Universidade Estadual de Londrina

WALTER AQUILES SEPÚLVEDA LOYOLA

THEORETICAL FOUNDATIONS AND FUTURE PERSPECTIVES FOR NEW EVALUATIONS AND CLINICAL INTERVENTIONS IN INDIVIDUALS WITH COPD WITH SARCOPENIA:

THE INTERACTION BETWEEN GERONTOLOGY AND PULMONOLOGY

Londrina
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Tese apresentada ao Programa de Pós-graduação em Ciências da reabilitação (Programa associado entre Universidade Estadual de Londrina (UEL) e Universidade Norte do Paraná (UNOPAR)), como requisito parcial para a obtenção do título de Doutor em Ciências da Reabilitação.

Orientadora: Prof[a]. Dr[a]. Vanessa Suziane Probst

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2022

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

T396s Sepúlveda-Loyola, Walter Aquiles .
THEORETICAL FOUNDATIONS AND FUTURE PERSPECTIVES FOR NEW EVALUATIONS AND CLINICAL INTERVENTIONS IN INDIVIDUALS WITH COPD WITH SARCOPENIA: THE INTERACTION BETWEEN GERONTOLOGY AND PULMONOLOGY / Walter Aquiles Sepúlveda-Loyola. - Londrina, 2022.
299 f. : il.

Orientador: Vanessa Suziane Probst.
Tese (Doutorado em Ciências da Reabilitação) - Universidade Estadual de Londrina, Centro de Ciências da Saúde, Programa de Pós-Graduação em Ciências da Reabilitação, 2022.
Inclui bibliografia.

1. DPOC - Tese. 2. Sarcopenia - Tese. 3. Sindromes Geriatricas - Tese. 4. Exercicio - Tese. I. Probst, Vanessa Suziane . II. Universidade Estadual de Londrina. Centro de Ciências da Saúde. Programa de Pós-Graduação em Ciências da Reabilitação. III. Título.

CDU 615.8

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Londrina, 03 de junho de 2022.

DEDICATÓRIA

Dedico a mi familia, a mis padres y hermanos, especialmente a mis abuelos y a mi bisabuela que está en el cielo, que el amor que ellos me han entregado en mi vida, me motivó a trabajar en el area de la geriatría.
También dedico este trabajo a mis profesores y amigos, quienes siempre creyeron en mí. Finalmente, agradezco a Dios quien abrió todas las oportunidades para estar en Brasil, y haber recibido los medios para poder estudiar, conocer diferentes paises, aprender idiomas y ser el primer estudiante extranjero de este programa. Dios me ha permito hacer historia.

SEPÚLVEDA-LOYOLA, Walter Aquiles. **Theoretical foundations and future perspectives for new evaluations and clinical interventions in individuals with copd with sarcopenia: the interaction between gerontology and pulmonology**. 2022, 294 p. PhD Thesis. Program of Masters and Doctoral degree in Rehabilitation Sciences, State University of Londrina (UEL) and University North of Paraná (UNOPAR), Londrina, Brazil, 2022.

