Cardiometabolic risk modulation in normal-weight COPD

Rosanne Johanna Hubertine Christine Guillaumine Beijers

School of Nutrition and Translational Research in Metabolism

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Rosanne Johanna Hubertine Christine Guillaumine Beijers
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Promotor
Prof. dr. A.M.W.J. Schols

Co-promotor
Dr. H.R. Gosker
Dr. B. van den Borst

Beoordelingscommissie
Prof. dr. J. Plat (voorzitter)
Prof. dr. W.J. Janssens (Katholieke Universiteit Leuven, België)
Prof. dr. J.W.M. Muris
Prof. dr. C.D.A. Stehouwer
Prof. dr. M.C. Steiner (University of Leicester, United Kingdom)

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Chapter 1

General introduction

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a common, preventable and treatable lung disease, which is characterized by persistent airflow limitation resulting from inflammation and remodeling of the airways [1]. Symptoms of COPD include dyspnea, cough, sputum production and exercise limitation and patients commonly suffer from acute exacerbations. During the course of their disease, many patients with COPD are frequently hospitalized not only for exacerbations but also for a wide range of non-respiratory causes [2, 3], resulting in high healthcare costs [4]. According to the World Health Organization, sixty-five million people suffer from COPD worldwide and in 2012 more than 3 million people died of COPD [5]. Currently, COPD is the fourth leading cause of mortality and is expected to be the 3rd by 2020 as a result of an increasing prevalence of COPD due to a higher smoking prevalence and aging populations [1].

Tobacco smoking is the main risk factor for the development of COPD [1]. However, non-smokers may also develop COPD because other identified risk factors include genetics and environmental exposures to particles such as toxic gasses, air pollution and biomass fuel [1]. The airflow limitation is caused by thickening of the airway walls causing increased mucus production (chronic bronchitis) and/or destruction of the alveolar walls (emphysema). In any person who has dyspnea, chronic cough or sputum production and/or a history of exposure to the described risk factors, COPD should be considered. The diagnosis of COPD is based on the presence of airflow limitation measured by post-bronchodilator spirometry. Furthermore, COPD severity is graded by combining severity scores for airflow limitation, symptoms and past exacerbations [1]. COPD cannot be cured, but measures can be undertaken aiming to stabilize lung function and improve symptoms. Smoking cessation is the cornerstone of COPD treatment to prevent further damage to the lungs. Pharmacological therapy includes inhaled bronchodilators with or without corticosteroids to reduce inflammation, and oxygen therapy may be prescribed depending on the severity of hypoxemia. For those patients that remain symptomatic despite these measures, integrated pulmonary rehabilitation is recommended as the next step in treatment. Finally, non-invasive ventilation, lung volume reduction and lung transplantation may be considered in patients with very severe COPD.

SYSTEMIC MANIFESTATIONS AND COMORBIDITIES IN COPD

COPD is a heterogeneous disease that often coexists with systemic manifestations and other diseases (comorbidities). These systemic manifestations and comorbidities affect symptom burden, functional performance and health status, increase the risk of hospitalization and mortality and significantly increase healthcare costs [6, 7]. The most prevalent systemic manifestations and comorbidities are cardiovascular diseases, skeletal muscle dysfunction, osteoporosis, lung cancer and psychological disorders [8]. These comorbidities are linked

by shared risk factors such as older age, systemic inflammation and an unhealthy lifestyle including smoking, physical inactivity and poor dietary quality. The prevalence of comorbidities in COPD has been shown to be independent of the degree of airflow limitation [9, 10].

The presence of multiple chronic comorbidities (multimorbidity) is common in patients with COPD [1]. Recent unbiased statistical approaches have identified several comorbidity clusters in COPD and demonstrated that muscle wasting and osteoporosis is mainly present in emphysematous patients prone to weight loss while atherosclerosis, dyslipidemia, hyperglycemia, and hypertension are more common in overweight to obese patients [10].

INCREASED CARDIOMETABOLIC RISK IN COPD

Cardiovascular disease is the leading cause of mortality in patients with mild-to-moderate COPD [11, 12]. Indeed, patients with COPD have a 2 to 5 times higher risk of major cardiovascular disease compared with controls which is independent of shared risk factors such as age and smoking [13, 14]. More specifically, congestive heart failure, coronary heart disease, arrhythmias, stroke and peripheral artery disease were associated with COPD [14]. In addition, the COPD comorbidome described by Divo et al. showed that especially congestive heart failure, coronary artery disease and atrial fibrillation were strongly associated with mortality in patients with COPD [15].

A large cohort study including 505 COPD patients referred to pulmonary rehabilitation showed a high prevalence of abdominal obesity (78%) [16]. Another large cohort study including 213 patients referred to pulmonary rehabilitation showed a high prevalence of hyperglycemia (54%), hypertension (48%) and dyslipidemia (36%) [10]. These metabolic abnormalities are included in the metabolic syndrome, a constellation of metabolic risk factors that increase the risk of developing cardiovascular diseases and diabetes. Based on the high prevalence of the independent metabolic risk factors and the increased cardiovascular disease risk of patients with COPD, the prevalence of metabolic syndrome is expected to be high and more common in patients with COPD compared to healthy controls.

The increased cardiovascular and metabolic (cardiometabolic) disease risk in COPD is not fully explained by smoking [8], suggesting that other factors are involved. A decreased skeletal muscle oxidative capacity and an increased abdominal visceral adipose tissue have been suggested to be two major phenotypic drivers of the increased cardiometabolic risk profile of patients with COPD [17].

Loss of skeletal muscle mass and oxidative phenotype

Loss of skeletal muscle mass is an important systemic manifestation in COPD not only due to its contribution to decreased physical performance and health status [18, 19], but also

because it is a determinant of mortality [20]. In addition to loss of muscle mass, intrinsic alterations in the peripheral skeletal muscle of COPD patients have been identified [21]. A shift from oxidative type I towards more glycolytic type II muscle fibers has been identified in mild-to-moderate [22] and is even more pronounced in patients with advanced COPD [23]. Furthermore, patients with COPD frequently have decreased oxidative enzyme capacity and mitochondrial dysfunction [24], which is associated with decreased muscle endurance, exercise capacity and mechanical efficiency [25, 26]. The loss of skeletal muscle oxidative phenotype has been suggested to play a major role in "metabolic inflexibility" [27], in which the capacity to increase fat oxidation in response to increased fatty acid availability is decreased and the capacity to switch from fat to glucose oxidation in response to nutritional circumstances is impaired [28]. This may lead to the accumulation of intramyocellular lipids which has been associated with insulin resistance [27, 29]. Altogether, skeletal muscle alterations seem to contribute to the cardiometabolic risk profile in patients with COPD. Recently, it has been shown that COPD patients with sarcopenia, defined as low muscle mass and muscle function, have a more pronounced decrease in type I muscle fiber proportion compared to non-sarcopenic patients [30]. However, to what extend this low skeletal muscle mass in COPD contributes to an increased cardiometabolic risk still needs to be investigated.

Abdominal obesity

If normal-to-overweight patients with COPD are diagnosed with low muscle mass, by definition it means that these patients have relatively high fat mass. In a large Dutch cohort study including 505 patients with COPD eligible for pulmonary rehabilitation, 336 patients were normal-to-overweight [16]. In these normal-to-overweight patients, sarcopenia was prevalent in 92.9% and coexistence of abdominal obesity and sarcopenia was even prevalent in 78.9% of the patients. The group of patients with combined abdominal obesity and sarcopenia displayed higher physical functioning than patients with low muscle mass without abdominal obesity. Another study performed in The Netherlands included 564 COPD patients referred for pulmonary rehabilitation and showed that patients with combined abdominal obesity and low muscle mass consumed most often a poor-quality diet [31]. Adipose tissue excretes adipokines which contribute to low-grade systemic inflammation in many chronic diseases, and appears to be also relevant in COPD. Indeed, combined loss of muscle mass and adiposity, termed sarcopenic obesity, was associated with higher systemic inflammatory burden compared normal body composition and sarcopenia in clinically stable patients with COPD [32]. Persistence of systemic inflammation defined as more than 2 elevated systemic inflammatory biomarkers has been shown to be associated with higher BMI in patients with COPD [33]. More specifically, a positive relation between low-grade systemic inflammation and total abdominal fat mass has been shown, independent of body mass index (BMI) [34]. COPD patients with high C-reactive protein (CRP) levels had significantly greater adipose tissue macrophage infiltration than patients with low CRP, suggesting a relation between

low-grade systemic inflammation and adipose tissue inflammation [35]. Therefore, it was suggested that adipose tissue is a significant contributor to the systemic inflammatory load in clinically stable COPD.

Abdominal obesity, in particularly visceral adiposity, is receiving increasing attention in COPD. Several studies have shown an excessive visceral fat mass in patients with COPD independent of age, BMI, subcutaneous fat mass, abdominal circumference and whole body fat mass [36-39]. In general, visceral fat appears to be associated with cardiometabolic disorders, including hypertension and insulin resistance [40, 41]. Also in COPD, the increased visceral fat mass was associated with a higher prevalence of diabetes, myocardial infarction and low-grade systemic inflammation [37, 39, 42]. Low-grade systemic inflammation was associated with cardiovascular mortality in older subjects with obstructive lung disease [37]. To what extend low muscle mass combined with abdominal obesity adversely influence the cardiometabolic risk profile of patients with COPD still needs to be determined.

EXERCISE TRAINING TO MODULATE THE CARDIOMETABOLIC RISK IN COPD

Patients with COPD are characterized by an inactive lifestyle compared to healthy controls [22]. In general, physical inactivity predisposes to incident cardiovascular disease and diabetes [43-46]. Also in COPD reduced physical activity probably contributes to the increased cardiometabolic risk in COPD as was shown by lower physical activity levels in COPD patients with metabolic syndrome compared to patients without metabolic syndrome [47]. Therefore, we hypothesized that patients with COPD benefit from exercise training to improve their cardiometabolic risk profile.

In healthy overweight and obese persons without COPD, exercise training has been shown to improve muscle mitochondrial biogenesis and improve insulin sensitivity even in individuals with insulin resistance [48]. Furthermore, a recent large meta-analysis including 160 randomized controlled trials showed that exercise training significantly improved cardiorespiratory fitness and cardiometabolic biomarkers [49]. People with an increased cardiometabolic risk profile (people with type 2 diabetes, hypertension, dyslipidemia or metabolic syndrome) seemed to benefit even more from exercise training. In addition, another recent meta-analysis showed that exercise training can reduce visceral adipose mass and despite smaller effects on reducing body weight exercise training tended to have superior effects on reducing visceral adipose mass compared with diet interventions [50]. Furthermore, meta-analyses showed decreased CRP and blood pressure after exercise training in adults with or without comorbidities [51, 52].

In patients with mild to moderate COPD, acute exercise caused similar upregulation of oxidative gene expression in skeletal muscle compared to healthy controls, despite lower baseline oxidative capacity in the COPD patients [53]. This indicates that the regulatory

response to acute exercise is not impaired in patients with COPD, suggesting that muscle oxidative phenotype could still be improved in COPD. Exercise training is considered to be the cornerstone of pulmonary rehabilitation in COPD [54]. Although the primary aim of exercise training in these state-of-the art pulmonary rehabilitation programs is to improve dyspnea, functional capacity and quality, it has also great potential to modulate the drivers of the increased cardiometabolic risk [17]. However, studies investigating the efficacy of exercise training in COPD specifically focusing on the increased cardiometabolic risk are scarce. Therefore, more studies are needed to investigate the modulating potential of exercise training on the cardiometabolic risk profile in patients with COPD.

NUTRITIONAL INTERVENTIONS TO MODULATE THE CARDIOMETABOLIC RISK IN COPD

Exercise training seems to be a promising intervention to modulate the cardiometabolic risk profile of patients with COPD. However, due to ventilatory restrictions and symptoms such as dyspnea and fatigue, the cardiometabolic benefit of exercise training may be limited in COPD. Therefore, interventions that might enhance or mimic the effects of exercise training are relevant to explore. Two interesting nutritional candidates are dietary nitrate and resveratrol.

Dietary nitrate

Nitrate can be derived via two sources: the endogenous L-arginine-nitric oxide (NO) synthase pathway and the diet. The L-arginine-NO synthase pathway is oxygen dependent and generates NO via the oxidation of the amino acid L-arginine in a reaction catalyzed by NO synthase (NOS) enzymes. Subsequently, NO is oxidized to nitrite and nitrate [55]. In the diet, nitrate is highly present in green leafy vegetables, such as spinach and rocket, and in root vegetables, such as beetroot [56]. Nitrate can be reduced to nitrite via nitrate reductase activity of facultative anaerobic bacteria in the mouth and can subsequently be converted to nitritc oxide (NO), usually in the acidic environment such as in the stomach [57]. Mainly under anaerobic conditions, such as during exercise, nitrate will be converted via this oxygen independent nitrate-nitrite-NO-pathway.

As a result of dietary nitrate intake, NO availability will be increased which can have vasodilatory effects that can lower the blood pressure and modulate muscle-related processes including muscle contractility, glucose homeostasis, blood flow, mitochondrial respiration and biogenesis [55]. Indeed, recent meta-analyses have shown a reduction in blood pressure, improved endurance exercise capacity and lower oxygen consumption during submaximal exercise after dietary nitrate supplementation in healthy adults [58-61]. For this reason, we hypothesized that patients with COPD might also benefit from dietary nitrate supplementation, not only because it might lower the blood pressure, but also because it

might improve mechanical efficiency and exercise performance. If dietary nitrate improves exercise performance, it might be a useful nutritional supplement as adjunct to pulmonary rehabilitation to enhance the effects of exercise training.

Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a natural polyphenol produced by plants and is present in various dietary components such as grapes, peanuts, mulberries, and red wine. Already in 1992 resveratrol was supposed to explain some of the cardioprotective effects of red wine and was suggested to be an important factor in the French paradox, describing a low incidence of cardiovascular disease in the French population, despite a diet high in saturated fat [62, 63]. After that, interest in resveratrol increased and chemo-preventive, anti-inflammatory and anti-oxidant properties of resveratrol were identified [63]. Therefore, it is thought that resveratrol can contribute to health benefits, including cardiovascular disease risk [64].

Resveratrol has been suggested to mimic the effects of calorie restriction and exercise due to the activation of sirtuin 1, a human deacetylase that promotes cell survival [65]. Subsequently, sirtuin 1 activates peroxisome proliferator-activated receptor gamma coactivator 1-alpha, a master regulator of mitochondrial metabolism and biogenesis. Hereby, resveratrol might improve skeletal muscle mitochondrial function. Resveratrol has never been tested in patients with COPD but might be an interesting supplement, not only to improve skeletal muscle metabolism and to modulate the cardiometabolic risk profile but also to decrease inflammation and oxidative stress in the lungs.

AIM AND OUTLINE OF THIS THESIS

Prevention of cardiometabolic diseases in COPD requires a detailed understanding of the risk factors and phenotypic characteristics involved. Abnormal body composition has been suggested to contribute to an increased cardiometabolic risk profile in patients with COPD, even in those with normal weight. In this light, cardiometabolic risk modulation strategies need to be investigated. Therefore, the general aim of this thesis is to investigate the cardiometabolic risk profile of normal weight patients with COPD and to study the effectiveness of exercise and nutritional interventions on the cardiometabolic risk profile in these patients.

In **Chapter 2** we analyzed risk factors, including cardiometabolic risk parameters, for all-cause hospitalizations in older patients with obstructive lung disease.

In **Chapter 3** we systematically reviewed the prevalence of the metabolic syndrome and its components in patients with COPD compared to controls.

Chapter 4 reports a prospective observational study investigating the cardiometabolic risk

profile of severe COPD patients with normal muscle mass compared to patients with low muscle mass. Furthermore, in this study the cardiometabolic effects of a short-term high-intensity rehabilitation program in these severe COPD patients were investigated.

In **Chapter 5** a prescheduled analysis of the NUTRAIN-trial is described in which we investigated the cardiometabolic risk profile of normal weight patients with COPD with low muscle mass stratified by abdominal obesity. In addition, the responsiveness to 4 months high-intensity exercise training was assessed in normal weight COPD patients with low muscle mass stratified by abdominal obesity.

Chapter 6 describes data of a double-blind, randomized cross-over placebo-controlled trial investigating the acute and 7-day effects of dietary nitrate supplementation on mechanical efficiency, exercise performance and cardiac biomarkers of patients with COPD.

We review in **Chapter 7** available evidence for effects of resveratrol on lung injury, muscle metabolism and cardiometabolic risk profile and discuss if resveratrol supplementation would be beneficial in patients with COPD.

Finally, the results of the studies described in the current thesis will be discussed and placed in a broader context in **Chapter 8**.

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Chapter 2

A Multidimensional Risk Score to Predict All-Cause Hospitalization in Community-Dwelling Older Individuals With Obstructive Lung Disease

Rosanne J.H.C.G. Beijers
Bram van den Borst
Anne B. Newman
Sachin Yende
Stephen B. Kritchevsky
Patricia A. Cassano
Douglas C. Bauer
Tamara B. Harris
Annemie M.W.J. Schols
For the Health, Aging and Body Composition study

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ABSTRACT

Background: Both respiratory and nonrespiratory hospitalizations are common and costly events in older individuals with obstructive lung disease. Prevention of any hospitalization in these individuals is essential. We aimed to construct a prediction model for all-cause hospitalization risk in community-dwelling older individuals with obstructive lung disease.

Methods: We studied 268 community-dwelling individuals with obstructive lung disease (defined as FEV_1/FVC<LLN) who participated in the observational Health, Aging and Body Composition Study and constructed a prediction model for 9-year all-cause hospitalization risk using a weighted linear combination based upon beta coefficients.

Results: There were 225 individuals with 1 or more hospitalizations and 43 individuals free from hospitalization during the follow-up. Heart and vascular disease (H), objectively measured lower extremity dysfunction (O), systemic inflammation (S), dyspnea (P), impaired renal function (I), and tobacco exposure (T) were independent predictors for all-cause hospitalization (ALL). These factors were combined into the HOSPITALL score (0-23 points), with an area under the curve in ROC analysis of 0.70 (p<0.001). The hazard ratio for all-cause hospitalization per one-point increase in the HOSPITALL score was 1.15 (95% confidence interval, 1.11-1.19, p=0.001). Increasing HOSPITALL score was further associated with shorter time to first admission, increased admission rate, and more respiratory admissions.

Conclusion: The HOSPITALL score is a multidimensional score to predict all-cause hospitalization risk in community-dwelling older individuals with obstructive lung disease, that may aid in patient counseling and prevention to reduce burden and health care costs.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) in particular is common in aged persons, and is associated with significant functional limitations [1, 2] and high health care costs [3]. During the course of their disease, patients with COPD are frequently hospitalized not only for exacerbations or pneumonia [4, 5], but also for a wide range of non-respiratory causes [6, 7]. All-cause hospitalization rates in COPD range from approximately 0.4 to 0.7 admissions per person per year, depending on the population studied [6-10]. Whereas hospital stays drive direct costs of COPD-related care [11], hospitalizations for nonrespiratory reasons constitute the greatest expense in patients with COPD [3]. Moreover, 72% of the 30-day readmissions after exacerbated COPD are primarily for non-respiratory problems [12].

Irrespective of the primary reason for admission, hospitalization may trigger a progressive physical decline [13, 14], which has recently also been shown in patients with COPD [10]. Patients with COPD hospitalized for respiratory and nonrespiratory causes experienced an equal rate of accelerated decline in 6- minute walking distance after discharge compared to nonhospitalized patients [10]. Furthermore, all-cause hospitalization in COPD is associated with high mortality [15]. Preventing any hospitalization in patients with COPD is therefore crucial. Although several studies have suggested that the physical activity level and exercise capacity may be predictors of all-cause hospitalization in patients with COPD [10, 16, 17], a thorough investigation of potential risk factors has not been undertaken. Moreover, these were studies conducted in secondary and tertiary care center populations, whereas strategies to prevent hospitalization should ideally commence in the preclinical setting.

In the current study, we analyzed baseline and 9-year follow-up data from community-dwelling older subjects with obstructive lung disease participating in the observational Health, Aging and Body Composition (ABC) Study to identify risk factors for all-cause hospitalization. We subsequently constructed a risk prediction model that may aid in patient counseling in the preclinical setting. The Health ABC Study cohort was selected as it provides a rich characterization of community-dwelling older individuals with long follow-up data available.

METHODS

A detailed methodology can be found in the supplemental material.

Study population

This study was performed using data from the Health ABC Study which is a longitudinal observational study of 3075 community-dwelling black and white men and women, 70 to 79 years of age, residing in Pittsburgh, Pennsylvania and Memphis, Tennessee. Participants were included if they reported no difficulty walking a quarter mile, climbing 10 steps without

resting, or performing mobility-related activities of daily living. The Health ABC Study protocol was approved by the Institutional Review Boards of the clinical sites. All participants gave written informed consent.

For the current analyses we used baseline data, obtained in 1997/1998 through in-person interview and clinic based examination, and 9-year follow-up hospitalization data. We analyzed the individuals (n=268) who met the criterion for obstructive lung disease (reduced [i.e. < lower limit of normal, LLN] forced expiratory volume in 1 second [FEV_1]/forced vital capacity [FVC] as determined by age, sex, and race-normalized values) [18] at baseline. Pre-bronchodilator lung function was assessed according to international standards [19].

Hospitalizations and survival

Participants were asked to report any hospitalizations and every 6 months they were asked directed questions about interim events. When an event was reported, medical records were collected (admission and discharge dates, and primary reason for hospitalization [supplemental material]).

Covariates at baseline

As previously reported [18, 20], clinic site, gender, race (black/white), age, tobacco exposure, dyspnea, body mass index (BMI), fat free mass index (FFMI), daily physical activity, knee extensor strength, hand grip strength, Short Physical Performance Battery (SPPB), and plasma C-reactive protein (CRP) were determined following standardized methodology. Comorbid heart and vascular disease (coronary heart disease, cerebrovascular disease, and congestive heart failure), chronic kidney disease (CKD) [21], diabetes, cancer, and cognitive impairment [22] were recorded. Furthermore, all-cause hospitalizations in the year before inclusion were reported by the Centers for Medicare and Medicaid Sevices.

Statistical analyses

Baseline differences between individuals with 1 or more hospitalizations and individuals free from hospitalizations during the follow-up were tested using Student t-test for continuous variables, χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables with skewed distributions. Univariate Cox proportional hazards models using bootstrap estimation (1000 replications; resampling with replacement) were performed to identify the association of candidate variables with all-cause hospitalization. All covariates with a *p*-value ≤0.10 were considered for inclusion in a multivariable model with a backward elimination approach using bootstrap estimation again allowing variables with a *p*-value ≤0.10 to be retained in the model. FEV_1, physical activity, BMI and CRP were modeled in categories [23-25]. The bias-corrected beta coefficients from the final multivariate model were subsequently standardized and rounded to the closest integer in order to assign weighted scores to each of the remaining

variables [26]. Summation of these scores led to the final risk score. Receiver-Operating Curve (ROC) analysis was performed for the newly developed risk score to estimate its sensitivity and specificity in classifying persons by means of the Cox model as either hospitalized or not. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 22 for Windows, SPSS Inc., Chicago, IL).

RESULTS

Of the 268 individuals, 225 (84%) had 1 or more hospitalizations and 43 (16%) were free from hospitalization during the 9-year follow-up, with a total of 1944 person years. In total, those with 1 or more hospitalizations were hospitalized 811 times for any cause. Most of these hospitalizations were for nonrespiratory reasons (72.3%). Participants had a median number of 3 (interquartile range [IQR] 1-5) hospitalizations, corresponding to 0.44 (IQR 0.22-0.78) hospitalizations per year. The median length of stay per hospitalization was 5 (IQR 4-9) days, and the median time to the first all-cause hospitalization was 2.2 (IQR 1.0-4.4) years.

Compared with individuals free from hospitalization, those with 1 or more hospitalizations had a higher prevalence of heart and vascular disease, a lower prevalence of cancer (particularly driven by a lower prevalence of prostate cancer [data not shown]) and a tendency toward a higher prevalence of CKD (Table 1). The latter group also had higher CRP levels and were more often ever-smokers, whereas no differences were found in body composition or muscle strength.

Univariate Cox proportional hazards analysis indicated that all-cause hospitalization was significantly predicted by clinic site, previous all-cause hospitalization, heart and vascular disease, FEV_1, dyspnea, CRP, daily physical activity level, SPPB, CKD, and tobacco exposure (Table 2).

Table 1. Baseline characteristics of the 268 subjects with obstructive lung disease.

	Total (n=268)	Subjects with ≥1 hospitalizations (n=225)	Subjects free from hospitalizations (n=43)	*p*-value
General characteristics				
Age, y	73.2 ± 2.9	73.2 ± 2.8	73.0 ± 3.1	0.580
Gender, %male	57.5	58.2	53.5	0.565
Race, %white	55.6	55.1	58.1	0.714
Site, %Memphis	51.1	49.8	58.1	0.315
Previous all-cause hospitalization, %	13.1	13.8	9.3	0.419
Tobacco exposure				
Ever, %	82.8	85.8	67.4	**0.003**
Never, %	17.2	14.2	32.6	
Lung function				
FEV_1, %pred[a]	63 ± 18	62 ± 18	67 ± 20	0.121
Dyspnea				
None, %	50.6	48.6	60.5	0.245
Mild, %	39.2	40.0	34.9	
Moderate, %	10.3	11.4	4.7	
Physical functioning				
Physical activity, kcal/kg/wk[b]	65 (36-100)	62 (35-95)	77 (44-106)	0.068
Inactive	27.6	30.2	14.0	0.085
Lifestyle active	56.3	54.7	65.1	
Exercisers	16.0	15.1	20.9	
Grip strength, kg	64 ± 22	63 ± 21	66 ± 23	0.421
SPPB, %<10	31.3	33.8	21.4	0.115
Body composition				
BMI, kg/m²	25.4 ± 4.7	25.3 ± 4.7	26.0 ± 4.8	0.379
FFMI, kg/m²	17.0 ± 2.5	17.0 ± 2.5	17.1 ± 2.4	0.666
Systemic inflammation				
CRP, µg/ml[b]	2.06 (1.16-3.64)	2.16 (1.24-3.78)	1.17 (0.79-2.39)	**<0.001**
Comorbidities				
Heart and vascular disease, %	27.2	30.5	14.3	**0.032**
Chronic kidney disease, %	20.1	22.6	9.5	0.054
Diabetes, %	11.6	12.5	7.0	0.300
Cancer, %	17.2	15.1	27.9	**0.041**
Cognitive impairment, %	13.1	12.9	14.3	0.806

Data are shown as mean ± SD unless indicated otherwise. [a]From reference equations [27]. [b]Median (IQR). Abbreviations: BMI, body mass index; CRP, C-reactive protein; FEV1, forced expiratory volume in 1 second; FFMI, fat free mass index; FMI, fat mass index; IL-6, interleukin-6; SPPB, short physical performance battery; TNF-α, tumor necrosis factor-α.

Table 2. Investigated covariates in 268 subjects with obstructive lung disease as predictors for 9-year all-cause hospitalization using univariate Cox proportional hazards regression analysis.

Variable	Category	No. of hospitalizations	No. at risk	HR (95% CI)	*p*-value
Age, y	-	225	268	1.03 (0.98-1.07)	0.258
Gender	Female	94	114	1.00	
	Male	131	154	1.05 (0.81-1.37)	0.708
Race	White	124	149	1.00	
	Black	101	119	1.19 (0.91-1.55)	0.214
Site	Memphis	112	137	1.00	
	Pittsburgh	113	131	1.38 (1.06-1.79)	**0.012**
Previous all-cause hospitalization	No	193	232	1.00	
	Yes	31	35	1.58 (1.08-2.31)	**0.048**
Tobacco exposure	Never	32	46	1.00	
	Ever	193	222	1.69 (1.16-2.47)	**0.019**
FEV_1 %pred	Mild	38	47	1.00	
	Moderate	125	149	1.29 (0.89-1.85)	0.142
	Severe	62	72	1.56 (1.04-2.34)	**0.037**
Dyspnea	None	107	133	1.00	
	Mild	99	103	1.20 (0.90-1.59)	0.238
	Moderate	25	27	2.49 (1.60-3.86)	**0.004**
Physical activity	Exercisers	34	43	1.00	
	Lifestyle active	123	151	1.02 (0.70-1.49)	0.914
	Inactive	68	74	1.63 (1.08-2.47)	**0.020**
Quadriceps strength	-	198	236	1.00 (0.99-1.00)	0.154
Grip strength	-	223	265	1.00 (0.99-1.00)	0.216
SPPB	≥10	147	180	1.00	
	<10	75	84	1.78 (1.34-2.35)	**0.001**
BMI	≤20.0 kg/m²	21	24	1.21 (0.75-1.94)	0.377
	20.0-24.9 kg/m²	99	115	1.00	
	25.0-29.9 kg/m²	67	85	0.90 (0.66-1.22)	0.479
	≥30.0 kg/m²	38	44	1.07 (0.74-1.55)	0.704
FFMI	-	222	265	1.00 (0.95-1.06)	0.904
CRP	<5.0 µg/ml	186	224	1.00	
	≥5.0 µg/ml	35	38	1.70 (1.18-2.44)	**0.010**
Heart and vascular disease	No	153	189	1.00	
	Yes	67	73	1.86 (1.39-2.48)	**0.002**
Chronic kidney disease	No	171	209	1.00	
	Yes	50	54	1.49 (1.08-2.04)	**0.013**
Diabetes	No	196	236	1.00	
	Yes	28	31	1.27 (0.85-1.88)	0.263

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; FEV1, forced expiratory volume in 1 second; FFMI, fat free mass index; HR, hazard ratio; SPPB, short physical performance battery.

In the multivariate Cox regression model heart and vascular disease, SPPB, CRP, dyspnea, CKD, and tobacco exposure were retained as significant predictors (Table 3). These variables were subsequently combined into the HOSPITALL score (Heart and vascular disease [H], Objectively measured lower extremity dysfunction [O], Systemic inflammation [S], dysPnea [P], Impaired renal function [I], and Tobacco exposure [T] predict ALL-cause hospitalization [ALL]). Each individual received points based on the presence of heart and vascular disease, SPPB (<10 or ≥10), CRP (<5.0 or ≥5.0 µg/ml), dyspnea (none/mild or moderate), the presence of CKD, and tobacco exposure (ever- or never smokers) (Table 3). None or mild dyspnea as well as current and former smoking were taken together as their beta-coefficients were comparable (data not shown). The final HOSPITALL score for each individual was obtained by summing the points corresponding to each variable. HOSPITALL scores ranged from 0 to 23. As expected, individuals with 1 or more hospitalizations had higher HOSPITALL scores than those free from hospitalization (6.2±2.7 vs 4.4±2.1, p<0.001). Per 1-point increase of the HOSPITALL score the all-cause hospitalization risk increased by 15% (hazard ratio [HR] 1.15 [95% confidence interval [CI] 1.11-1.19], p=0.001). The final model did not substantially change when additionally adjusting for clinic site (data not shown).

Table 3. Final multivariable model as 9-year risk predictor for all-cause hospitalization: HOSPITALL score.

Covariate	Coefficient	Hazard Ratio (95% CI)	*p*-value	HOSPITALL points
Heart and vascular disease				
No		1.00		0
Yes	0.44	1.55 (1.14-2.10)	**0.011**	3
Short Physical Performance Battery				
≥10		1.00		0
<10	0.55	1.73 (1.28-2.35)	**0.001**	4
CRP				
<5.0 µg/ml		1.00		0
≥5.0 µg/ml	0.37	1.45 (0.99-2.13)	0.058	3
Dyspnea				
None or mild		1.00		0
Moderate	0.98	1.71 (1.71-4.17)	**0.001**	7
Chronic kidney disease				
No		1.00		0
Yes	0.30	1.34 (0.96-1.88)	0.070	2
Tobacco exposure				
Never		1.00		0
Ever	0.55	1.73 (1.16-2.58)	**0.018**	4

Abbreviation: CRP, C-reactive protein.

