categories in Healthy Adults. Med Sci Sports Exerc. 2017;49(5):1022-8.
90. Pickard AS, Wilke C, Jung E, Patel S, Stavem K, Lee TA. Use of a preference-based measure of health (EQ-5D) in COPD and asthma. Respir Med. 2008;102(4):519-36.
91. Lowe C, Rabbitt P. Test / re-test reliability of the CANTAB and ISPOCD neuropsychological batteries: theoretical and practical issues. Neuropsychologia. 1998;36(9):915-23.
92. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behav Res Ther. 1995;33(3):335-43.
93. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70.

94. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of Health and Social Behaviour. 1983;24(4):385-96.
95. Schwabe L, Haddad L, Schachinger H. HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology. 2008;33(6):890-5.
96. O'Brien KM, Tronick EZ, Moore CL. Relationship between hair cortisol and perceived chronic stress in a diverse sample. Stress and health : journal of the International Society for the Investigation of Stress. 2013;29(4):337-44.
97. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. Journal of gerontology. 1994;49(2):M85-94.
98. Patel MS, Mohan D, Andersson YM, Baz M, Samantha Kon SC, Canavan JL, et al. Phenotypic characteristics associated with reduced short physical performance battery score in COPD. Chest. 2014;145(5):1016-24.
99. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, et al. Midlife hand grip strength as a predictor of old age disability. Jama. 1999;281(6):558-60.
100. Rantanen T, Harris T, Leveille SG, Visser M, Foley D, Masaki K, et al. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. J Gerontol A Biol Sci Med Sci. 2000;55(3):M168-73.
101. Laboratories ATSCoPSfCPF. A. T. S. Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7.
102. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. Journal of psychosomatic research. 1994;38(5):383-92.
103. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
104. Pelsers MM, Namiot Z, Kisielewski W, Namiot A, Januszkiewicz M, Hermens WT, et al. Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. Clin Biochem. 2003;36(7):529-35.
105. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32(9):920-4.
106. Van de Bool C, Rutten EPA, Van Helvoort A, Franssen F, Wouters EF, Schols AM. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. Journal of Cachexia, Sarcopenia and Muscle. 2017.
107. Han B, Enas NH, McEntegart D. Randomization by minimizatioin for unbalanced treatment allocation. Statistics in Medicine. 2009;28(27):3329-46.
108. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975;31(1):103-15.

Figure legend

Figure 1. Study design.

Measurements at baseline, after 3 months and at the end of the study are indicated as M1, M2 and M3 respectively. After a hospitalization for a COPD exacerbation (H) the recovery phase of 4 weeks will be monitored (T1 and T2). In case patients will be recruited during a hospitalization for a COPD exacerbation, M1 will take place 4 weeks after discharge. In case the hospitalization will be within 3 months after baseline, M2 will not take place.

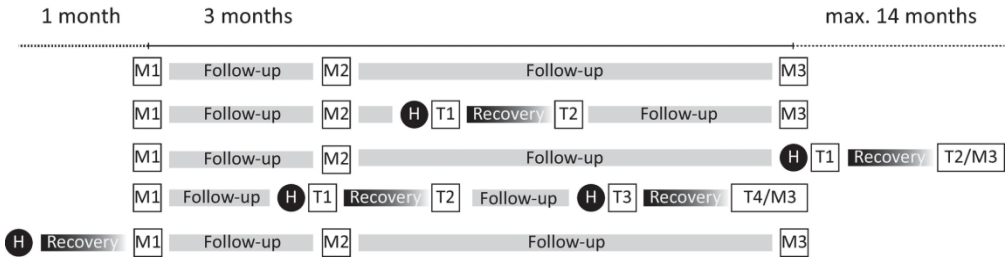

Figure 1: Study design

181x46mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	p. 2
	2b	All items from the World Health Organization Trial Registration Data Set	N.A.
Protocol version	3	Date and version identifier	N.A.
Funding	4	Sources and types of financial, material, and other support	p. 23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p. 1
	5b	Name and contact information for the trial sponsor	p. 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N.A.
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N.A.

bmjopen-2021-059252 on 16 March 2022. Downloaded from

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	p. 7
	6b	Explanation for choice of comparators	p. 7
Objectives	7	Specific objectives or hypotheses	p. 7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	p. 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	p. 10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	p. 12
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	p. 13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	p. 13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	p. 17
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N.A.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p. 20
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	p. 10

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p. 21
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	p. 21
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	p. 21
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p. 21
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p. 21
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N.A.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	N.A.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N.A.

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p. 22
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p. 22
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p. 22
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p. 22
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p. 22
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p. 22
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p. 22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p. 22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p. 23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N.A.

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p. 23
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N.A.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	p. 23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p. 23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	p. 23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N.A.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	p. 23
	31b	Authorship eligibility guidelines and any intended use of professional writers	N.A.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N.A.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N.A.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N.A.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The effect of targeted nutrient supplementation on physical activity and health related quality of life in COPD: study protocol for the randomized controlled NUTRECOVER trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-059252.R1
Article Type:	Protocol
Date Submitted by the Author:	03-Feb-2022
Complete List of Authors:	Beijers, Rosanne; Maastricht University Medical Centre+, Department of Respiratory Medicine van Iersel, Lieke E.J.; Maastricht University Medical Centre+, Department of Respiratory Medicine Schuurman, Lisanne; Maastricht University Medical Centre+, Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism Hageman, Robert J.J.; Danone Nutricia Research Simons, Sami; Maastricht University Medical Centre+, Respiratory Medicine van Helvoort, Ardy; Maastricht University Medical Centre+, Department of Respiratory Medicine; Danone Nutricia Research Gosker, Harry; Maastricht University Medical Centre+, Department of Respiratory Medicine Schols, Annemie; Maastricht University Medical Centre+, Department of Respiratory Medicine
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Respiratory medicine
Keywords:	RESPIRATORY MEDICINE (see Thoracic Medicine), NUTRITION & DIETETICS, Chronic airways disease < THORACIC MEDICINE

The effect of targeted nutrient supplementation on physical activity and health related quality of life in COPD: study protocol for the randomized controlled NUTRECOVER trial

Rosanne J.H.C.H. Beijers[1], Lieke E.J. van Iersel[1], Lisanne T. Schuurman[1], Robert J.J. Hageman[2], Sami O. Simons[1], Ardy van Helvoort[1,2], Harry R. Gosker[1], Annemie M.W.J. Schols[1]

[1] Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, Netherlands.

[2] Danone Nutricia Research, Utrecht, Netherlands.

Corresponding author: Rosanne J.H.C.G. Beijers

P.O. Box 5800

6202 AZ Maastricht, Netherlands

Tel: 0031 43 3882990

Fax: 0031 43 3875051

E-mail: r.beijers@maastrichtuniversity.nl

Abstract

Introduction: Physical and mental health is often affected in COPD adversely affecting disease course and quality of life. Abnormalities in whole body and cellular energy metabolism, dietary and plasma nutrient status, and intestinal permeability have been well established in these patients as systemic determinants of functional decline and underexplored treatable traits. The aim of this study is to investigate the efficacy of one-year targeted nutrient supplementation on physical activity level and health-related quality of life in patients with COPD.

Methods and analysis: This study is a single-center randomized, placebo-controlled, double-blind trial in 166 patients with COPD recruited from multiple hospitals in the Netherlands. The intervention group will receive a multi-nutrient supplement, including vitamin D, tryptophan, long-chain polyunsaturated fatty acids, and prebiotic dietary fibers as main components (94 kCal per daily dose). The control group will receive an isocaloric isonitrogenous placebo. Both groups will ingest one portion per day for at least 12 months and will additionally receive counselling on healthy lifestyle and medical adherence over the course of the study. Co-primary outcomes are physical activity assessed by triaxial accelerometry and health related quality of life measured by the EuroQol-5D questionnaire. Secondary outcomes are cognitive function, psychological wellbeing, physical performance, patient-reported outcomes, and the metabolic profile assessed by body composition, systemic inflammation, plasma nutrient levels, intestinal integrity and microbiome composition. Outcomes will be measured at baseline and after 12 months of supplementation. In case patients are hospitalized for a COPD exacerbation, a subset outcome panel will be measured during a 4-week recovery period after hospitalization.

Ethics and dissemination: This study was approved by the local Ethics Committee of Maastricht University. Subjects will be included after written informed consent is provided. Study outcomes will be disseminated through presentations at (inter)national conferences and through peer-reviewed journals.

Trial registration: NCT03807310.

Word count: 4569

Strengths and limitations of this study

- A double-blind, randomized, placebo-controlled clinical trial investigating the effect of a novel nutritional intervention product relative to placebo on objective and subjective health status in patients with COPD.
- The study design resembles a real-life setting.
- Next to investigating overall efficacy, the study design allows additional insight in the relative efficacy of the nutritional intervention in clinically stable disease as well as in response to acute disease flare-ups.
- The study product is a newly developed nutritional supplement targeting nutrient status and metabolic alterations in COPD.
- The study population targets patients with moderate to very severe COPD; inclusion, long-term compliance and outcome assessment may be challenging.