ABSTRACT

Introduction: The following doctoral thesis aimed to answer the main gaps in the literature related to the relationship between COPD and sarcopenia, its clinical impact and associated biological factors. Additionally, we have described and defined a new geriatric syndrome and proposed a new exercise program based on functional tasks in order to prevent and treat the functional impact caused by sarcopenia and other geriatric syndromes. **Methods:** Seven studies were included in this thesis, developed in collaboration with different international research centers. In the first study the clinical impact, prevalence and criteria to diagnose sarcopenia in individuals with COPD was investigated. In the second study the main biological mechanisms associated with the prevalence of sarcopenia in individuals with COPD were investigated. The third and fourth studies analysed the relationship between oxidative stress and some clinical variables related to the presence of sarcopenia in individuals with COPD. In the fifth study the criteria and clinical impact of a new geriatric syndrome called osteosarcopenia were presented. The sixth and seventh studies proposed a new exercise intervention method to treat older adults with geriatric syndromes. **Results:** The first study reported that overall sarcopenia prevalence varied from 15% to 34%, being greater in people with more severe condition [37% (95%CI 24.8–50.4)]. In addition, the presence of sarcopenia has a negative impact upon important clinical outcomes. The second study found that oxidative stress, chronic inflammation, genetic and metabolic factors are the main biological mechanism related to the presence of sarcopenia in individuals with COPD. The third and fourth studies reported that the total radical trapping antioxidant parameter (TRAP) and advanced oxidation protein products (AOPP) are important biomarkers related to clinical variables associated to sarcopenia in individuals with COPD and that antioxidant capacity was correlated with muscle mass and strength in this population (r from 0.5 to 0.64). The fifth study reported that osteosarcopenia (using the severe sarcopenia classification) conferred an increased rate of falls [odds ratios (ORs) from 2.83 to 3.63; P <.05 for all] and fractures (ORs from 3.86 to 4.38; P < .05 for all). The sixth and seventh studies found that the exercise intervention method improves the clinical variables of sarcopenia in community older adults. **Conclusion:** Sarcopenia is highly prevalent in individuals with COPD, generating a negative impact on various clinical variables. Its origin is due to the presence of various factors such as oxidative stress and systemic inflammation related to COPD. Sarcopenia may also be associated with low bone mineral mass, a syndrome called osteosarcopenia, which has a greater impact than sarcopenia alone. Functional physical exercise could be a great intervention to reduce the risk of sarcopenia and other geriatric syndromes. Since this exercise method has been studied in community older adults, future clinical randomized control trials are needed in individuals with COPD and other comorbidities.

LIST OF ILUSTRATIONS

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LIST OF ABBREVIATIONS

ACCI	Age-adjusted Charlson comorbidity index
ADLs	Activities of daily living
AOPP	Advanced oxidation protein products
ASM	Appendicular skeletal mass
ASMI	Appendicular skeletal mass index
AWSG	Asian Working Group for Sarcopenia
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BODE index	Body-mass index, airflow obstruction, dyspnea, and exercise capacity index
CACI	Charlson Age-Comorbidity Index
CAT	COPD Assessment Test
CAT	Catalase activity
CIs	Confidence intervals
CC	Calf circumference
COPD	Chronic Obstructive Pulmonary Disease
CoP	Center of pressure
CRP	C-reactive protein
DEXA	Dual-energy x-ray absorptiometry
EQ-5D index	EuroQol five-dimensiona
EWSGOP	European Working Group on Sarcopenia in Older People
FEV_1	Forced expiratory volume in 1 second
FEC	Functional exercise circuit
FFM	Fat free mass
FFMI	Fat free mass index
FNIH	Foundation for the National Institutes of Health
FVC	Forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GS	Gait speed
GST	Glutathione-S-transferase
HDL-C	high-density lipoprotein cholesterol

HGF	Hand grip force
HR	Hazard ratio
ICD-10	International Statistical Classification of Diseases and Related Health Problems of the World Health Organization
IGF-1	Insulin-like growth factor 1
IL-6	Interleukin-6
IL-8	Interleukin-8
ISWT	Incremental shuttle walk test
LDL-C	Low-density lipoprotein cholesterol
MDA	Melondiadehyde
NF-kB	Nuclear factor-Kb
NOX	Nitric oxide metabolites
OS	Oxidative stress
MEP	Maximal expiratory pressure
METS	Metabolic Syndrome
MIP	Maximal inspiratory pressure
MRC	Medical Research Council
PON1	Paraoxonase 1
QF	Quadriceps force
QS	Quadriceps strength
ROS	Reactive oxygen species
SCWD	Society on Sarcopenia, Cachexia and Wasting Disorders
SH	Sulfhydryl group
SGRQ	St George’s respiratory disease questionnaire
SIG	Special Interest group
SM	Skeletal muscle mass
SMI	Skeletal muscle mass index
SOD	Superoxide dismutase activity
SD	Standard deviation
SPPB	Short physical performance battery
SPSS	Statistical Package for the Social Sciences
TARBS	Thiobarbituric acid reactive substances
TNF-α	Tumor necrosis factor alpha

TRAP	Total radical trapping antioxidant parameter
3.4MGS	3.4-meter gait speed
4MGS	4-meter gait speed
5STS	5-repetition sit-to-stand test

SUMMARY

INTRODUCTION

The following doctoral thesis aimed to answer the main gaps in the literature related to the relationship between chronic pulmonary disease (COPD) and sarcopenia, its clinical impact and associated biological factors. Additionally, we have described and defined a new geriatric syndrome and proposed a new exercise program based on functional tasks in order to prevent and treat the functional impact caused by sarcopenia and other geriatric syndromes.