The area under the curve (AUC) of the ROC curve for the HOSPITALL score was 0.70 (95% CI 0.62-0.78, p<0.001). For comparison, we also analyzed the AUCs for 2 previously validated mortality predictor scores in COPD and for FEV_1 alone. In the same Health ABC Study cohort, Mehrotra *et al*. described the PILE index (a combination score of FEV_1, interleukin [IL]-6, and knee extensor strength) and a modified version of the BODE index (mBODE, a combination score of BMI, FEV_1, dyspnea, and time to complete 400 meter walking).[20] The AUCs for the

PILE index, mBODE index and FEV_1 to predict time to first all-cause hospitalization were 0.60 (95% CI 0.50-0.70, *p*=0.052), 0.58 (95% CI 0.49-0.67, *p*=0.105), and 0.44 (95% CI 0.35-0.53, *p*=0.216), respectively, indicating superiority of the HOSPITALL score in this population.

When we divided the individuals into 3 HOSPITALL score risk strata (low, average and high risk with HOSPITALL scores of 0-4, 5-8, and ≥9, respectively), we identified significant differences between these groups not only in risk for all-cause hospitalization (Figure 1), but also in time to the first admission, hospitalization rate, and primary cause for hospitalization (Table 4).

Table 4. Stratification of HOSPITALL scores.

	HOSPITALL score groups			*p*-value
	Low risk	Average risk	High risk	
n	101	80	66	
HOSPITALL score	0-4	5-8	≥9	
Risk of all-cause hospitalization, HR[a]	0.60 (0.43-0.84)[b]	1	2.36 (1.66-3.34)[c]	-
Time to first all-cause hospitalization, y	3.06 (1.26-5.87)	2.92 (1.16-4.38)	1.27 (0.52-2.15)	**<0.001**
All-cause hospitalizations per person per year, n	0.11 (0.00-0.39)	0.40 (0.13-0.73)	0.66 (0.35-1.23)	**<0.001**
Primary cause of first hospitalization				
Respiratory, n (%)	8 (10.7)	17 (24.6)	21 (32.8)	**0.006**
Non-respiratory, n (%)	67 (89.3)	52 (75.4)	43 (67.2)	

Data are shown as median (IQR) unless indicated otherwise. [a]HR (95%CI), [b]*p*=0.002, [c]*p*<0.001.

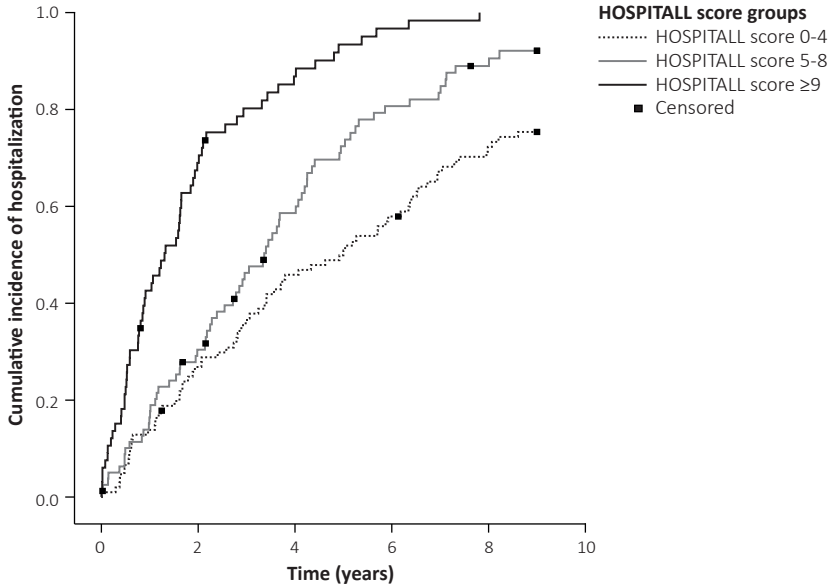

Figure 1. Kaplan-Meier plot using HOSPITALL score groups as stratum (*p*<0.001 by log-rank test).

DISCUSSION

Preventing hospitalizations is key in the management of patients with obstructive lung disease. The alarming admission and readmission rates urge physicians to break and preferably prevent this vicious cycle of hospitalizations. In community-dwelling older individuals with obstructive lung disease participating in the Health ABC Study, we constructed the HOSPITALL score to predict all-cause hospitalization. The score comprises heart and vascular disease, lower extremity dysfunction, systemic inflammation, dyspnea, CKD, and tobacco exposure. The score may aid in the guidance for risk reduction in a population of older patients with obstructive lung disease in the preclinical setting. The HOSPITALL score was more discriminative in predicting all-cause hospitalization than existing multidimensional risk scores. Although the HOSPITALL risk factors may not necessarily be disease-specific and may also predict hospitalization and associated poor outcomes in general populations, the score underlines the broad multidimensional scope needed in obstructive lung disease care.

Moderate dyspnea was the strongest predictor among the HOSPITALL risk factors. Mild dyspnea did not increase the risk above no dyspnea. The definition of moderate dyspnea as applied in the Health ABC Study is comparable to a modified Medical Research Council grade of 2 [28]. In our population the percentage of individuals with moderate dyspnea was relatively low with 10.3% as was to be expected given the community-dwelling nature of the population and that individuals needed to be well-functioning on inclusion. Our results stress the need for documenting dyspnea severity in primary care individuals with obstructive lung disease as it is a simple and sensitive measure that provides insight into the risk of a variety of poor outcomes including all-cause hospitalization.

Heart and vascular disease is the most prevalent comorbidity in COPD in most studies, ranging from 28-70% [29, 30]. Furthermore, a recent meta-analysis showed a 2 to 5 times higher risk of major cardiovascular diseases in patients with COPD compared with a non-COPD population [31]. Further stressing the clinical importance of comorbid heart and vascular disease in COPD, we found that it was a strong predictor in the HOSPITALL score. This suggests that preventing the development of heart and vascular disease in COPD may decrease the risk of hospitalization. Early identification of cardiovascular risk factors in COPD is therefore essential. Not only should we focus on common risk factors such as smoking and age, but metabolic syndrome status, systemic inflammation, adipose tissue distribution, and skeletal muscle oxidative capacity have been proposed as key mediators in COPD [32], that need further investigation in future studies. Also, interventions to modify cardiovascular risk in COPD are urgently warranted.

We found that objectively measured lower extremity dysfunction measured by the SPPB was a strong predictor for all-cause hospitalization. The SPPB is commonly used in older age populations but has recently also been shown to be a valid and simple assessment tool to

measure functional impairment in COPD, independent of FEV_1 [33]. SPPB scores less than 10 have been associated with disability in aged persons [34], with hyperinflation and with an increased proportion of type 2 quadriceps muscle fibers in patients with COPD indicative of decreased oxidative capacity in skeletal muscle [33]. Also, SPPB scores at discharge after hospitalization have been inversely correlated with the rate of decline in activity of daily living performance in older persons [35]. Clinical use of the SPPB test in patients with COPD warrants further investigation.

It is well established that persistent low-grade systemic inflammation is present in some patients with COPD but its origin is still unclear [36]. Recent studies indicate that plasma levels of the clinical inflammatory marker CRP are at least partly influenced by adipose tissue mass [37, 38], which is modifiable by lifestyle adaptations. In addition to previously reported associations between high CRP and low exercise capacity [39], respiratory hospitalizations and increased mortality [40], the current study also shows that high levels of CRP are predictive of all-cause hospitalization risk.

Although CKD is less common in patients with COPD than heart and vascular disease, it has recently been shown that patients with COPD and CKD had the highest incidence of all-cause emergency room visits that led to hospitalizations, and had the highest incidence of all-cause hospitalizations [29]. In addition, that study also showed that all-cause total health care costs were highest in COPD patients with CKD compared with other comorbidities [29]. Therefore, future studies need to increase the understanding of the relation between COPD and CKD. In our study, CKD was defined based on the CKD-Epidemiology Collaboration creatinine-cystatin C equation, which, in comparison with equations based on creatinine or cystatin C alone, has been shown to be more precise and accurate in estimating the glomerular filtration rate (GFR) across the range of GFR [21].

It is well known that smoking is a major risk factor for developing COPD and many other chronic diseases, but smokers in this population still had an additional risk for all-cause hospitalization. This may be related to known effects of smoking on other organ systems and could reflect the influence of an overall unhealthy lifestyle.

Strengths and weaknesses

The unique design of the Health ABC Study enabled us to thoroughly investigate risk factors for all-cause hospitalization during a long follow-up of 9 years in community-dwelling older individuals with obstructive lung disease. An advantage of the long follow-up duration was that we could identify individuals free from any hospitalization during the entire follow-up. By comparing their characteristics to those with at least one all-cause hospitalization we were able to construct a solid risk score. Also, it was possible to compare the HOSPITALL score with other existing multidimensional risk scores showing superiority of the HOSPITALL score.

A limitation of this study is that no COPD diagnosis based on Global Initiative of Obstructive Lung Disease criteria could be given because post-bronchodilator pulmonary function was not available. To define obstructive lung disease, we used stringent criteria based on age-, sex-, and race-adjusted LLN cutoffs, as recommended by previous studies [27]. Nevertheless, it would be of interest to validate the HOSPITALL score in comparable cohorts with post-bronchodilator spirometry. Furthermore, it should be noted that the Health ABC Study included individuals without disability or mobility impairment which has implications for the generalizability of our results.

CONCLUSION

The HOSPITALL score is a multidimensional score to predict the risk of all-cause hospitalization in community-dwelling older individuals with obstructive lung disease. The HOSPITALL score may aid in patient counseling and prevention to reduce burden and health care costs.

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SUPPLEMENTAL MATERIAL

Methods

Study population and lung function

This study was performed using data from the Health ABC Study which is a longitudinal observational study of 3075 community-dwelling black and white men and women, 70 to 79 years of age, residing in Pittsburgh, Pennsylvania and Memphis, Tennessee. Participants were included if they reported no difficulty walking a quarter mile, climbing 10 steps without resting, or performing mobility-related activities of daily living. Exclusion criteria of the Health ABC Study were any life-threatening condition, participation in any research study involving medications or modification of eating or exercise habits, plans to move from the geographic area within 3 years, and difficulty in communicating with the study personnel or cognitive impairments. The Health ABC Study protocol was approved by the Institutional Review Boards of the clinical sites. All participants gave written informed consent.

For the current analyses we used baseline data, obtained in 1997/1998 through in-person interview and clinic based examination, 9-year follow-up hospitalization, and mortality data from those who met the criterion for obstructive lung disease (i.e. < lower limit of normal (LLN) forced expiratory volume in 1s (FEV_1)/forced vital capacity (FVC) as determined by age, sex, and race-normalized values) at baseline [1]. Participants with normal lung function (FEV_1/FVC≥LLN and FVC≥LLN), restrictive lung disease (FEV_1/FVC≥LLN and FVC<LLN) or missing lung function were excluded from the analysis.

Covariates at baseline

Height was measured using a wall mounted stadiometer. Body weight was assessed using a standard balance beam scale and body mass index (BMI) was calculated as weight/height² (kg/m²). Whole body dual energy X-ray absorptiometry (DXA, Hologic 4500A software version 8.21, Waltham, MA) was applied to retrieve total fat and fat free masses (FM and FFM, respectively) [1]. Fat-free mass index (FFMI) was calculated as FFM/height².

Dyspnea was assessed by self-report and classified as none, mild, or moderate based on the following questions [2]. Subjects were asked whether they stopped for breath when hurrying on a level surface or walking up a slight hill, or whether or not they have to stop for breath when walking at their own pace on a level surface. Subjects who answered ‘‘yes’’ to both questions were classified as having moderate dyspnea, while those answering ‘‘no’’ to both questions were classified as having no dyspnea. Individuals answering “yes” only to the first question were classified as having mild dyspnea.

Physical activity in the preceding seven days was assessed by questionnaire and was defined as the sum of time spent on gardening, heavy household chores, light house work, grocery

shopping, laundry, climbing stairs, walking for exercise, walking for other purposes, aerobic exercise, weight or circuit training, high-intensity exercise activities, and moderate intensity exercise activities [3, 4]. Also, information of the intensity level of each activity performed was obtained. To each of the activity categories approximate metabolic equivalent unit values were assigned to calculate a weekly energy expenditure estimate in kcal/kg [3, 4].

Tobacco exposure status was determined based on self-report. Two questions were used: "Have you smoked at least 100 cigarettes in your entire life?" and "Do you smoke cigarettes now?" Subjects who answered both questions with no are never smokers, subjects who answered the first question with yes and the second with no are former smokers and subjects who answered both questions with yes are current smokers.

Maximal isokinetic strength of the quadriceps muscle was assessed by Kin-Com 125 AP Dynamometer (Chattanooga Group, Chattanooga, TN) at 60 degrees per second [1]. Hand grip strength was measured using a Jamar Hydraulic Hand Dynamometer [1]. The Short Physical Performance Battery (SPPB) was assessed, measuring gait speed, standing balance, and time to rise from a chair five times [5, 6]. In the SPPB, each item was scored using a five-point scale (0 = inability to complete test, 4 = highest level of performance) leading to a combined 0-12-point summary scale.

Plasma concentrations of C-reactive protein (CRP) were determined in frozen stored plasma obtained from a venipuncture after an overnight fast [7].

Prevalent heart and vessel disease (prevalent coronary heart disease, cerebrovascular disease, and congestive heart failure) was defined based on self-report or by medical records of coronary heart disease and/or stroke. Chronic kidney disease (CKD) was defined as an estimated glomular filtration rate less than 60 ml/min/1.73m^2 based on serum creatinine and cystatin C by using the CKD-Epidemiology Collaboration (CKD-EPI) creatinine-cystatin C equations [8]. Diabetes and cancer were also defined by self-report, by medical records or by diabetes medication use. Cognitive impairment was defined based on the Modified Mini-Mental State Examination (3MS), which is an extended, 100 point version of the Mini Mental State Examination (MMSE) [9]. Consistent with other Health ABC studies, a cutoff of 80 was used indicating poor cognitive function [10-12].

Hospitalizations

Subjects were asked to report any hospitalizations and every 6 months they were asked directed questions about interim events. When an event was reported, medical records were collected (admission and discharge dates and primary reason for hospitalization.

Supplemental Table 1. Categories of primary causes for hospitalizations.

Category	Causes for hospitalizations
Respiratory	COPD, emphysema, asthma
	Pneumonia
	Other disease of respiratory system
Cardiovascular	Myocardial infarction
	Angina, coronary insufficiency or other ischemic heart disease
	Congestive heart failure or congestive cardiomyopathy
	Carotid artery disease
	Peripheral arterial disease (aorta, iliac arteries, or below)
	Stroke
	Transient ischemic attack
	Other disease of circulatory system
	Primary coronary revascularization
Cancer	Cancer
Gastro-intestinal	Upper or lower gastro-intestinal disease
	Gallbladder disease
	Other diseases of digestive system
Musculoskeletal	Osteoarthritis, joint surgery for osteoarthritis
	Fracture
	Disease of musculoskeletal system, connected tissue (not osteoarthritis)
Endocrine, metabolic and nutrition	Diabetes mellitus
	Endocrine, nutrition, metabolic and immunity disorders
Mental	Depression
	Dementia
	Mental disorders (not dementia and depression)
Other	Abdominal hernia
	Benign prostatic hyperplasia
	Infectious diseases
	Neoplasms (benign)
	Disease of blood and blood forming organs
	Disease of nervous system and sense organs
	Disease of genitourinary system (not benign prostatic hyperplasia)
	Disease of skin
	Symptoms, signs and ill-defined condition
	Injury and poisoning (not fractures)

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Chapter 3

The Prevalence of Metabolic Syndrome in Chronic Obstructive Pulmonary Disease: A Systematic Review

Nanca Cebron Lipovec
Rosanne J.H.C.G. Beijers
Bram van den Borst
Wolfram Doehner
Mitja Lainscak
Annemie M.W.J. Schols

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) and cardiovascular diseases (CVD) are common in patients with chronic obstructive pulmonary disease (COPD). Prevention of these co-morbidities in COPD requires knowledge on their risk factors. Metabolic syndrome (MetS) predisposes to the development of T2DM and CVD but its prevalence in COPD remains unclear. The aim of this review was to assess the prevalence of MetS and its components in COPD patients compared to controls and to investigate the contribution of clinical characteristics to MetS prevalence.

Methods: We systematically searched PubMed and EMBASE for original studies in COPD that have investigated the prevalence of MetS and its components.

Results: In total, 19 studies involving 4208 COPD patients were included. The pooled MetS prevalence was 34%. Compared to controls, the prevalence was higher in COPD (10 studies, 32% and 30%, p=0.001). The three most prevalent components in both COPD and controls were arterial hypertension (56% and 51%), abdominal obesity (39% and 38%) and hyperglycemia (44% and 47%). Compared to COPD patients without MetS, those with MetS had higher body mass index (BMI) (29.9 and 24.6 kg/m^2, p<0.001), higher forced expiratory volume in one second (FEV_1) % predicted (54 and 51, p<0.001) and were more frequently female (31% and 25%, p=0.011).

Conclusion: In conclusion, the prevalence of MetS in COPD patients is high and hypertension, abdominal obesity and hyperglycemia are the most prevalent components. Further studies are needed to evaluate the impact of lifestyle factors and medications on MetS in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) represents a major cause of morbidity and mortality worldwide and is estimated to become the fourth leading cause of death in 2030 [1]. Although COPD is primarily characterized by airflow obstruction and pulmonary inflammation, its effects reach beyond the lungs. Systemic manifestations such as osteoporosis, depression, cardiovascular disease (CVD) and Type 2 Diabetes Mellitus (T2DM) are highly prevalent in these patients and significantly contribute to symptom burden and health status [2, 3]. CVD and T2DM are present across all COPD disease stages [2] and increase the risk of hospitalization and mortality [4]. In fact, in COPD patients with mild-to-moderate airflow obstruction, CVD is even the leading cause of mortality [5].

Prevention of incident T2DM and CVD in patients with COPD requires a detailed understanding of their risk factors, which could be generic or could reflect an interaction between lifestyle and disease specific determinants. Metabolic syndrome (MetS) is a constellation of metabolic risk factors that increase the risk developing T2DM and CVD. The diagnosis of MetS is based on the presence of central obesity, hypertension, dyslipidemia, and hyperglycemia. Various MetS definitions are available and differ in specific cut points of the components (Table 1). The prevalence of MetS in the general population varies from 21%-31% in Asia [6, 7], to 34% in the USA [8], and increases with increasing age and body mass index (BMI) [9]. Predisposing factors associated with MetS development are smoking [10, 11] and a sedentary lifestyle [12, 13], which are well-described features in COPD patients [3]. Moreover, specific factors relating to COPD as a primary lung disease, such as relative hypoxaemia and steroid use may also contribute to the MetS [14]. Therefore, the prevalence of MetS in COPD is hypothesized to be higher compared to the general population. Furthermore, knowledge on predisposing factors may aid in characterizing the patients with the highest risk of developing MetS and it may give more insight into targets for interventions aiming to reduce MetS prevalence, development of T2DM and eventually CVD mortality in COPD.

In this review we systematically searched the literature for observational studies that analyzed MetS prevalence in COPD patients and preferentially in control populations as well. Furthermore, we investigated the prevalence of the individual MetS components and studied associations with general clinical characteristics including age, gender, BMI, disease severity, inflammatory profile, medication use and lifestyle characteristics.

Table 1: Most widely used definitions of metabolic syndrome.

	WHO (1998) [15]	EGIR [16]	NCEP ATP III (2001 and 2005) [17, 18]	AACE (2003) [19]	IDF [20]	Alberti (2009) [21]
Central obesity	BMI >30 kg/m² and/or WHR >0.90 in men or >0.85 in women	WC ≥94 cm in men and ≥80 cm in women	WC ≥102 cm in men and >88 cm in women	BMI >25 kg/m²	WC ≥94 cm for Europid men and ≥80 cm for Europid women, with ethnicity specific values for other groups[a]	WC ≥102 cm in men and ≥88 cm in women, with ethnicity specific values for other groups
Hyperglycemia	IGT, IFG, or T2DM or lowered insulin sensitivity	IGT or IFG	≥110 mg/dl [b c]	IGT or IFG	≥100 mg/dl	≥100 mg/dl[c]
Dyslipidemia	TG ≥150 mg/dl and/or HDL <35 mg/dl in men or <39 mg/dl in women	TG ≥150 mg/dl and/or HDL <38 mg/dl in men or women	TG ≥150 mg/dl[c] HDL <40 mg/dl in men or <50 mg/dl in women[c]	TG >150 mg/dl and HDL <40 mg/dl in men or <50 mg/dl in women	TG >150 mg/dl or specific treatment HDL <40 mg/dl in men or <50 mg/dl in women or specific treatment	TG ≥150 mg/dl[c] HDL <40 mg/dl in men or <50 mg/dl in women[c]
Hypertension	≥160/90 mmHg	-	≥130/85 mmHg[c]	>130/85 mmHg	≥130/85 mmHg or treatment of previously diagnosed hypertension	≥130/85 mmHg[c]
Other	Microalbuminuria[d]	-	-	Other features of IR[e]	-	-
MetS diagnose	Hyperglycemia plus any two of the other criteria	Plasma insulin >75th percentile plus two other criteria	Three or more of the five criteria	Hyperglycemia plus any of the other criteria	Central obesity plus any two of the other criteria	Three or more of the five criteria

[a]Ethnic specific cutoffs are: Europids, Sub-Saharan Africans, Mediterranean and Middle East: male ≥94cm, female ≥80cm; South Asians, Chinese and South and Central Americans: male ≥90cm, female ≥80cm; Japanese: male ≥85cm, female ≥90cm. [b]In the revised NCEP ATP III definition of 2005 criterion for hyperglycemia was glucose level of ≥100 mg/dl. [c]In the revised NCEP ATP III definition of 2005 and in the definition of Alberti, treatment for elevated glucose, elevated triglycerides, reduced HDL and antihypertensive drug treatment in a patient with a history of hypertension were also positive criteria.[d]Urinary albumin excretion of ≥20 µg/min or albumin-to-creatinine of ≥30 mg/g.[e]Includes family history of type 2 diabetes mellitus, hypertension and cardiovascular disease, polycystic ovary syndrome, sedentary lifestyle, advancing age, and ethnic groups susceptible to type 2 Diabetes Mellitus. Abbreviations: AACE, American Association of Clinical Endocrinologists; BMI, body mass index; EGIR, European Group for Study of Insulin Resistance; HDL, high density lipoprotein; IDF, International Diabetes Foundation; IFG, impaired fasting glucose; IGT, impaired glucose intolerance; IR, insulin resistance; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; T2DM, Type 2 Diabetes Mellitus; TG, triglycerides; WC, waist circumference; WHO, World Health Organization; WHR, waist-to-hip ratio.

METHODS

Data sources and search strategy

This systematic review was performed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [22]. Pubmed and EMBASE databases (through April 2015) were used to find relevant articles. The search strategy consisted of terms on MetS and COPD (see supplemental material for terms used and search strategies). In addition, reference lists of retrieved articles were scanned for additional publications.

Study selection and data extraction

A first screening was independently done by two researchers (NCL and RB) based on title and abstract. In case of disagreement, a third person (BB) decided whether to include or exclude the study. Articles were considered for inclusion when they were original studies in patients with COPD, reported on the definition and prevalence of MetS and were written in English. MetS definition, prevalence of MetS and its individual components, age, gender, BMI, forced expiratory volume in 1 second (FEV_1), Global initiative for Obstructive Lung Disease (GOLD) stage, information about systemic inflammation, lifestyle characteristics (smoking and physical inactivity), and medication use were extracted. Original authors were contacted in case of missing data. If we were unable to gain information on the MetS definition applied, the study was excluded.

Statistical analyses

The overall pooled prevalence was calculated by summing the number of COPD patients with MetS divided by the total number of COPD patients. Means were weighted by sample size to calculate the pooled means. We compared the prevalence of MetS, prevalence of its components and characteristics (i.e. age, gender, BMI and FEV_1 % predicted) between COPD patients and controls as well as between COPD patients with and without MetS using Chi-square test for discrete variables and Welch test for continuous variables due to heterogeneity of variance. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22. A p-value <0.05 was considered statistically significant.

RESULTS

Study selection

A flow chart of article selection is presented in Figure 1. The searches in Pubmed and EMBASE yielded 107 and 269 hits, respectively. Of these 376 articles, 69 were duplicates and 278 were excluded based on title and abstract. Of the remaining 29 articles, 6 were excluded because

they were not original studies, and 4 were excluded because no prevalence of MetS was mentioned. Finally, 19 observational studies were included, of which 10 included a control group.

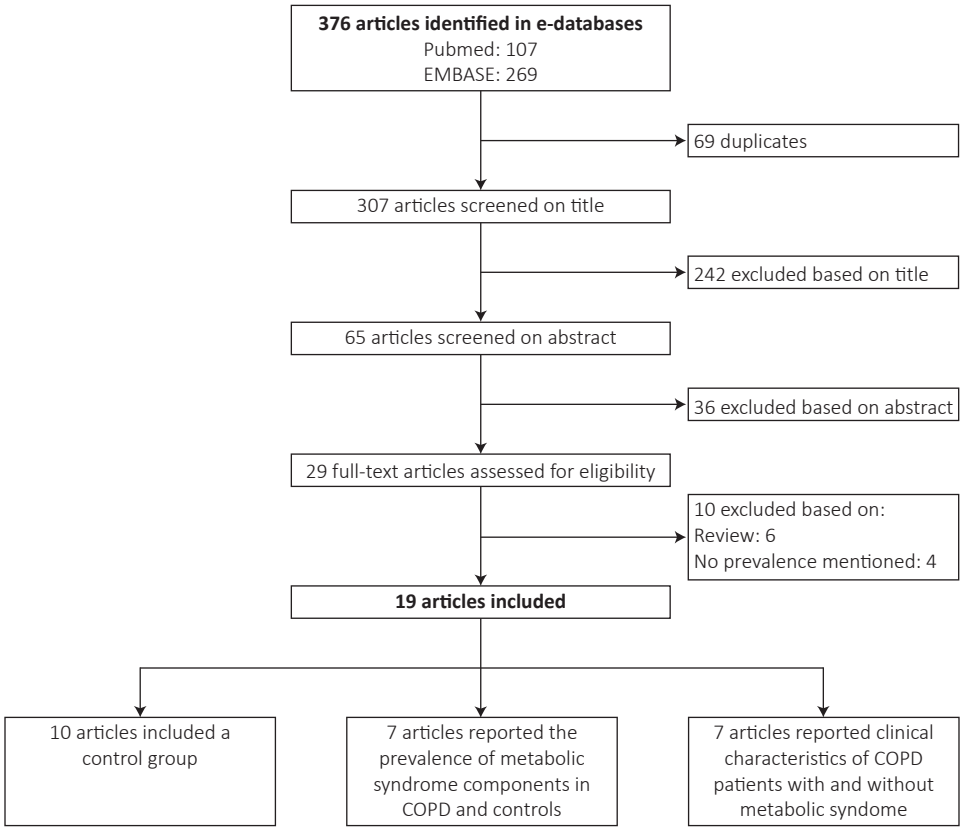

Figure 1. Flow chart of article selection.

General characteristics and prevalence of MetS in COPD patients

Characteristics of the COPD patients of the included 19 studies are presented in Table 2. In total, studies reported on 4208 COPD patients. These patients had a pooled mean age of 65±4 years (mean ± standard deviation), a BMI of 25.1±2.0 kg/m² a FEV_1% predicted of 66±14% and were mostly men (72%). The overall mean prevalence of MetS was 34% (21-58%) and varied between geographical areas (North America 53%, South America 36%, Europe 41%, Middle East 38% and East and Northeast Asia 28%). Studies differed in the MetS definition used: 6 studies applied NCEP ATP III 2001, 5 studies used NCEP ATP III 2005, 5 studies used IDF and 4 studies applied Alberti *et al*.[21].

Table 2. Characteristics of COPD patients in included papers and prevalence of metabolic syndrome and its components.

First author	Population (setting)	n	Age, y	Sex, %M	BMI, kg/m²	FEV_1, %pred	GOLD, %	Definition	Prevalence, %	
							I/II/III/IV		MetS	WC/BG/BP/TG/HDL
North America										
Marquis [23]	Patients entering a 12-week PR	38	66±7	61	28±5	43±16	-	NCEP ATP III 2001	47	61/13/82/63/24
Park [24]	NHANES subjects with physician-diagnosed emphysema or chronic bronchitis	223	70±9	51	29±7	-	-	Alberti	55	67/66/79/72/51
Park [25]	NHANES subjects with physician diagnosed emphysema or chronic bronchitis with FEV_1/FVC <0.7	94	62±10	45	27±6	67±21	-	Alberti	58	53/68/66/49/52
Poulain [26]	Patients previously engaged in PR	28	65±5[a]	100	28±4[a]	42±16[a]	29/36/36/-	NCEP ATP III 2005	29	-
South America										
Tanni [27]	Patients with mild to very severe COPD	115	65±10	68	26±6	59±25	18/38/14/30	Alberti	36	33/-/48/33/-
Diez-Manglano [28]	Patients admitted for COPD exacerbation	375	74±9[a]	89	27±5[a]	43±12[a]	-/34/54/13	Alberti[b]	43	22[c]/80/70/28/30
Minas [29]	Outpatients with mild to very severe airflow limitation	114	66 (62-71)[c]	100	25±5	55 (35-66)[c]	-	NCEP ATP III 2001	21	28/25/25/23/17
Breyer [30]	Clinically stable COPD patients from the CIROCO study	228	64±7	59	26±5	53±19	-	IDF	57	79/49/47[d]/31/10
Watz [31]	Stable outpatients	170	64±7[a]	75	26±5[a]	56±8[a]	20/34/25/21	IDF	47	78/38/75/37/31
Fumagalli [32]	Patients referred to pulmonary wards of hospitals	169	74±8	73	27±5	56±20	-	NCEP ATP III 2005	21	-

Table 2. Continued.

First author	Population (setting)	n	Age, y	Sex, %M	BMI, kg/m²	FEV_1, %pred	GOLD, % I/II/III/IV	Definition	Prevalence, % MetS	Prevalence, % WC/BG/BP/TG/HDL
Skyba [33]	Outpatients free from exacerbations for ≥8 weeks	44	62±7	86	27±7	54±23	-	IDF	39	66/39/57/20/9
Middle East										
Akpinar [34]	Outpatients with stable COPD	91	64±9	86	-	-	14/58/22/7	NCEP ATP III 2001	45	52/47/77/35/25
Küpeli [35]	Patients reported for regular follow-up at pulmonary department	106	67±9[a]	86	28±6[a]	63±24[a]	27/37/30/6	NCEP ATP III 2001	27	-
Ozgen Alpaydin [36]	Outpatients	50	61±6	90	27±5	46±17	-	IDF	44	-
Hosny [37]	COPD patients from the chest unit	50	58±8	88	27±4	54±16	-	NCEP ATP III 2001	40	-
East and Northeast Asia										
Park [38]	Newly diagnosed airflow obstruction in KNHANES II	133	61±10[a]	75	24±3[a]	-	59/38/4/-	NCEP ATP III 2001[e]	37	38/19/53/49/44
Lam [39]	Patients with airflow obstruction from the GHHARE	496	64±6[a]	26	-	-	-	IDF	23	34/34/57/30/16
Funakoshi [40]	Subjects who underwent a health screening	645	62±9[a]	100	23±3[a]	80±10[a]	46 GOLD I 54 GOLD II-IV	NCEP ATP III 2005[e]	23	24/54/52/33/5
Chung [41]	KHNANES subjects	1039	65±10[a]	73	23±3[a]	77±16[a]	46/48/6/1	NCEP ATP III 2005[e]	32	-

Data are shown as mean ± SD unless specified otherwise. [a]Pooled mean ± SD. [b]Body mass index >30 kg/m² instead of waist circumference criteria. [c]Median (Interquartile range). [d]Pooled prevalence of high systolic and diastolic blood pressure. [e]Blood glucose ≥100 mg/dL instead of ≥110 mg/dL. Abbreviations: BG, blood glucose; BMI, body Mass Index; BP, blood pressure; COPD, Chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global initiative for Obstructive Lung Disease; HDL, high density lipoprotein; IDF, International Diabetes Foundation; MetS, metabolic syndrome; n, number of subjects; NCEP-ATP III 2001, National Cholesterol Education Program Adult Treatment Panel; NCEP ATP III 2005, Revised National Cholesterol Education Program Adult Treatment Panel; TG, triglycerides; WC, waist circumference.