Keywords: COPD, nutritional supplementation, lifestyle, exacerbations

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow limitation resulting from loss and remodeling of the airways (1). During the course of the disease, patients with COPD experience periods of acute worsening of respiratory symptoms called exacerbations. During severe COPD exacerbations (requiring hospitalization) disease-related detrimental factors such as systemic inflammation, hypoxia, physical inactivity, malnutrition and treatment with glucocorticoids and antibiotics, converge and intensify (2). Patients with frequent exacerbations constitute a specific disease phenotype with a worse prognosis, specifically a faster decline in lung function and muscle function, a greater worsening of health status and a substantial reduction in daily physical activity (3-6). In turn, physical inactivity drives further physical impairments, thereby increases the risk of recurrent exacerbations, leading to a vicious cycle of deterioration (6). It is this circle that has to be broken and adapting a more healthy lifestyle may help achieving this. Adapting a healthier lifestyle, however, is not always easy and may need additional support, especially in the affected health domains.

Symptoms of dyspnea and fatigue in patients with COPD often affect their lifestyle. Alongside with disease exacerbations and malnutrition, a sedentary lifestyle contributes to extrapulmonary manifestations including depression, cognitive decline loss of muscle mass and function and gastro-intestinal problems (7-11), causing a vicious cycle towards physical inactivity. Overall high percentages of impaired cognitive function (25%) and depression (27%) have been observed in patients with COPD (12-14) and are negatively influenced by chronic stress and systemic inflammation (15, 16). The risk of developing cognitive dysfunction or depression is even further increased in advanced disease stages (17, 18). Skeletal muscle weakness is another well-established extrapulmonary manifestation of COPD resulting from loss of muscle mass and a decreased muscle oxidative phenotype (19). The latter is characterized by a muscle fiber type shift from oxidative type I towards more glycolytic type II fibers and decreased expression of oxidative enzymes and regulatory proteins (e.g. peroxisome proliferator-

activated receptors (PPARs) and their coactivator 1α (PGC-1α)) (20), which render the skeletal muscle more susceptible to fatigue (9, 10). Systemic inflammation and physical inactivity have also been identified as determinants of muscle mass loss in COPD (20-22). During an exacerbation, enhanced systemic inflammation and physical inactivity lead to acute muscle wasting, which may not be fully restored during recovery as there is evidence for an impaired regenerative potential in these patients (6). Disturbances of intestinal integrity manifested by increased gastro-intestinal (GI) permeability and alterations in intestinal fatty acid binding protein (IFABP) have also been reported in COPD (11). These disturbances are not only observed in stable COPD, but also during hospitalization for a COPD exacerbation (23). Moreover, several factors associated with COPD, including age, gender, smoking, use of corticosteroids and antibiotics, BMI and diet, are known to cause dysbiosis in the gut microbiome (24). However, up till now no published studies of the COPD gut microbiome are available.

The extrapulmonary manifestations in COPD are linked by the cross-talk between the gut-muscle-brain axis which includes the vagus nerve, gut hormone signaling, the immune system, serotonin and tryptophan metabolism and microbial metabolites such as short chain fatty acids (25). All routes might be disturbed in patients with COPD, but especially serotonin and tryptophan metabolism has recently been shown to be impaired in patients with COPD leading to unfavorable higher kynurenine/tryptophan ratios in the circulation (26, 27). By modulating tryptophan availability, the microbiota can regulate the kynurenine pathway decreasing plasma kynurenine levels. In muscle, kynurenine can also be metabolized and cleared by kynurenine aminotransferases that are regulated by PGC-1α. Interestingly, a recent study of our group showed reduced expression levels of these aminotransferases in skeletal muscle of patients with COPD, which might explain the elevated plasma kynurenine levels (27). Impairments in the kynurenine pathway have been linked to anxiety, depression and cognitive decline (28).

Extrapulmonary manifestations as well as impairments in the cross-talk between these extra-pulmonary organs could adversely affect daily physical activity level and quality of life (29), accelerating

the vicious cycle towards physical inactivity and deterioration of physical and mental status. Interventions facilitating a more physically active lifestyle in patients with COPD are therefore needed. For instance, physical activity coaching interventions may enhance physical activity levels in patients with COPD (30). However, current pulmonary rehabilitation strategies mainly focus on improving physical performance by exercise training and counselling targeting respiratory and skeletal muscle function (31), but behavioral translation of improved physical performance to a more active lifestyle in COPD has been disappointing (32-35). Therefore, additional interventions need to be investigated.

Nutritional supplementation targeting impairments of skeletal muscle and gut, by preventing or restoring disease-specific deficiencies in patients with COPD and modulation of the function of the gut-brain axis, might be beneficial in patients with COPD. As systematically summarized in **Table 1**, omega-3 polyunsaturated fatty acids (N-3 PUFAs), tryptophan, vitamin D, and nutritional fibers are particularly interesting nutritional components to explore. Intake of these components is generally low in patients with COPD, and we hypothesize that supplementation of these nutrients has positive effects on different pathways of the gut-muscle-brain axis which may jointly facilitate adaptation to a more physically active lifestyle and improved health status. To minimize risk of other nutritional deficiencies and to ensure presence of essential co-factors, supportive micronutrients are provided together with these core ingredients in the supplement.

We hypothesize that this newly developed multi-nutrient product facilitates maintenance of a more active lifestyle in patients with COPD and improves objective and subjective physical and mental health status relative to placebo. Therefore, the primary aim of this study is to investigate the effect of one-year targeted nutrient supplementation compared to placebo supplementation on daily physical activity level as well as general health status in patients with COPD. Secondary aims are to investigate the effect of targeted nutrient supplementation on potential mediators; cognitive function, psychological wellbeing, physical performance, patient-reported outcomes and the metabolic profile assessed by body

composition, systemic inflammation, plasma nutrient levels, intestinal integrity and microbiome composition in patients with COPD. As an additional explorative aim, we will investigate the relative effect of targeted nutrient supplementation during the recovery phase after hospitalization for a COPD exacerbation.

Table 1. Rationale for the core components to be included in the multimodal nutritional intervention

Component	Proposed effects and underlying mechanisms	Intake, status and supplementation in COPD
N-3 PUFAs	*Proposed effect:* Lowers the risk on depression, improves cognitive function, muscle function and muscle mass as well as mitochondrial function (36-39). *Possible underlying mechanisms:* - Substrate inhibition of pro-inflammatory prostaglandin E2 (40) and 4 series leukotrienes EPA and DHA give rise to resolvins and lipid related signaling molecules such as protectins (41). - N-3 PUFAs are natural ligands for PPAR gamma that attenuates NF-kB activation and inflammatory gene expression and NF-kB activation reducing systematic inflammation (42, 43). - Altering membrane lipid composition (44) and increasing membrane fluidity which facilitate the activation of receptors e.g. adenosine A2A and dopamine D2 receptors in the brain (45). - Decreasing Indoleamine-2,3-Dioxigenase expression and increasing hippocampal serotonin, a neurotransmitter in the central and enteric nervous system (46); - Stimulating skeletal muscle anabolism via the Akt-mTOR-p70S6K pathway (47); - Stimulating muscle oxidative metabolism and boosting mitochondrial function (39).	*Intake and status in COPD:* • Dietary intake of PUFAs is generally low (48-50); • High intake is positively associated with lung function (51). • Blood levels decrease in tune with disease progression in patients with COPD (52). *Supplementation in COPD:* • As an adjunct to exercise training, PUFAs significantly enhanced improvement in exercise performance and physical activity level in COPD in an 8-week and 4-months placebo-controlled RCT respectively (35, 53).
Tryptophan	*Proposed effect*: Lowers the risk for depression, improves mood, behavior, and cognitive function (54-56). *Possible underlying mechanisms:* - Being a precursor of serotonin (54-56); - Being a precursor for melatonin which may improve sleep quality which can improve functional capacity, skeletal muscle strength, cognitive function and general QoL in COPD (57, 58); - Being a precursor for niacin (vitamin B3) which for example is essential for mitochondrial function (59).	*Intake and status in COPD:* • Plasma tryptophan levels are decreased during acute exacerbations (60); • Circulating KYN and KYN/tryptophan ratios are elevated compared to controls and is associated with disease severity (26), KYN clearance might be reduced in skeletal muscle tissue of patients with COPD due to reduced PPAR/PGC-1α-mediated KAT expression (27).