COPD is a preventable and treatable pulmonary disease, which is characterized by persistent respiratory symptoms and airflow limitation due to airway and /or alveolar abnormalities caused by significant exposure to noxious particles or gases[1]. Additionally, there are extra pulmonary changes, such as declining in aerobic capacity, endurance, strength and balance, which affect the performance in activities of daily living (ADLs), impairing quality of life[2–6]. Individuals with COPD have lower levels of physical activity, which can decrease muscle mass and produce muscle weakness[7,8]. Muscle weakness increases in the presence of chronic diseases and is directly related to sarcopenia[9]. This happens since chronic diseases, such as COPD, produce systemic inflammation[10] and oxidative stress[11], which contribute to age-related deficits in muscle mass[12], through increased damage to the muscle cell [9,13].

Sarcopenia is a disease characterized by lower muscle mass, muscle strength and physical performance[14,15]. It is a significant contributor to frailty in the elderly population which is associated with hospitalization, disability and mortality[16,17]. In COPD, the presence of sarcopenia has been associated with different clinical implications such as changes in body composition, physical activity, exercise capacity, quality of life, systemic inflammation, oxidative stress and health status[10,18–21]. In addition, sarcopenia is more prevalent among those individuals with worse disease severity and is related to poorer prognosis in COPD[18,22].

The prevalence of sarcopenia is approximately 5%-13% in the elderly population[9,14]. However, in subjects with COPD, sarcopenia is reportedly higher, with a range between 15%[22] to 55%[23]. The variability in these prevalence estimates can be attributed to the different criteria used to define

sarcopenia[18,22,24]. Although international recommendations exist for the diagnosis of sarcopenia in older people[14,17,25], consensus has not yet been reached regarding the best criteria to apply to adults with COPD and its clinical relevance. Therefore, the first gap in the literature that this PhD thesis addressed was the identification of the main diagnostic criteria for sarcopenia, its prevalence and clinical impact in individuals with COPD (Chapter II).

COPD is a systemic disease and there is strong evidence showing that pathophysiological changes are related to an imbalance between oxidant and antioxidant substances, producing oxidative stress (OS)[11]. However, according to a systematic review, there are some contradicting results about a few biomarkers in COPD compared with control groups, such as advanced oxidation protein products (AOPP), sulfhydryl group (SH), superoxide dismutase activity (SOD) and catalase activity (CAT)[26]. In addition, the total radical trapping antioxidant parameter (TRAP), which has been associated to increase in muscle strength in older people[27], has been little studied in COPD. Hence, the second gap in the literature that this thesis addressed was the association between theses blood biomarkers with sarcopenia in individuals with COPD (Chapter III).

Although there is evidence showing that OS induces more inflammation[9,13], muscular atrophy[28] and sarcopenia[28], no direct associations have been shown between clinical measurements of sarcopenia (muscle mass, muscle strength and physical performance) and OS biomarkers in subjects with COPD. On the other hand, oxidative stress biomarkers play an important role in the multiple comorbidities associated with COPD[29], such as metabolic syndrome (MetS)[30]. MetS can be considered as a “clustering” of risk factors for sarcopenia, associated with insulin resistance, dyslipidemia, central obesity, hypertension, and impaired glucose tolerance [31]. A recent systematic review showed that the prevalence of MetS in patients with COPD is around 34%, being higher compared with controls[32]. Both MetS as well as COPD have been associated with increase in reactive oxygen species (ROS), causing OS. The relationship between these biomarkers, the presence of OS and its impact on measurements related to sarcopenia and MetS in patients with COPD had not yet been clarified. Therefore, this PhD thesis studied this third gap in the literature (Chapter III).