General characteristics of COPD patients with MetS and without Mets

In total 10 studies compared characteristics of COPD patients with MetS to COPD patients without MetS [24, 25, 27, 29-31, 34-36, 42]. Patients with MetS had a higher BMI and had higher FEV_1% predicted compared to patients without MetS (Table 3). The prevalence of male patients was significantly lower in the MetS group compared to the non-MetS group. Furthermore, four studies reported higher serum C-reactive protein [31, 34-36] and serum interleukin-6 [31] in COPD patients with MetS compared with patients without MetS (data not shown).

Table 3. General characteristics of chronic obstructive pulmonary disease patients with and without metabolic syndrome.

	MetS	Non-MetS	*p*-value
Age, y	69±4 (n=537, 6 studies)	68±5 (n=604, 6 studies)	0.135
Sex, %female	31 (n=561, 7 studies)	25 (n=694, 7 studies)	**0.011**
BMI, kg/m²	29.9±1.7 (n=561, 7 studies)	24.6±1.7 (n=694, 7 studies)	**<0.001**
FEV_1, %predicted	54±8 (n=414, 5 studies)	51±9 (n=504, 5 studies)	**<0.001**

Data are shown as mean ± SD, unless otherwise indicated. Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1s; MetS, metabolic syndrome; n, number of subjects.

Differences in prevalence of MetS and its components between COPD patients and controls

In total, 10 studies compared COPD patients (n=2864) with a control group (n=24532) [25, 30, 34, 36-41]. Characteristics of COPD patients are shown in Table 2, characteristics of the controls are presented in Supplemental Table 1. COPD patients were significantly older, had a significantly lower BMI and were more often males (data not shown). As presented in Figure 2, a higher prevalence of MetS among COPD patients compared to controls was found in 9 out of 10 studies [23, 25, 30, 34, 36-39, 41], and was significant in 5 studies [23, 30, 34, 37, 38]. The overall mean prevalence of MetS in COPD patients was 32% (23-58%) versus 30% (17-54%) in controls (p=0.001). In total, 7 studies reported the prevalence of MetS components in COPD patients (N=1725) and controls (N=18380). As shown in Figure 3, the three most prevalent components in both COPD patients and controls were hypertension (56% and 51%), hyperglycemia (44% and 47%) and abdominal obesity (39% and 38%). Prevalence of hypertension and low HDL cholesterol was significantly different between COPD patients and controls while prevalence of other components was comparable between both groups.

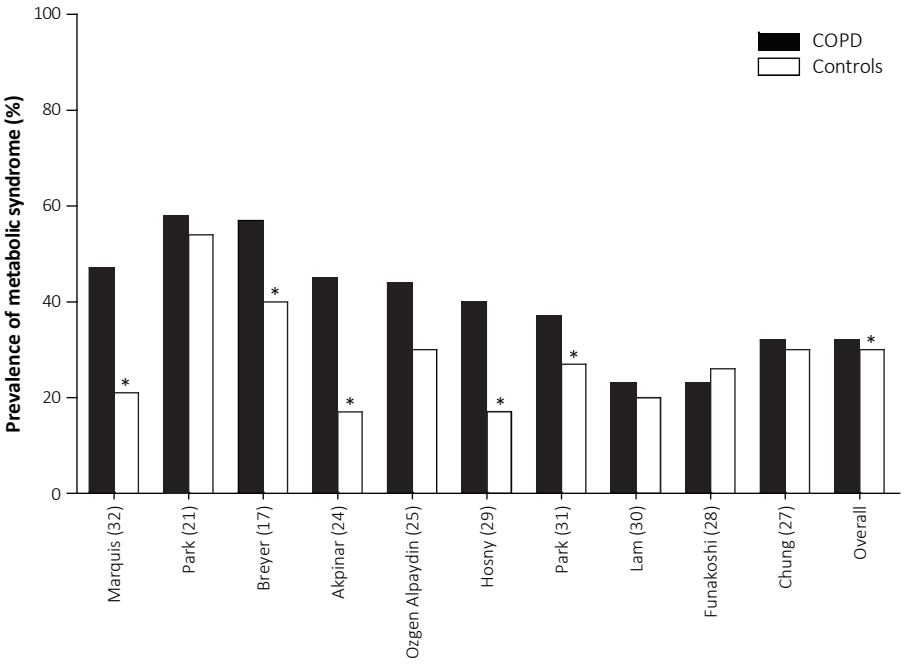

Figure 2. Prevalence of metabolic syndrome in patients with COPD versus controls.
Absolute numbers included in the studies: Marquis [23] n=38 COPD patients n=34 controls; Park [25] n=94 COPD patients and n=3661 controls, Breyer [30] n=228 COPD patients, n=156 controls; Akpinar [34] n=91 COPD patients, n=42 controls; Ozgen Alpaydin [36] n=50 COPD patients, n=40 controls; Hosny[37] n=50 COPD patients, n=35 controls; Park [38] n=133 COPD patients, n=1082 controls; Lam [39] n=496 COPD patients, n=6861 controls; Funakoshi [40] n=645 COPD patients, n=6544 controls; Chung [41] n=1039 COPD patients, n=6077 controls. An asterisk (*) indicates significant difference in prevalence of metabolic syndrome between COPD patients and controls.

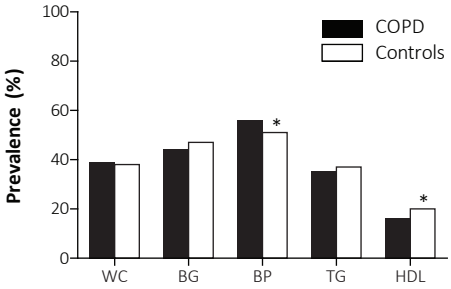

Figure 3. Pooled prevalence of metabolic syndrome components in COPD and controls.
Prevalence of components of metabolic syndrome was based on 1725 COPD patients and 18380 controls. An asterisk (*) indicates significant difference in prevalence of metabolic syndrome between COPD patients and controls. Abbreviations: BG, blood glucose; BP, blood pressure; COPD, Chronic obstructive pulmonary disease; HDL, high density lipoprotein; TG, triglycerides; WC, waist circumference.

Contributing factors of MetS in COPD

Smoking status in MetS and non-MetS patients was reported in 5 studies [24, 28-30, 35]. Four studies found no significant difference [24, 28, 30, 35] and one study showed significantly less smoking in the MetS group expressed by pack-years [29]. Physical activity level was assessed in three studies [24, 25, 31] by measuring the physical activity level with an accelerometer or by measuring the sedentary time. One study found no significant difference in sedentary time between the MetS group and non-MetS group [25], however, the other studies showed significantly reduced physical activity levels and lower activity intensity in the COPD patients with MetS compared to the patients without MetS [24, 31]. Medication use between MetS and non-MetS patients with COPD was reported in three studies [28, 30, 35]. These studies showed more inhaled or oral steroid use, more statin use, more beta-blockers use and more antihypertensives in the MetS group compared to the non-MetS group. No differences were found in use of anti-diabetics, insulin and long term oxygen therapy [28, 30].

DISCUSSION

This systematic review of current literature shows that the prevalence of MetS in COPD is significantly higher in COPD patients compared to controls. MetS is more prevalent in overweight and obese patients with less advanced airflow obstruction and seems to occur more frequently in female patients. The three most prevalent MetS components in both COPD patients and controls are hypertension, abdominal obesity and hyperglycemia. Smoking does not seem to be a discriminative factor between patients with and without MetS, while further studies are needed to assess the impact of other lifestyle factors and medications on MetS prevalence.

To our knowledge, this is the first systematic review on the prevalence of MetS and its components in COPD patients. Our findings are strengthened by the large number of included subjects in both the patient and control groups and the review provides some new insights about the risk profile for MetS in COPD.

A high MetS prevalence implies a significant risk for development of T2DM with or without CVD. Previous research has indeed shown that both CVD and T2DM are frequent comorbidities in COPD [4, 5]. Furthermore, COPD patients with CVD, hypertension and T2DM were shown to be at increased risk for hospitalizations and all-cause mortality [4]. The high MetS prevalence in lower disease stages coincides with the reported high cardiovascular-related mortality in mild to moderate disease [5]. Furthermore, recent studies have identified a so called “co-morbidity predominant subtype” of COPD patients, which is characterized by a cluster of metabolic co-morbidities, including obesity, CVD and T2DM [43, 44]. This seems to coincide with the most prevalent MetS components found in our review.

COPD patients with MetS were more frequently females, had higher BMI and had higher FEV_1 compared to COPD patients without MetS. The later was also shown by many studies reporting the highest MetS prevalence in patients with GOLD stage II compared to higher GOLD stages [28, 29, 32, 34, 36]. This observation could be due to a relatively higher influence of lifestyle on body composition and metabolic health in less advanced disease compared to COPD induced triggers on the wasting process in advanced disease [45]. Secondly, if we assume that MetS in COPD is also related to higher CVD risk, these patients might die earlier of CVD mortality, not reaching end-stage COPD.

Smoking is an established risk factor for COPD and has been associated with increased MetS prevalence and increased CVD risk [11]. It would thus be expected that the prevalence of smokers is higher in COPD patients with MetS compared to patients without MetS. Furthermore, physical inactivity and sedentary activity are also associated with MetS [12, 13] and typical for COPD patients [3]. However, limited studies reported smoking prevalence and physical activity level and future studies addressing MetS in COPD should include detailed assessment of lifestyle factors including smoking behavior and physical activity level as well as dietary quality which was recently shown to be poor in these patients [46].

Medications can directly influence the prevalence of MetS. Accordingly, the NCEP ATP III and IDF definitions of MetS include medication use for dyslipidemia, hypertension or diabetes as fulfillment of the selected criteria (Figure 1). However, not all studies clearly reported use of medications as a positive criterion for MetS. This inconsistency may explain the vast differences in the prevalence of hyperglycemia and dyslipidemia among studies. Furthermore, oral glucocorticoids can increase blood glucose levels, HDL levels and appetite, and cause muscle atrophy and abdominal obesity [47]. Indeed, of the four studies in COPD reporting medication use, two found a significantly higher use of steroids in the group with MetS [28, 31]. Other common medications in COPD, such as anti-depressants can cause impaired glucose tolerance [48], further contributing to MetS. Medications can thus influence MetS prevalence in COPD and need to be considered in future studies.

MetS and components were prevalent in COPD patients, but the prevalence varied greatly among studies, which could be due to differences in study design and setting. As MetS is a predictor for CVD and DM, existing co-morbidities can greatly affect its' prevalence. Minas *et al*., which excluded COPD patients with DM and CVD with the exception of hypertension, reported the lowest MetS prevalence (22%), whereas Diez-Manglano *et al*. and Breyer *et al*. found the highest MetS prevalence in COPD patients with more co-morbidities [28, 30]. Furthermore, included studies used different MetS definitions (Table 1), which are similar, but apply slightly different criteria for diagnosing MetS. Moreover, the average prevalence of MetS differed in different regions, with a lower MetS prevalence in the Asian studies (28%) compared to European (41%) and American studies (53%). This is consistent with findings from population studies, which found lower prevalence in Asia (21% in China [6] and 31%

in Korea [7]) compared to USA (34%) [8]. Furthermore, the difference in MetS prevalence between COPD patients and controls was small. This could be explained by the fact that the control group included subjects without COPD, but with other co-morbidities. Altogether, the study diversity has probably contributed to the broad range of reported MetS prevalence and components prevalence observed in our review and should be considered when interpreting the results.

The concept of MetS has received criticism on its applicability in scientific research and it has been questioned whether it better predicts cardiometabolic risk compared to its individual components [49]. Studies assessing individual MetS components or insulin resistance as the cornerstone of MetS might help unveil the background of increased cardiometabolic risk in COPD patients and allow us to specifically target these factors. While the relative influence of lifestyle versus disease specific determinants and medication is still unclear, we know that exercise training may improve MetS in other risk populations [50]. Pulmonary rehabilitation is an established intervention in COPD focusing on exercise training but the effects on modification of MetS is surprisingly not yet investigated in detail. Studies assessing the effect of such interventions on the cardiometabolic risk in COPD patients would contribute importantly to the understanding of MetS and to reducing disease burden for both patients and the healthcare system.

CONCLUSION

The prevalence of MetS is higher in COPD patients compared to controls. Its most prevalent components are abdominal obesity, hypertension and hyperglycemia. MetS is more prevalent in female patients, patients with less severe COPD and high BMI. Smoking does not seem to be discriminative for MetS in COPD. Data is lacking on the contribution of physical activity and medications to MetS prevalence in COPD. Future longitudinal and interventional studies are needed to unveil the relation of lifestyle and disease to MetS prevalence as well as the best management possibilities.

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SUPPLEMENTAL MATERIAL

Appendix 1: Search terms used in Pubmed

Search in Pubmed on April 17, 2015 yielding 107 results

((“Metabolic syndrome” [All Fields] OR “syndrome x” [All Fields] OR “insulin resistance syndrome” [All Fields]) AND (“COPD” [All Fields] OR “chronic obstructive pulmonary disease” [All Fields]))

Search in Embase on April 17, 2015 yielding 269 results:

1. Exp COPD/
2. Exp chronic obstructive pulmonary disease/
3. Exp metabolic syndrome/
4. Exp syndrome x/
5. Exp insulin resistance syndrome/
6. 1 or 2
7. 3 or 4 or 5
8. 6 and 7

Supplemental Table 1. Characteristics of controls of the included COPD papers and prevalence of metabolic syndrome and its components.

First author	Population (setting)	n	Age, y	Sex, %M	BMI, kg/m²	FEV_1, %pred	Definition	Prevalence, %	
								MetS	WC/BG/BP/TG/HDL
North America									
Marquis [1]	Age-matched participants without COPD or cardiovascular disease	34	63±6	59	29±5	-	NCEP ATP III 2001	21	32/12/59/32/15
Park [2]	NHANES subjects with no COPD, ≥40 years and spirometry results	3661	57±11	51	29±6	95±16	Alberti *et al.* 2009	54	62/61/56/45/44
Europe									
Breyer [3]	Healthy subjects of two observational studies	156	60±7	45	27±4	120±16	IDF	40	79/45/61[a]/22/8
Middle East									
Akpinar [4]	Smokers or non-smokers, age and sex matched, with normal spirometry and without infectious or inflammatory diseases	42	63±6	83	-	-	NCEP ATP III 2001	17	15/10/37/27/44
Ozgen Alpaydin [5]	Age matched healthy controls from family members of hospitalized patients	40	58±8	85	28±5	-	IDF	30	-
Hosny [6]	Healthy nonsmokers without history of chronic chest troubles	35	56±9	91	28±4	-	NCEP ATP III 2001	17	-
East and Northeast Asia									
Park [7]	KNHANES II subjects	1082	51±9[b]	40	24±3[b]	-	NCEP ATP III 2001[c]	27	39/15/37/35/51
Lam [8]	Patients from the GHHARE	6861	61±7[b]	27	-	-	IDF	20	33/37/53/33/17
Funakoshi [9]	Subjects of a health screening with normal lung function	6544	56±8	100	24±3	96±10	NCEP ATP III 2005[c]	26	29/55/48/36/4
Chung [10]	KHNANES IV subjects	6077	55±11[b]	39	24±3[b]	98±10	NCEP ATP III 2005[c]	30	-

Data are shown as mean ± SD unless specified otherwise. [a]Pooled prevalence of high systolic and diastolic blood pressure. [b]Pooled mean±standard deviation. [c]Used region specific cutoffs for waist circumference which were ≥90 cm for male and ≥80 cm for female. Abbreviations: BG, blood glucose; BMI, Body Mass Index; BP, blood pressure; FEV1, forced expiratory volume in 1 second; HDL, high density lipoprotein; IDF, International Diabetes Foundation; MetS, metabolic syndrome; N, Number of subjects; NCEP-ATP III 2001, National Cholesterol Education Program Adult Treatment Panel; NCEP ATP III 2005, revised National Cholesterol Education Program Adult Treatment Panel; TG, triglycerides; WC, waist circumference.

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Chapter 4

Sarcopenia in Advanced COPD Affects Cardiometabolic Risk Reduction by Short-Term High-Intensity Pulmonary Rehabilitation

Nanca Cebron Lipovec
Annemie M.W.J. Schols
Bram van den Borst
Rosanne J.H.C.G. Beijers
Tatjana Kosten
Daniel Omersa
Mitja Lainscak

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ABSTRACT

Background: Sarcopenia is common in chronic obstructive pulmonary disease (COPD) and may contribute to increased cardiometabolic risk. Interventions to reduce cardiometabolic risk in advanced COPD have been scarcely studied. We have investigated the cardiometabolic effect of a short-term high-intensity rehabilitation program in sarcopenic and non-sarcopenic patients with advanced COPD.

Methods: This was a prospective observational study including 112 stable COPD patients (66±8 years, 85% GOLD III/IV, 66% men) of an inpatient 4-week short–term high-intensity pulmonary rehabilitation program at the University Clinic Golnik, Slovenia. Blood biomarkers were assessed at baseline and after rehabilitation. Sarcopenia was assessed at baseline (skeletal muscle index <7.23 kg/m2 for men and <5.67 kg/m^2 for women, as measured by whole-body dual energy X-ray absorptiometry. Insulin resistance (IR) was defined as homeostasis model assessment of insulin resistance (HOMA-IR) above 2.5.

Results: IR and sarcopenia were detected in 59% and 55% of patients, respectively. In contrast to sarcopenic patients, rehabilitation decreased HOMA-IR (2.8 to 1.9, p=0.031), fat mass index (10.1 to 9.7 kg/m^2, p=0.013), waist circumference (103 to 101 cm, p=0.002) and LDL cholesterol (3.2 to 3.0 mmol/l, p=0.034) in non-sarcopenic patients. A decrease in total cholesterol levels was observed in both groups.

Conclusions: Sarcopenia affects the modification of cardiometabolic risk markers by short-term high-intensity pulmonary rehabilitation in advanced COPD patients.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease in terms of clinical manifestations and disease progression. The manifestations can be driven by pulmonary events but may also be the result of systemic consequences such as unintended weight loss and muscle weakness, and/or comorbidities such as osteoporosis, diabetes and cardiovascular disease. Comorbidities significantly increase the risk of hospitalizations [1] and mortality in COPD patients [2] and pose an additional burden to the patients and to the healthcare system [3].

Sarcopenia is defined as a syndrome characterized by progressive loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death [4]. Patients with COPD commonly develop sarcopenia, which is often present in conjunction with weight loss, osteoporosis and emphysema [5]. However, sarcopenia accompanied by (hidden) fat accumulation can also be present in normal and overweight patients [6]. The accumulation of adipose tissue may go unnoticed as it can be balanced by the loss of muscle mass. Thus, although total body weight remains stable, the metabolic profile can alter unfavorably into sarcopenic obesity, which is associated with various risks and poor clinical outcomes.

Muscle weakness in COPD is partly attributable to loss of muscle mass, but it is also related to a shift in muscle fiber composition towards a less oxidative type. These metabolic alterations result in decreased muscle oxidative capacity [7]. The fiber type switch reduces the capacity for fat oxidation in response to increased fatty acid availability, this in turn alters energy substrate preference from primarily fat to glucose utilization [8]. Given that skeletal muscle is the primary site of glucose utilization [9], sarcopenia can promote insulin resistance directly and independently of COPD [10]. Therefore, sarcopenia represents a vulnerable COPD phenotype as it not only affects physical functioning, but may also increase cardiometabolic risk.

Few studies have investigated the cardiometabolic risk in patients with advanced COPD. It is unknown whether cardiometabolic risk should be a therapeutic target and what the optimal management strategy should be. Exercise training is known to improve cardiometabolic risk profiles [11], however, the degree of improvement is at least partially dependent on the type of exercise. It is unknown if state-of-the-art pulmonary rehabilitation programs influence cardiometabolic risk profiles in addition to enhancing exercise performance and improving quality of life in COPD patients [12].

In the present study, we aimed to investigate the cardiometabolic effects of short-term high-intensity pulmonary rehabilitation in patients with advanced COPD with and without sarcopenia.

METHODS

Study Design

In this prospective observational study we recruited COPD patients from the in-patient pulmonary rehabilitation unit at the University Clinic Golnik between June 2012 and August 2014. Patients were required to be in a stable disease state, free from exacerbation in the previous 4 weeks prior to start of the program. Patients were excluded from the study if their metabolic risk factor data were unavailable for baseline analysis. Patients experiencing an exacerbation during the rehabilitation program were excluded from follow-up analysis as they did not attend the whole rehabilitation program (Figure 1). The study protocol was reviewed and approved by the Slovenian National Medical Ethics Committee. All patients received written and verbal study information and informed consent was obtained. The study is registered at clinicaltrials.gov (NCT02550808).

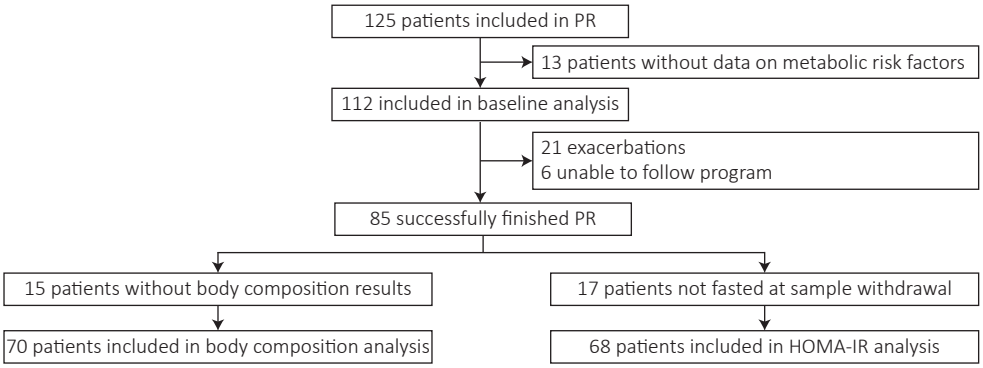

Figure 1. Study design outline.
Abbreviations: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; PR, pulmonary rehabilitation.

Pulmonary Rehabilitation Program

The outline of the inpatient rehabilitation is presented in Figure 2. The 5-week program consisted of an extensive baseline assessment, 4 weeks of intervention and a postrehabilitation assessment [13]. In total, patients received 20 training days with at least one session of combined endurance and resistance training daily with the following modalities: (1) interval training on a cycle-ergometer with alternating 40%-50% maximal capacity and 80% maximal capacity, 30 minutes per day; (2) treadmill training with alternating slope, 20 to 30 minutes per day; (3) transcutaneous electrostimulation of thigh muscles; (4) upper-limb and trunk muscles training; (5) respiratory muscle training. Exercise intensity was adjusted weekly during patient supervision visits according to patient performance. In addition to exercise training, patients also received nutritional assessment with counseling and support through diets tailored to

patient needs and preferences as well as counseling to quit smoking. Furthermore patients also received social and psychological support in line with the latest European Respiratory Society and American Thoracic Society recommendations on pulmonary rehabilitation [12].

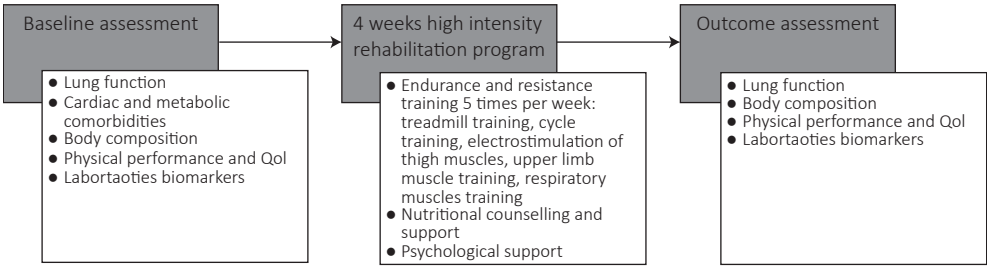

Figure 2. Rehabilitation program outline.

At baseline, all patients had cardiology referral with noninvasive imaging and exercise testing to rule out any relevant cardiovascular contraindications for exercise training and rehabilitation. Training program and procedures were tailored to the cardiovascular and pulmonary performance levels of the individual patients in order to meet the patients' training objectives. During the rehabilitation, patients were under daily medical supervision.

Baseline Clinical Assessment

The baseline clinical assessment included lung function testing, body composition analysis, physical performance testing, cardiovascular system evaluation and nutritional assessment. Furthermore, multiple laboratory biomarkers were routinely assessed from fasting blood samples before and after rehabilitation.

Lung function

Spirometry was performed to determine forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and their ratio (FEV1/FVC) according to European Respiratory Society guidelines [14]. Based on these measurements, the patients' disease severity was classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [15].

Metabolic and cardiac comorbidities

At baseline, participants underwent diagnostic workup for selected metabolic and cardiac comorbidities. Diabetes mellitus type 2 (T2DM) was diagnosed using International Diabetes Federation (IDF) guidelines [16]. Arterial hypertension, heart failure, ischemic heart disease, and valve disease were diagnosed per relevant European Society of Cardiology guidelines [17].

Body composition

Body height, and waist and hip circumference were measured to the nearest centimeter. Body weight was measured to the nearest 0.1 kg using a standard balance beam scale. Body mass index (BMI) was calculated as weight/height squared. Patients were categorized as underweight (BMI lower than 20.00 kg/m2), normal weight (BMI between 20.00 and 24.99 kg/m2), overweight (BMI between 25.00 and 30.00 kg/m2) and obese (BMI higher than 30.00 kg/m2).

Body composition was assessed using dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance (BIA). DXA was assessed at baseline using Hologic QDR Series Explorer bone densitometer (Hologic Inc, Marlborough, MA). Appendicular skeletal muscle index (ASMI) was calculated as the lean mass of the extremities divided by body height squared. Sarcopenia was defined by the ASMI cut-offs (<7.23 kg/m2 for men and <5.67 kg/m2 for women) [4].

BIA was performed at baseline and at the end of the rehabilitation program using a multifrequency tetra-polar technique (QuadScan 4000; Bodystat, Douglas, United Kingdom) with 4 electrodes. Patients were lying in a supine position on a flat surface. Two electrodes were placed on the right wrist with one just proximal to the third metacarpophalangeal joint (positive) and one on the wrist next to the ulnar head (negative). Two electrodes were placed on the right ankle with one just proximal to the third metatarsophalangeal joint (positive) and one between the medial and lateral malleoli (negative). Multifrequency (5, 50, 100, and 200 kHz) currents were introduced from the positive leads and traveled throughout the body to the negative leads.

Physical performance and quality of life

Physical performance was evaluated with the 6-minute walking test (6MWT) according to standardised protocol [18]. Quality of life (QoL) was determined through the Saint George's Respiratory Questionnaire (SGRQ) scores.

Laboratory biomarkers

Blood samples were collected after an 8-hour overnight fast. Plasma glucose, insulin, cholesterol and triglyceride levels were measured. Routine biochemistry was performed in the University Clinic Golnik laboratory according to standard procedures. Fasting insulin levels were determined from frozen serum samples using Immuno-chemiluminescention-assay. The analysis was performed in the Department of Clinical Chemistry, Reinier de Graaf Hospital in Delft, The Netherlands.

Rehabilitation Outcome Evaluation

The effectiveness of the rehabilitation program was evaluated by measures of physical performance and quality of life. The improvement in physical performance and quality of life was calculated as the absolute value as well as the percentage of participants attaining the minimal clinically important difference (MCID; 25 m for 6MWT [19], 4 points for SGRQ [20]).

Metabolic Risk Factors

Metabolic risk was based on the presence of metabolic syndrome and its components, IR and lipids profile. Metabolic syndrome was defined using the IDF definition [16]. Insulin resistance was defined based on the Homeostatic Model Assessment of IR (HOMA-IR) from (fasting glucose (mmol/L)*fasting insulin (mU/mL))/22.5. Insulin resistance was defined as HOMA-IR above 2.5 [21].

Statistical Analysis

Continuous variables are presented as mean values ± standard deviation. Categorical variables are presented as absolute numbers. To compare values between the 2 groups, independent samples t-test was used for continuous variables and Chi-square for categorical variables. For comparison of data before and after rehabilitation, paired samples t-test was used to compare normally distributed data and Wilcoxon sign-rank test for nonnormally distributed data. In correlation analysis, Pearson correlation coefficient was reported for normally distributed data and Spearman rank correlation coefficient for nonnormally distributed data. All analyses were performed with SPSS 21.0 (IBM Corp, Armonk, NY) and a p-value of <0.05 was considered statistically significant.

RESULTS

General Characteristics and Body Composition Parameters at Baseline

Of 125 patients referred to the rehabilitation program, 112 were included in the study. Most patients were elderly men of GOLD stage III/IV. The majority of patients were current or ex-smokers. Approximately half of the patients were overweight or obese, whereas a minority was underweight. Sarcopenia was present in 61 (55%) patients. Physical performance and reported quality of life was significantly lower in sarcopenic patients compared with nonsarcopenic patients (Table 1). Sarcopenia was most prevalent in underweight patients but also present in more than half of normal and overweight patients. Sarcopenic patients did not differ from nonsarcopenic patients in gender, age, degree of airflow obstruction or prevalence of cardiovascular disease.

Table 1. General characteristics.

	All patients (n=112)	Sarcopenia (n=61)	No sarcopenia (n=51)	p-value
General characteristics				
Sex, %male	66	72	59	0.141
Age, years	66±8	66±9	66±8	0.822
Smokers, %current	82	80	83	0.636
Lung function				
FVC, %pred	81 ± 19	79 ± 20	83 ± 18	0.267
FEV_1, %pred	38 ± 14	36 ± 13	40 ± 15	0.129
FEV_1/FVC	36 ± 13	35 ± 13	37 ± 14	0.376
GOLD stage II/III/IV, %	17/52/31	17/50/33	18/54/28	0.450
Body composition				
BMI, kg/m^2	25 ± 5	23 ± 4	29 ± 5	**<0.001**
BMI category, %				**<0.001**
Underweight	14	23	2	
Normal weight	39	51	25.5	
Overweight	23	13	25.5	
Obese	24	3	47	
Total body mass, kg	71 ± 16	65 ± 13	79 ± 15	**<0.001**
FMI, kg/m^2	8.6 ± 3.1	7.4 ± 1.9	10.0 ± 3.7	**<0.001**
FFMI, kg/m^2	16.7 ± 3.1	15.7 ± 2.9	18.1 ± 2.8	**<0.001**
ASMI, kg/m^2	6.6 ± 1.2	5.9 ± 0.8	7.4 ± 1.0	**<0.001**
Bone mass, kg	2.1 ± 0.6	2.0 ± 0.6	2.3 ± 0.6	**0.014**
Waist circumference, cm	97 ± 13	93 ± 11	103 ± 13	**<0.001**
Waist-hip ratio	0.98 ± 0.08	0.97 ± 0.08	0.98 ± 0.07	0.398
Systemic inflammation				
CRP, mg/l[a]	3.0 (1.2-5.3)	3.3 (1.0-6.4)	2.8 (1.5-4.8)	0.651
Comorbidities, %				
Diabetes Mellitus Type 2	23	22	24	0.981
Heart failure	25	20	31	0.157
Atrial fibrillation	7	9	6	0.588
Ischemic heart disease	10	12	8	0.470
Drug use, %				
ICS	70	66	75	0.594
LABA	80	75	86	0.617
SABA	71	64	78	0.358
LAMA	71	67	75	0.412
Theophylline	15	16	14	0.427
ACE inhibitors	32	26	37	0.672
ARBs	7	7	8	0.489
CCBs	10	8	12	0.278
Diuretics	27	27	28	0.948
Beta-blockers	14	12	18	0.785
Statins	21	13	31	0.098
Oral antidiabetics	9	10	8	0.584
Physical performance and quality of life				
6 minute walking test, m	335 ± 105	316 ± 113	357 ± 92	**0.046**
QoL, SGRQ points	40 ± 13	43 ± 13	36 ± 13	**0.012**

Data are shown as mean ± SD unless indicated otherwise. [a]Median (IQR). Abbreviations: ACE, angiotensin-converting-enzyme; ARBs, angiotensin II receptor blockers; ASMI, appendicular skeletal muscle index; BMI, body Mass Index; CCBs, calcium-chanel blockers; FEV1, forced Expiratory Volume in 1 second; FFMI, fat-free mass index; FMI, fat mass index; FVC, forced Vital Capacity; ICS, inhaled corticosteroids; LABA, long-acting beta-agonists; LAMA, long-acting muscarinic antagonists; QoL, quality of life; SABA, short-acting beta-agonists; SGRQ, St. George's respiratory questionnaire.