		Supplementation in COPD: Not available
Vitamin D	*Proposed effect:* Low plasma vitamin D concentrations are associated with depressive symptoms and low muscle function (61-63). *Possible underlying mechanisms:* - An increased region-specific expression of vitamin D receptors in brain areas play a key role in mood regulation (64); - Has anti-inflammatory effects leading to neuroprotective properties (65, 66); - Maintaining a normal calcium and phosphorus balance in skeletal muscle; low vitamin D levels reduce calcium reuptake into sarcoplasmic reticulum, impairing muscle function (61). - Improving mitochondrial function, dynamics and enzyme function in skeletal muscle (62).	*Intake and status in COPD:* • Vitamin D levels are low and deficiency is highly prevalent (67, 68). • Deficiency is associated with COPD severity, osteoporosis, depression and lower muscle strength (69-73). *Supplementation in COPD:* • Significantly improves inspiratory muscle strength, maximal oxygen uptake and QoL in patients with COPD in a 3-months and 8-weeks placebo-controlled RCT (74, 75); • Supplementation reduces number of acute exacerbations in patients with COPD with low vitamin D plasma levels as shown in two meta-analyses (76, 77).
Prebiotic fibers	*Proposed effect*: Improve gut function (78-80). *Possible underlying mechanisms:* - Fibers are substrate for intestinal microbes and support their growth (79); - Can be converted into SCFA that support gastro-intestinal integrity and fuel coloncytes (80); - Change gut microbial composition towards a less inflammatory profile (78). - A mixture of prebiotic fiber may prevent alveolar wall destruction, right ventricle hypertrophy and neutrophil infiltration into the lungs after LPS instillation in mice (81).	*Intake and status in COPD:* • Dietary intake is generally low (48); • Low fiber intake is associated with reduced measures of lung function (82) and high intake with a 30% lower risk of COPD (83); • Dietary intake has an inverse relationship with poor lung function and COPD risk (82). *Supplementation in COPD:* Not available

Abbreviations: COPD, chronic obstructive pulmonary disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; KAT, kynurenine aminotransferase; KYN, kynurenine; LPS, lipopolysaccharide; NF-kB, nuclear factor kappa-light chain enhancer of activated B cells; PPARs, peroxisome proliferator-activated receptors; PUFA, polyunsaturated fatty acids; QoL, quality of life; RCT, randomized controlled trial; SCFA, short-chain fatty acids.

METHODS AND ANALYSIS

Study design

The study design was developed according to the SPIRIT 2013 recommendations (84). In a single-center randomized, placebo-controlled, double-blind clinical trial the active nutritional supplement will be compared to a placebo supplement in patients with COPD. Before, after three months and after 12 months (extended in case of hospitalization in the previous 4 weeks) measurements will be performed at the Maastricht University Medical Centre+ (**Figure 1**). A one-year follow-up period is chosen to investigate the long-term effect of nutritional supplementation in a real-life setting during which COPD exacerbations might occur. We will investigate in an explorative analysis the relative effect of targeted nutrient supplementation on clinical outcome during a 4-week recovery phase after hospitalization for a COPD exacerbation. First of all, because disturbances in the gut-muscle-brain axis as well as specific nutrient deficiencies might be affected by severe COPD exacerbations (3-6, 69). Secondly, because the recovery period after severe COPD exacerbations is a completely neglected phase for nutritional interventions thus far (2). For this, we will perform a subset panel of outcome measures within one week after discharge and one month later (recovery phase) each time a patient is hospitalized for a COPD exacerbation during the intervention period. In case the last measurement day will take place within the post-exacerbation recovery phase, the intervention period will be lengthened until the end of the recovery phase, with a maximum of 2 months. See figure 1 for these different scenarios. The study has been started in October 2020 and is planned to last 3 years.

Participants

The study population will consist of patients with moderate to very severe, but medically stable, COPD according to the GOLD criteria (1) (see Table 2 for detailed in- and exclusion criteria). Participants will be recruited via respiratory physicians from multiple hospitals in the southeast of the Netherlands or via

advertisements. Potential eligible participants will receive detailed information of the study including the informed consent form (Appendix 1). Patients that are willing to participate will be screened by the researchers and study physician based on the eligibility criteria. The first participant was enrolled in October 2020.

Table 2. Eligibility criteria

Eligibility criteria	
Inclusion criteria	• Moderate to very severe COPD according to GOLD criteria (i.e. GOLD stage II-IV); • Medically stable (no hospital admission <4 weeks prior to the start of the study and no temporary oral steroid or antibiotics use due to a COPD exacerbation in the last 4 weeks);
Exclusion criteria	• Age <18 years; • Allergy or intolerance to components of the study product; • Other acute or unstable chronic diseases that will compromise the study outcome (e.g. active cancer requiring treatments); • Participation in any other study involving investigational or marketed products concomitantly or within four weeks prior to entry into the study; • Terminal illness; • Lung malignance in the previous 5 years; • Diagnosis of dementia or neurodegenerative disease (e.g. Alzheimer's disease, Parkinson's disease, Huntington's chorea, frontotemporal dementia) in the medical records; • Recent diagnosis of cerebral conditions (<1 year e.g. cerebral infarction, hemorrhage, brain tumors, transient ischemic attack) in the medical records; • Any medical condition that significantly interferes with digestion and/or gastro-intestinal (GI) function (e.g. short bowel syndrome, inflammatory bowel disease, gastric ulcers, gastritis, (gastro)-enteritis, GI cancer) as judged by the investigator.

Trial interventions

Nutritional intervention

One group will receive the targeted nutrient supplement, and one group will receive an isocaloric isonitrogenous placebo for at least 12 months. Both study products will be provided as a flavored powder in labelled sachets that are blinded for both the participants and investigators. Participants will be randomly assigned to one of the intervention groups (see randomization and blinding). Both supplements will be produced and supplied by Danone Nutricia Research, Utrecht, Netherlands. The active supplement consists of 4 g proteins and free amino acids, 4 g fat (fish oil; N6/N3 ratio = 0.12), 8 g carbohydrates and 6 g fibers, providing 1 g of N-3 long-chain PUFAs (600 mg EPA and 400 mg DHA), 200 mg tryptophan, 20 µg vitamin D, and 6 g of prebiotic fibers as core components (Table 1). To minimize risk of other nutritional deficiencies and to ensure presence of essential co-factors, supportive micronutrients are provided at dosages present in a normal diet and below no observed adverse effect levels (NOAEL) together with these core ingredients in the supplement. The dose of components are all within the recommendations and safety levels as provided by the European Food and Safety Authority (EFSA), as published in their report of December 2017 (85). The active supplement includes a daily dose of 94 kCal. The placebo product is isocaloric and isonitrogenous where protein and free amino acid levels are compensated with L-alanine as neutral amino acid that is known to not modulate outcome parameters in patients with COPD, fish oil is replaced by vegetable oils (palm and soy oil; N6/N3 ratio = 12.3) without EPA and DHA, and fiber/energy is compensated by maltodextrin. The micronutrients will not be added to the placebo powder. . Each day, one sachet will be consumed after suspension in 60 mL water or any other drink. In case patients are hospitalized during the intervention period, patients will have to quit supplement ingestion temporarily until discharge in order to avoid interactions of the supplement with the medical care. The subjects will receive the nutritional supplements for a period of two months. New nutritional supplements will be provided at home by the investigators or send by mail.

Healthy lifestyle counselling

Both groups will receive counselling on healthy lifestyle (in particular physical activity and smoking cessation), weight management (loss or gain) and medical adherence. Counselling is based on the Self-Determination Theory (SDT) and will be operationalized through motivational interviewing by one of the trained researchers (86). The counselling aims at increasing self-regulation skills of the participants, as well as competence and autonomy. Briefly, at baseline subjects will be informed about the results of their physical activity level, body composition and dietary intake. Subsequently, subjects will be asked to construct specific learning goals based on their physical activity level, smoking status and weight management. Every month, one of the counselors will contact the subjects and perform a short interview in which the subjects will be asked to recall and name the specific learning goals. Furthermore, the learning goals will be discussed and potentially adjusted using motivational interviewing. During this interview, researchers will also record any adverse events and researchers will ask the subjects the number of supplements they have left in order to check for compliance. This monthly contact will additionally give the researchers the opportunity to keep the subjects motivated for participation in this study.

Study procedures and outcomes

Table 3 gives an overview of measurements taking place at each measurement day in order to assess the study outcomes. **Figure 2** provides a timeline of a baseline or end measurement day (M1 and M3). The subset of measurements included at M2, T1 and T2 will be performed in the same order as depicted in Figure 2.

Primary outcomes

In line with the joint American Thoracic Society/European Respiratory Society taskforce on outcome measures in COPD, which recommends a multi-outcome approach in trials with COPD, this study includes both daily physical activity level and general health status as co-primary outcomes (87). Both primary outcomes will be measured on each measurement day (**Table 3**). Physical activity level will be measured using the activPAL™ (PAL Technologies Ltb., Glasgow, Scotland), which is capable of recording step count and physical activity pattern and intensity for a continuous period. The activPAL™ has been validated in patients with COPD (88). The activPAL™ will be attached to the center of the right thigh and subjects have to wear this device for seven consecutive days, 24 hours per day. The activPal™ calculates total amount of steps which is one of the primary outcomes in this study. Additionally, the activPAL™ calculates body posture as sitting, lying, standing, and stepping in hours per day, and energy expenditure in metabolic equivalent of tasks (METs value) using static and dynamic acceleration information (89), which will be analyzed as secondary outcomes. Following the recommendation by Demeyer *et al*., only measurements including a minimum of three completely measured days will be considered valid to be included in the analysis (90).

General health status will be assessed by the EuroQol five dimensions (EQ-5D), which is a generic classification system used to characterize current health states of patients. The EQ-5D consists of five domains (mobility; self-care; usual activity; pain/discomfort; anxiety/depression) and a Visual Analogue Scale (EQ-VAS). Subjects will be asked to indicate their level of health by checking one of three levels of functioning for each domain. For the VAS, subjects draw a line from a box to the point on the thermometer like-scale corresponding to their health state, 0-100 (100 is best health state) (91).