Cardiometabolic Risk Markers at Baseline

On the group level, IR was present in 59% of the patients and 23% of the patients were diagnosed with T2DM. Of all patients 50% had metabolic syndrome, whereas approximately 10% had dyslipidemia. No difference was observed in indices of hyperglycemia and IR between sarcopenic and non-sarcopenic patients. Metabolic syndrome prevalence was lower, but was still present in 37% of the sarcopenic patients (Table 2).

Table 2. Prevalence of cardiometabolic risk markers at baseline.

	All patients (n=112)	**Sarcopenia (n=61)**	**No sarcopenia (n=51)**	***p*-value**
Insulin resistance, %	59	54	65	0.231
HOMA-IR[a]	3.2 (1.7-6.7)	2.7 (1.5-6.0)	3.4 (2.1-7.3)	0.140
Fasting glucose, mmol/L[a]	5.9 (5.3-6.9)	5.9 (5.2-6.9)	5.9 (5.4-7.0)	0.505
Fasting insulin, mIU/L[a]	11.1 (6.6-23.6)	9.5 (6.1-19.8)	11.9 (7.7-27.3)	0.112
Metabolic syndrome, %	47	37	57	**0.038**
Abdominal obesity, %	72	63	83	**0.025**
Arterial hypertension, %	86	82	92	0.569
Hyperglycemia, %	69	68	69	0.569
Hypertriglyceridemia, %	12	6	19	0.062
Low HDL-cholesterol, %	10	8	13	0.509
Total cholesterol, mmol/L	5.1 ± 1.1	5.0 ± 1.1	5.2 ± 1.1	0.496
LDL-cholesterol, mmol/L	3.1 ± 1.0	3.0 ± 1.0	3.2 ± 1.0	0.432
HDL-cholesterol, mmol/L[a]	1.7 (1.4-1.7)	1.8 (1.4-2.1)	1.7 (1.3-2.1)	0.642
Triglycerides, mmol/L	1.2 ± 0.5	1.1 ± 0.4	1.3 ± 0.6	**0.044**

Data are shown as mean ± SD unless indicated otherwise. [a]Median (IQR). Abbreviations: HDL, high density lipoprotein; LDL, Low Density Lipoprotein.

Effect of Rehabilitation on Body Composition Parameters and Cardiometabolic Risk Markers in Sarcopenic and Nonsarcopenic Patients

Eighty-five patients successfully completed the 4-week high-intensity training (42 sarcopenic patients (69%), 43 nonsarcopenic patients (84%), p=0.057). More than 50% of both sarcopenic and nonsarcopenic patients improved in physical performance beyond MCID and more than 75% of patients improved in QoL beyond MCID.

At the group level, fasting glucose significantly decreased, but the prevalence of IR showed almost no change. However, in nonsarcopenic patients, the IR prevalence decreased by 10% and was accompanied by a significant decrease in HOMA-IR and fasting glucose. In sarcopenic patients the opposite was observed, the prevalence of IR slightly increased, along with an increase in fasting insulin levels and HOMA-IR (Figure 3). The difference in the change of HOMA-IR values between sarcopenic and nonsarcopenic patients was statistically significant (median, IQR: 0.06 (-0.86 – 1.78) in sarcopenic patients versus-0.63 (-2.26 – 0.15) in nonsarcopenic patients, p=0.031).

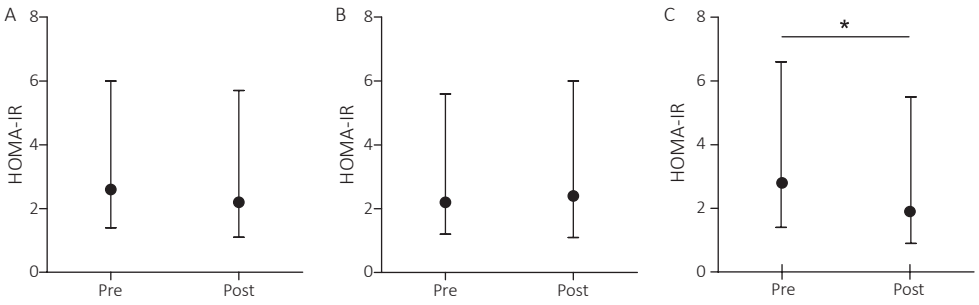

Figure 3. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index pre- and postrehabilitation (median and IQR).
A) All patients (n=68). B) Sarcopenic patients (n=34). C) Nonsarcopenic patients (n=34). *p<0.05.

Neither group showed a significant change in lean mass, while the nonsarcopenic group had decreased body fat and waist circumference (Figure 4). A significant decrease in total cholesterol levels as well as high-density lipoprotein (HDL) cholesterol levels was observed at the group level. Nonsarcopenic patients also decreased low-density lipoprotein (LDL) cholesterol levels (Figure 5).

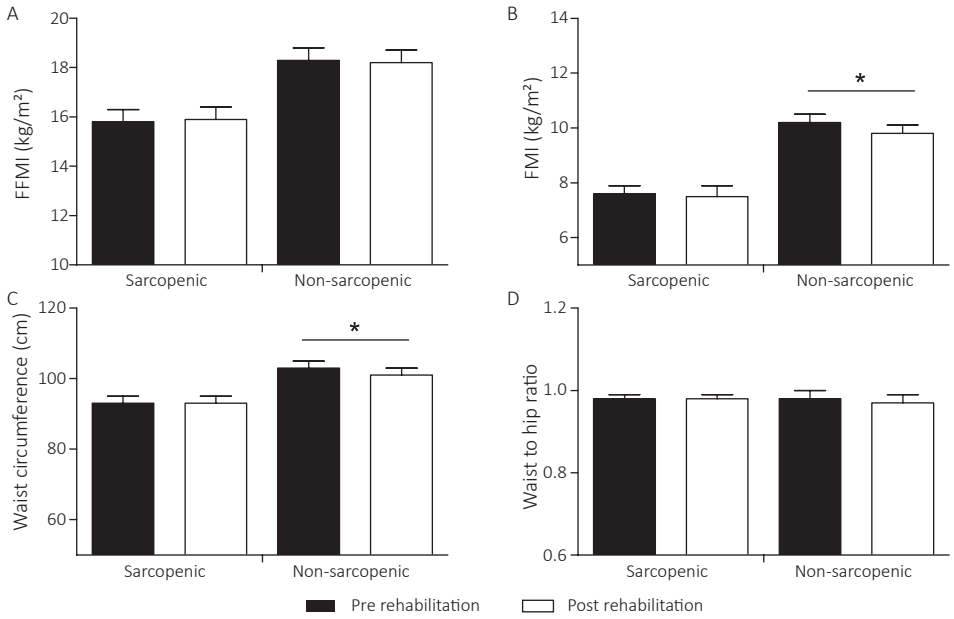

Figure 4. Changes to body composition after short-term high-intensity rehabilitation (mean and SEM).
A) Fat-free mass index (FFMI). B) Fat mass index (FMI). C) Waist circumference. D) Waist to hip ratio. N=35 for sarcopenic patients, n=35 for nonsarcopenic patients. *p<0.05.

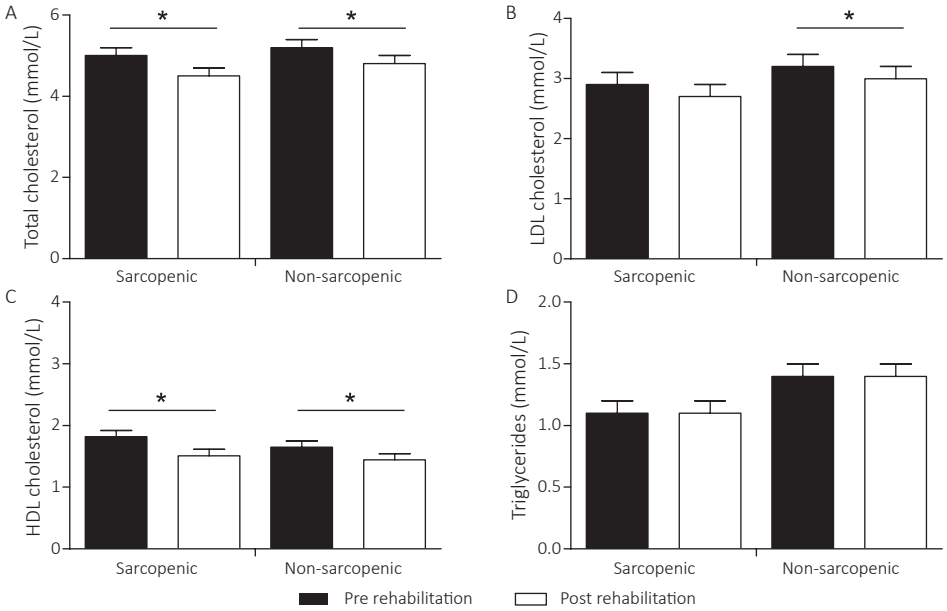

Figure 5. Changes in fasting lipid values after short-term high-intensity rehabilitation (mean and SEM).
A) Total cholesterol. B) Low-density lipoprotein (LDL) cholesterol. C) High-density lipoprotein (HDL). D) Triglycerides. N=35 for sarcopenic patients, n=39 for nonsarcopenic patients. *p<0.05.

DISCUSSION

This study reports the prevalence of cardiometabolic risk in advanced COPD and to our knowledge, is the first to report the effect of short-term high-intensity pulmonary rehabilitation on cardiometabolic risk in this population. Our main finding is that the rehabilitation-induced changes in cardiometabolic risk factors are sarcopenia-dependent. Insulin resistance was common in both sarcopenic and nonsarcopenic patients, but the HOMA-IR decreased in nonsarcopenic patients only. Short-term high-intensity pulmonary rehabilitation also resulted in decreased adiposity indices in the non-sarcopenic group and in decreased total cholesterol and LDL-cholesterol and, surprisingly, HDL cholesterol, in both sarcopenic and nonsarcopenic patients.

Recently, sarcopenic obesity has been receiving increasing attention in the research field. Besides the negative effects of obesity on cardiovascular risk, increasing evidence is available on its relation with muscle metabolism. In the elderly population, obesity is linked to decreased muscle metabolic health and muscle lipid availability has been suggested to contribute to anabolic resistance in insulin-resistant healthy subjects [22, 23]. In COPD, data on sarcopenic obesity and IR are scarce. A recent study associated skeletal muscle weakness with IR in COPD patients [24]. Increasing data is available on the higher prevalence of both sarcopenia and IR

in COPD compared with controls [25, 26]. Despite the reported high prevalence, no data are available on the effect of exercise on metabolic risk factors in these phenotypes.

The beneficial effects of exercise training on glucose metabolism are well known. A recent meta-analysis on the effect of high-intensity training on markers of glucose regulation showed a significant decrease in fasting glucose and body weight in T2DM patients and other chronic patients [27]. A combination of aerobic and resistance training has also shown to be effective in reducing IR in metabolic syndrome and obesity [28] and to improve glycemic control in T2DM [29]. Similarly, our short-term high-intensity combined exercise program resulted in a reduction of selected metabolic risk factors in patients with advanced COPD. In the nonsarcopenic group, HOMA-IR significantly decreased and resulted in decreased IR prevalence. Studies have shown that increased HOMA-IR is related to increased cardiovascular mortality [30], hence we could speculate that any decrease in this marker in patients with high cardiometabolic risk can be regarded beneficial. Interestingly, however, the change in glycemic markers differed in sarcopenic and nonsarcopenic patients. Nonsarcopenic patients had significantly decreased HOMA-IR, whereas sarcopenic patients showed a nonsignificant increase in HOMA-IR. This might be related to lower baseline insulin levels in COPD patients with reduced BMI as previously reported by Franssen et al. [31]. The IR reported in sarcopenic patients might also be related to the skeletal muscle fiber-type switch from slow to fast twitch, as has been observed in patients with T2DM [32]. IR in COPD has been linked to low-grade systemic inflammation [33], however recent studies point toward different inflammatory markers involved in the obese and cachectic profile [5]. These differences may imply a different pathophysiological mechanism of IR in these 2 patient groups, perhaps – driven by muscle loss and/or by abdominal obesity. The increase in IR in muscle-wasted patients might also be an anabolic response to training and hence potentially beneficial. Future studies are needed to explain the different cardiometabolic response to pulmonary rehabilitation in sarcopenic patients. More specifically for example, these studies could focus on the influence of abdominal obesity, increasing the amount of endurance exercise and adding nutritional supplements or pharmacologic agents to the rehabilitation. Assessing the biological significance of changes in HOMA-IR would also be of value.

Besides alterations in IR indices, the short-term high-intensity rehabilitation also resulted in changes in body composition. Nonsarcopenic patients decreased in fat mass and waist circumference, whereas sarcopenic patients showed a trend toward increased lean mass. Similar improvements have been observed after an 8-week training program in normal weight advanced COPD patients [34]. The minor changes in body composition and the lack of increase in lean mass are probably related to the short duration of the pulmonary rehabilitation program in our study.

Total cholesterol and HDL cholesterol decreased on the group level. This is in line with Gale et al., who evaluated the effect of pulmonary rehabilitation on cardiometabolic risk in a small

group of COPD patients, and showed a decrease in fasting glucose, total cholesterol and HDL-cholesterol [35]. Only 10% of our patients had low HDL-cholesterol at baseline. Elevated HDL-cholesterol levels in advanced COPD patients have been reported previously and have been partly attributed to steroid use [36]. Indeed, a recent study revealed a mechanistic relationship between steroids use and elevated HDL-cholesterol [37]. Moreover, studies in other chronic patient populations have reported changes in HDL-cholesterol structure in patients with profoundly elevated HDL-cholesterol, which has led to the questioning of HDL's protective function [38, 39]. Hence we believe that the significant decrease in HDL-levels observed after our pulmonary rehabilitation program may not be an indicator of increased cardiovascular risk, as would be expected in the general population. However, further research is needed to evaluate these speculations.

Our study does not provide a causal relationship between IR and sarcopenia, and does not provide an explanation for the different IR responses in sarcopenic and nonsarcopenic patients. To answer these questions, studies should focus on pathophysiological mechanisms behind IR and sarcopenia, and should use a more accurate evaluation of IR, such as the euglycemic clamp. However, elucidating the pathophysiological mechanisms was beyond the scope of this study. Furthermore, HOMA-IR is less invasive and thus more applicable in large cohort studies such as ours.

We have shown that short-term high-intensity pulmonary rehabilitation exhibits beneficial effects beyond reduction of symptoms, improvements in exercise performance and an increase in QoL. Its effects on cardiometabolic risk seem to differ depending on body composition. Further research is needed to better understand the causal relationships between these metabolic abnormalities and to evaluate the best treatment options. Furthermore, our findings support the recent call for a more personalized pulmonary rehabilitation program [40, 41].

CONCLUSION

Our study shows a high prevalence of both sarcopenia and IR in advanced COPD patients eligible for rehabilitation. Sarcopenia seems to affect the modification of cardiometabolic risk markers by short-term high-intensity pulmonary rehabilitation in this patient population.

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Chapter 5

Normal Weight but Low Muscle Mass and Abdominal Obese: Implications for the Cardiometabolic Risk Profile in COPD

Rosanne J.H.C.G. Beijers
Coby van de Bool
Bram van den Borst
Frits M.E. Franssen
Emiel F.M. Wouters
Annemie M.W.J. Schols

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ABSTRACT

Background: It is well established that low muscle mass affects physical performance in chronic obstructive pulmonary disease (COPD). We hypothesize that combined low muscle mass and abdominal obesity may also adversely influence the cardiometabolic risk profile in COPD, even in those with normal weight. The cardiometabolic risk profile and the responsiveness to 4 months high-intensity exercise training was assessed in normal weight COPD patients with low muscle mass stratified by abdominal obesity.

Methods: This is a cross-sectional study including 81 clinically stable patients with COPD (age 62.5±8.2 years; 50.6% males; forced expiratory volume in 1 second 55.1±19.5 percentage predicted) with fat-free mass index <25th percentile eligible for out-patient pulmonary rehabilitation. Body composition, blood biomarkers, blood pressure, physical activity level, dietary intake, and physical performance were assessed at baseline and in a subgroup after 4 months of exercise training.

Results: Mean body mass index was 22.7±2.7 kg/m² and 75% of patients had abdominal obesity. Abdominally obese patients had higher glucose, insulin, homeaostatic model assessment for insulin resistance (HOMA-IR), branched chain amino acids and a higher prevalence of metabolic syndrome compared with those without abdominal obesity. Exercise training improved cycling endurance time and quadriceps strength, but did not yield a clinically meaningful improvement of the cardiometabolic risk profile. Triglycerides showed a significant decrease, while the HOMA-IR increased.

Conclusion: Abdominal obesity is highly prevalent in normal-weight patients with COPD with low muscle mass who showed an increased cardiometabolic risk compared with patients without abdominal obesity. This cardiometabolic risk profile was not altered after 4 months of exercise training.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow limitation resulting from inflammation and remodeling of the airways [1]. Besides respiratory impairment, systemic disease manifestations influence disease burden and mortality risk [1]. It is well-established that both musculoskeletal impairment and elevated cardiovascular risk are common in COPD. Low muscle mass in COPD not only contributes to decreased physical performance and decreased health status [2, 3], but it is also a determinant of mortality [4]. In normal-to-overweight patients with COPD, low muscle mass is accompanied by a relative abundance of fat mass. There is increasing evidence that adiposity significantly contributes to the systemic inflammatory load in COPD [5], independent of body mass index (BMI) [6]. Furthermore, several studies reported an excessive abdominal visceral fat mass in COPD, independent of BMI, subcutaneous fat mass or abdominal circumference [7-9]. Disproportionally large abdominal visceral fat mass has been associated with low-grade systemic inflammation, which in turn was strongly predictive for the risk of all-cause and cardiovascular mortality in older persons with airflow obstruction [8].

In the current obesogenic society, low muscle mass may frequently coexist with abdominal obesity, also in normal-weight patients with COPD. In healthy persons the 'metabolically obese normal-weight' (MONW) phenotype, characterized by normal BMI but increased body fat and reduced muscle mass, was associated with metabolic abnormalities [10, 11]. Furthermore, the MONW phenotype is characterized by increased amount of visceral fat, increased liver and muscle fat content and adipose tissue inflammation [12-14]. We hypothesize that next to diet and physical activity [15, 16], (a history of) smoking [1] and specific disease induced triggers (e.g. inflammation and muscle wasting) may render in particular patients with COPD susceptible to develop the MONW phenotype. Exercise training is a cornerstone of pulmonary rehabilitation (PR) to improve physical functioning in COPD. In other conditions (e.g. obesity and diabetes) exercise training is also an established intervention to enhance cardiometabolic health.

The cardiometabolic consequences of relative adiposity, however, have not been investigated in this subgroup of patients with COPD, as the focus has primarily been on adverse effects of low muscle mass on muscle function and exercise performance. Next to adiposity, loss of skeletal muscle oxidative capacity has been suggested to play a major role in 'metabolic inflexibility' [17], in which the capacity to switch from fat to carbohydrate oxidation in response to nutritional circumstances is reduced [18]. Metabolic inflexibility has been associated with insulin resistance (IR) [17]. Muscle oxidative capacity is decreased in many COPD patients due to a type I-to-II muscle fiber type shift [19, 20]. In COPD patients with low appendicular muscle mass, skeletal muscle oxidative capacity is even more affected [21]. Furthermore, Maddocks *et al*. showed in normal weight patients with COPD an inverse correlation between

type I muscle fibres and intramuscular fat infiltration assessed by computed tomography analysis [22].

The objective of the present study was to investigate the cardiometabolic risk profile of normal-weight COPD patients with low muscle mass stratified by abdominal obesity and to explore the responsiveness to 4 months of high-intensity exercise training.

METHODS

A detailed methodology can be found in the supplemental material.

Study design and participants

The research question was incorporated as prescheduled analysis of the NUTRAIN-trial investigating the efficacy of targeted nutrition as adjunct to exercise training [23]. Patients were recruited from CIRO Horn, The Netherlands, between 2011 and 2014. The study population included a total of 81 COPD patients with low muscle mass eligible for outpatient PR. Low muscle mass was defined as a fat-free mass index (FFMI) under the sex- and age-specific 25th percentile values [24], assessed by Dual Energy X-Ray Absorptiometry (DEXA, Lunar Prodigy system; GE Healthcare, Madison, WI, USA). The study was registered at clinicaltrials.gov (NCT01344135) and ethical approval was granted by the Medical Ethics Committee from Maastricht University Medical Centre + (NL34927068.10/MEC 11-3-004). Written informed consent was obtained from all patients. Cross-sectional analysis involved the total study group, but only the placebo group of the NUTRAIN trial was included to investigate the cardiometabolic response to exercise training as the intervention group received nutritional supplementation that could have modified this response.

Measurements

Measurements were performed at CIRO during pre- and post-rehabilitation assessment. BMI was calculated and the ratio of percentage fat mass (FM) in the android to the gynoid region measured by DEXA was used as a measure for abdominal FM. Abdominal obesity was defined by android/gynoid %FM >1.0 for men and >0.8 for women, as previously reported [15, 25, 26].

Cardiometabolic risk markers were measured including, glucose, insulin, homeostatic model assessment to estimate insulin resistance (HOMA-IR), triglycerides, high and low density lipoprotein cholesterol, high-sensitive C-reactive protein and branched chain amino acids (BCAAs). Blood pressure was measured and metabolic syndrome was defined [27]. Furthermore, medication use and comorbidities were recorded based on medical history and self-report, respectively.

Lung function was measured using forced spirometry and the diffusing capacity of the lung for carbon monoxide (DLCO) was measured using the single-breath method. Smoking status was based on self-report and was categorized as smokers, former smokers and never smokers. Physical performance was measured by cycle endurance time (CET), isometric muscle strength and 6 minute walk distance (6MWD). Physical activity level was measured using accelerometers. Dietary intake was assessed using a validated cross-check dietary history method [28].

High-intensity exercise training

According to the latest PR recommendations [29], patients received 40 supervised exercise training sessions, 2 to 3 times a week, including progressive high-intensity interval and endurance exercise by cycle ergometry and treadmill walking.

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS version 22 for Windows, SPSS Inc. Chicago, IL). The Shapiro-Wilk test was used to assess data for normal distribution. Parametric data were presented as mean ± standard deviation and nonparametric data were presented as median (interquartile range). Differences between groups were compared using Student t-test for continuous variables, χ^2-test for categorical variables and Kruskal-Wallis test for continuous variables with skewed distributions. Correlations were tested using Pearson's r or Spearman's ρ. The paired t-test or Wilcoxon signed-rank test was used to assess the effect of exercise training on cardiometabolic risk parameters. Differences were considered to be statistically significant at $p<0.05$.

RESULTS

Mean ± standard deviation age of the patients was 63±8 years and 50.6% were male patients. The proportion of smokers, former smokers and never smokers was 25.0%, 73.8% and 1.3%, respectively. The majority of the patients had moderate-to-severe COPD (Global Initiative for Chronic Obstructive Lung Disease I/II/III/IV: 11.1%/49.4%/30.9%/8.6%). The average DLCO was 49.4±14.6 %predicted, indicative of emphysema [30, 31].

Abdominal vs non-abdominal obesity

Abdominal obesity was present in 61 of the 81 patients (75%) (Figure 1). Forced expiratory volume in 1 second and DLCO were comparable between groups, but patients with abdominal obesity had lower residual volume, total lung capacity and intra-thoracic gas volume (Table 1). Although patients with abdominal obesity had a higher BMI, their FFMI was not significantly different from those without abdominal obesity. Furthermore, the groups were not significantly different in bone mineral density.

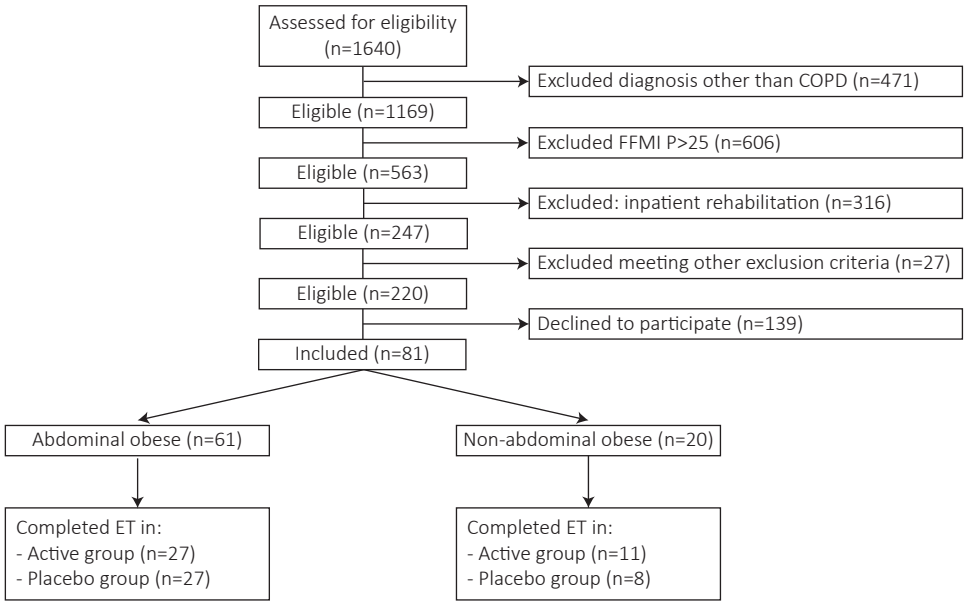

Figure 1. Participant flowchart.
Abbreviations: ET, exercise training; FFMI, fat free mass index.

Table 1. Characteristics of COPD patients with low muscle mass stratified by abdominal obesity.

	Abdominal obese (n=61)	**Non-abdominal obese (n=20)**	***p*-value**
General characteristics			
Age, y	63.3 ± 8.1	60.0 ± 8.2	0.111
Males, %	54.1	40.0	0.274
Smoking status[a]			
Current smoker, %	23.0	31.6	0.574
Former smoker, %	75.4	68.4	
Never smoker, %	1.6	0.0	
Lung function			
FEV_1, %pred	54.1 ± 17.9	58.1 ± 24.0	0.432
FVC, %pred	97.8 ± 16.3	98.2 ± 16.9	0.924
FEV_1/FVC, %	39.3 ± 11.0	43.9 ± 12.8	0.215
DLCO, %pred	49.0 ± 15.1	50.7 ± 13.0	0.680
RV,% pred	141.8 ± 42.7	166.8 ± 50.4	**0.037**
TLC, %pred	112.1 ± 16.2	122.7 ± 16.6	**0.021**
ITGV, %pred	136.6 ± 28.9	161.2 ± 32.5	**0.002**
Body composition			
BMI, kg/m²	23.6 ± 2.4	20.0 ± 0.8	**<0.001**
FFMI, kg/m²	15.9 ± 1.6	15.6 ± 1.6	0.424
SMI, kg/m²	6.3 ± 0.9	6.1 ± 0.8	0.332
Fat percentage, %	32.4 ± 8.0	22.0 ± 9.0	**<0.001**
BMD, g/cm²	1.1 ± 0.1	1.0 ± 0.1	0.154

Data are shown as mean ± SD unless indicated otherwise. [a]Only available for 80 patients. Abbreviations: BMD, bone mineral density; BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1s; %pred, percentage of predicted; FFMI, fat free mass index; FVC, forced vital capacity; ITGV, intra-thoracic gas volume; RV, residual volume; SMI, skeletal muscle mass index; TLC, total lung capacity.

Abdominally obese COPD patients had higher glucose and insulin levels and higher HOMA-IR than those without abdominal obesity (Table 2), resulting in a higher prevalence of IR (32% vs 7%, *p*=0.055). Furthermore, almost one-half of the abdominally obese patients had the metabolic syndrome vs 1 patient in the non-abdominally obese group (*p*=0.001). Plasma concentration of BCAAs was significantly higher in abdominally obese patients compared with those without abdominal obesity and significantly correlated with HOMA-IR in the group as a whole (ρ=0.49 *p*<0.001, Figure 2). The prevalence of cardiovascular disease (27.9% vs 40.0%) and diabetes (6.6% vs 0%) as well as cardiometabolic medication use was not significantly different between patients with and without abdominal obesity (Supplemental Table 1).

Table 2. Cardiometabolic risk profile in COPD patients with low muscle mass stratified by abdominal obesity.

	Abdominal obese (n=61)	Non-abdominal obese (n=20)	*p*-value
Cardiometabolic risk parameters			
Glucose, mmol/L[a]	5.2 (4.9-5.7)	4.9 (4.4-5.3)	**0.003**
Insulin, mIU/L[a]	8.0 (5.6-12.0)	4.9 (3.3-8.1)	**0.007**
HOMA-IR[a]	1.9 (1.3-2.8)	1.1 (0.7-1.8)	**0.005**
Triglycerides, mmol/L[a]	1.3 (1.0-1.7)	1.1 (0.9-1.3)	0.065
HDL cholesterol, mmol/L[a]	1.5 (1.2-1.9)	1.8 (1.3-2.2)	0.181
LDL cholesterol, mmol/L	3.2 ± 1.1	3.1 ± 1.1	0.793
Systolic blood pressure, mmHg[b]	124.7 ± 18.4	122.5 ± 13.7	0.638
Diastolic blood pressure, mmHg[b]	73.4 ± 11.2	70.3 ± 9.0	0.277
Metabolic syndrome, %[c]	44.1	5.0	**0.001**
Hs-CRP, mg/L[a]	2.5 (1.0-6.1)	2.0 (0.2-5.6)	0.273
BCAAs, µm/L[a]	469 (421-507)	415 (377-468)	**0.013**

Data are shown as mean ± SD unless indicated otherwise. [a]Median (IQR). [b]Only measured in 53 abdominal obese patients. [c]Only measured in 59 abdominal obese patients. Abbreviations: BCAAs, branched chain amino acids; HDL, high density lipoprotein; HOMA-IR, Homeostatic model assessment to estimate insulin resistance; Hs-CRP, high-sensitive C-reactive protein, LDL, low density lipoprotein.

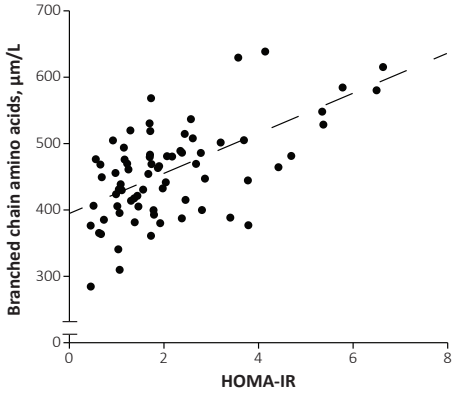

Figure 2. Correlation between HOMA-IR and branched chain amino acids in COPD patients (ρ=0.492 *p*<0.001).

Peak work rate, CET, maximal isometric strength and 6MWD were comparable between patients with and without abdominal obesity (Table 3). Total physical activity level was significantly lower in abdominally obese patients. Furthermore, abdominally obese patients tended to spend more time sedentary and less time in moderate to vigorous physical activity (MVPA) compared with the patients without abdominal obesity. No significant differences were seen in dietary intake or dietary quality.

Table 3. Physical performance, physical activity and dietary intake of COPD patients with low muscle mass stratified by abdominal obesity.

	Abdominal obese (n=61)	Non-abdominal obese (n=20)	*p*-value
Physical performance			
Peak work rate, Watt	78 ± 28	81 ± 34	0.754
CET, sec	277 ± 178	279 ± 172	0.969
Maximal isometric strength, Nm	123 ± 39	108 ± 41	0.166
6MWD, m	487 ± 79	512 ± 93	0.254
Physical activity level			
Total activity, counts/min	172.95 ± 77.49	217.13 ± 88.19	**0.042**
Time spent in sedentary PA, % of wear time	71.67 ± 7.77	67.62 ± 10.34	0.077
Time spent in lifestyle PA, % of wear time	21.91 ± 5.10	23.74 ± 6.41	0.213
Time spent in MVPA, % of wear time	6.42 ± 3.97	8.64 ± 4.91	0.051
Dietary intake			
Total energy, kcal[a]	2050 (1779-2487)	2378 (1681-3499)	0.121
Protein, E%	15.9 ± 3.5	14.6 ± 2.0	0.056
Carbohydrates, E%	44.3 ± 9.8	44.5 ± 10.1	0.937
Fat total, E%	36.3 ± 7.5	39.3 ± 9.0	0.154

Data are shown as mean ± SD unless indicated otherwise. Physical activity level was measured in 55 abdominal obese patients and 19 non-abdominal obese patients. [a]Median (IQR). Abbreviations: 6MWD, six minute walking distance; CET, cycling endurance time; E%, energy percentage; MVPA, moderate to vigorous physical activity; PA, physical activity.