Secondary outcomes

Cognitive function

Cognitive function will be measured by the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition Ltb, Cambridge, UK) which has been widely used in a large range of clinical studies, and has been thoroughly documented, replicated and validated (92). The CANTAB will be performed on a tablet computer under supervision of the researchers and will include the following tasks: motor screening task, reaction time task, paired associated learning, delayed matching-to-sample, spatial working memory and stop signal task.

Psychological wellbeing

Multiple questionnaires will be used to assess depression, anxiety and stress. The Depression Anxiety Stress Scale 21 (DASS-21) and the Hospital Anxiety and Depression Scale (HADS) will be used to assess vulnerability to depression. The DASS-21 is a clinical assessment that measures the three related states of depression, anxiety and stress (93). It consists of 21 negative emotional symptoms and subjects will be asked how often they have experienced each symptom over the past week, on a 4-point severity/frequency scale. The HADS is a 14-item instrument designed to detect the presence and severity of mild degrees of mood disorder, anxiety and depression in hospital and community settings and outside (94). It will allow us to measure domain-specific quality of life. The Cohen's Perceived Stress Scale (PSS), which is a measure of the degree to which situations in one's life are appraised as stressful (95), will be used to assess the level of stress. Ten items assess how unpredictable, uncontrollable and overloaded respondents have found their lives to be over the last month. The scale also includes a number of direct queries about current stress levels.

Acute as well as chronic cognitive stress susceptibility will be measured. To assess acute stress, the socially evaluated cold-pressor test (SECPT) following the protocol described by Schwabe *et al.* will be used (96). Briefly, the subject will immerse his or her right hand including the wrist into ice water (0-4°C) for a maximum of 3 minutes. Before and afterwards, blood pressure, heart rate and cortisol in the saliva

will be measured. Furthermore, subjects will be asked to rate how stressful, painful and unpleasant this has been, on a scale from 0 ("not at all") to 100 ("very"). To assess chronic stress cortisol levels in hair samples will be measured, since this reflects the long-term impact of stress (97). Therefore, a small piece of hair will be sampled before the SECPT to determine cortisol level in hair as a marker for long-term stress. Both saliva and hair cortisol will be determined using liquid chromatography with tandem mass spectrometry (98).

Physical performance

To measure physical performance, the lower extremity performance will be measured by the short physical performance battery (SPPB) and the three individual types of physical maneuvers: the balance test, the gait speed test, and the chair stand test (99). The SPPB is commonly used in older populations, but has also been shown to be a valid and simple assessment tool to measure functional impairment in COPD, independent of the severity of airflow obstruction (100).

Muscle strength will be measured by the handgrip strength test, which has been widely used as a general indicator of frailty with predictive validity for both mortality and functional limitation (101, 102). The handgrip strength will be measured in the dominant hand using a hydraulic grip strength dynamometer.

The six-minute walking test (6MWT) will be used to measure functional exercise performance. First, a practice test will be performed as recommended by the American Thoracic Society whereupon the highest walking distance will be reported (103). Before and after the 6MWT dyspnea and overall fatigue using the Borg scale will be rated. The 6MWT is commonly used in clinical practice to assess impairment and functional level in patients with pulmonary disease (103).

The MicroRPM monitor™ (Micro Medical/CareFusion, Basingstoke, United Kingdom), will be used to assess the maximum inspiratory and expiratory mouth pressures (MIP/MEP), as a measure for

respiratory muscle strength (104). To measure MIP, the subjects will be asked to exhale to residual volume and then perform a 'Mueller' maneuver, a forced inhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds). To measure MEP subjects will be asked to inhale to total lung capacity and then perform a 'Valsalva' maneuver, a forced exhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds). Both MIP and MEP procedures are repeated three times, of which the highest value is used.

Body composition

To assess whole body composition a dual energy x-ray absorptiometry (DEXA)-scan will be performed. Using the DEXA-scan lean mass, fat mass and bone mass can be measured at whole body level and for the extremities. Furthermore, height and weight will be measured using a wall mounted stadiometer and a standard balance beam scale.

Systemic inflammation and nutrient levels

Blood samples (both serum and plasma) will be stored at -80 °Celsius and will be analyzed after completion of the trial. Markers of systemic inflammation (such as high-sensitive C-reactive protein, procalcitonin, interleukin-6, interleukin-8 and leucocyte levels), nutrient status (such as vitamin E, vitamin D, PUFAs, amino acids (tryptophan), and homocysteine) and mechanistic markers of gut-muscle-brain crosstalk (such as kynurenine and kynurenic acid) will be determined as previously described (27, 35). The nutrient levels will be used to check for compliance.

Patient related outcomes

The checklist individual strength (CIS) will be used to assess fatigue. The CIS is a 20-item self-report questionnaire that measures several aspects of fatigue: fatigue severity, concentration, motivation and physical activity level (105).

The visual analogue scale will be used to assess pain. In case patients experience pain in a specific part of the body, the location of the pain will be reported.

Pittsburgh Sleep Quality Index (PSQI) will be used to assess sleep quality. The PSQI is a self-report questionnaire to assess sleep quality. The questionnaire consists of 19 individual items, creating seven components that produce one global score (106).

Intestinal integrity and gut microbiome

To evaluate the presence and the extent of small intestine injury, plasma intestinal fatty acid binding protein (IFABP) will be determined in rest and after the 6MWT using an enzyme-linked immunosorbent assay (11) . IFABP is exclusively present in the gut, especially in the mature enterocytes of the small intestine and to a lesser extent in the colon. It rapidly diffuses through the interstitial space into the circulation upon enterocyte membrane integrity loss, making it an early and sensitive marker of small intestine injury (107). One hour after the 6MWT, IFABP will again be determined, since activities of daily living led to enterocyte damage in patients with COPD (11).

In addition, composition of the gut microbiome will be measured in selected stool samples. Stool sampling is voluntary for each subject. Preferably, the day before the measurement day subjects will gather stool samples at home in a container and store them in a cool environment. The consistency of the stool samples will be scored using the Bristol stool form scale (108), ranging from 1 (i.e. hard lumpy) to 7 (i.e. watery/liquid stools). Stool samples will be aliquoted and stored at -80°C until microbiome composition and functionality is analyzed using state-of-the-art metagenome sequencing (109).

Other study outcomes

In addition to the secondary outcomes, lung function will be measured using post-bronchodilator spirometry according to the GOLD criteria (1)., Additionally, questionnaires will be used to determine disease impact on wellbeing and daily life (110), COPD specific health status (111) and breathlessness (112). Medication use medical history and history of exacerbations will be recorded based on self-report and based on medical records. Furthermore, subjects will be asked to record everything they eat and drink for three entire days (3-day food diary) in order to assess the food intake. For everything they consume subjects are asked to record the time of consumption, the type of food, the brand name and a detailed description, the amount consumed and anything that was added to the food (e.g. sugar, salt). Additionally, subjects underlying motivational regulations for being physically active and eating healthy will be determined using multiple SDT based questionnaires (113-117). In case a subject will be hospitalized for a COPD exacerbation, details of the exacerbation (e.g. duration, viral/bacterial infection based on sputum and blood markers including C-reactive protein and eosinophils, blood gases) will be recorded from medical records.

Table 3. Overview of study procedures at each measurement day.

Outcome	Measurement	M1	M2	T1	T2	M3
Primary outcomes						
Physical activity level	Accelerometry	X	X	X	X	X
General health status	EQ-5D*	X	X	X	X	X
Secondary outcomes						
Cognitive function						
Cognitive function	CANTAB	X		X	X	X
Psychological wellbeing						
Depression and anxiety	DASS-21*	X		X	X	X
Vulnerability to depression	HADS*	X		X	X	X
Level of stress	PSS*	X		X	X	X
Acute stress	SECPT	X				X
Chronic stress	Hair cortisol	X		X	X	X
Physical performance						
Lower extremity performance	SPPB	X		X	X	X
Handgrip strength	Hydraulic dynamometer	X		X	X	X
Mouth pressure	MicroRPM monitor	X		X	X	X
Exercise performance	6MWT	X				X
Body composition						
Body composition	DEXA-scan	X				X
	Weight	X		X	X	X
	Length	X				X
Blood markers						
Systemic inflammation	Hs-CRP, procalcitonin, IL-6, IL-8, leukocytes	X	X	X	X	X
Nutrient levels	Vitamin E and D, PUFAs, amino acids, homocysteine	X	X	X	X	X
Patient related outcomes						
Fatigue	CIS*	X		X	X	X
Pain	Pain VAS*	X		X	X	X
Sleep quality	PSQI*	X		X	X	X
Intestinal function and gut microbiome						
Intestinal integrity	IFABP rest	X		X	X	X
	IFABP after exercise	X				X
Microbiome composition	Stool sampling (optional)	X	X			X
Other						
Lung function	Spirometry and body plethysmography	X				X
Diffusion capacity	Diffusion capacity	X				X
Impact of COPD	CAT*	X		X	X	X
COPD health status	CCQ*	X		X	X	X
Breathlessness	Modified MRC-scale*	X		X	X	X
Nutritional supplement use#	Self-report	X	X	X	X	X
Medication use	Medical records and self-report	X	X	X	X	X
Medical history	Medical records and self-report	X	X	X	X	X
Food intake	3-day food diary	X				X
Motivation	SDT*	X		X	X	X

*Measurements at baseline, after 3 months and at the end of the study are indicated as M1, M2 and M3 respectively. The recovery period after a hospitalization for a COPD exacerbation is indicated as T1 and T2. * Questionnaire, #Subjects will be requested to refrain from the use of nutritional supplements during the course of the study. In subjects that do not stop due to their medical condition or other arguments, frequency, dose and type of nutritional supplement is recorded. Abbreviations: 6MWT, six minute walking test; CANTAB, Cambridge Neuropsychological Test Automated Battery; CAT, COPD Assessment Test; CCQ, Clinical COPD Questionnaire; CIS, Checklist Individual Strength; DASS-21, Depression Anxiety Stress Scale; DEXA, Dual-Energy X-ray absorptiometry; EQ-5D, EuroQol 5 dimensions; HADS, Hospital Anxiety and Depression Scale; Hs-CRP, High-sensitive C-reactive protein; IFABP, intestinal fatty-acid binding protein; IL; interleukin; MRC, Medical Research Council; PSS, Perceived Stress Scale; PUFAs, Poly unsaturated fatty acids; PSQI, Pittsburgh Sleep Quality Index; SDT, Self-determination theory; SECPT, Socially Evaluated Cold-Pressor Test; SPPB, Short Physical Performance Battery; VAS, Visual Analogue Scale.*

Statistical analysis

Sample size calculation

The recruitment target is calculated to ensure adequate statistical power to detect a difference in the primary outcomes step count and general health status after one year supplementation with the active nutritional supplement compared to the placebo supplement. Both outcomes have been assessed in our previous NUTRAIN-trial (52, 118), so we expect a mean difference between the intervention and placebo group of 900 steps/day (which is also considered a clinically important difference (119)) with a standard deviation of 1790 steps/day and a mean difference in EQ-5D index score of 0.0775 with a standard deviation of 0.153. Using a power of 80% and a significance level of 0.05, we conclude that 62 patients per group finishing the intervention will be sufficient. Based on our experiences with the NUTRAIN-trial, we estimate the drop-out rate will be 25%. Therefore, 83 patients per group will be included.

Randomisation and blinding

Participants meeting the eligibility criteria will be randomised to the active or placebo supplement by an independent researcher. A block randomisation will be conducted to ensure equal allocation of patients in the intervention and control group. The block size will be 10, including five interventions and five controls. The independent researcher will use the minimisation method to maintain an equal distribution

in age, gender, lung function and history of exacerbations of the subjects (120, 121). Briefly, the independent researcher will regularly check (after every 25 included subjects) if these factors are statistically comparable between both groups. In case of large differences, the independent researcher will influence the randomization scheme by manually appointing future subjects to one of the groups until groups are comparable again. The independent researcher will provide the investigator with a number corresponding to a batch of sachets which the investigator will provide to the subjects. The dependent investigators as well as the subjects will be blinded throughout the study.

Data analysis and statistical methods

Data analyses will be conducted using the statistical package IBM SPSS Statistics for Windows (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov normality test will be used to evaluate normal distributions. If necessary, data will be transformed with appropriate functions to achieve normality. In normally distributed data baseline values for the two groups will be compared by the independent sample t-test. If transformation does not result in normally distributed data, non-parametric methods will be used. Chi-square analysis will be performed for categorical variables. Generally, all analyses will be performed according to the intention-to-treat (ITT) principle. Only subjects compliant to the intervention will be included in the per protocol analysis, which will be used as a sensitivity analysis for the ITT analyses. A second per protocol analysis will be performed excluding subjects that used nutritional supplements other than the study products. Within the subset of patients that have been hospitalized for a COPD exacerbation, the effect of nutritional supplementation on the recovery phase (T1 and T2) will be investigated as an explorative analysis. To determine the effects of the nutritional supplementation on the primary, secondary and other parameters, repeated measures analysis will be used to compare mean changes between M1 and M2, M1 and M3 and between T1 and T2. Two-sided *p*-values <0.05 will be claimed as statistically significant.

Safety assessment

Adverse events and serious adverse events will be recorded during the study. An independent data safety monitoring board (DSMB) will be assigned to perform analysis on the serious adverse events data. The DSMB will meet after 25%, 50% and 75% of the inclusion is completed or when principal investigators do striking observations that need further investigation. The DSMB will check whether serious adverse events are related to the study product and will subsequently give the principal investigators advice on the continuation of the study.

Patient and Public Involvement

A patient advisory panel is included in the current study and gave feedback on the study design and patient information during a face-to-face meeting that was organized by the researchers. During the study, this panel will be informed about the study progression and at the end of the study they will be involved in interpretation and dissemination of the results towards the patient population. Additionally, all individual participants will be informed about their personal results after a measurement day. After the full study has been finished, all participants will be informed about the total study results.

ETHICS AND DISSEMINATION

The study has been approved by the Medical Ethics Committee from Maastricht University Medical Centre+ (MUMC+ [NL66543.068.18/METC18-011]) and is registered at clinicaltrials.gov (NCT03807310). The study will be conducted according to the principles laid down in the Declaration of Helsinki (Brazil, October 2013). Subjects will be provided with at least one week to consider their participation and will be given an opportunity to ask questions before they will sign the informed consent. Subjects will be informed that they can leave the study at any time for any reason. Handling of the personal data in this study will conform to the General Data Protection Regulation. Data will be handled confidentially. To trace data of

individual subjects, the study will use a subject identification code list that is linked to the data of the participating subjects. Only the principal investigators have access to this code list. The results of this study will be disseminated through presentations at national and international respiratory and nutritional conferences and through publications in peer-reviewed journals.

Acknowledgments: The researchers want to thank the patient advisory panel for their involvement thus far in the development of the current study.

Contributors: RB, HG, AvH, RH and AS developed the protocol. RB and LvI drafted the manuscript. All authors (RB, LvI, LS, RH, SS, AvH, HR, AS) reviewed the protocol as well as this manuscript.

Funding: This work was supported by a LSH-TKI Lung Foundation grant 10.2.16.119, 2017 'Food for thought and active lifestyle in COPD'.

Competing interests: AvH is employed by Danone Nutricia Research; RH is retired from Danone Nutricia Research; AvH and RH report that a patent might derive from the research described in this paper; Dr. Simons reports grants and personal fees from GlaxoSmithKline, personal fees from Chiesi, grants from Boehringer Ingelheim, grants from AstraZeneca, all outside the submitted work; HG and AS were granted PPP Allowance made available by Health~Holland, Top Sector Life Sciences & Health to Lung Foundation Netherlands & matching by UM, UU, Nutricia Reasearch; all other authors report no competing interests.

REFERENCES

1. (2020-2021): GSCM, Vogelmeier CF, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease (2021 report). 2021 [Available from: https://goldcopd.org/.
2. Lainscak M, Gosker HR, Schols AM. Chronic obstructive pulmonary disease patient journey: hospitalizations as window of opportunity for extra-pulmonary intervention. Curr Opin Clin Nutr Metab Care. 2013;16(3):278-83.
3. Demeyer H, Costilla-Frias M, Louvaris Z, Gimeno-Santos E, Tabberer M, Rabinovich RA, et al. Both moderate and severe exacerbations accelerate physical activity decline in COPD patients. Eur Respir J. 2018;51(1).
4. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax. 2002;57(10):847-52.
5. Miravitlles M, Ferrer M, Pont A, Zalacain R, Alvarez-Sala JL, Masa F, et al. Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. Thorax. 2004;59(5):387-95.
6. Gosker HR, Langen RC, Simons SO. Role of acute exacerbations in skeletal muscle impairment in COPD. Expert Rev Respir Med. 2020:1-13.
7. Falck RS, Davis JC, Liu-Ambrose T. What is the association between sedentary behaviour and cognitive function? A systematic review. Br J Sports Med. 2017;51(10):800-11.
8. Teychenne M, Costigan SA, Parker K. The association between sedentary behaviour and risk of anxiety: a systematic review. BMC Public Health. 2015;15:513.
9. Zhai L, Zhang Y, Zhang D. Sedentary behaviour and the risk of depression: a meta-analysis. Br J Sports Med. 2015;49(11):705-9.
10. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. Journal of Thoracic Disease. 2015;7:E418-E38.
11. Rutten EPA, Lenaerts K, Buurman WA, Wouters EFM. Disturbed intestinal integrity in patients with COPD: effects of activities of daily living. Chest. 2014;145(2):245-52.
12. Matte DL, Pizzichini MM, Hoepers AT, Diaz AP, Karloh M, Dias M, et al. Prevalence of depression in COPD: A systematic review and meta-analysis of controlled studies. Respir Med. 2016;117:154-61.
13. Hung WW, Wisnivesky JP, Siu AL, Ross JS. Cognitive decline among patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2009;180(2):134-7.
14. Yohannes AM, Chen W, Moga AM, Leroi I, Connolly MJ. Cognitive Impairment in Chronic Obstructive Pulmonary Disease and Chronic Heart Failure: A Systematic Review and Meta-analysis of Observational Studies. J Am Med Dir Assoc. 2017;18(5):451 e1- e11.
15. van Beers M, Janssen DJA, Gosker HR, Schols A. Cognitive impairment in chronic obstructive pulmonary disease: disease burden, determinants and possible future interventions. Expert Rev Respir Med. 2018;12(12):1061-74.
16. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33(5):1165-85.
17. Poot B, Travers J, Weatherall M, McGinty M. Cognitive function during exacerbations of chronic obstructive pulmonary disease. Intern Med J. 2019;49(10):1307-12.
18. Schneider C, Jick SS, Bothner U, Meier CR. COPD and the risk of depression. Chest. 2010;137(2):341-7.

19. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare 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(9):e15-62.
20. Remels AH, Gosker HR, Langen RC, Schols AM. The mechanisms of cachexia underlying muscle dysfunction in COPD. Journal of Applied Physiology. 2013;114:1253-62.
21. Langen RC, Haegens A, Vernooy JH, Wouters EF, de Winther MP, Carlsen H, et al. NF-kappaB activation is required for the transition of pulmonary inflammation to muscle atrophy. American journal of respiratory cell and molecular biology. 2012;47(3):288-97.
22. Remels AH, Gosker HR, Schrauwen P, Hommelberg PP, Sliwinski P, Polkey MI, et al. TNF-alpha impairs regulation of muscle oxidative phenotype: implications for cachexia? FASEB. 2010;24:5052-62.
23. Sprooten RTM, Lenaerts K, Braeken DCW, Grimbergen I, Rutten EP, Wouters EFM, et al. Increased Small Intestinal Permeability during Severe Acute Exacerbations of COPD. Respiration. 2018;95(5):334-42.
24. Vaughan A, Frazer ZA, Hansbro PM, Yang IA. COPD and the gut-lung axis: the therapeutic potential of fibre. J Thorac Dis. 2019;11(Suppl 17):S2173-S80.
25. Foster JA, Rinaman L, Cryan JF. Stress & the gut-brain axis: Regulation by the microbiome. Neurobiol Stress. 2017;7:124-36.
26. Zinellu A, Fois AG, Zinellu E, Sotgiu E, Sotgia S, Arru D, et al. Increased kynurenine plasma concentrations and kynurenine-tryptophan ratio in mild-to-moderate chronic obstructive pulmonary disease patients. Biomark Med. 2018;12(3):229-37.
27. Gosker HR, Clarke G, de Theije CC, Cryan JF, Schols A. Impaired Skeletal Muscle Kynurenine Metabolism in Patients with Chronic Obstructive Pulmonary Disease. J Clin Med. 2019;8(7).
28. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology. 2017;112(Pt B):399-412.
29. Duenas-Espin I, Demeyer H, Gimeno-Santos E, Polkey MI, Hopkinson NS, Rabinovich RA, et al. Depression symptoms reduce physical activity in COPD patients: a prospective multicenter study. Int J Chron Obstruct Pulmon Dis. 2016;11:1287-95.
30. Coultas DB, Jackson BE, Russo R, Peoples J, Singh KP, Sloan J, et al. Home-based Physical Activity Coaching, Physical Activity, and Health Care Utilization in Chronic Obstructive Pulmonary Disease. Chronic Obstructive Pulmonary Disease Self-Management Activation Research Trial Secondary Outcomes. Ann Am Thorac Soc. 2018;15(4):470-8.
31. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester 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(8):e13-64.
32. Egan C, Deering BM, Blake C, Fullen BM, McCormack NM, Spruit MA, et al. Short term and long term effects of pulmonary rehabilitation on physical activity in COPD. Respir Med. 2012;106(12):1671-9.
33. Mador MJ, Patel AN, Nadler J. Effects of pulmonary rehabilitation on activity levels in patients with chronic obstructive pulmonary disease. J Cardiopulm Rehabil Prev. 2011;31(1):52-9.
34. Steele BG, Belza B, Cain KC, Coppersmith J, Lakshminarayan S, Howard J, et al. A randomized clinical trial of an activity and exercise adherence intervention in chronic pulmonary disease. Arch Phys Med Rehabil. 2008;89(3):404-12.
35. van de Bool C, Rutten EPA, van Helvoort A, Franssen FME, Wouters EFM, Schols A. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. J Cachexia Sarcopenia Muscle. 2017;8(5):748-58.
36. Nurk E, Drevon CA, Refsum H, Solvoll K, Vollset SE, Nygard O, et al. Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study. Am J Clin Nutr. 2007;86(5):1470-8.

37. Grosso G, Micek A, Marventano S, Castellano S, Mistretta A, Pajak A, et al. Dietary n-3 PUFA, fish consumption and depression: a systematic review and meta-analysis of observational studies. Journal of Affective Disorders. 2016;205:269-81.
38. Ma WJ, Li H, Zhang W, Zhai J, Li J, Liu H, et al. Effect of n-3 polyunsaturated fatty acid supplementation on muscle mass and function with aging: A meta-analysis of randomized controlled trials(). Prostaglandins Leukot Essent Fatty Acids. 2021;165:102249.
39. Remels AH, Langen RC, Schrauwen P, Schaart G, Schols AM, Gosker HR. Regulation of mitochondrial biogenesis during myogenesis. Mol Cell Endocrinol. 2010;315(1-2):113-20.
40. Yaqoob P, Calder P. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. Cell Immunol. 1995;163(1):120-8.
41. Duvall MG, Levy BD. DHA- and EPA-derived resolvins, protectins, and maresins in airway inflammation. Eur J Pharmacol. 2016;785:144-55.
42. Remels AH, Langen RC, Gosker HR, Russell AP, Spaapen F, Voncken JW, et al. PPARgamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. Am J Physiol Endocrinol Metab. 2009;297(1):E174-83.
43. Giudetti AM, Cagnazzo R. Beneficial effects of n-3 PUFA on chronic airway inflammatory diseases. Prostaglandins Other Lipid Mediat. 2012;99(3-4):57-67.
44. Chapkin RS, Akoh CC, Miller CC. Influence of dietary n-3 fatty acids on macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis. J Lipid Res. 1991;32(7):1205-13.
45. Guixa-Gonzalez R, Javanainen M, Gomez-Soler M, Cordobilla B, Domingo JC, Sanz F, et al. Membrane omega-3 fatty acids modulate the oligomerisation kinetics of adenosine A2A and dopamine D2 receptors. Sci Rep. 2016;6:19839.
46. Carabelli B, Delattre AM, Waltrick APF, Araujo G, Suchecki D, Machado RB, et al. Fish-oil supplementation decreases Indoleamine-2,3-Dioxygenase expression and increases hippocampal serotonin levels in the LPS depression model. Behav Brain Res. 2020;390:112675.
47. McGlory C, Calder PC, Nunes EA. The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. Front Nutr. 2019;6:144.
48. Van de Bool C, Mattijssen-Verdonschot C, Van Melick PP, Spruit MA, Franssen FM, Wouters EF, et al. Quality of dietary intake in relation to body composition in patients with chronic obstructive pulmonary disease eligible for pulmonary rehabilitation. European Journal of Clinical Nutrition. 2014;68:159-65.
49. Varraso R, Fung TT, Barr RG, Hu FB, Willett W, Camargo CA, Jr. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US women. Am J Clin Nutr. 2007;86(2):488-95.
50. Varraso R, Fung TT, Hu FB, Willett W, Camargo CA. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US men. Thorax. 2007;62(9):786-91.
51. Hirayama F, Lee AH, Binns CW, Hiramatsu N, Mori M, Nishimura K. Dietary intake of isoflavones and polyunsaturated fatty acids associated with lung function, breathlessness and the prevalence of chronic obstructive pulmonary disease: possible protective effect of traditional Japanese diet. Mol Nutr Food Res. 2010;54(7):909-17.
52. van Beers M, Rutten-van Molken M, van de Bool C, Boland M, Kremers SPJ, Franssen FME, et al. Clinical outcome and cost-effectiveness of a 1-year nutritional intervention programme in COPD patients with low muscle mass: The randomized controlled NUTRAIN trial. Clin Nutr. 2020;39(2):405-13.
53. Broekhuizen R, Wouters EF, Creutzberg EC, Weling-Scheepers CA, Schols AM. Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. Thorax. 2005;60(5):376-82.

54. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 2017;357(6349).
55. Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. International journal of tryptophan research : IJTR. 2009;2:45-60.
56. Young SN. The effect of raising and lowering tryptophan levels on human mood and social behaviour. Philosophical Transactions of the Royal Society of London. 2013;368(1615):20110375.
57. Vardar-Yagli N, Saglam M, Savci S, Inal-Ince D, Calik-Kutukcu E, Arikan H, et al. Impact of sleep quality on functional capacity, peripheral muscle strength and quality of life in patients with chronic obstructive pulmonary disease. Expert Rev Respir Med. 2015;9(2):233-9.
58. Cleutjens FA, Pedone C, Janssen DJ, Wouters EF, Incalzi RA. Sleep quality disturbances and cognitive functioning in elderly patients with COPD. ERJ Open Res. 2016;2(3).
59. Song SB, Park JS, Chung GJ, Lee IH, Hwang ES. Diverse therapeutic efficacies and more diverse mechanisms of nicotinamide. Metabolomics. 2019;15(10):137.
60. Gulcev M, Reilly C, Griffin TJ, Broeckling CD, Sandri BJ, Witthuhn BA, et al. Tryptophan catabolism in acute exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2016;11:2435-46.
61. Dzik KP, Kaczor JJ. Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. Eur J Appl Physiol. 2019;119(4):825-39.
62. Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS, et al. 1alpha,25-Dihydroxyvitamin D3 Regulates Mitochondrial Oxygen Consumption and Dynamics in Human Skeletal Muscle Cells. J Biol Chem. 2016;291(3):1514-28.
63. Spedding S. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. Nutrients. 2014;6(4):1501-18.
64. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. Journal of chemical neuroanatomy. 2005;29(1):21-30.
65. Buell JS, Dawson-Hughes B. Vitamin D and neurocognitive dysfunction: preventing "D"ecline? Mol Aspects Med. 2008;29(6):415-22.
66. van Etten E, Stoffels K, Gysemans C, Mathieu C, Overbergh L. Regulation of vitamin D homeostasis: implications for the immune system. Nutr Rev. 2008;66(10 Suppl 2):S125-34.
67. Mahlin C, von Sydow H, Osmancevic A, Emtner M, Gronberg AM, Larsson S, et al. Vitamin D status and dietary intake in a Swedish COPD population. Clin Respir J. 2014;8(1):24-32.
68. Zhu M, Wang T, Wang C, Ji Y. The association between vitamin D and COPD risk, severity, and exacerbation: an updated systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis. 2016;11:2597-607.
69. Zhu B, Zhu B, Xiao C, Zheng Z. Vitamin D deficiency is associated with the severity of COPD: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis. 2015;10:1907-16.
70. Romme EA, Smeenk FW, Rutten EP, Wouters EF. Osteoporosis in chronic obstructive pulmonary disease. Expert Rev Respir Med. 2013;7(4):397-410.
71. Persson LJ, Aanerud M, Hiemstra PS, Hardie JA, Bakke PS, Eagan TM. Chronic obstructive pulmonary disease is associated with low levels of vitamin D. PLoS One. 2012;7(6):e38934.
72. Kokturk N, Baha A, Oh YM, Young Ju J, Jones PW. Vitamin D deficiency: What does it mean for chronic obstructive pulmonary disease (COPD)? a compherensive review for pulmonologists. Clin Respir J. 2018;12(2):382-97.
73. Carson EL, Pourshahidi LK, Madigan SM, Baldrick FR, Kelly MG, Laird E, et al. Vitamin D status is associated with muscle strength and quality of life in patients with COPD: a seasonal prospective observation study. Int J Chron Obstruct Pulmon Dis. 2018;13:2613-22.

74. Hornikx M, Van Remoortel H, Lehouck A, Mathieu C, Maes K, Gayan-Ramirez G, et al. Vitamin D supplementation during rehabilitation in COPD: a secondary analysis of a randomized trial. Respiratory Research. 2012;13:84.
75. Alavi Foumani A, Mehrdad M, Jafarinezhad A, Nokani K, Jafari A. Impact of vitamin D on spirometry findings and quality of life in patients with chronic obstructive pulmonary disease: a randomized, double-blinded, placebo-controlled clinical trial. Int J Chron Obstruct Pulmon Dis. 2019;14:1495-501.
76. Jolliffe DA, Greenberg L, Hooper RL, Mathyssen C, Rafiq R, de Jongh RT, et al. Vitamin D to prevent exacerbations of COPD: systematic review and meta-analysis of individual participant data from randomised controlled trials. Thorax. 2019;74(4):337-45.
77. Martineau AR, Jolliffe DA, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. Health Technol Assess. 2019;23(2):1-44.
78. Pelgrim CE, Peterson JD, Gosker HR, Schols A, van Helvoort A, Garssen J, et al. Psychological co-morbidities in COPD: Targeting systemic inflammation, a benefit for both? Eur J Pharmacol. 2018;842:99-110.
79. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125(6):1401-12.
80. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54(9):2325-40.
81. Janbazacyabar H, van Bergenhenegouwen J, Verheijden KAT, Leusink-Muis T, van Helvoort A, Garssen J, et al. Non-digestible oligosaccharides partially prevent the development of LPS-induced lung emphysema in mice. Pharmanutrition. 2019;10.
82. Hanson C, Lyden E, Rennard S, Mannino DM, Rutten EP, Hopkins R, et al. The Relationship between Dietary Fiber Intake and Lung Function in the National Health and Nutrition Examination Surveys. Ann Am Thorac Soc. 2016;13(5):643-50.
83. Szmidt MK, Kaluza J, Harris HR, Linden A, Wolk A. Long-term dietary fiber intake and risk of chronic obstructive pulmonary disease: a prospective cohort study of women. Eur J Nutr. 2020;59(5):1869-79.
84. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krleza-Jeric K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-7.
85. European Food Safety Authority. Dietary reference values for nutrients. Summary report. 2017 [Available from: https://www.efsa.europa.eu/sites/default/files/2017_09_DRVs_summary_report.pdf.
86. Resnicow K, Dilorio C, Soet JE, Ernst D, Borrelli B, Hecht J. Motivational interviewing in health promotion: it sounds like something is changing. Health Psychol. 2002;21(5):444-51.
87. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J. 2008;31(2):416-69.
88. Ng LWC, Jenkins S, Hill K. Accuracy and responsiveness of the stepwatch activity monitor and ActivPAL in patients with COPD when walking with and without a rollator. Disability and Rehabilitation. 2012;34(15):1317-22.
89. Lyden K, Keadle SK, Staudenmayer J, Freedson PS. The activPALTM Accurately Classifies Activity Intensity Categories in Healthy Adults. Med Sci Sports Exerc. 2017;49(5):1022-8.
90. Demeyer H, Mohan D, Burtin C, Vaes AW, Heasley M, Bowler RP, et al. Objectively Measured Physical Activity in Patients with COPD: Recommendations from an International Task Force on Physical Activity. Chronic Obstr Pulm Dis. 2021;8(4):528-50.
91. Pickard AS, Wilke C, Jung E, Patel S, Stavem K, Lee TA. Use of a preference-based measure of health (EQ-5D) in COPD and asthma. Respir Med. 2008;102(4):519-36.

92. Lowe C, Rabbitt P. Test / re-test reliability of the CANTAB and ISPOCD neuropsychological batteries: theoretical and practical issues. Neuropsychologia. 1998;36(9):915-23.
93. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behav Res Ther. 1995;33(3):335-43.
94. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta psychiatrica Scandinavica. 1983;67(6):361-70.
95. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of Health and Social Behaviour. 1983;24(4):385-96.
96. Schwabe L, Haddad L, Schachinger H. HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology. 2008;33(6):890-5.
97. O'Brien KM, Tronick EZ, Moore CL. Relationship between hair cortisol and perceived chronic stress in a diverse sample. Stress and health : journal of the International Society for the Investigation of Stress. 2013;29(4):337-44.
98. van Ockenburg SL, Schenk HM, van der Veen A, van Rossum EF, Kema IP, Rosmalen JG. The relationship between 63days of 24-h urinary free cortisol and hair cortisol levels in 10 healthy individuals. Psychoneuroendocrinology. 2016;73:142-7.
99. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. Journal of gerontology. 1994;49(2):M85-94.
100. Patel MS, Mohan D, Andersson YM, Baz M, Samantha Kon SC, Canavan JL, et al. Phenotypic characteristics associated with reduced short physical performance battery score in COPD. Chest. 2014;145(5):1016-24.
101. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, et al. Midlife hand grip strength as a predictor of old age disability. Jama. 1999;281(6):558-60.
102. Rantanen T, Harris T, Leveille SG, Visser M, Foley D, Masaki K, et al. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. J Gerontol A Biol Sci Med Sci. 2000;55(3):M168-73.
103. Laboratories ATSCoPSfCPF. A. T. S. Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7.
104. American Thoracic Society/European Respiratory S. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med. 2002;166(4):518-624.
105. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. Journal of psychosomatic research. 1994;38(5):383-92.
106. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
107. Pelsers MM, Namiot Z, Kisielewski W, Namiot A, Januszkiewicz M, Hermens WT, et al. Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. Clin Biochem. 2003;36(7):529-35.
108. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32(9):920-4.
109. Ramiro-Garcia J, Hermes GDA, Giatsis C, Sipkema D, Zoetendal EG, Schaap PJ, et al. NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes. F1000Res. 2016;5:1791.
110. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009;34(3):648-54.