Responsiveness to high-intensity exercise training

Of the placebo group in the NUTRAIN-trial 35 patients (90%) completed the exercise training program, of which 27 had abdominal obesity whereas 8 had no abdominal obesity. Quadriceps strength ($p<0.001$) and CET ($p<0.001$) significantly increased, while 6MWD did not change after completion of the exercise training (Table 4). However, no changes were found in glucose, high and low density lipoprotein cholesterol, systolic and diastolic blood pressure, high-sensitive C-reactive protein and BCAAs. Triglycerides showed a significant decrease ($p=0.011$), while the HOMA-IR increased ($p=0.042$) because of an increase in insulin.

Table 4. Effect of 4 months high-intensity exercise training on physical performance, physical activity, dietary intake, and the cardiometabolic risk profile in low muscle mass COPD patients with abdominal obesity.

	Baseline (n=27)	After 4 months (n=27)	*p*-value
Physical performance			
CET, sec	237 ± 77	533 ± 388	**<0.001**
Maximal isometric strength, Nm	126 ± 40	139 ± 41	**<0.001**
6MWD, m	487 ± 72	490 ± 96	0.781
Physical activity[a]			
Total activity, counts/min	165.94 ± 73.21	165.20 ± 93.90	0.949
Time spent in sedentary PA, % of wear time	74.04 ± 5.58	73.79 ± 8.28	0.845
Time spent in lifestyle PA, % of wear time	19.79 ± 3.35	19.87 ± 5.00	0.949
Time spent in MVPA, % of wear time	6.17 ± 3.60	6.33 ± 4.27	0.773
Dietary intake			
Total energy, kcal[b]	2176 (1930-2590)	2155 (1656-2773)	0.532
Protein, E%	15.7 ± 3.7	15.6 ± 3.5	0.886
Carbohydrates, E%	45.4 ± 11.4	43.4 ± 8.4	0.273
Fat total, E%	35.1 ± 8.0	36.8 ± 7.4	0.262
Cardiometabolic risk parameters			
Glucose, mmol/L[b]	5.2 (5.0-5.8)	5.5 (4.9-6.0)	0.515
Insulin, mIU/L[b]	7.7 (6.3-11.2)	9.1 (7.0-11.9)	0.074
HOMA-IR[b]	1.9 (1.4-2.7)	2.4 (1.8-3.0)	**0.042**
Triglycerides, mmol/L[b]	1.4 (1.0-1.8)	1.0 (0.9-1.4)	**0.011**
HDL cholesterol, mmol/L[b]	1.6 (1.1-1.9)	1.5 (1.2-1.8)	0.572
LDL cholesterol, mmol/L[b]	3.2 (2.4-4.0)	3.0 (2.2-4.0)	0.149
Systolic blood pressure, mmHg[a]	130 ± 20	128 ± 22	0.761
Diastolic blood pressure, mmHg[a]	77 ± 13	76 ± 15	0.853
Hs-CRP, mg/L[b]	2.8 (1.3-3.7)	1.9 (0.5-8.4)	0.895
BCAAs, µm/L[b]	470 (449-508)	461 (443-519)	0.428

Data are shown as mean ± SD unless indicated otherwise. [a]Only measured in 22 patients after 4 months of training. [b]Median (IQR). Abbreviations: BCAAs, branched chain amino acids; CET, cycling endurance time; HOMA-IR, Homeostatic model assessment to estimate insulin resistance; Hs-CRP, high-sensitive C-reactive protein.

DISCUSSION

To our knowledge this is the first study investigating the cardiometabolic risk profile in normal-weight COPD patients with low muscle mass stratified by abdominal obesity. The main finding is that abdominal obesity frequently concurs with low muscle mass in patients with COPD eligible for outpatient PR and that these patients are characterized by higher glucose, insulin, HOMA-IR and BCAAs levels, and lower total physical activity level compared to those without abdominal obesity. No differences were seen in other lifestyle factors including smoking behavior and dietary quality. The prevalence of metabolic syndrome was high in patients with abdominal obesity. This metabolic profile resembles the recently highlighted MONW phenotype. The cardiometabolic risk profile of the abdominally obese patients, however, was not altered immediately after 4 months of high-intensity exercise training and HOMA-IR, but not BCAAs, actually increased because of an increase in insulin.

Within this selected population of COPD patients with low muscle mass, the proportion of those with abdominal obesity was high (75%). This seems to be more related to lifestyle factors, in particular low physical activity level, than to specific disease characteristics. Although we did not characterize emphysema by high-resolution computed tomography, patients with and without abdominal obesity were comparable in terms of airflow limitation and diffusion capacity as proxy for emphysema. The slightly lower static hyperinflation in the abdominally obese patients might be related to effects of adiposity on functional residual capacity as shown in studies by O'Donnell *et al.* [32-34].

Differences in physical activity level were characterized by a 20% lower total physical activity level. Furthermore, the time spent sedentary and in MVPA seems to be higher and lower, respectively, in the abdominally obese patients. It would be worthwhile to explore how the amount of sedentary time in COPD can be decreased. In diabetes-oriented research a shift has occurred in physical activity targets from more time in MVPA toward sitting less and increasing standing and stepping as means to improve cardiometabolic health [35]. However, efficacy and feasibility of such interventions and eventual added value in other chronic diseases such as COPD needs to be established.

Metabolic syndrome is a constellation of risk factors that has been shown to increase the risk of developing type II diabetes mellitus and cardiovascular disease in the general population. The prevalence of metabolic syndrome in this group of patients with low muscle mass was 34.2% which corresponds to the recently reported prevalence (34%) in normal-to-overweight patients with COPD in a systematic review [36]. Although metabolic syndrome was determined based on the National Cholesterol Education Program's Adult Treatment Panel III definition with no obligatory component for abdominal obesity, the high proportion of abdominal obesity appears to be the main driver, because only 1 patient in the group without abdominal obesity had metabolic syndrome.

In line with the low prevalence of metabolic syndrome in COPD patients without abdominal obesity, the prevalence of IR, based on the HOMA-IR, was also low in this group (7%). Compared to the patients without abdominal obesity, the abdominally obese group had a significantly higher prevalence of IR. Although we are aware HOMA-IR is not a definitive tool to assess IR, we also found higher levels of BCAAs in patients with abdominal obesity, which was significantly correlated with HOMA-IR. The causality of BCAAs and IR is still unclear but BCAAs have recently been positioned as biomarker of IR [37, 38]. Several potential mechanisms have been proposed to explain the contribution of BCAAs to IR [37, 39]. BCAAs are proposed to activate the mammalian target of rapamycin complex 1 signaling pathway, which could lead to an impaired insulin action. Furthermore, increased BCAAs are proposed to be a biomarker of impaired BCAA metabolism causing β-cell dysfunction and IR. In addition, increased BCAAs can cause secretion of the 3-hydroxyisobutyrate from muscle, which activates endothelial fatty acid transport and uptake, resulting in lipid accumulation in muscle and IR [39]. These are

interesting observations, which seem clinically relevant, but because the biological and clinical relevance of the HOMA-IR has been questioned, euglycaemic clamp studies are needed to gain more insight into the pathophysiology of IR and increased BCAA levels in this phenotype.

Despite the fact that exercise training has been shown to improve the cardiometabolic risk profile in overweight and obese persons without COPD [40-42]. less is known about its effects in the MONW phenotype. We therefore investigated as proof-of-concept the effects of high-intensity exercise training on the cardiometabolic risk profile of the abdominally obese COPD patients. A remarkable finding was the raised HOMA-IR after this high-intensity exercise training. This is in line with a recent study by Cebron *et al*. showing significant improvements in HOMA-IR after short-term (4 weeks) high-intensity exercise training only in COPD patients with normal muscle mass, whereas COPD patients with low muscle mass did not improve [43]. Furthermore, in line with the current study the non-IR COPD patients with low muscle mass even increased in HOMA-IR, because of an increase in insulin (personal communication). It could be speculated that the increase in HOMA-IR, primarily because of increased insulin levels, reflects an adaptive anabolic response to the exercise training, as increased insulin signaling stimulates muscle anabolism. No studies are available in literature that have related changes in plasma insulin and HOMA-IR to muscle insulin/insulin-like growth factor 1 signaling after exercise training in patients with COPD. This could be further investigated in future research.

Some limitations of the current study deserve discussion. First, the cardiometabolic profile was assessed in cross-sectional design. This was suitable to answer our research question but not to unravel the cause of the elevated cardiometabolic risk and the development of cardiometabolic diseases over time. Another limitation is that the proof-of-concept exercise intervention was studied in a subset of patients that nevertheless showed a consistent response.

CONCLUSION

In conclusion, this study showed that abdominal obesity is highly prevalent in COPD patients with low muscle mass who showed an increased cardiometabolic risk in comparison to COPD patients without abdominal obesity. The effectiveness of the current exercise program in terms of maximizing exercise performance was confirmed as shown by significant improvements in CET and quadriceps strength. However, no clinically meaningful alterations in cardiometabolic risk parameters were observed. Future studies should give more insight into the underlying pathophysiology of the elevated cardiometabolic profile in COPD. Subsequently the modulating potential of different exercise training types and intensities and nutritional or pharmacologic interventions on the cardiometabolic profile could be opportunistic to investigate.

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SUPPLEMENTAL MATERIAL

Measurements

Measurements were performed at CIRO during pre- and post-rehabilitation assessment. Height and total body weight were measured to calculate the BMI as weight/height^2. Whole body composition was measured using DEXA. The ratio of the percentage fat mass (FM) in the android region (waist) to the percentage FM in the gynoid region (hip) was used as a measure for abdominal FM. Abdominal obesity was defined by android/gynoid %FM >1.0 for men and >0.8 for women, as previously reported [1-3].

After an overnight fast, peripheral blood was obtained to determine cardiometabolic risk markers including glucose, insulin, triglycerides, high and low density lipoprotein (HDL and LDL, respectively) cholesterol, high-sensitive C-reactive protein (Hs-CRP) and branched chain amino acids (BCAAs), as high levels of BCAAs tend to be increased in IR and type 2 diabetes mellitus, and have been linked to the metabolic syndrome and cardiovascular disease [4]. Homeostatic model assessment (HOMA) was applied to estimate insulin resistance (IR) by HOMA-IR=(Fasting insulin * Fasting glucose)/22.5 [5]. IR was defined as HOMA-IR higher than 2.5 [6].

Resting blood pressure was measured by a digital sphygmomanometer (Suntech, Tango, NC, USA). Furthermore, metabolic syndrome was defined according to the NCEP ATP III definition (The National Cholesterol Education Program's Adult Treatment Panel III) [7]. Medication use and comorbidities were recorded based on medical history and self-report, respectively.

Lung function testing was performed using weekly calibrated standardized equipment (Masterlab®; Jaeger, Würzburg, Germany). Forced spirometry was used to assess forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), total lung capacity (TLC), intra-thoracic gas volume (ITGV) and residual volume (RV). The single-breath method was used to assess the diffusing capacity of the lung for carbon monoxide (DLCO). Smoking status was based on self-report and was categorized as smokers, former smokers and never smokers.

At baseline the peak work rate was retrieved from a maximal cycling test on an electronically braked cycle ergometer (Carefusion, Houten, The Netherlands). On the next day, cycle endurance time (CET) was determined during constant work rate endurance test (CWRT) at 75% of the peak load. This CET was also performed after the rehabilitation programme. The maximal volitional isometric muscle strength was measured by dynamometry (Biodex System 4 Pro; Biodex Medical Systems, Inc., New York, USA). Furthermore, the best of two six minute walk distance (6MWD) tests was used for analysis of functional exercise capacity.

Physical activity level was measured using a tri-axial GT3X Actigraph accelerometer (ActiGraphTM, LLC, Fort Walton Beach, FL, USA). Patients were instructed to wear the accelerometer for 7 days, before and after the exercise training, during the time they were

not asleep, except when showering or bathing. Data of the accelerometer were analyzed as previously reported [8].

Dietary intake was assessed by trained dieticians using a validated cross-check dietary history method and calculated using the Dutch Food Composition Database. Total energy intake was reported in kilocalories and intake of macronutrients were expressed in energy percentage (%).

High-intensity exercise training

According to the latest American Thoracic Society and European Respiratory Society statement on PR [9], patients received 40 supervised exercise training sessions, 2-3 times a week, including progressive high-intensity interval and endurance exercise by cycle ergometry and treadmill walking. Interval training (2 minutes exercise, 1 minute rest) was performed at 70-100% of peak load for 20 minutes and endurance training was performed at 35-55% of peak load for 20 minutes. In addition, patients received progressive resistance exercises of upper and lower body parts at 75% of the 1 repetition maximum.

Supplemental Table 1. Cardiometabolic comorbidities and medication use of low muscle mass COPD patients with and without abdominal obesity.

	Abdominal obese (n=61)	Non-abdominal obese (n=20)	*p*-value
Comorbidities			
Cardiovascular disease, %[a]	27.9	40.0	0.308
Acute myocardinfarct, %	6.6	15.0	0.244
Peripheral vascurlar disease, %	6.6	10.0	0.610
Cerebrovascular disease, %	11.5	10.0	0.855
Other cardiovascular disease, %	19.7	15.0	0.641
Diabetes, %	6.6	0.0	0.240
Medication use			
Antihypertensives, %	44.3	30.0	0.260
ACE or ARB, %	29.5	20.0	0.407
Beta-blockers, %	16.4	10.0	0.485
Calcium blockers, %	13.1	15.0	0.831
Anti-arrhythmia, %	16.4	15.0	0.883
Nitrates, %	8.2	10.0	0.803
Diuretics, %	13.1	15.0	0.831
Cholesterol lowering drugs, %	29.5	25.0	0.698
Antiaggregants, %	21.3	30.0	0.426
Coumarins, %	6.6	0.0	0.240
Oral antidiabetics or insulin, %	8.2	0.0	0.186

[a]None of the patients had chronic heart failure. Abbreviations: ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker.

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Chapter 6

The Effect of Acute and 7-days Dietary Nitrate on Mechanical Efficiency, Exercise Performance and Cardiac Biomakers in Patients with Chronic Obstructive Pulmonary Disease

Rosanne J.H.C.G. Beijers
Stephanie M.D. Huysmans
Coby van de Bool
Boris R.M. Kingma
Lex B. Verdijk
Luc J.C. van Loon
Steven J.R. Meex
Harry R. Gosker
Annemie M.W.J. Schols

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ABSTRACT

Background: Many COPD patients have a reduced exercise capacity and mechanical efficiency and are at increased cardiometabolic risk. This study aimed to assess acute and 7-days effects of dietary nitrate on mechanical efficiency, exercise performance and cardiac biomarkers in patients with COPD.

Methods: This double-blind, randomized cross-over placebo controlled trial included 20 mild-to-moderate COPD patients (66.6±7.5 years) with moderate exercise impairments and decreased mechanical efficiency, normal BMI (26±3 kg/m²) but high prevalence of abdominal obesity (83.3%). Subjects were randomly allocated to the treatment order of 7 days sodium nitrate ingestion (~8 mmol/day) and 7 days placebo (NaCl solution) or vice versa, separated by a washout period. Before (Day-1) and after (Day-7) both intervention periods resting metabolic rate and the metabolic response during submaximal cycle ergometry, cycling endurance time, plasma nitrate and nitrite levels, cardiac plasma biomarkers (e.g. cardiac troponin T, Nt-proBNP and creatinine kinase) and blood pressure were measured. Subsequently, gross, net and delta mechanical efficiency were calculated.

Results: Plasma nitrate and nitrite concentrations increased at Day-1 and Day-7 after sodium nitrate but not after placebo ingestion. Systolic and diastolic blood pressure did not change following nitrate ingestion. Furthermore, no differences were observed in gross, net, and delta mechanical efficiency during submaximal exercise, cycling endurance time and cardiac biomarkers between nitrate and placebo on Day-1 and Day-7. Meta-analysis of all available studies in COPD also showed no beneficial effect of beetroot juice on systolic and diastolic blood pressure.

Conclusion: Acute as well as 7-days sodium nitrate supplementation does not modulate mechanical efficiency, blood pressure or cardiac biomarkers in mild-to-moderate COPD patients.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow obstruction resulting from enhanced inflammation in the airways [1]. Besides the respiratory impairment, extrapulmonary manifestations and comorbidities influence disease burden and mortality [1]. Common comorbidities include cardiovascular disease and metabolic syndrome [2, 3]. Cardiometabolic risk is not only increased in obese patients but also in normal weight COPD patients with low muscle mass and abdominal obesity [4, 5]. Furthermore, patients with COPD have lower mechanical efficiency, i.e. the proportion of work accomplished to energy expended, compared to healthy controls [6-8], possibly due to an increased oxygen cost of breathing [6] and impaired muscle mitochondrial metabolism [8-11]. As a lower mechanical efficiency can contribute to impaired exercise performance and hamper efficacy of aerobic exercise training, patients with COPD might benefit from interventions targeting mechanical efficiency.

Nitrate is an interesting nutrient that might improve both mechanical efficiency and cardiovascular health in COPD. Dietary nitrate is reduced to nitrite which is subsequently converted to nitric oxide (NO) [12]. As a result, NO availability will be increased which can have vasodilatory effects that can lower blood pressure and affect body temperature [13]. Indeed, two meta-analyses showed lowered blood pressure in adults with or without comorbidities after inorganic nitrate or beetroot juice (BRJ) supplementation [14, 15]. Besides beneficial effects on blood pressure, increased NO availability can modulate muscle-related processes including muscle contractility, glucose homeostasis, blood flow, mitochondrial respiration and biogenesis [16]. A side effect of an increased blood flow in the extremities is that it may warm the skin and facilitate extra heat loss, which has to be compensated by a higher metabolic rate, or may lead to a reduction in body core temperature. Recently, a meta-analysis demonstrated improved endurance exercise performance following dietary nitrate ingestion in healthy adults [17]. Furthermore, oxygen cost of exercise has been shown to decrease after dietary nitrate intake, without affecting resting metabolic rate [18]. We therefore hypothesized that dietary nitrate might also modulate mechanical efficiency in COPD.

Dietary nitrate is mostly present in green leafy and root vegetables and in most research it is applied in the form of BRJ. Although beneficial physiological effects of BRJ are ascribed to the high nitrate content [19], it could be argued that nitrate is not the active nutrient after all or that nitrate interacts with other compounds in BRJ which cause the beneficial effects. In order to investigate the effects of nitrate alone, without needing to account for unknown interactions with other interventional compounds in the solution, sodium nitrate would be preferred. In healthy adults, a multiple dosing day strategy was more efficacious for improving exercise performance than an acute dose [20]. If this is also the case in patients with COPD it would be interesting to compare the acute effect with the multiple dosing day effects. We hypothesized that sodium nitrate modulates mechanical efficiency, improves

exercise performance and improves cardiac biomarkers. Therefore, the aim of this study was to assess the acute and 7-days effects of sodium nitrate supplementation on mechanical efficiency, exercise performance and cardiac biomarkers in patients with COPD.

METHODS

Study design and subjects

This study was a double-blind, randomized cross-over placebo controlled trial including 20 clinically stable COPD patients with a decreased mechanical efficiency based on screening of the ratio between peak oxygen consumption (VO_2)/maximal work load (Wmax) during incremental cycling test (≥10 mL/min/W)[21]. Patients were recruited via advertisements in local newspapers, between 2015 and 2016. Exclusion criteria were sodium intake limitation, long-term oxygen therapy, severe renal impairment (glomerular filtration rate <30 mL/min), medications that might develop a risk for hypotension in combination with nitrate (i.e. PDE-5 inhibitors and nitrate-containing/releasing medication) and contra-indications for performing (sub-)maximal cycle test. The study was registered at clinicaltrials.gov (NCT02084758) and was approved by the Medical Ethics Committee from Maastricht University Medical Centre + (MUMC+ [NL47701.068.1/MEC 14-3-016]). All patients gave their written informed consent.

Supplementation protocol

Before the start of the study, subjects visited the laboratory for a screening to measure lung function using forced spirometry (Masterlab, Jaeger, Würzburg, Germany) and to perform an incremental cycling ergometry test on an electromagnetic braked cycle ergometer (Ergoselect 200, Ergoline, Blitz, Germany) (see supplemental material for detailed methodology). Eligible subjects were randomly allocated to the treatment order of ingesting a daily dose of sodium nitrate (NaNO3 [BASF, Ludwigshafen, Germany]) and placebo (NaCl [Frisia Zout BV, Harlingen, The Netherlands) dissolved in 140 mL water. Randomisation was performed by an independent researcher from the MUMC+ and both subjects and researchers were blinded for the treatments till the end of the study. The nitrate intervention period consisted of a daily dose of 680 mg NaNO3 (which equals 496 mg or ~8 mmol of nitrate) ingestion for 7 days. The placebo was provided in an equal daily dose of 680 mg NaCl ingestion for 7 days. Both intervention periods were separated by at least 7 days wash-out (Figure 1). The first supplemental bolus was consumed in the laboratory at the first test-day, 2.5 h before the submaximal cycling test (Figure 2). The last bolus was consumed in the laboratory at day 7, also 2.5 h before the submaximal cycling test. Test-days were separated by the subjects consuming a supplemental bolus for 5 consecutive days.

Subjects were requested to abstain from foods naturally high in nitrate and to avoid using antibacterial mouthwash during the intervention periods [22].

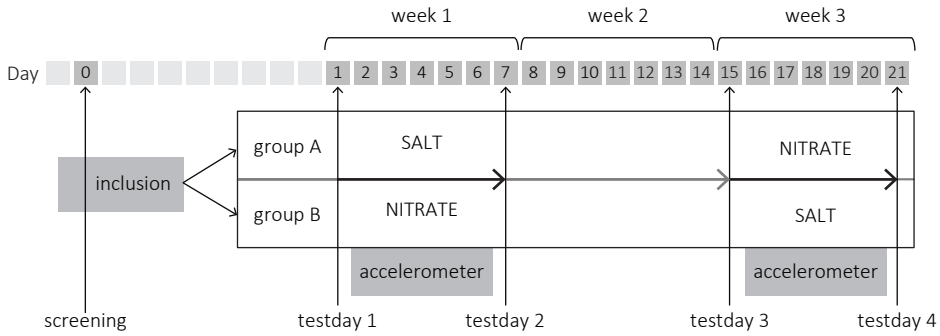

Figure 1. Schematic illustration of the study protocol.

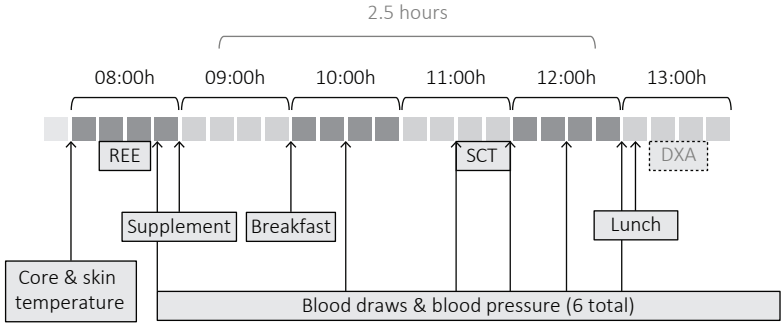

Figure 2. Schematic illustration of the test-day.
Abbreviations: DXA, Dual-energy X-ray absorptiometry; REE, Resting energy expenditure; SCT, submaximal cycling test.

Test-days

On the morning of each test-day, subjects came to the laboratory in a fasted state. Subjects orally ingested a temperature telemetry medical grade capsule (EQ02 SEW, Philips Respironic Massachusetts, USA) to measure the core temperature and afterwards iButton® dataloggers (DS1923, Maxim USA) were attached to 20 skin sites to measure the mean skin temperature. Subsequently, the ventilated hood system (Omnical; Maastricht University, Maastricht, The Netherlands) was used to measure resting energy expenditure (REE), an automated blood pressure monitor was used to measure the blood pressure (Omron Healthcare Inc, Field Court Lake Forest, USA) and an intravenous cannula was inserted into an antecubital vein to obtain a fasted blood sample (T0). After this, subjects received their supplemental beverage (Figure 2) and one hour later they received a standardized liquid breakfast (125 mL Respifor, Nutricia, Zoetermeer, Netherlands). A submaximal cycle ergometry test at 50% Wmax for 10 min, at a fixed pedal rate of 60-70 RPM was performed 2.5 h after ingestion of the beverage. After 10 min, workload was increased to 70% Wmax and subjects were instructed to cycle until (symptom limited) exhaustion with a maximum of 20 min. Gross,

net, and delta mechanical efficiency were calculated according to Ettema *et al*. [23]. The abbreviated Weir formula was used to calculate energy expenditure during exercise [24]. Repeated blood pressure measurements and blood draws were performed 90 (T1) and 150 (T2) min after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 min (T5).

Only on the first test-day Dual Energy X-Ray Absorptiometry (DEXA, Hologic, Discovery A, QDR Series, Bedford, MA, USA) was applied to assess body composition. During the 5 supplementation days, patients wore an accelerometer to verify similar physical activity (PA) levels during both intervention periods.

Plasma analysis procedures

Blood was sampled in Lithium-Heparin S-Monovette® tubes (Sarstedt, Nümbrecht, Germany). Tubes were immediately centrifuged at 1000g for 10 min, at 4°C after which the aliquots were snap-frozen in liquid nitrogen and stored at -80°C for subsequent analysis of plasma nitrate and nitrite using gas-phase chemiluminescence technique as was previously described [25]. Briefly, nitrate and nitrite concentrations were determined based on their reduction to NO. Upon the NO reaction with ozone, nitrogen dioxide is formed and during this production NO is quantified by detecting the light emitted using a thermoelectrically cooled, red-sensitive photomultiplier tube, housed in a gas-phase chemiluminescence NO analyzer (Sievers Instruments, NOATM 280i, Analytix). Furthermore, plasma was used to determine cardiac biomarkers high sensitive troponin T (Hs-TNT), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and creatine kinase (CK) (see supplemental material for details). Furthermore, baseline glucose and lipid profiles, estimated glomerular filtration rate (CKD-EPI) and high-sensitive C-reactive protein (Hs-CRP) were determined.

Statistical analyses

Mechanical efficiency and temperature data were analyzed by two-way repeated measures ANOVA with treatment (nitrate and placebo) and test-day (day 1 and day 7) as within subject factors. Statistical analysis of all plasma and blood pressure data were performed using three-way repeated measures ANOVA with treatment (nitrate and placebo), test-day (day 1 and day 7) and time (T0, T1, T2, T3, T4 and T5) as within subject factors. All data were analyzed using Statistical Package for the Social Sciences (SPSS version 22 for Windows, IBM Corp., Armonk, USA) and data are presented as mean ± standard deviation (SD). A p-value<0.05 was considered statistically significant.

In order to compare results of the current study with previous literature in COPD a meta-analysis was performed as described in the supplemental material. Briefly, Pubmed database was used to find relevant articles on dietary nitrate in COPD. Data on systolic and diastolic blood pressure of both nitrate and placebo group were extracted. If a study did not show

mean ± SD for the suggested outcomes, original authors were contacted for additional information. Subsequently, standardized mean differences (95% confidence intervals (CI)) have been calculated as Hedges' g due to the small sample sizes in the studies. A random effects model was used because of the considerable variability in several experimental factors (e.g. dose, duration and measurement) across studies [26]. The meta-analysis was performed with the Stata software package (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

RESULTS

In total 20 subjects were randomized in the study and 18 eventually finished the full study (Figure 3). Subject characteristics are presented in Table 1. The study population comprised a normal to overweight group (BMI 25.9±3.4 kg/m²) with mild-to-moderate COPD (FEV_1%pred 69.2±16.3). Baseline gross mechanical efficiency during the incremental cycling test was 21.3±3.4%, which was significantly lower than a healthy age matched control group from a previous study of our group (FEV_1: 113.3±14.6 %predicted, gross mechanical efficiency: 24.8±6.1%, *p*=0.049) [10]. Abdominal obesity was present in 83.3% of the patients and four patients had low muscle mass. Plasma markers of lipid and glucose metabolism, kidney function and Hs-CRP were within normal range. Subjects reported that they had consumed all doses of the supplements. Both supplements were well-tolerated and no patients reported any deleterious side effects.

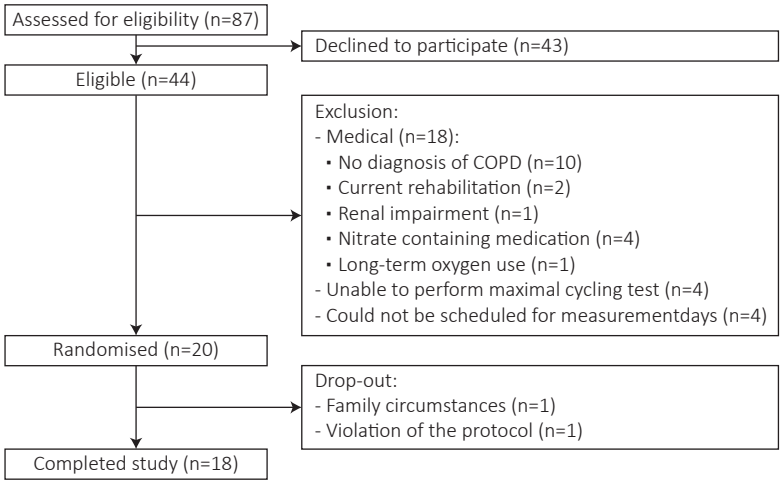

Figure 3. Inclusion flowchart.

Table 1. Subject characteristics (n=18)

	N=18
General characteristics	
Age, y	66.6 ± 7.5
Males, %	72.2
Smoking status	
Current smokers, %	44.4
Former smokers, %	55.6
Lung function	
FEV_1, %pred	69.2 ± 16.3
FVC, %pred	97.8 ± 18.5
FEV_1/FVC, %	54.2 ± 9.8
Body composition	
BMI, kg/m²	25.9 ± 3.4
FFMI, kg/m²	17.3 ± 1.9
SMI, kg/m²	7.3 ± 0.9
Low SMI, %	22.2
Fat percentage, %	30.7 ± 5.6
Abdominal obesity, %	83.3
BMD, g/cm²	1.1 ± 0.1
Exercise performance	
Peak VO_2, ml/min/kg	19.7 ± 4.0
Peak VO_2, %pred[a]	79 (70-87)
Wmax, W	115 ± 34
Wmax, %pred	76.1 ± 18.9
Maximal heart rate, beats/min	136 ± 17
Cardiometabolic risk profile	
Cholesterol, mmol/L	4.87 ± 0.92
HDL cholesterol, mmol/L[a]	1.2 (1.1-1.5)
LDL cholesterol, mmol/L	2.75 ± 0.88
Triglycerides, mmol/L[a]	1.2 (0.9-1.7)
Glucose,mmol/L[a]	5.96 (5.55-6.72)
Insulin, mU/L	5.7 ± 3.2
HOMA-IR	1.7 ± 1.0
Kreatinine, µmol/L	81.8 ± 19.9
CKD-epi, ml/min/1.73m²	80.0 ± 14.2
Hs-CRP, mg/L[a]	2.6 (0.9-6.0)

Data are shown as mean ± SD unless indicated otherwise. [a]Median (IQR). Abbreviations: BMD, bone mineral density; CKD-epi, Chronic Kidney Disease Epidemiology Collaboration; FEV_1, forced expiratory volume in 1 second; FFMI, fat free mass index; FVC, forced vital capacity; Hs-CRP, high-sensitive C-reactive protein; SMI, skeletal muscle mass index; VO_2, oxygen consumption; Wmax, maximal work load.

Plasma nitrate and nitrite

Baseline (T=0) plasma concentration of nitrate was ~2-fold increased at Nitrate day 7 (133±106 µM) compared to Nitrate day 1 (58±36 µM, p=0.006), while no differences were observed in plasma nitrite (Figure 4). Following ingestion of nitrate, plasma nitrate increased to the same extent at Nitrate day 1 (T0 vs T1; 331±54 µM, p<0.001) and day 7 (T0 vs T1; 339±57 µM, p<0.001) and remained elevated throughout the test-day. Baseline plasma nitrite was ~2-fold increased after the nitrate ingestion at Nitrate day 1 (T0 to T1; 256±132 to 634±345 nM; p<0.001) as well as Nitrate day 7 (T0 vs T1; 245±165 to 501±358 nM; p=0.003) and also remained elevated throughout the test-day. Plasma nitrate and nitrite levels were not different at Placebo day 1 and 7.

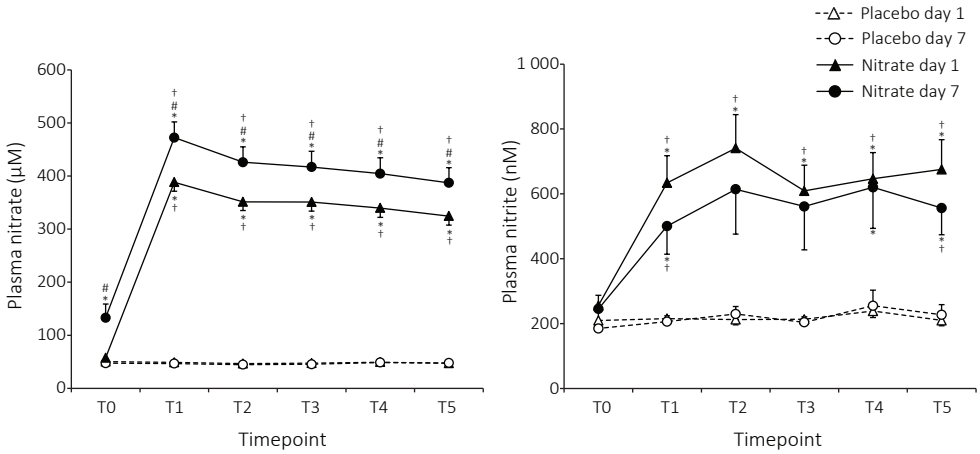

Figure 4. Plasma nitrate and nitrite concentrations on day 1 and day 7 for the placebo and nitrate intervention at different timepoints.

Blood draws were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 minutes (T5). Values are mean ± SEM; n=17. *Significantly different from placebo (p<0.05). #Significantly different from nitrate day 7 (p<0.05). †Significantly different from T0 (p<0.05).

Submaximal cycling, resting energy expenditure and body temperatures

During cycling at 50% and 70% Wmax, baseline gross mechanical efficiency (placebo day 1) was 15.9±2.8% and 17.6±2.8% and baseline net mechanical efficiency was 20.4±3.0% and 21.4±2.9%, respectively. Following nitrate ingestion, both gross and net mechanical efficiency were not different at nitrate day 1 and day 7, both at 50% and 70% Wmax, compared to placebo (Table 2). Delta mechanical efficiency was also not different between the nitrate and placebo treatment. Furthermore, no differences in VO_2, VCO_2, respiratory exchange rate and energy expenditure in rest as well as during cycling at 50% and 70% Wmax were observed between nitrate and placebo, both at day 1 and day 7. Furthermore, the cycling endurance time was not different between the interventions. Skin and core temperature during the REE measurement and the submaximal cycling test were not different between nitrate and placebo at both day 1 and day 7.

Table 2. Steady-state values of energy expenditure during rest and submaximal cycling after nitrate and placebo ingestion.

	Nitrate		Placebo		
	Day 1	**Day 7**	**Day 1**	**Day 7**	***p*-value**
Resting energy expenditure					
VO_2, mL/min	231 ± 31	233 ± 31	234 ± 30	237 ± 34	0.825
VCO_2, mL/min	196 ± 28	194 ± 23	193 ± 21	198 ± 26	0.062
VO_2, mL/min/kg	3.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	0.823
VCO_2, mL/min/kg	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	2.6 ± 0.3	0.083
RER	0.85 ± 0.05	0.84 ± 0.05	0.83 ± 0.04	0.84 ± 0.05	0.195
Energy expenditure, kcal/min	1.12 ± 0.15	1.12 ± 0.14	1.13 ± 0.14	1.14 ± 0.16	0.538
Core temperature, °C[†]	36.8 ± 0.2	36.8 ± 0.3	36.9 ± 0.3	36.7 ± 0.6	0.313
Skin temperature, °C	32.9 ± 0.5	33.0 ± 0.5	33.0 ± 0.4	33.0 ± 0.5	0.827
Submaximal cycling at 50% Wmax					
VO_2, mL/min	1034 ± 182	1032 ± 191	1039 ± 182	1033 ± 191	0.806
VCO_2, mL/min	945 ± 184	950 ± 195	949 ± 175	953 ± 180	0.951
VO_2, mL/min/kg	13.3 ± 2.4	13.3 ± 2.5	13.4 ± 2.3	13.3 ± 2.2	0.622
VCO_2, mL/min/kg	12.2 ± 2.4	12.2 ± 2.6	12.2 ± 2.3	12.2 ± 2.1	0.761
RER	0.91 ± 0.04	0.92 ± 0.06	0.91 ± 0.04	0.92 ± 0.04	0.744
Energy expenditure, kcal/min	5.07 ± 0.91	5.07 ± 0.96	5.10 ± 0.90	5.08 ± 0.94	0.840
Gross ME, %	16.0 ± 2.8	16.0 ± 2.7	15.9 ± 2.8	16.0 ± 3.1	0.626
Net ME, %	20.6 ± 3.2	20.6 ± 2.8	20.4 ± 3.0	20.7 ± 3.4	0.647
Core temperature, °C	37.0 ± 0.3	36.9 ± 0.4	37.1 ± 0.4	36.7 ± 0.7	0.089
Skin temperature, °C	31.8 ± 0.4	31.9 ± 0.6	31.9 ± 0.6	32.0 ± 0.6	0.651
Submaximal cycling at 70% Wmax					
VO_2, mL/min	1302 ± 274	1315 ± 291	1319 ± 273	1323 ± 275	0.633
VCO_2, mL/min	1246 ± 298	1260 ± 307	1239 ± 278	1269 ± 276	0.506
VO_2, mL/min/kg	16.7 ± 3.7	16.9 ± 3.9	16.9 ± 3.6	16.9 ± 3.9	0.558
VCO_2, mL/min/kg	16.0 ± 3.9	16.1 ± 4.0	15.9 ± 3.7	16.2 ± 3.5	0.583
RER	0.95 ± 0.05	0.95 ± 0.05	0.93 ± 0.06	0.96 ± 0.04	0.195
Energy expenditure, kcal/min	6.45 ± 1.39	6.51 ± 1.47	6.51 ± 1.37	6.56 ± 1.37	0.868
Gross ME, %	17.8 ± 2.7	17.6 ± 2.6	17.6 ± 2.8	17.5 ± 3.0	0.839
Net ME, %	21.7 ± 2.8	21.5 ± 2.5	21.4 ± 2.9	21.3 ± 3.1	0.757
Delta ME, %	26.9 ± 4.8	24.7 ± 3.6	26.6 ± 6.8	24.3 ± 3.9	0.997
Cycling time, min	1186 ± 402	1316 ± 440	1331 ± 426	1300 ± 387	0.077
Core temperature, °C	37.2 ± 0.3	37.2 ± 0.4	37.4 ± 0.4	37.0 ± 0.9	0.066
Skin temperature, °C	32.0 ± 0.5	32.0 ± 0.6	32.0 ± 0.4	32.1 ± 0.6	0.763

Data are shown as mean ± SD. Abbreviations: ME, mechanical efficiency; VCO_2, carbon dioxide production; VO_2, oxygen consumption; RER, respiratory exchange rate.

Blood pressure

Baseline systolic blood pressure was 140±14 mmHg and baseline diastolic blood pressure was 81±10 mmHg. Following acute and 7-days nitrate ingestion blood pressure as well as heart rate did not change compared to placebo (Table 3). At both Nitrate and Placebo day 1 and day 7, systolic blood pressure decreased significantly (T0 vs T4; -13±12 mmHg, p<0.001) during the resting period after the submaximal cycling test compared to baseline while diastolic blood pressure remained unchanged. Furthermore, heart rate was significantly increased (T0 vs T3; 41±15 beats/min, p<0.001) directly after the submaximal cycling test and remained elevated during the resting period after cycling (T0 vs T4; 13±9 and T0 vs T5; 8±7 beats/min, respectively, both p<0.001) at both day 1 and 7 of nitrate and placebo ingestion.

Table 3. Blood pressure and heart rate after nitrate or placebo ingestion (n=18).

	Nitrate		Placebo		
	Day 1	**Day 7**	**Day 1**	**Day 7**	***p*-value**
Systolic blood pressure, mmHg					
T0	137 ± 15	135 ± 18	137 ± 15	137 ± 21	0.613
T1	132 ± 17	130 ± 18	134 ± 15	138 ± 21	0.138
T2	135 ± 19	135 ± 18	135 ± 18	138 ± 20	0.655
T3	152 ± 33	148 ± 22	145 ± 31	151 ± 26	0.165
T4	123 ± 19*	122 ± 16*	124 ± 17*	123 ± 16*	0.820
T5	125 ± 17*	125 ± 15*	126 ± 13*	130 ± 15	0.278
Diastolic blood pressure, mmHg					
T0	79 ± 9	78 ± 11	79 ± 13	79 ± 11	0.639
T1	74 ± 10*	74 ± 11	75 ± 10	77 ± 12	0.368
T2	78 ± 8	78 ± 9	80 ± 10	78 ± 10	0.348
T3	81 ± 13	80 ± 14	77 ± 11	78 ± 11	0.635
T4	77 ± 13	73 ± 9	78 ± 11	76 ± 10	0.535
T5	76 ± 11	76 ± 12	77 ± 10	81 ± 10	0.188
Heart rate, beats/min					
T0	64 ± 8	64 ± 8	64 ± 8	64 ± 7	0.862
T1	69 ± 11	68 ± 11	70 ± 9*	67 ± 9	0.236
T2	68 ± 9*	68 ± 10	67 ± 9	67 ± 10	0.762
T3	106 ± 12*	106 ± 17*	106 ± 13*	104 ± 18*	0.676
T4	77 ± 10*	76 ± 10*	78 ± 13*	77 ± 12*	0.980
T5	72 ± 10*	71 ± 9*	72 ± 13*	72 ± 12*	0.771

Data are shown as mean ± SD. Measurements were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 minutes (T5). *Significantly different from T0 (p<0.05).

Cardiac biomarkers

No differences were observed in cardiac markers Hs-TNT, NT-proBNP and CK following acute and 7-days nitrate ingestion compared to placebo (Figure 5). All cardiac markers were increased after exercise (T2 vs T3; Hs-TNT 0.4±0.1 ng/mL, p=0.011; NT-proBNP 1.7±0.5 pmol/L, p=0.003; CK 8.1±1.7, p<0.001). Furthermore, Hs-TNT was higher at baseline (T0) compared to other time points at the test-day.

Physical activity levels

Total physical activity level as well as physical activity pattern was not different during nitrate and placebo ingestion (Table 4).

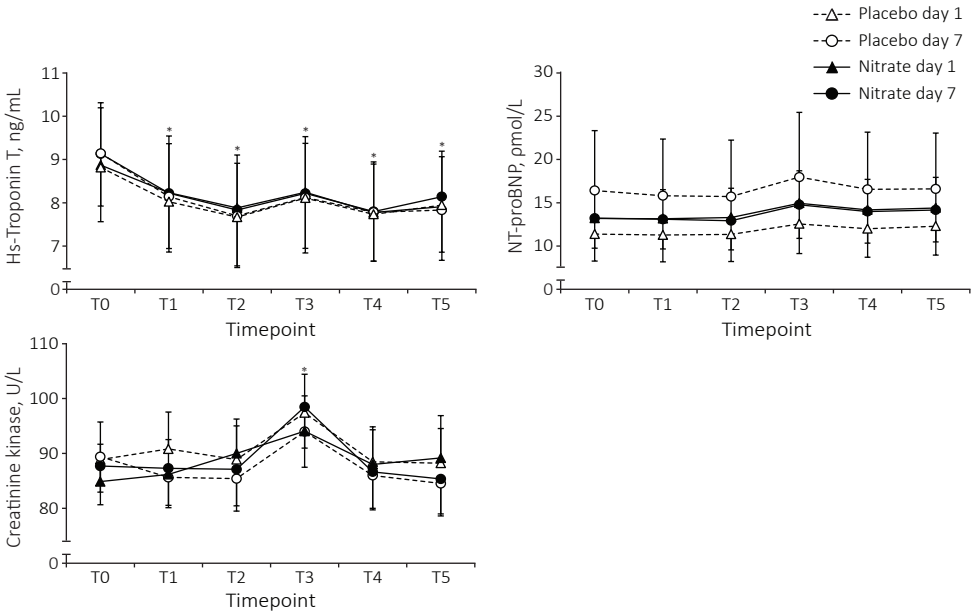

Figure 5. Plasma high sensitive troponin T, NT-proBNP and creatinine kinase on day 1 and day 7 for the placebo and nitrate intervention at different timepoints.

Blood draws were performed at baseline (T0), 90 (T1) and 150 (T2) minutes after the beverage ingestion, immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 minutes (T5). Values are mean ± SEM; n=17. No significant differences were observed between nitrate versus placebo and day 1 versus day 7. *Significantly different from T0 (p<0.05). Abbreviation: NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 4. Physical activity levels during nitrate and placebo intervention (n=17).

	Placebo	Nitrate	*p*-value
Total activity, counts/min	204 ± 106	214 ± 104	0.528
Time spent in sedentary PA, % of wear time	68.5 ± 12.6	68.7 ± 9.6	0.866
Time spent in lifestyle PA, % of wear time	23.8 ± 8.1	23.2 ± 5.4	0.573
Time spent in MVPA, % of wear time	7.8 ± 5.4	8.1 ± 5.0	0.605

Data are shown as mean ± SD. Abbreviations: MVPA, moderate to vigorous physical activity; PA, physical activity.

Meta-analysis

Main characteristics of the studies are summarized in Supplemental Table 1. In total 94 patients participated in the included studies. The mean age range was 65-70 years and patients were normal to overweight (mean BMI: 25-29 kg/m²) with mild-to-moderate COPD (mean FEV_1: 43-62 %predicted). All studies used beetroot juice as a nitrate-rich supplement, with a nitrate-dose ranging between 6.77-12.0 mmol/day. Following the meta-analysis the standardized mean difference was-0.03 (95% CI-0.32 to 0.26) for systolic blood pressure and -0.24 (95% CI-0.55 to 0.08) for diastolic blood pressure, showing a small but non-significant effect in favour of dietary nitrate (Figure 6).

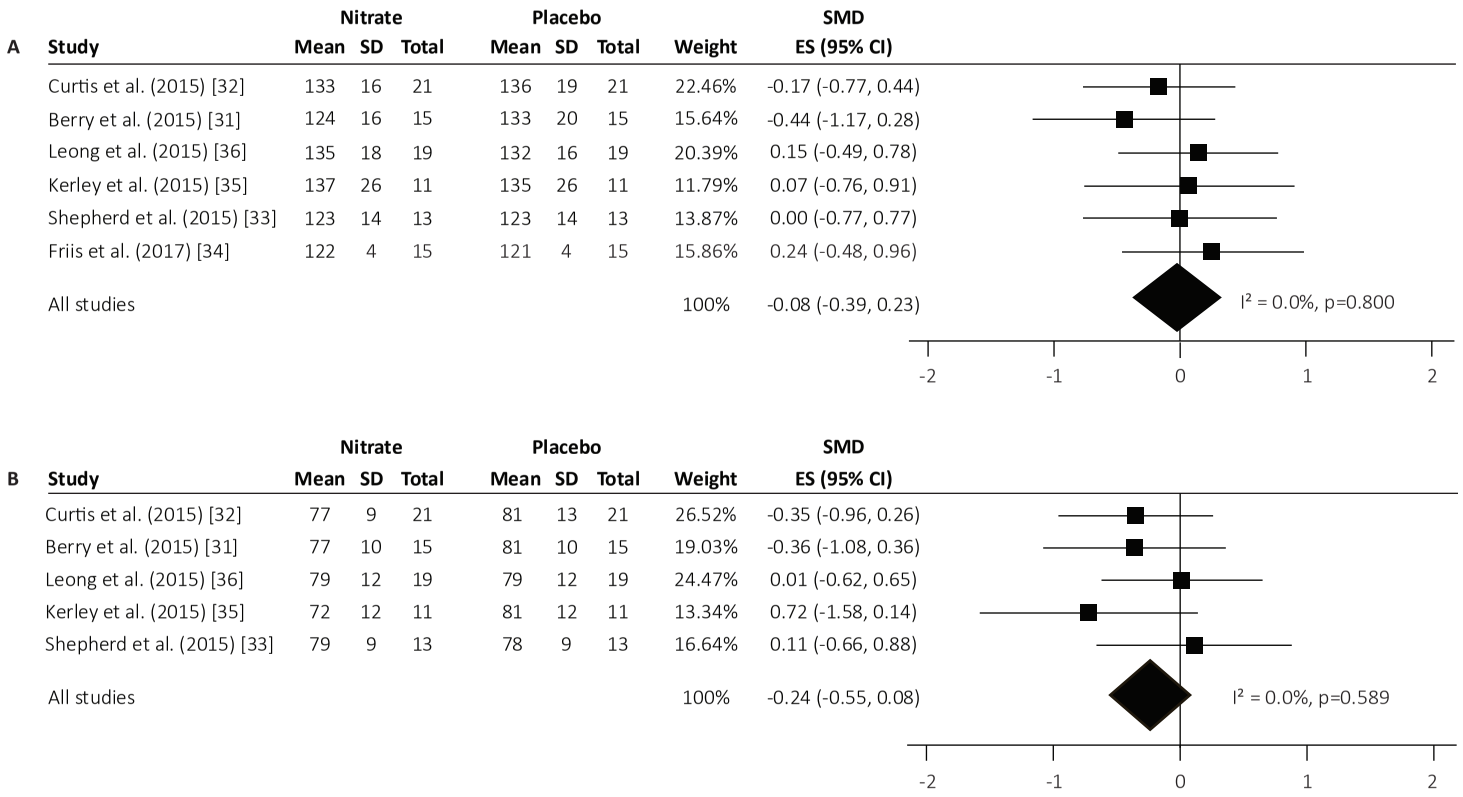

Figure 6. Effect size forest plots for the effect of dietary nitrate supplementation on A) systolic blood pressure (mmHg) and B) diastolic blood pressure (mmHg) in patients with chronic obstructive pulmonary disease.

DISCUSSION

To our knowledge this is the first study investigating the acute as well as the 7-days effects of sodium nitrate supplementation on mechanical efficiency and cardiac biomarkers in mild-to-moderate COPD patients. Acute as well as 7-days nitrate supplementation did not alter mechanical efficiency, despite clear and anticipated elevated plasma nitrate and nitrite levels. Furthermore, exercise performance, blood pressure, cardiac biomarkers Hs-TNT, NT-proBNP and CK, skin and core temperatures and finally REE were not affected by sodium nitrate. These findings were in contrast to our hypothesis.

We expected improved mechanical efficiency after sodium nitrate ingestion as nitrate is suggested to decrease VO_2 during exercise in healthy subjects, without alterations in REE [18]. However, in the current study both mechanical efficiency and VO_2 during submaximal exercise were not affected after sodium nitrate ingestion. Since most studies showing beneficial effects of dietary nitrate on oxygen requirements during exercise are performed in healthy young individuals it is possible that VO_2 kinetics cannot be altered at an older age or in a clinically compromised older population. Two studies have been performed in healthy older subjects with conflicting results [27, 28]. After nitrate rich BRJ, one study showed a reduced VO_2 mean response time in the transition from standing rest to treadmill walking which might indicate a reduced reliance on nonoxidative metabolic processes across the transition from a lower to a higher metabolic rate [27]. However, the oxygen cost of exercise remained unchanged, corresponding to another study in which no changes in resting, submaximal and maximal VO_2 during incremental cycling were found [28]. Furthermore, in older patients with heart failure [29], type 2 diabetes mellitus [30] and COPD [31-34] most studies could not find improved VO_2 kinetics after BRJ ingestion. Only one study showed slightly decreased isotime VO_2 during submaximal cycling following acute BRJ ingestion [32]. Since patients with COPD spend probably more time walking instead of cycling it could be questioned whether the cycling test was the most optimal test to find beneficial effects of nitrate on mechanical efficiency in COPD. However, a previous study showed good reproducibility of the submaximal cycling test to measure mechanical efficiency in patients with COPD [6]. Furthermore, previous studies in COPD investigating the effect of dietary nitrate on walking performance showed no beneficial effect of dietary nitrate [33-36]. Overall, previous studies in COPD show no convincing improvements on VO_2 kinetics after nitrate ingestion supporting the results of the current study (Supplemental Table 1).

Nitrate-rich supplements have increased in popularity among elite and recreational athletes as they have been shown to improve exercise capacity [17]. For this reason we hypothesized that nitrate could be a useful intervention in COPD as adjunct to pulmonary rehabilitation (PR) to increase the effects of exercise training. However, the current study showed no improvement in cycling endurance time. This corresponds to previous studies in COPD (Supplemental Table 1), in which almost no effects of BRJ were found on exercise performance. Only two studies

showed significant improvements in submaximal cycling time and walking distance after acute ingestion of BRJ [31, 35]. However, these studies used prune juice and blackcurrant cordial as placebo, which is not a robust placebo for BRJ. The effect of nitrate on exercise performance has been shown to be enhanced supplemented in combination with exposure to ultraviolet A radiation (UVA) and might be influenced by seasonal differences [37]. Despite this study showed no effect of nitrate on exercise performance in absence of UVA radiation there seemed to be a trend towards reduced oxygen consumption during exercise after nitrate ingestion without exposure to UVA radiation. Therefore, we believe differences in UVA radiation do not affect the results of the current study.

In the current study we recruited patients via advertisements, which resulted in inclusion of normal-weight mild-to-moderate COPD patients with moderate exercise impairment. Although patients were not referred to PR, all patients had a sedentary lifestyle corresponding to the PA levels of patients referred to outpatient PR [4]. Besides, patients had moderate decreased mechanical efficiency, which was significantly lower compared to healthy controls from a previous study by our group [10]. It has recently been shown that gross efficiency is declined in COPD with increasing disease severity [38], which might suggest that more severe diseased COPD patients might still benefit from dietary nitrate. However, in the current study the response of sodium nitrate intake on mechanical efficiency was not correlated with baseline mechanical efficiency (data not shown), suggesting the results would be similar in case more severe patients would be included. Therefore, we believe dietary nitrate is not the promising adjunct to PR to elevate the training effects.

It has been established that in healthy and mostly young subjects dietary nitrate supplementation can lower blood pressure [14, 15]. In the current study, nitrate supplementation caused no changes in blood pressure and the meta-analysis also showed no beneficial effects of BRJ on blood pressure in the studies in COPD so far (Figure 6). Results may be influenced by current use of antihypertensive medication. Two studies did not find significant reductions in blood pressure in older hypertensive subjects that were on antihypertensive medication [39, 40], suggesting that an additional reduction in blood pressure might not occur in a group of patients whose blood pressure is already well-controlled. In the current study 7 patients were on antihypertensive medication. However, excluding these patients from analyses did not influence the results (data not shown). Another possible explanation for the lack of changes in blood pressure might be the vascular ageing process in which the capacity to convert nitrate to NO is possibly reduced and the sensitivity of vascular smooth muscle cells to the vasodilatory effects of NO might be diminished [41]. This might also be the case in COPD and it could be speculated that higher doses of nitrate may be required to detect beneficial effects on blood pressure. Directly after the cycling test an increase in blood pressure was observed, while after this phase (T4 and T5) the systolic blood pressure was significantly lower compared to baseline (T0) which was consistent between both experimental groups at both days. This drop in blood pressure is the post-exercise hypotension phenomenon which has previously been

reported [42-44]. During the recovery phase after exercise several mechanisms contribute to a lower blood pressure including the mediated decreases in sympathetic nerve activity, a decreased signal transduction from sympathetic nerve activation into vasoconstriction as well as local vasodilator mechanisms [45].

In the current study a dose of ~8 mmol nitrate per day was applied. This dose was based on a dose-response study that described a dose of 8.4 mmol/L was needed to significantly change oxygen parameters in recreationally active men [46]. Previous studies have used a wide range in both the amount (i.e. dose and duration) and source of nitrate supplemented and found contradictory results [14, 15, 17, 18], even in COPD [31-36]. In the current study we used sodium nitrate as nitrate-rich supplement, since we were interested in the effects of nitrate without needing to account for unknown interactions with other interventional compounds in the solution. Furthermore, sodium nitrate also reduced the oxygen costs of exercise and lowered the blood pressure in healthy adults [47-49]. Nevertheless, the current study shows that sodium nitrate does not affect mechanical efficiency and blood pressure in COPD. All previous studies in COPD used BRJ as a source of nitrate. Although beneficial effects on physiological responses of BRJ are ascribed to the high nitrate content [19], it is still possible that nitrate is not the active nutrient after all or that nitrate interacts with other compounds (e.g. vitamin C, potassium and polyphenols) in BRJ, that cause the beneficial effects. Indeed recent studies showed greater blood pressure lowering and oxygen consumption lowering effects of BRJ, rocket salad beverage or spinach beverage compared to sodium nitrate in healthy adults [50, 51]. However, the meta-analysis performed in the current study shows no beneficial effect of BRJ on blood pressure in patients with COPD. Therefore, the results of the current study suggest no beneficial effect of dietary nitrate on blood pressure in patients with COPD and question the efficacy on oxygen consumption and exercise performance.

In the current study the effect of sodium nitrate intake on cardiac biomarkers Hs-TNT, NT-proBNP and CK was investigated. These biomarkers are known for the diagnosis of myocardial injury and heart failure, are associated with increased cardiovascular and all-cause mortality risk and are known to increase following exercise [52, 53]. In the current study we indeed show elevated levels of the cardiac biomarkers after the cycling test, however, the changes were unaffected by sodium nitrate ingestion. Note that the observed higher Hs-TNT levels at the first blood sampling (early in the morning) can be ascribed to the diurnal rhythm of Hs-TNT [54]. This study is also one of the first studies investigating the effect of nitrate on skin and core temperature. Only the effect of BRJ on oxygen cost of desert marching was previously investigated and reported an elevated rise in core temperature after nitrate ingestion without a change in skin temperature [55]. Based on the expected vasodilatory effect of NO we expected that core and skin temperatures would have been affected. However, no changes in both body temperatures were observed. More studies are needed to investigate the acute as well as the longer term effects of dietary nitrate on cardiac markers and body temperatures.

CONCLUSION

In conclusion, both acute and 7-days sodium nitrate supplementation does not increase mechanical efficiency, lower blood pressure and modulate cardiac markers of mild-to-moderate patients with COPD. Dietary nitrate does not seem to be a promising adjunct to PR to enhance the effects of exercise training.

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SUPPLEMENTAL MATERIAL

Screening

During the screening, lung function was measured using forced spirometry (Masterlab, Jaeger, Würzburg, Germany) to determine forced vital capacity and forced expiratory volume in 1 second. Smoking status was based on self-report and was categorized as smokers, former smokers and never smokers. Furthermore, subjects performed a maximal incremental cycling test on an electrically braked cycle ergometer (Ergoselect 200, Ergoline, Blitz, Germany). Expired gases were investigated using breath by breath analysis through a breathing mask (Oxycon Pro, Jaeger, Würzburg, Germany). Heart rate, blood pressure and percutaneous oxygen saturation were monitored. After one minute of unloaded cycling, power was increased based on the predicted Wmax [1], in order to achieve a cycling duration of 8-12 minutes. Peak VO_2 and Wmax were used to determine the mechanical efficiency at baseline.

Test-days

On the morning of each test-day, subjects reported to the laboratory after an overnight fast. After subjects orally ingested a temperature telemetry medical grade capsule (EQ02 SEW, Philips Respironic Massachusetts, USA) to measure the core temperature, iButton® dataloggers (DS1923, Maxim USA) were attached at 20 skin sites to measure the mean skin temperature. Furthermore, 4 iButtons were placed next to the subject at different heights to measure the air temperature. After this, resting energy expenditure (REE) was measured for 30 minutes in a supine position by indirect calorimetry using an open-circuit ventilated hood system (Omnical; Maastricht University, Maastricht, The Netherlands). The abbreviated Weir equation was used to calculate REE of the last 20 minutes [2]. The precision of the ventilated-hood system was weekly checked with a methanol combustion test. Following the REE measurement, blood pressure was measured using an automated blood pressure monitor (Omron Healthcare Inc, Field Court Lake Forest, USA) and an intravenous cannula was inserted into an antecubital vein to obtain a fasted blood sample (T0). Subsequently, subjects received their supplemental beverage. Repeated blood pressure measurements and blood draws were performed 90 (T1) and 150 (T2) minutes after the beverage ingestion (start cycle ergometry test), immediately after the cycle test (T3), and subsequently after 30 (T4) and 60 minutes (T5). After the 2.5 hour rest period subjects performed a submaximal cycle ergometry test at 50% Wmax for 10 minutes at a fixed pedal rate of 60-70 RPM. After 10 minutes, workload was increased to 70% Wmax and subjects were instructed to cycle until (symptom limited) exhaustion with a maximum of 20 minutes. Gross and net mechanical efficiency were calculated using formulas 1.1 and 1.2:

1.1: Gross mechanical efficiency = (Load (W) of exercise·0.01433(kcal·min-1) / (energy expenditure during exercise)(kcal·min-1))·100%

1.2: Net mechanical efficiency = (Load (W) of exercise*0.01433(kcal/min) / (energy expenditure during exercise – REE)(kcal/min))*100%

Both gross and net mechanical efficiency were calculated during the maximal incremental cycling test at the screening and during cycling at 50% and 70% of Wmax. The difference in mechanical efficiency between cycling at 50% and 70% of Wmax was calculated as the delta mechanical efficiency (formula 1.3):

1.3: Delta mechanical efficiency = ((Load (W) of 70% Wmax exercise) – (Load (W) of 50% Wmax exercise)*0.01433(kcal/min)) / (energy expenditure during 70% Wmax exercise) – (energy expenditure during 50% Wmax exercise)(kcal/min))*100%

The abbreviated Weir formula was used to calculate energy expenditure during exercise from the steady state values of VO_2 and VCO_2 [2]. Only on the first test-day Dual Energy X-Ray Absorptiometry (DEXA, Hologic, Discovery A, QDR Series, Bedford, MA, USA) was applied to assess whole body composition. The ratio of the percentage fat mass (FM) in the android region (waist) to the percentage FM in the gynoid region (hip) was used as a measure for abdominal FM. Abdominal obesity was defined by android/gynoid %FM >1.0 for men and >0.8 for women, as previously reported [3-5]. Fat free mass index was calculated as fat free mass/height² and skeletal muscle mass index as lean appendicular mass/height². The prevalence of low skeletal muscle mass was explored by applying the criteria for sarcopenia (SMI<7.23 kg/m² for men; <5.67 kg/m² for women) [6].

During both intervention periods, physical activity was measured using a trial-axial GT3X accelerometer (ActiGraphTM, LLC, Fort Walton Beach, FL, USA). Subjects were instructed to wear an accelerometer during the time they were not asleep, except when showering or bathing. Data of the accelerometer were analyzed as previously reported [7].

Plasma analysis procedures

Blood samples were collected in S-Monovette® Lithium-Heparin containing tubes (Sarstedt, Nümbrecht, Germany) and immediately centrifuged at 1000 g for 10 min, at 4°C after which the aliquots were frozen in liquid nitrogen and stored at -80°C for subsequent analysis of plasma nitrate and nitrite using chemiluminescence, as described previously [8]. Cardiac markers high sensitive troponin T (Hs-TNT) N-terminal pro-B-type natriuretic peptide (NT-proBNP) and creatine kinase (CK) were measured on the Cobas 8000 analyzer (Roch Diagnostics, Mannheim, Germany) with lower limits of detection (LLOD) of 5 ng/L, 0.6 pmol/L and 7 U/L, respectively. In addition, baseline high-sensitive C-reactive protein (Hs-CRP) was determined with the CardioPhase® hs-CRP kit (Siemens Healthcare Diagnostics Inc., Neward, NI) (LLOD 0.18 mg/L). Baseline glucose was measured on the Cobas 8000 analyzer (Roche diagnostics, LLOD 0.11 mmol/L) and insulin was measured by IMMULITE® 2000 immunoassay system (Siemens Healthineers, Erlangen, Germany). Homeostatic model assessment (HOMA) was

applied to estimate insulin resistance (IR) by HOMA-IR=(fasting insulin * fasting glucose)/22.5 [9]. Baseline lipid metabolism, i.e. HDL, LDL and total cholesterol and triglycerides as well as creatinine, were also measured using Roche Diagnostics assays on the Cobas 6000 analyzer. Creatinine was measured to calculate the estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [10].