111. van der Molen T, Willemse BW, Schokker S, ten Hacken NH, Postma DS, Juniper EF. Development, validity and responsiveness of the Clinical COPD Questionnaire. Health and quality of life outcomes. 2003;1:13.
112. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax. 1999;54(7):581-6.
113. Markland D. Self-determination moderates the effects of perceived competence on intrinsic motivation in an exercise setting. J Sport Exercise Psy. 1999;21(4):351-61.
114. Markland D, Ingledew DK. The measurement of exercise motives: Factorial validity and invariance across gender of a revised exercise motivations inventory. Brit J Health Psych. 1997;2:361-76.
115. Markland D, Tobin V. A Modification of the Behavioral Regulation in Exercise Questionnaire to include an assessment of amotivation. Journal of Sport and Exercise Psychology. 2004;26:191-6.
116. Rose EA, Markland D, Parfitt G. The development and initial validation of the Exercise Causality Orientations Scale. J Sport Sci. 2001;19(6):445-62.
117. Mcauley E, Duncan T, Tammen VV. Psychometric Properties of the Intrinsic Motivation Inventory in a Competitive Sport Setting - a Confirmatory Factor-Analysis. Res Q Exercise Sport. 1989;60(1):48-58.
118. Van de Bool C, Rutten EPA, Van Helvoort A, Franssen F, Wouters EF, Schols AM. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. Journal of Cachexia, Sarcopenia and Muscle. 2017.
119. Demeyer H, Burtin C, Hornikx M, Camillo CA, Van Remoortel H, Langer D, et al. The Minimal Important Difference in Physical Activity in Patients with COPD. PLoS One. 2016;11(4):e0154587.
120. Han B, Enas NH, McEntegart D. Randomization by minimizatioin for unbalanced treatment allocation. Statistics in Medicine. 2009;28(27):3329-46.
121. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975;31(1):103-15.

Figure legends

Figure 1. Study design

Measurements at baseline, after 3 months and at the end of the study are indicated as M1, M2 and M3 respectively. After a hospitalization for a COPD exacerbation (H) the recovery phase of 4 weeks will be monitored (T1 and T2). In case patients will be recruited during a hospitalization for a COPD exacerbation, M1 will take place 4 weeks after discharge. In case the hospitalization will be within 3 months after baseline, M2 will not take place.

Figure 2. Timeline of measurement day

This outline provides a timeline of a measurement day at M1 and M3. During M2, T1 and T2 a selection of these measurements will be performed as described in Table 3. Measurements will be performed in this order unless logistically not possible.

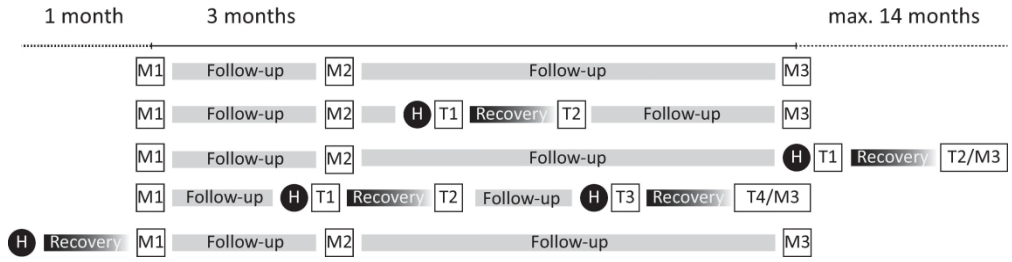

Figure 1: Study design

181x46mm (600 x 600 DPI)

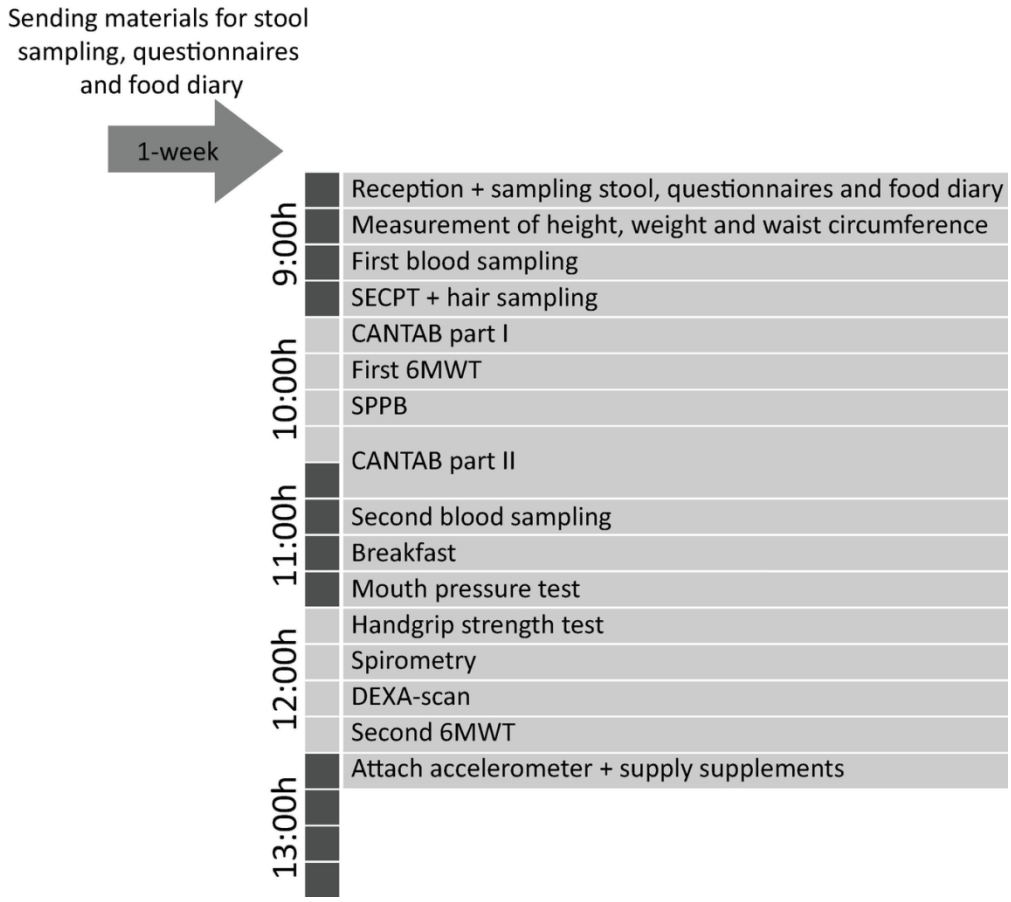

Figure 2: Timeline of measurement day.

114x101mm (300 x 300 DPI)

Bijlage 4: Toestemmingsverklaring

Voor deelname aan het wetenschappelijk onderzoek:
Het effect van een voedingsinterventie op de dagelijkse lichamelijke activiteiten en de gezondheidstoestand van patiënten met COPD
(NUTRECOVER trial) (NL66543.068.18; versie 8 / 13/09/2021)

Ik heb de informatiebrief gelezen (Versie 6). Ook kon ik vragen stellen. Ik had genoeg tijd om te beslissen of ik meedoe. Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.

- Ik geef toestemming voor het informeren van mijn huisarts en behandelend arts dat ik meedoe aan dit onderzoek.
- Ik geef toestemming voor het opvragen van informatie bij mijn huisarts/specialist(en) die mij behandelt voor COPD.
- Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens, bloedmonsters en lichaamsmateriaal voor de beantwoording van de onderzoeksvraag in dit onderzoek.
- Ik weet dat voor controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Die mensen staan vermeld in de informatiebrief. Ik geef toestemming voor
  die inzage voor deze personen.
- Ik geef toestemming voor het informeren van mijn huisarts en/of behandelend specialist van onverwachte bevindingen die van belang (kunnen) zijn voor mijn gezondheid.
- Ik geef **wel/geen** (doorhalen wat **niet** van toepassing is) toestemming om mijn persoonsgegevens langer te bewaren en te gebruiken voor toekomstig onderzoek op het gebied van leefstijlveranderingen in COPD.
- Ik geef **wel/geen** (doorhalen wat **niet** van toepassing is) toestemming om mijn lichaamsmateriaal na afloop van dit onderzoek te bewaren. En om dit later nog voor ander onderzoek te gebruiken, zoals in de informatiebrief staat.
- Ik geef **wel/geen** (doorhalen wat **niet** van toepassing is) toestemming om mij na dit onderzoek te benaderen voor een vervolgonderzoek.
- Ik wil **wel/niet** (doorhalen wat **niet** van toepassing is) geïnformeerd worden over welke behandeling ik heb gehad/in welke groep ik zat.
- Ik wil meedoen aan dit onderzoek.

Naam proefpersoon: ……

Handtekening: …… Datum: _ _ / _ _ / _ _ _ _

In te vullen door de onderzoeker: Ik verklaar hierbij dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek. Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker: ……

Handtekening: …… Datum: _ _ / _ _ / _ _ _ _

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	p. 2
	2b	All items from the World Health Organization Trial Registration Data Set	N.A.
Protocol version	3	Date and version identifier	N.A.
Funding	4	Sources and types of financial, material, and other support	p. 23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p. 1
	5b	Name and contact information for the trial sponsor	p. 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N.A.
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N.A.

http://bmjopen-2021-059252 on 16 March 2022. Downloaded from

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	p. 7
	6b	Explanation for choice of comparators	p. 7
Objectives	7	Specific objectives or hypotheses	p. 7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	p. 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	p. 10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	p. 12
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	p. 13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	p. 13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	p. 17
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N.A.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p. 20
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	p. 10

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p. 21
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	p. 21
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	p. 21
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p. 21
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p. 21
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N.A.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	N.A.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N.A.

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p. 22
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p. 22
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p. 22
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p. 22
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p. 22
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p. 22
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p. 22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p. 22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p. 23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N.A.

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p. 23
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N.A.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	p. 23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p. 23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	p. 23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N.A.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	p. 23
	31b	Authorship eligibility guidelines and any intended use of professional writers	N.A.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N.A.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N.A.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N.A.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.