Meta-analysis

Pubmed database (through July 2017) was used to find relevant articles. The following search terms were used: nitrates (All Fields) OR nitrate (All Fields) or beetroot juice (All Fields) AND COPD (All Fields) OR chronic obstructive pulmonary disease (All Fields). In addition, reference lists of retrieved articles were scanned for additional publications. Only randomized, placebo-controlled studies including patients with COPD were included in the meta-analysis. In total this search yielded 113 hits of which 6 studies were included in the meta-analysis (Table 1 supplemental material).

Data (mean ± standard deviation (SD)) on systolic and diastolic blood pressure of the nitrate and the placebo group were extracted systematically. If a study did not show mean ± SD for the suggested outcomes, original authors were contacted for additional information. Subsequently, the standardized mean differences (95% confidence intervals (CI)) were calculated as Hedges' g because of the relative small sample sizes in the studies. A random effects model was applied for the meta-analysis because of the considerable variability in several experimental factors (e.g. dose, duration and measurement) across studies [11]. The meta-analysis was performed with the Stata software package (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Supplemental Table 1. Randomized placebo-controlled crossover studies investigating the effect of dietary nitrate in patients with COPD.

Ref	Study population						Nitrate		Placebo	Duration			Main outcomes				
	N	Age (y)	BMI (kg/m²)	FEV_1 %pred	SBP (mmHg)	DBP (mmHg)	Form	Dose nitrate (mmol)	Form	Acute	Multiple days	Wash-out	↓ SBP	↓ DBP	Improved exercise performance	Improved resting VO_2	Improved isotime VO_2	Improved end-exercise VO_2
Curtis [12]	21	68±5	25±6	50±22	137±19	79±7	BRJ	12.9	ND-BRJ	Y	-	7 d	N	Y	N	N	Y	-
Leong [13]	19	67±8	29±7	62±7	131±17	80±11	BRJ	4.8 (twice daily)	ND-BRJ	-	3 d	4 d	N	N	N	-	-	-
Berry [14]	15	70±9	29±6	62±17	124 (115-133)	77 (72-83)	BRJ	3.79 (twice)	Prune juice	Y	-	7 d	Y	Y	Y	N	N	N
Kerley [15]	11	69±7	27±6	43±19	131±17	73±17	BRJ + blackcurrant cordial	12.9	Water + blackcurrant cordial	Y	-	7 d	Y	Y	Y	-	-	-
Shepherd [16]	13	65±8	29±8	57±9	132±15	85±10	BRJ	6.77 (twice daily)	ND-BRJ	-	2 d	7 d	N	N	N	N	-	N
Friss [17]	15	63±13	26±5	45±15	129±4	-	BRJ	4.8 (twice daily)	ND-BRJ	-	7 d	7 d	N	N	N	N	-	-

Abbreviations: %pred; Percentage predicted; BMI; Body mass index; D, days; DBP, Diastolic blood pressure; FEV1, Forced expiratory volume in 1 second; N, no; ND-BRJ, Nitrate-depleted beetroot juice; SBP, systolic blood pressure; VO_2, oxygen consumption; Y, yes.

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Chapter 7

Resveratrol for Patients with Chronic Obstructive Pulmonary Disease: hype or hope?

Rosanne J.H.C.G. Beijers
Harry R. Gosker
Annemie M.W.J. Schols

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ABSTRACT

Purpose of review: Chronic obstructive pulmonary disease (COPD) is a progressive lung disease with a high prevalence of extrapulmonary manifestations and, frequently, cardiovascular comorbidity. Resveratrol is a food derived compound with anti-inflammatory, antioxidant, metabolic and cardioprotective potential. Therefore, resveratrol might improve the pulmonary as well as extrapulmonary pathology in COPD. In this review we will evaluate knowledge on the effects of resveratrol on lung injury, muscle metabolism and cardiovascular risk profile and discuss if resveratrol is a hype or hope for patients with COPD.

Recent findings: Experimental models of COPD consistently show decreased inflammation and oxidative stress in the lungs after resveratrol treatment. These beneficial anti-inflammatory and antioxidant properties of resveratrol can indirectly also improve both skeletal and respiratory muscle impairment in COPD. Recent clinical studies in non-COPD populations show improved mitochondrial oxidative metabolism after resveratrol treatment, which could be beneficial for both lung and muscle impairment in COPD. Moreover, preclinical studies suggest cardioprotective effects of resveratrol but results of clinical studies are inconclusive.

Summary: Resveratrol might be an interesting therapeutic candidate to counteract lung and muscle impairments characteristic to COPD. However, there is no convincing evidence that resveratrol will significantly decrease the cardiovascular risk in patients with COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow obstruction and lung inflammation primarily caused by inhalation of cigarette smoke [1]. The pulmonary inflammation involves macrophages, epithelial cells, dendritic cells, neutrophils, eosinophils and T- and B-lymphocytes which release many inflammatory mediators that contribute to the pathophysiology of COPD [2]. Furthermore, anti-oxidant capacity is reduced in COPD whereas oxidant release from increased activated inflammatory cells is increased [2]. The resulting oxidative stress is even further increased during exacerbations and may be associated with increased inflammation, airway remodeling and corticosteroid resistance [3].

Next to the respiratory impairment, extrapulmonary manifestations and comorbidities contribute to disease burden and mortality [1]. Cardiovascular disease is a common comorbidity in patients with COPD and is even the leading cause of mortality in patients with mild-to-moderate COPD [4]. Furthermore, skeletal muscle wasting is highly prevalent in COPD, in particular in patients with emphysema [5], and is associated with intrinsic muscular abnormalities [6]. A shift from less oxidative type-I towards more glycolytic type-II muscle fibers has been consistently reported in lower limb muscles of patients with COPD [7] and markedly decreased phosphorylated adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) protein levels were observed in muscle biopsies of COPD patients [8-10]. In addition, these patients rely more on the metabolically less-efficient complex-II driven mitochondrial respiration [11]. Moreover, increased reactive oxygen species production has been observed in skeletal muscle mitochondria of patients with COPD compared with controls [12]. The respiratory muscles are also affected in patients with COPD, mainly due to lung hyperinflation and increased airway resistance and obstruction but also by systemic factors such as inflammation and oxidative stress [13]. In contrast to lower limb muscle, the diaphragm has an increased proportion of type-I fibers and increased mitochondrial and capillary content [13]. Boosting muscle oxidative metabolism could be beneficial for patients with COPD by stimulating adaptive changes in respiratory muscles and/or reversing altered cellular energy metabolism in limb muscle.

The natural polyphenol resveratrol (3,5,4'-trihydroxystilbene) might be an interesting therapeutic candidate for COPD by simultaneously targeting both pulmonary as well as extrapulmonary pathology. Interest in resveratrol started when cardioprotective effects of red wine were identified [14]. Nowadays, resveratrol is receiving increasing interest because of its potential to improve metabolic health and its anti-inflammatory and anti-oxidant properties [15-17], which might also benefit the lungs and muscles. Beneficial effects of resveratrol have been reviewed in relation to aging, obesity or diabetes [15, 18, 19], but not in relation to COPD. Here we will therefore evaluate available evidence regarding the effects of resveratrol

on lung injury, muscle metabolism and cardiovascular risk profile and discuss if resveratrol supplementation is just a hype or could give hope to patients with COPD.

THE MECHANISMS OF ACTION OF RESVERATROL

Resveratrol exerts its beneficial health effects through several signaling pathways which have been reviewed elsewhere [15, 18, 20-22]. Here we will briefly summarize the processes and molecular pathways affected by resveratrol and relevant for COPD, focusing on molecular targets to decrease lung and muscle impairment. The most relevant target for the downstream effects of resveratrol is Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide-dependent histone deacetylase that promotes cell survival, which is activated by resveratrol directly or indirectly via the activation of AMPK [15]. Activation of SIRT1 has been proposed to be involved in a number of beneficial health effects of resveratrol. First of all, SIRT1 activates PGC-1α, a master regulator of mitochondrial metabolism and biogenesis [15]. This beneficial effect of resveratrol may improve mitochondrial function which is known to be compromised in muscles and lungs of patients with COPD [10, 23]. Second, SIRT1 activation can modulate several stress response transcription factors such as forkhead box O (FOXO)3 which regulates autophagy [18, 21]. Autophagy is the process of eliminating organelles and proteins through the lysosomal degradation pathway; it is increased in both lungs and skeletal muscle of patients with COPD [24, 25]. Third, SIRT1 downregulates the transcription factor nuclear factor kappa B (NF-κB) which is involved in the anti-inflammatory properties of resveratrol [22]. Activation of SIRT1 inhibits degradation of the inhibitor of κB, inhibiting the translocation of NF-κB to the nucleus and reducing the expression of inflammatory and immune genes including proinflammatory cytokines, chemokines, inflammatory enzymes, and adhesion molecules [22]. Other putative pathways responsible for the anti-inflammatory properties of resveratrol have been described elsewhere [22], but since most inflammatory proteins upregulated in COPD macrophages are regulated by NF-κB pathway [24], this pathway would be most interesting for resveratrol in COPD.

In addition, resveratrol exerts its antioxidant effects mainly through regulation of nuclear factor erythroid-2-related factor-2 (Nrf2). By inhibiting pro-inflammatory cytokines and oxidative stress Nrf2 plays a protective role against inflammation in cells [20]. Normally Nrf2 is activated by oxidative stress, however, in COPD activation is not appropriate despite high levels of oxidative stress in the lungs [24]. Activation of Nrf2 leads to the induction of many antioxidant enzymes including heme-oxigenase-1, superoxide dismutase, glutathione peroxidase, and catalase and can be protective against damage, inflammation and oxidative cell death [18]. The activation of Nrf2 is also suggested to be mediated by SIRT1 [26]. The described anti-inflammatory and antioxidant properties of resveratrol can also contribute to cardiovascular protective effects as reviewed elsewhere [27].

EFFECTS OF RESVERATROL ON LUNG DAMAGE

Anti-inflammatory and antioxidant properties of resveratrol in the lungs have been demonstrated in preclinical models. Resveratrol causes a reduction in lung tissue neutrophilia and proinflammatory cytokines in a rodent model of acute lipopolysaccharide (LPS) induced airway inflammation [28]. Furthermore, in vitro treatment with resveratrol inhibited the release of inflammatory cytokines from bronchoalveolar lavage fluid macrophages and human bronchial smooth muscle cells isolated from COPD patients [29-32]. These anti-inflammatory effects of resveratrol were ascribed to the inhibition of NF-κB activation [32]. Resveratrol has also been shown to inhibit autophagy in vitro in human bronchial epithelial cells and in vivo in a LPS and cigarette smoke-induced COPD mice model by reversing the decrease in SIRT1 and FoxO3a expression and by decreasing the production of Beclin1 protein [33-35].

Long-term cigarette smoke exposure caused persistent oxidative stress-induced impairments in mitochondrial structure and function in human bronchial epithelial cells [36]. This mitochondrial dysfunction is also involved in the pathogenesis of COPD and may be related to a reduction in PGC-1α [23]. Resveratrol has been shown to attenuate cigarette smoke-induced oxidative stress in human lung epithelial cells via nuclear translocation of Nrf2 [37, 38], possibly mediated by SIRT1 [38]. Moreover, intratracheal instillation of resveratrol in mice caused increased SIRT1 levels and maintained PGC-1α in alveolar epithelial cells [39]. These mitochondrial effects were correlated with maintenance of lung structure and function.

Corticosteroids are able to suppress the release of inflammatory mediators in macrophages, but fail to sufficiently suppress airway inflammation in stable COPD [40]. Furthermore, long-term treatments with oral corticosteroids bear high risks of significant adverse effects such as increasing blood glucose levels, muscle atrophy and abdominal obesity [41]. Resveratrol could be an alternative treatment for corticosteroids in COPD. Indeed, in cultured human airway smooth muscle cells exposed to tumor necrosis factor (TNF)-α resveratrol reduced the release of inflammatory mediators more efficiently than dexamethasone [31]. Furthermore, resveratrol was superior to dexamethasone in reducing COPD-associated cytokines and matrix-metalloprotease-9 in human airway smooth muscle cells and alveolar macrophages [30, 42]. These potential effects of resveratrol have led to the development of a spray-dried resveratrol powder [43, 44], which showed anti-inflammatory activities in vitro [44].

The various reported experimental models consistently show beneficial effects of resveratrol on inflammatory processes and oxidative stress markers in the lungs. However, up till now no clinical proof-of-concept studies are available to confirm these results in a clinical setting.

EFFECTS OF RESVERATROL ON SKELETAL MUSCLE MASS AND MITOCHONDRIAL HEALTH

The described mechanisms of action suggest that resveratrol can both improve skeletal muscle oxidative metabolism and maintain skeletal muscle mass. Timmers *et al*. were the first to confirm improved mitochondrial metabolism in a clinical proof-of-concept study, as increased muscle protein expression of AMPK, SIRT1, PGC-1α and citrate synthase were observed after 30 days of 150 mg/day resveratrol supplementation in healthy obese men [45]. Although another study including 10 subjects with type 2 diabetes mellitus (T2DM) also found increased SIRT1 after 12 weeks of resveratrol (3g/day) [46], three other studies including T2DM, obese and nonobese patients did not find increased mitochondrial biogenesis markers after resveratrol treatment, despite comparable dosage and duration [45, 47, 48]. In addition, improved muscle mitochondrial respiration on the electron input of both complexes I and II and increased maximal capacity of the electron transport chain was found after resveratrol supplementation in overweight and obese patients [45, 49] and in T2DM patients [50]. In these studies no differences in mitochondrial content were found, suggesting that mitochondria became more efficient. Contradictory, another study found an increased mitochondrial number after 6 weeks of resveratrol in older adults, despite no changes in mitochondrial size and morphology [51]. However, a higher dose of resveratrol (2-3 g/day) was used compared with the studies that did find an improvement in mitochondrial respiration (80-150 mg/day) [45, 49, 50]. In addition, an increment in the oxidative type-I myosin heavy chain protein in primate soleus muscle was found after long-term resveratrol treatment [52]. To our knowledge, only one study investigated the effect of resveratrol on skeletal muscle mRNA and protein expression levels in a model of COPD [53]. In rats exposed to cigarette smoke and LPS, supplementation with resveratrol lowered serum and muscle TNF-α accompanied by an increase in AMPK. Altogether, these results show promising results of resveratrol on skeletal muscle oxidative metabolism.

Preliminary data also suggest several beneficial effects of resveratrol on skeletal muscle mass maintenance. In C2C12 myotubes and in mice, muscle atrophy, induced by TNF-α or glucocorticoids, was inhibited by resveratrol through inhibition of the atrogenes downstream of the Akt/mTOR/FOXO1 signaling pathway [54-56]. In addition, resveratrol reduced the expression of palmitate-induced inflammation in C2C12 myoblasts by mechanisms involving the inhibition of oxidative stress and decreasing the activity of NF-κB [57]. Moreover, loss of oxidative capacity is postulated to accelerate the process of muscle loss [58]. Therefore, improving the skeletal muscle oxidative capacity might maintain skeletal muscle mass. These preclinical effects of resveratrol on skeletal muscle maintenance have not been observed in human clinical studies yet [48, 49, 59], possibly because the studied populations (i.e. healthy obese or nonobese individuals with metabolic syndrome) were not characterized with muscle wasting.

EFFECTS OF RESVERATROL ON THE CARDIOVASCULAR RISK PROFILE

Preclinical animal studies have suggested beneficial effects of resveratrol for the treatment of cardiovascular diseases as was recently reviewed [27, 60, 61]. However, these reviews also revealed that clinical studies produced inconsistent results and were not as promising as preclinical data. Nevertheless, a recent meta-analysis including overweight and obese participants showed that SBP, fasting glucose and total cholesterol were significantly lower after resveratrol supplementation, whereas other cardiovascular risk parameters remained unaltered [62]. More specifically, a subgroup analysis showed that beneficial effects of resveratrol were found at a high dose of resveratrol (≥300 mg/day). The variability in dosage (8-3000 mg/day) and duration (2 weeks to 6 months) between studies and the low bioavailability of resveratrol [63], can contribute to the variation in results between studies. The fact that some cardiovascular risk parameters were lowered in a group of individuals at risk for cardiovascular disease still implicates that there is potential for resveratrol to decrease the cardiovascular risk. In COPD not only obese patients but even normal weight patients are already at an increased cardiovascular risk [64, 65].

RESVERATROL IN COPD

Resveratrol could be an interesting intervention for COPD as it targets both lung and muscle via overlapping mechanisms (Figure 1). First of all, the anti-inflammatory properties of resveratrol can reduce both lung and muscle impairment and may potentially even reduce the need for anti-inflammatory drugs with their negative side-effects. Systemic inflammation, measured by NF-κB, TNF-α and matrix-metalloprotease-9 protein expression in lymphocytes, was increased in COPD patients compared with healthy controls and was reduced after resveratrol treatment [66]. This increased systemic inflammation may affect both the lungs and muscles [24]. Moreover, increased macrophage numbers in the lungs play a key role in the pathophysiology of COPD and most inflammatory proteins upregulated in these macrophages are regulated by NF-κB [24]. Inflammatory modulation in the lungs would therefore be beneficial for COPD. Since systemic inflammation has been implicated in skeletal muscle wasting, loss of lower limb mitochondrial capacity and deterioration of respiratory muscles [13, 67], the anti-inflammatory effects of resveratrol would also benefit the muscular impairments in COPD.

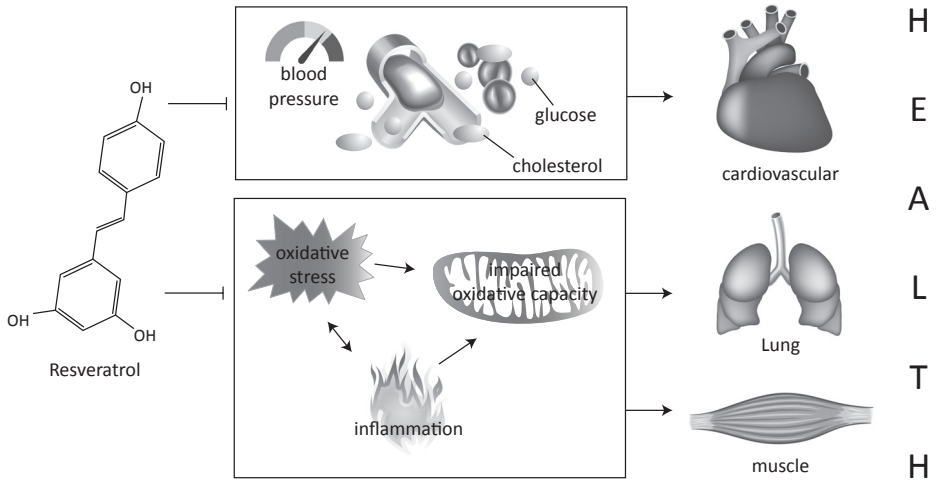

Figure 1. Schematic overview of the beneficial effects of resveratrol on lung impairment, muscle metabolism and cardiovascular disease risk in patients with COPD.

Resveratrol not only improves mitochondrial oxidative capacity indirectly via its anti-inflammatory properties, but also directly via the AMPK-SIRT1-PGC-1α axis. Again this beneficial effect of resveratrol can affect both the muscle and the lung since SIRT1 levels and PGC-1α are decreased in both lung and muscle tissue of patients with COPD [9, 10, 23, 68]. Preclinical findings suggest that inhaled resveratrol can improve mitochondrial function in the lungs and subsequently maintain lung structure and function in COPD [39]. In muscle, nutritional supplementation with resveratrol showed improved mitochondrial function accompanied by improved mitochondrial respiration in obese men [45].

Finally, the anti-oxidant properties of resveratrol may affect both the lungs and the skeletal muscle as well. In COPD patients, high levels of oxidative stress have been observed in the lungs and have been associated with increased inflammation, airway remodeling, autoimmunity and corticosteroid resistance [3]. Reducing oxidative stress would therefore be beneficial to decrease lung injury in COPD. Moreover, many studies indicate an important role of oxidative stress, in skeletal and respiratory muscle dysfunction and loss of skeletal muscle mass in patients with COPD [6, 13, 69].

Because of the overlapping mechanisms in lungs and skeletal muscle that can be improved by resveratrol, treatment with resveratrol would be even more interesting for specific phenotypes of patients with COPD. For example, the cachectic COPD phenotype, characterized by high prevalence of emphysema and muscle wasting [5], display more severe abnormalities in muscle oxidative phenotype which could be improved by resveratrol [58]. Furthermore, patients with COPD who often experience exacerbations would be another

interesting population for resveratrol treatment, since exacerbations are often accompanied by pulmonary and systemic inflammation and are associated with increased susceptibility to muscle wasting [70].

On top of the beneficial effects of resveratrol on the lungs and muscle, preclinical studies suggest cardioprotective effects of resveratrol. Cardiovascular risk modification is currently underappreciated in COPD management and should receive more attention. Early assessment of the cardiovascular risk profile is expected to result in the initiation of risk-lowering therapies. Despite overwhelming attention to resveratrol in the cardiovascular risk context we conclude that clinical evidence for resveratrol is inconclusive due to the large variability in dosage and duration. Therefore, it is still unclear whether resveratrol can be used as adjunct or alternative for proven lifestyle interventions including smoking cessation, physical activity and nutritional and dietary modulation, to improve the cardiovascular risk in COPD.

CONCLUSION

Resveratrol seems a promising candidate to decrease lung injury and to improve skeletal muscle mitochondrial function which is known to be compromised in COPD. However, there is no convincing evidence that resveratrol will significantly decrease the cardiovascular risk in patients with COPD.

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Chapter 8

General discussion

Cardiovascular disease is a frequent comorbidity in patients with COPD and is associated with disease progression and mortality [1, 2]. Adiposity and the loss of skeletal muscle oxidative capacity have been suggested to be drivers of the increased cardiometabolic risk in patients with COPD [3]. This thesis shows that even normal-weight patients with COPD may be at an increased cardiometabolic risk, due to a proportionally abundance of fat mass in combination with a low muscle mass. This finding stresses the importance of assessing body composition in normal-weight patients with COPD for cardiovascular risk assessment and exploration of interventions that may aid in the modulation of the cardiometabolic risk profile.

CARDIOMETABOLIC RISK IN NORMAL-WEIGHT PATIENTS WITH COPD

Nutritional assessment based on body weight and body composition is important in patients with COPD and up till now, most nutritional attention has been given to underweight and obese COPD patients [4]. Unintentional weight loss and low BMI (≤20 kg/m²) in patients with COPD have been consistently associated with an increased mortality risk relative to overweight and even obese patients [5, 6], while it is well-established that obesity (BMI≥30 kg/m²) predisposes to an increased cardiometabolic risk [4]. Less attention has been given to normal and overweight COPD patients (BMI 20-30 kg/m²), despite the fact that the majority of patients fall within this BMI range. For example, in a large Dutch cohort of 317 COPD patients recruited from primary care, 69.7% was normal to overweight while only 12.3% was underweight and 18.0% obese [7]. The normal BMI in these patients might hide a disturbed body composition, i.e. combined low muscle mass and high fat mass. Indeed, low muscle mass (i.e. fat free mass index <17 kg/m² for men and <15 kg/m² for women) with coexisting abdominal obesity is highly prevalent in normal-weight COPD patients admitted to pulmonary rehabilitation (78.9%) [8]. Low muscle mass has been associated with decreased exercise capacity, poor quality of life and mortality, irrespective of body weight [9-11]. In the current thesis we found an increased cardiometabolic risk profile in normal-weight patients witch COPD, particularly in patients with combined low muscle mass and abdominal obesity.

Loss of skeletal muscle oxidative capacity and increased visceral adipose tissue have been suggested to be implicated in driving cardiometabolic risk in COPD [3]. Loss of skeletal muscle oxidative capacity is more pronounced in patients with sarcopenia [12], suggesting that the cardiometabolic risk profile might be further increased in COPD patients with low muscle mass. However, we did not find any difference in cardiometabolic risk profile between COPD patients with low muscle mass compared to patients with normal muscle mass. The role of muscle oxidative capacity loss in the increased cardiometabolic risk profile of normal-weight patients with COPD still needs to be investigated. It is also possible that the cardiometabolic risk in normal-weight patients with COPD is mainly affected by the presence of abdominal obesity on top of the low muscle mass. Abdominal obesity has been associated with low-

grade systemic inflammation in COPD and especially when the adipose tissue is located in the visceral compartment it has been associated with an increased cardiometabolic risk [13-16]. It is, however, still unknown whether an increased inflammatory load by visceral fat mass abundance or also an increased inflammatory activity of the visceral fat tissue leads to an increased cardiometabolic risk. Visceral fat mass is not usually assessed in patients with COPD due to the lack of a clinical indication. It can be measured by magnetic resonance imaging (MRI), however this measurement technique is expensive and time-consuming. Another technique that has been shown to be able to assess body composition and distinguish between different fat depots is computed tomography (CT) [17]. Although CT imaging is associated with ionizing radiation, it is increasingly obtained in patients with COPD due to the overlapping risk factors and clinical manifestations of COPD with other chest diseases such as lung cancer, bronchiectasis or pulmonary nodules [18, 19]. However, these routine clinical images in patients with chest diseases are usually taken at a higher level than the golden standard to measure visceral fat mass (lumbar vertebra 4-5). Although visceral fat mass measured at a higher level (lumbar vertebra 1-3) is still associated with metabolic syndrome [20] and although studies report that a single image in the upper abdomen is reliable to measure total visceral adipose tissue [21, 22], the question remains whether this is also the case in patients with COPD, in particular those with hyperinflation and downward displacement of the diaphragm in which the abdominal structures are translocated to a more distal level. Still the use of CT imaging might be a step forward to easily assess the visceral fat mass in patients with COPD and relate it to their cardiometabolic health. In the near future the same CT scan of the thorax might even be used to assess other parameters relevant for cardiometabolic risk profiling in patients with COPD (such as coronary calcification, peri- and epicardial fat mass, left ventricle hypertrophy and steatosis).

The increased cardiometabolic risk profile of normal-weight patients with COPD described in the current thesis is based on increased cardiometabolic risk parameters in cross-sectional analyses. Although these parameters are well-known predictors for cardiovascular events or the development of cardiovascular disease in the general population, the interpretation might differ in an accelerated aging population such as COPD due to the “reverse epidemiology” phenomenon [23]. This phenomenon describes that in the elderly population several conventional cardiovascular risk factors such as elevated BMI, serum cholesterol and blood pressure lower the risk of death instead of increasing it. Whether this reverse epidemiology also appears in the course of COPD needs to be confirmed in longitudinal studies.

TARGETED INTERVENTIONS TO MODULATE THE CARDIOMETABOLIC RISK IN COPD

Exercise training

Cardiometabolic risk parameters have been shown to improve after exercise training in the general population and other conditions including obesity and diabetes [24-26]. However, studies investigating the efficacy of exercise training specifically focusing on the increased cardiometabolic risk in COPD are scarce and results are inconsistent. In three small studies significant improvements were found in cardiovascular and metabolic risk parameters after aerobic exercise training [27-29]. One study showed decreased blood pressure, arterial stiffness and total cholesterol after 7 weeks pulmonary rehabilitation in 22 moderate-severe (FEV1 45 %predicted) normal-weight COPD patients (BMI 25 kg/m²), while fasting glucose levels remained unchanged [27]. Another study also showed decreased systolic blood pressure, arterial stiffness and fasting glucose without changes in inflammatory (C-reactive protein) and oxidative stress markers (total antioxidant status) in a comparable group of 10 COPD patients (FEV1 47 %predicted and BMI 23 kg/m²) after 4 weeks aerobic exercise training [28]. Furthermore, a larger and longer-term study including 36 severe normal-weight patients with COPD (FEV1 36 %predicted and BMI 23 kg/m²) showed significant improvements in cardiovascular risk factors including heart rate, blood pressure and high-density lipoproteins after 96 weeks exercise training [29]. In contrast, these results could not be confirmed in two other studies [30, 31]. One study failed to show improvements in blood pressure after 12 weeks of exercise training in 6 severe but normal-weight COPD patients (FEV1 39 %predicted and BMI 24 kg/m²) [30] and another larger study also found no beneficial effects of pulmonary rehabilitation, including exercise training, on the arterial stiffness and blood pressure in 129 mild-moderate normal-weight COPD patients (FEV1 51 %predicted and BMI 25 kg/m²) [31]. We also found no clinically relevant beneficial effects of high-intensity exercise training on the cardiometabolic risk profile of 112 and 27 normal-weight COPD patients, as described in Chapters 4 and 5. All of the above mentioned studies applied a high-intensity training program with most of them having at least three training sessions per week. Except for two studies [28, 30], all training programs consisted of a combination of endurance training and resistance training. Furthermore, the duration of the training programs varied from 4 to 96 weeks. The large variation between these studies however does not allow for drawing firm conclusions on the cardiometabolic effects of exercise training in patients with COPD.

It is possible that the high-intensity exercise training using a combination of endurance and resistance training is not the most optimal training program to improve the cardiometabolic risk profile in patients with COPD. Perhaps the ventilatory limitations of patients with COPD limit their training capacity to achieve cardiometabolic health benefits of high-intensity exercise training. Since high-intensity exercise training also did not show additional benefits on cardiovascular risk factors in 303 patients with type 2 diabetes (not hampered by ventilatory

limitation) compared to low-intensity exercise training [32], the latter might be better to improve the cardiometabolic health in patients with COPD. It should also be noted that most studies described the exercise training as part of a pulmonary rehabilitation program which is primarily designed to improve dyspnea and physical performance [33]. It could be questioned whether improving physical performance alone is sufficient for improving the cardiometabolic risk profile in COPD. Perhaps improving physical functioning by a combination of improving physical performance and increasing daily physical activity levels, might be more beneficial. High-intensity exercise training per se does not always translate in increased daily physical activity [34-37]. For example, van de Bool *et al.* showed in COPD patients with low muscle mass that physical activity levels decreased after 4 months high-intensity exercise training despite concomitant increased physical performance [37]. Another study also showed increased exercise capacity without significant differences in daily steps, time spent sedentary and daily physical activity in 47 patients with COPD after a seven week outpatient pulmonary rehabilitation program [34]. Comparable results were found in two other studies [35, 36]. These studies suggest that the moderate-to-vigorous physical activity level during the exercise training might be compensated with more sedentary behavior during the rest of the day which might abolish positive cardiometabolic effects of exercise training. Therefore, adopting a more active lifestyle might be more beneficial for patients with COPD to improve the cardiometabolic risk profile than supervised exercise training only.

Nutritional supplements

Alternatively or on top of exercise training a targeted approach using nutritional supplements that might enhance or mimic the effects of exercise training might be beneficial for patients with COPD to improve cardiometabolic health. In the current thesis we found no beneficial effects of dietary nitrate on cardiovascular risk parameters in patients with COPD. In addition, dietary nitrate did not improve exercise performance or decrease oxygen consumption during exercise suggesting that it is not a promising therapeutic for patients with COPD to enhance the effects of exercise training. Also the meta-analysis of previous studies in patients with COPD did not show beneficial effects of beetroot juice on blood pressure suggesting that dietary nitrate will not improve cardiometabolic health. Furthermore, in the current thesis the natural polyphenol resveratrol was suggested to be an interesting candidate for patients with COPD due to its cardioprotective, anti-inflammatory and anti-oxidant properties and its beneficial effect on skeletal muscle mitochondrial function. However, no clinical study in COPD has been published confirming these expected effects of resveratrol. Therefore, we performed a clinical proof-of-concept study investigating the effect of 4 weeks resveratrol on skeletal muscle oxidative metabolism, adipose tissue inflammation and secondary cardiometabolic clinical outcomes in patients with COPD. Recently, data collection of this study has been finished and analyses are ongoing.

Next to dietary nitrate and resveratrol there are several other nutrients that are receiving a lot of attention as an exercise mimetic and in relation to the cardiometabolic effects. The review by Fan et al. describes several exercise mimetics that might activate key regulators of the oxidative metabolism, including AMPK and SIRT1 [38]. These exercise mimetics might enhance energy expenditure and fatty acid oxidation and could be beneficial to decrease the cardiometabolic risk. Since most of these exercise mimetics are still in pre-clinical stages they need further investigation to confirm that they will be beneficial in humans. Nutritional supplements that receive a lot of attention due to their cardiometabolic effects are for example epigallocatechin-3-gallate (EGCG), plant sterol and stanol enriched foods and polyunsaturated fatty acids (PUFAs). The polyphenol EGCG is mostly present in green tea and has, just like resveratrol, anti-inflammatory and anti-oxidant effects [39]. Moreover, a randomized controlled trial including 56 obese hypertensive subjects showed decreased systolic and diastolic blood pressure, decreased plasma glucose, insulin, TNF-α, total cholesterol and LDL cholesterol and increased HDL cholesterol after 3 months green tea capsules containing 208 mg EGCG [40]. The consumption of plant sterol and stanol enriched foods have been consistently shown to lower low density lipoprotein (LDL) cholesterol [41]. A large meta-analysis in which 113 randomized controlled trials were included showed that intake of 2 grams per day of plant sterols or stanols could result in a lowering of LDL cholesterol up to 10% [42]. Such a decrease in LDL cholesterol would be effective in lowering the number of new cardiovascular events [43]. The specific PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to lower blood pressure [44]. Despite a favorable impact on blood pressure, long-term data on prevention of cardiovascular events with the use of PUFAs have not been confirmed. Additionally, PUFAs are interesting nutraceuticals for patients with COPD as they are hypothesized to boost the decreased skeletal muscle oxidative capacity since they are the natural ligands of peroxisome proliferator-activated receptors (PPARs) that promote mitochondrial biogenesis and a slow-twitch fibre-type I muscle phenotype.

Although there are many more nutritional supplements that might also be beneficial to modulate the cardiometabolic risk profile [45-47], a question that remains unanswered is whether a single component strategy would be useful in a complex disease such as COPD or whether a multicomponent strategy targeting cardiometabolic health would be more beneficial, since the cause of the increased cardiometabolic risk profile seems multifactorial.

LIFESTYLE AS A MODULATOR OF THE CARDIOMETABOLIC RISK IN COPD

Patients with COPD are often characterized by an unhealthy lifestyle. It is, however, not clear whether abdominal obesity in normal-weight patients with COPD reflects an overall poor lifestyle or is disease-induced and whether the two act synergistically [3]. Without improvement of patients' lifestyles, interventions aimed at reducing cardiometabolic risk

are probably ineffective in the longer term. Therefore, addressing lifestyle modifications in an integrated approach might result in a behavioral change leading to an improved cardiometabolic health.

Smoking cessation

Smoking is the most important lifestyle factor that increases the risk of developing both COPD and cardiovascular diseases. Furthermore, various studies have reported that compared to non-smokers, smokers have more central adiposity, poorer skeletal muscle function, decreased skeletal muscle oxidative capacity and decreased skeletal muscle insulin sensitivity [48-51]; all of which may contribute to an increased cardiometabolic risk [3]. Smoking cessation is therefore essential in COPD, not only for respiratory reasons but also for cardiometabolic health. However, fat mass gain after smoking cessation is often reported and has been shown to be especially located in the visceral fat compartment [52], which can further affect the cardiometabolic risk. These contradictions suggest that smoking cessation alone seems not sufficient and stresses the need for an integrated approach with more lifestyle interventions.

Reducing sedentary behavior

The Dutch health council recommends that adults and elderly should be active on a moderate to vigorous intensity for at least 150 minutes per week, to do muscle and bone strength exercises which elderly need to combine with balance exercises at least two times a week, and to avoid sedentary behavior [53]. The overall advice is that physical activity is good, the more the better. Patients with COPD however are characterized by a physically inactive lifestyle compared to healthy age-matched controls [54]. In one study, only 1 out of 29 COPD patients complied with the recommendation of at least 150 min per week of moderate to vigorous physical activity and patients spend most of the time (67.4%) sedentary [54]. Physical inactivity can be interpreted as a consequence of the disease due to dyspnea, but is can also worsen disease progression. This leads to a downward spiral of inactivity which predisposes patients to reduced quality of life and increased rates of hospitalization and mortality [55-57]. Physical inactivity is also a risk factor for a hospitalization for exacerbation of COPD [55, 58], that subsequently contributes to a lowering of physical activity levels which may persist for weeks, months or even years after hospital discharge, further increasing the risk of a readmission for a subsequent exacerbation [59, 60]. Furthermore, a low physical activity level has been associated with the presence of metabolic syndrome [61]. Interventions that interrupt the physical inactivity spiral in patients with COPD are therefore important to investigate. Since the effects of exercise training on the cardiometabolic risk profile of patients with COPD are inconsistent, the focus should be shifted towards adapting a more active lifestyle by reducing the physical inactivity.

Sedentary behavior is associated with cardiovascular morbidity and mortality [62-64] and might also be an important target in patients with COPD to improve the cardiometabolic

health. In diabetes-oriented research this observation has already led to a shift in physical activity targets to improve cardiometabolic health [65]. This shift includes sitting less and increasing standing and walking as means instead of increasing time spend in moderate-to-vigorous physical activity. Several meta-analyses investigated the ability of interventions to reduce the amount of sedentary behavior and concluded that interventions with a specific focus on reducing sedentary behavior showed large and clinically meaningful reductions in sedentary time compared to interventions focusing on increasing the physical activity [66, 67]. The specific interventions included counseling interventions, software to stimulate participants to stand-up or walk or interventions to reduce the television viewing time. Although effective strategies to decrease the sedentary behavior have been proposed, only a few studies investigated the direct effect of reducing sedentary time on the cardiometabolic risk profile in overweight and diabetic adults [68, 69]. A recent study showed reduced sedentary time and increased light and moderate to vigorous physical activity as a result of a 4-month behavior intervention including theoretical and practical counseling in patients with diabetes [68]. These changes in physical activity patterns were accompanied by improvements in cardiometabolic risk parameters including HbA1c, glucose, body weight, waist circumference and circulating C-reactive protein levels. Another study showed improved insulin sensitivity, circulating lipids and diastolic blood pressure after substituting sitting with standing and walking in free-living conditions for 4 days in overweight adults [69]. Although these results seem promising, they need to be confirmed in longer term studies.

Reducing the sedentary behavior and maintaining it during the course of the disease is thus of great importance in COPD. Up till now, no studies have been performed to primarily decrease sedentary behavior and subsequently improve cardiometabolic risk parameters in COPD, but offering a program of exercise training coupled with a physical activity behavioral change is suggested to be the optimal approach [70, 71]. Although both guidelines for pulmonary rehabilitation and physiotherapy in COPD describe that health behavioral change is vital to optimization and maintenance of benefits from any intervention in chronic care [33, 72], a behavioral change into a more active lifestyle is not always achieved after completing a pulmonary rehabilitation program [56]. Future studies are therefore necessary to show how a reduction in sedentary behavior can be achieved in patients with COPD. A possible strategy would be to give patients real-time feedback on their physical activity levels using simple pedometers or an application on their mobile phone. Several studies investigating this research question have been performed in patients with COPD. A recent randomized controlled trial in 97 COPD patients showed that a 3-month physical activity pedometer-based program showed greater improvements in physical activity level than a standard program of physical activity encouragement [73]. Another randomized trial including 109 patients with COPD showed that a website added to pedometer use greater improved physical activity level compared to pedometer use alone [74]. Also a pilot-study of 21 COPD patients showed that the use of pedometers in combination with exercise counseling and the stimulation

of lifestyle physical activity is an effective strategy to increase physical activity level [75]. Although these studies show promising results of real-time feedback strategies to increase physical activity level in patients with COPD another recent study in 152 patients with COPD did not find enhanced physical activity levels after 8 weeks of pulmonary rehabilitation with or without additional pedometer-directed step targets. Therefore, strategies to adopt a more active lifestyle remain an interesting topic of investigation to enhance physical activity levels in patients with COPD. Several other studies investigating strategies to reduce the sedentary time in patients with COPD are currently ongoing. In one study a behavior change intervention is investigated that aims to reduce sedentary time through a process of guided goal setting [76]. The intervention includes two target behaviors, replacing sitting and lying down with light intensity physical activity where possible and stand up and move for two minutes after 30 minutes of continuous sedentary time. In another ongoing study the effect of a two week at-home intervention providing education and self-monitoring to reduce prolonged periods of sedentary behavior in patients with COPD discharged following an acute exacerbation is investigated [77]. Whether eventually a reduced sedentary behavior leads to an improved cardiometabolic health in COPD still needs to be investigated.

Improving the dietary quality

In 2015 the Dutch Health Council published new dietary guidelines [78]. These guidelines recommend a dietary pattern involving more plant-based and less animal-based food, sufficient intake of fruits and vegetables, whole grain products, legumes, unsalted nuts and fish and limited consumption of processed and red meat, salt and alcohol. Furthermore, the consumption of sugary drinks should be replaced by sugar-free drinks like tea, filtered coffee and water. These recommendations are in correspondence with guidelines of other countries [79, 80]. The Dutch dietary guidelines were based on the evidence regarding the risks of cardiovascular disease, diabetes and cancer. Moreover, the large Prospective Urban Rural Epidemiology (PURE) study including 135.335 individuals from 18 countries recently showed that fruit, vegetable, and legume intake is also associated with a lower risk of non-cardiovascular and total mortality [81].

The quality of dietary intake of patients with COPD is generally poor. A large cohort study in The Netherlands including 564 COPD patients referred for pulmonary rehabilitation showed that patients had a typically Western diet with insufficient intake of protein, carbohydrate, vitamins (especially vitamin D), and calcium, combined with a too high intake of (saturated) fat [82]. Furthermore, studies have consistently shown that COPD patients consume less dietary fiber, vegetables and fruit compared to control subjects [16, 83]. Since this dietary pattern is not in accordance with recommended guidelines it is hypothesized that dietary guidelines would also be beneficial for patients with COPD to modulate the cardiometabolic risk. The Dutch dietary guidelines include no specific recommendations for patients with COPD due to the lack of research data with clear evidence. Still, two recent studies add

evidence to this assumption [84, 85]. A study by Varraso et al. showed that high intakes of whole grains, polyunsaturated fatty acids, nuts, and long chain omega-3 fats and low intakes of red or processed meats, refined grains, and sugar sweetened drinks was associated with a lower risk of COPD in both women and men [85]. Another study by the same group in the same study population showed that more-frequent fish intake was inversely associated with risk of COPD [84]. Assuming that a dietary pattern characterizes a habit that has been going on for years, we hypothesize that this dietary pattern would characterize the dietary intake of patients with COPD. Therefore, also in patients with COPD more attention should be given to dietary guidelines to modulate the cardiometabolic risk.

EARLY RECOGNITION AND AWARENESS

General practitioners see the vast majority of patients with mild and clinically uncomplicated COPD in whom cardiovascular disease is the leading cause of mortality [86-88]. Despite the broad acceptance that cardiovascular diseases are highly prevalent in patients with COPD, there is still widespread under-recognition and under-treatment of comorbid cardiovascular diseases in COPD [89]. A possible reason might be the lack of appropriate guidelines for the management of comorbidities in patients with COPD is an important factor. In the Netherlands, newly diagnosed COPD patients in primary care receive education about COPD, are started on pulmonary medication, are offered assistance in smoking cessation, and may be referred to a physiotherapist or dietician. However, many patients are already more likely to have pre-existing cardiovascular disease, diabetes mellitus or a previous stroke and are at high risk of acute arteriovascular events [90]. Therefore, it would be interesting to assess the cardiometabolic risk profile of patients at the time of COPD diagnosis in order to start structural preventive cardiovascular risk management according to international guidelines as soon as possible [91]. This early recognition and awareness of the increased cardiometabolic risk in patients with COPD can contribute to adopting a healthier lifestyle.

CONCLUSION

In conclusion, this thesis shows an increased cardiometabolic risk profile in normal-weight patients with COPD due to a proportionally abundance of fat mass in combination with a low muscle mass. This further stresses the importance of assessing body composition also in normal-weight patients with COPD for cardiovascular risk assessment. Exercise training programs as currently employed in COPD are successful in improving exercise capacity but do not appear to beneficially modulate cardiometabolic risk. Intervention strategies to improve cardiometabolic health should therefore focus on lifestyle and behavior changes that aid in smoking cessation, improve dietary quality and reduce sedentary behavior. In

addition, a targeted approach using nutritional supplements may aid in the modulation of the cardiometabolic risk profile. Ultimately, awareness and early recognition in combination with personalized lifestyle intervention strategies based on individual body composition phenotypes may lead to decreased cardiometabolic burden in COPD.

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Summary

Chronic obstructive pulmonary disease (COPD) prevalence has reached epidemic proportions. It is currently the fourth most common cause of death and expected to be the third by 2020. COPD is primarily a lung disease but it often coexists with systemic manifestations and comorbidities such as cardiovascular and metabolic diseases that significantly contribute to disease burden and increase health care costs. Adiposity and the loss of muscle mass and oxidative capacity have been implicated in multifactorial pathways leading to an increased cardiometabolic risk in COPD, also in normal-weight patients in whom this adverse shift in body composition may go unnoticed. Preventing COPD patients from developing cardiometabolic diseases requires a detailed understanding of the risk factors and phenotypic characteristics involved, and calls for developing cardiometabolic risk modulation strategies. The general aim of this thesis was to investigate the cardiometabolic risk profile of normal-weight patients with COPD and to study the effectiveness of exercise and nutritional interventions on the cardiometabolic risk profile in these patients.

In **Chapter 2** we developed the HOSPITALL score, a multidimensional score to predict all-cause hospitalization in community-dwelling older individuals with obstructive lung disease. The HOSPITALL-score includes cardiovascular disease, objectively measured lower extremity dysfunction, systemic inflammation, dyspnea, impaired renal function and tobacco exposure as predictors for all-cause hospitalization in these patients. The finding that cardiovascular comorbid disease was among the strongest predictors for all-cause hospitalization further supports the need for initiatives to prevent cardiovascular disease in COPD.

Chapter 3 describes a systematic literature review of the prevalence of the metabolic syndrome in patients with COPD. The metabolic syndrome describes a constellation of metabolic risk factors that increase the risk of developing cardiovascular disease and type 2 Diabetes Mellitus. In this review we found that the prevalence of metabolic syndrome is significantly higher in COPD patients compared to controls. The most prevalent components of the metabolic syndrome in both COPD patients and controls were hypertension, hyperglycemia and abdominal obesity.

In **Chapter 4** we describe a prospective observational study investigating the effect of low muscle mass (sarcopenia) on the cardiometabolic risk profile in severe COPD patients. We found that cardiometabolic risk parameters were comparable between normal-weight patients with sarcopenia compared to overweight patients without sarcopenia. In addition, we report that 4-weeks of high-intensity training decreased adiposity and improved insulin sensitivity in the non-sarcopenic patients whereas no effects were found in sarcopenic patients. These data suggest that sarcopenia affects cardiometabolic risk and its modulation by exercise training.

In **Chapter 5** we studied the implications of abdominal obesity on the cardiometabolic risk profile of mild-to-moderate COPD patients with low muscle mass. This study demonstrated that abdominal obesity is highly prevalent (75%) in normal-weight COPD patients with low

muscle mass, which was associated with increased cardiometabolic risk. After 4 months of high-intensity exercise training, however, no major changes in cardiometabolic risk factors were observed despite improved exercise capacity.

Chapter 6 describes a randomized placebo controlled crossover study, in which we could not detect beneficial effects of sodium nitrate on mechanical efficiency, oxygen consumption, blood pressure, or cardiac biomarkers in patients with COPD. In addition, this chapter includes a meta-analysis of previous studies investigating the effect of dietary nitrate in the form of beetroot juice in COPD. This meta-analysis shows no effect on exercise performance, oxygen consumption and blood pressure. These results suggest that dietary nitrate is not the promising therapeutic for patients with COPD to enhance the effects of exercise training and to decrease the blood pressure that could result in a decreased cardiometabolic risk.

Another interesting nutritional intervention that might improve the cardiometabolic risk profile in patients with COPD is the natural polyphenol resveratrol. As reviewed in **Chapter 7** resveratrol has been suggested to mimic the effects of exercise training since it has been shown to include cardioprotective, anti-inflammatory and anti-oxidant properties. Although we found no convincing evidence that resveratrol will significantly decrease the cardiovascular risk directly, the critical review of the recent literature in this chapter suggests that resveratrol is a promising candidate to decrease lung injury and improve skeletal muscle mitochondrial function in patients with COPD which might indirectly modulate the cardiometabolic risk in COPD. A proof-of-concept study investigating the effect of resveratrol on the cardiometabolic risk profile in patients with COPD has been recently finished and data and tissue analyses are currently ongoing.

This thesis further stresses the clinical relevance of cardiometabolic disease in COPD and contributes to the understanding of the implications of body composition shifts on cardiometabolic risk in normal-weight COPD patients. Furthermore, exercise training programs as currently being employed in COPD rehabilitation are successful in improving exercise capacity but do not appear to majorly improve cardiometabolic risk profile. Ultimately, awarenesss and early recognition of increased cardiometabolic risk in COPD, lifestyle modifications and potentially further personalization of exercise training programs by additional nutritional interventions may lead to decreased cardiometabolic burden in COPD.

Samenvatting

COPD is een veelvoorkomende chronische ziekte waarvan verwacht wordt dat het in 2020 doodsoorzaak nummer 3 is. COPD is primair een chronisch obstructieve longziekte, maar gaat vaak gepaard met andere aandoeningen zoals hart- en vaatziekten en type 2 Diabetes Mellitus. Deze zogenaamde comorbiditeiten dragen significant bij aan de ziektelast en ziektekosten. Een toename van het lichaamsvet, verlies van spiermassa en een verminderde zuurstofafhankelijk metabolisme in de spier worden verondersteld bij te dragen aan een verhoogd cardiometabool risico in patiënten met COPD, zelfs in de patiënten met een normaal lichaamsgewicht waarbij deze verandering in lichaamssamenstelling vaak onopgemerkt gaat. Om de ontwikkeling van hart- en vaatziekten en type II Diabetes Mellitus in patiënten met COPD te voorkomen is het van belang dat strategieën ontwikkeld worden die het cardiometabole risico kunnen moduleren. Daarnaast is een beter begrip van de betrokken risicofactoren en patiëntkenmerken nodig. Het hoofddoel van dit proefschrift was het cardiometabole risicoprofiel van COPD patiënten met een normaal lichaamsgewicht te onderzoeken en de effectiviteit van trainings- en voedingsinterventies op het cardiometabole risicoprofiel van deze patiënten te bestuderen.

In **Hoofdstuk 2** hebben we de HOSPITALL score ontwikkeld, een multidimensionale score die het risico op een ziekenhuisopname, ongeacht de oorzaak, voorspelt in thuiswonende ouderen met een obstructieve longziekte. De HOSPITALL-score bestaat uit hart- en vaatziekten, verminderde beenspierfunctie, ontstekingsactiviteit in het bloed, kortademigheid, nierfunctie stoornis, en roken als voorspellers van een ziekenhuisopname in deze patiënten. De bevinding dat hart- en vaatziekten één van de sterkste voorspellers was voor een ziekenhuisopname bevestigde onze hypothese dat er behoefte is aan strategieën om het risico op hart- en vaatziekten te verlagen in patiënten met COPD.

Hoofdstuk 3 betreft een systematisch literatuuronderzoek naar het voorkomen van het metabool syndroom in patiënten met COPD. Het metabool syndroom is een combinatie van verschillende metabole factoren die het risico op het ontwikkelen van hart- en vaatziekten en type 2 Diabetes Mellitus verhogen. De metabole factoren zijn grote buikomvang ofwel abdominaal obesitas, hoge bloeddruk en verslechterde bloedwaarden waaronder verhoogd suikergehalte, verhoogd triglyceriden gehalte en te laag HDL-cholesterol. In dit literatuuronderzoek vonden we dat het metabool syndroom significant vaker voorkomt bij patiënten met COPD dan bij controles met een vergelijkbare leeftijd en geslacht. De metabool syndroom componenten die het meeste voorkwamen waren hoge bloeddruk, hoog glucose en teveel buikvet.

In **Hoofdstuk 4** hebben we in een prospectieve observationele studie het effect van een lage spiermassa op het cardiometabole risicoprofiel van patiënten met ernstig COPD onderzocht. We vonden dat cardiometabole risicoparameters vergelijkbaar waren tussen patiënten met een lage en een normale spiermassa. Daarnaast bleek dat de cardiometabole effecten van 4 weken intensieve lichamelijke training verschillend waren tussen COPD patiënten met een

lage en een normale spiermassa. In patiënten met een normale spiermassa verminderde de vetmassa en verbeterde de insuline gevoeligheid na 4 weken training, terwijl geen significante verbeteringen werden gevonden in patiënten met een lage spiermassa. Deze resultaten suggereren dat een lage spiermassa de beïnvloeding van het cardiometabole risico door training verstoord.

In **Hoofdstuk 5** hebben we de gevolgen van te veel buikvet op het cardiometabool risicoprofiel van mild-tot-matige COPD patiënten met een lage spiermassa onderzocht. Deze studie toonde aan dat 75% van de patiënten met een normaal lichaamsgewicht en een lage spiermassa te veel buikvet heeft. Een verhoogde hoeveelheid buikvet bleek geassocieerd te zijn met een verhoogd cardiometabool risico gebaseerd op een verlaagde insuline gevoeligheid en een hogere prevalentie van het metabool syndroom. Ondanks dat na 4 maanden intensieve training de inspanningscapaciteit was verbeterd, werden geen grote veranderingen gevonden op de cardiometabole risicofactoren.

Hoofdstuk 6 beschrijft een gerandomiseerde placebo gecontroleerde cross-over studie waarin we het effect van extra nitraat inname op de mechanische efficiëntie, het zuurstofgebruik tijdens inspanning, de bloeddruk en biomarkers van hartschade hebben onderzocht in patiënten met COPD. Op geen van deze parameters werd een gunstig effect van nitraat gevonden. Daarnaast hebben we in dit hoofdstuk een meta-analyse uitgevoerd van eerdere interventie studies waarin het effect van nitraat, in de vorm van rode bietensap, werd onderzocht in patiënten met COPD. Deze meta-analyse toonde geen effect van nitraat op het uithoudingsvermogen, de zuurstof consumptie tijdens inspanning en de bloeddruk in patiënten met COPD. Deze resultaten suggereren daarom dat nitraat niet een veelbelovend voedingssupplement is voor patiënten met COPD om het effect van training te vergroten. Daarnaast lijkt nitraat niet de bloeddruk te verlagen in patiënten met COPD waardoor het niet zal bijdragen aan een verlaagd cardiometabool risico.

Een andere interessante voedingsinterventie die het risico op cardiometabole aandoeningen in patiënten met COPD mogelijk zou kunnen verlagen is het natuurlijke polyfenol resveratrol. Zoals beschreven in ons review in **Hoofdstuk 7** kan resveratrol mogelijk de effecten van fysieke training nabootsen en lijkt het daarnaast beschermende eigenschappen te hebben tegen onder meer hart- en vaatziekten vanwege anti-inflammatoire en anti-oxidante eigenschappen van de voedingsstof. We vonden geen duidelijk bewijs voor een direct effect van resveratrol op het cardiometabole risico. De literatuur suggereert wel dat resveratrol een veelbelovend voedingssupplement is om longschade te verminderen en om de mitochondriële functie in de spier te verbeteren. Deze routes zouden indirect kunnen bijdragen aan een verminderd cardiometabool risico in patiënten met COPD. Een eerste proof-of-concept studie die het effect van resveratrol op het cardiometabole risicoprofiel en de mitochondriële functie van patiënten met COPD onderzoekt is recent door onze groep afgerond en de weefsels en resultaten worden op dit moment geanalyseerd.

Dit proefschrift benadrukt de klinische relevantie van een verhoogd cardiometabool risico in COPD en geeft meer inzicht in de gevolgen van veranderingen in lichaamssamenstelling hierop. Ondanks dat de trainingsprogramma's die momenteel worden gebruikt in longrevalidatie succesvol zijn in het verbeteren van de inspanningscapaciteit, lijken ze geen aanzienlijke verbeteringen in het cardiometabool risicoprofiel te geven. Bewustwording en vroegtijdige herkenning van het cardiometabole risico bij COPD, alsook leefstijlaanpassingen en eventueel verder gepersonaliseerde trainingsprogramma's met eventueel aanvullende voedingsinterventies kunnen leiden tot een verminderde cardiometabole ziektelast in patiënten met COPD.

Valorisation

SOCIETAL VALUE OF THE THESIS

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow limitation resulting from inflammation and remodeling of the airways [1]. It represents a major cause of morbidity and mortality worldwide resulting in a substantial and increasing economic and social burden. According to Global Initiative for Chronic Obstructive Lung Disease (GOLD) it is estimated to become the third leading cause of death in 2020 [2].

Although COPD is primarily a lung disease, it often coexists with systemic manifestations and comorbidities. The most prevalent comorbidities and systemic manifestations are cardiovascular diseases, skeletal muscle dysfunction, osteoporosis, lung cancer and psychological disorders [1]. These comorbidities affect symptom burden, functional performance and health status and are associated with increased risk for hospitalization [3, 4]. During the course of the disease patients with COPD are frequently hospitalized, not only for exacerbations or pneumonia [5, 6], but also for a wide range of non-respiratory causes [7, 8]. Whereas hospital stays drive direct costs of COPD-related care [9], hospitalizations for non-respiratory reasons constitute the greatest expense in patients with COPD [10]. Systemic manifestations and comorbidities as well as all-cause hospitalizations are associated with mortality and significantly increased health care costs [3, 4].

This thesis focuses on cardiovascular disease risk in COPD. Patients with COPD have a 2 to 5 times higher risk of major cardiovascular disease compared with controls and in mild-to-moderate COPD cardiovascular disease is even the leading cause of mortality [11-14]. This higher risk is independent of shared risk factors such as age and smoking [15], suggesting that other factors are involved and calls for developing cardiometabolic risk modulation strategies.

INNOVATION

In the current thesis a high prevalence of the metabolic syndrome was found in patients with COPD, which was significantly higher compared to controls. Metabolic syndrome is a constellation of metabolic risk factors that predict the risk of developing cardiovascular disease and type II Diabetes Mellitus. The high prevalence of the metabolic syndrome in patients with COPD further supports the increased cardiometabolic risk in patients with COPD. More interestingly, this thesis shows that even normal-weight patients with COPD may be at an increased cardiometabolic risk due to abundance of fat mass in combination with a low muscle mass. This finding stresses the importance of assessing body composition for cardiovascular risk assessment in COPD.

Another interesting innovative finding of the current thesis is that cardiovascular disease has been shown to be an important risk factor for all-cause hospitalizations in patients with COPD. Awareness and recognition of the increased cardiometabolic risk profile in patients

with COPD is therefore necessary to reduce the amount of hospitalizations in patients with COPD. Since non-respiratory hospitalizations constitute the greatest expense in patients with COPD, modulating the cardiometabolic risk profile is crucial to decrease disease burden and health care costs in COPD. This finding further stresses the importance of developing interventions that can modify the cardiometabolic risk in patients with COPD.

Based on literature in the general population and other conditions including obesity and diabetes, exercise training was suggested to be an interesting intervention to modulate the cardiometabolic risk profile in patients with COPD. However, as was shown in Chapter 3 and 4, exercise training programs as currently employed in COPD do not seem to improve cardiometabolic health, although they are successful in improving exercise capacity. Therefore, it has been suggested that intervention strategies should focus on lifestyle and behavior changes that aid in smoking cessation, improve dietary quality and reduce sedentary behavior. In addition, targeted nutritional supplements may aid in the modulation of the cardiometabolic risk profile. This thesis shows that dietary nitrate is not a promising nutritional supplement to improve cardiometabolic health. Furthermore, there is to date no convincing evidence that resveratrol will significantly decrease the cardiometabolic risk directly, but it may be a promising candidate to decrease lung injury and improve skeletal muscle mitochondrial function in patients with COPD. Targeted intervention strategies to modulate that cardiometabolic risk profile in patients with COPD need further investigation in future research.

KNOWLEDGE UTILIZATION

The present thesis provides important insights for health care providers, including general practitioners and chest physicians. In the Netherlands, newly diagnosed COPD patients in primary care receive education about COPD, are started on pulmonary medication, are offered assistance in smoking cessation, and may be referred to a physiotherapist or dietician. It would be interesting to assess the cardiometabolic risk profile of patients at the time of COPD diagnosis in order to start structural preventive cardiovascular risk management (CVRM) according to international guidelines as soon as possible. The CVRM guidelines describe a cardiovascular risk assessment that is obtained through history taking, physical examination and lab tests. The risk profile consists of: age, gender, pack years smoked, family history of cardiovascular disease, dietary composition, physical activity, alcohol use, blood pressure, body mass index, abdominal circumference, lipid levels, glucose level, and serum creatinine level. Based on this risk assessment, the general practitioner can decide whether or not to start pharmacological or non-pharmacological risk-lowering therapies. This early recognition and awareness of the increased cardiometabolic risk in patients with COPD can contribute to adopting a healthier lifestyle.

The current thesis also underlines the importance of dietary and physical activity guidelines for COPD. These guidelines not only need to be addressed by general practitioners but also by dieticians and physiotherapists. Assessment of dietary intake by dieticians is required, not only to estimate total intake of calories and to identify malnutrition in patients with COPD, but also to identify deficiencies and insufficient intake of macro- and micronutrients that contribute to an increased cardiometabolic risk. Primary aims for physiotherapy in patients with COPD are reducing dyspnea, improving exercise capacity, improving mucus clearance and improving knowledge, self-management and confidence to perform daily physical activities. To modulate the cardiometabolic risk profile in patients with COPD, physiotherapists should pay more attention on the education and self-management aspects of the physiotherapy in order to stimulate a more active lifestyle. An important target in this should be reducing the sedentary time instead of increasing the amount of moderate to vigorous physical activities. Such lifestyle modifications in an integrated approach are expected to result in a behavioral change leading to an improved cardiometabolic health.

The findings in the current thesis are also of great interest for the nutritional and pharmaceutical industry as this thesis provides leads for targeted nutritional and pharmacological interventions for cardiometabolic risk modulation, but also reports a 'hyped' ineffective nutritional intervention. Future studies should focus no only on development but also on implementation of lifestyle interventions in combination with personalized exercise training programs and/or nutritional supplementations. Eventually this should lead to the prevention of developing cardiometabolic diseases in this patient population and decrease the economic and social burden of COPD.

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Curriculum Vitae

Rosanne Beijers was born on September 21st, 1990 in Eijsden. In 2009 she graduated from secondary school Porta Mosana College in Maastricht. In the same year, she started the study Health Sciences at Maastricht University with a major in Human Movement Sciences and a minor in Bioregulation. In 2012 she obtained her bachelor's degree diploma after completing a research internship at Maastricht University, department of Human Movement Sciences. During this internship she investigated the reliability of measurements performed by physiotherapists in the Beweegkuur. In 2012, she started the Master Biology of Human Performance and Health at Maastricht University. During this study Rosanne completed a research internship at the department of Human Movement Sciences in which she investigated the effect of different artificial turfs on soft tissue vibrations in soccer players.

After obtaining her master's degree in 2013, Rosanne started her PhD trajectory at the department of Respiratory Medicine at Maastricht University under the supervision of Prof. Dr. Annemie Schols, Dr. Harry Gosker and Dr. Bram van den Borst. The main focus of her PhD was the cardiometabolic risk profile of normal-weight patients with COPD. Furthermore, she investigated the potential of nutritional and exercise interventions to modulate the cardiometabolic risk in patients with COPD. Currently, she is working as a postdoctoral fellow on a TKI Lung Foundation project, in which she will investigate the effect of targeted nutritional supplementation on adherence to an active lifestyle in patients with COPD.

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*authors contributed equally.