Sarcopenia is related to a high risk of falling[33] and its presence in individuals with COPD is probably one of the major factors related to the presence of balance problems in more advanced stages of the disease[34]. Severe sarcopenia in particular, defined as the presence of low muscle mass, physical performance and strength[14] may result in additional adverse outcomes including an increased risk of falls and fractures. Furthermore, there is increasing evidence of crosstalk between muscle and bone. In the case of sarcopenia, greater systemic inflammation and oxidative stress have been found, both of which are related to reduced bone mineral density (BMD)[35–37]. With regards to osteopenia and osteoporosis, both are strongly associated with fracture risk and frailty,[38] resulting in declines in quality of life and increased mortality, particularly in those elderly who experience hip fractures[39]. On the other hand, individuals with COPD presented several clinical risk factors for osteoporosis, including older age, emaciation, physical inactivity, and vitamin D deficiency[40]. The concomitant presence of osteoporosis or osteopenia combined with sarcopenia is defined as osteosarcopenia[41,42]. This new geriatric syndrome associates with higher disability, fracture and falls in older people[43,44] compared to either disease alone[45]. Although this new geriatric syndrome could probably impose a more pronounced negative impact on the functionality of older adults, its diagnostic criteria have not been well established yet. For this reason, as a fourth gap in the literature this thesis defined and described the main criteria and clinical impact of osteosarcopenia (Chapter IV).

In this PhD thesis, we have analyzed, for the first time, the functional impact of sarcopenia on individuals with COPD and the associated biological factors by developing a systematic review and two original studies. Additionally, we have defined and characterized a new geriatric syndrome (osteosarcopenia) that could be studied in individuals with COPD and other chronic conditions. Considering that the presence of geriatric syndromes such as sarcopenia causes great functional impact in older adults with COPD and other chronic conditions, we have proposed an exercise intervention program based on functional tasks called functional exercise circuit (FEC), which has improved the clinical variables of sarcopenia in community older adults. Future research should study the effects of this dual-task training protocol in older adults with COPD and other chronic

conditions or geriatric syndromes, such as frailty or osteosarcopenia and in other clinical contexts (nursing homes, hospitals, etc.) (Chapter V)

Our research developed during the PhD shines some light on the study of older adults with COPD, demonstrating the importance of evaluating and diagnosing geriatric syndromes in this population and carrying out exercise interventions not only focused on the aerobic capacity and muscle strength, but also considering the different functional alterations that these syndromes cause on balance, coordination, agility and power. Additionally, since some biomarkers of oxidative stress have been associated with the presence of sarcopenia in COPD, this opens a new field of study to clarify which biological biomarkers could be used to diagnose sarcopenia and help in developing a more tailored pharmacological and nutritional treatment for this population. On the other hand, we have initially analysed the effects of the functional exercise circuit on community older adults in clinical variables related to sarcopenia, which has been previously studied in individuals with COPD. Therefore, since positive effects have been reported in community older adults, we considered, as future perspectives, that randomized clinical trials are need in this field using our exercise protocol in individuals with COPD and other clinical conditions.

1. CHAPTER I. LITERATURE REVIEW

1.1 PRESENTATION

In order to avoid repeating information, in this section, we have made a brief presentation of the different topics covered in this thesis. The following topics will be discussed in more depth in the corresponding articles organized from chapter II to V:

1. *Clinical impact, prevalence and criteria to diagnose sarcopenia in individuals with COPD:* this topic will be presented as an article entitled "Diagnosis, prevalence and clinical impact of sarcopenia in COPD: a systematic review and meta-analysis" (published in Journal of Cachexia, Sarcopenia and Muscle) [46]
2. *Biological mechanisms related to sarcopenia in patients with COPD:* this topic will be presented as three different articles entitled: 1) "Pathophysiological mechanisms of Sarcopenia in COPD" (published in Revista Chilena de Enfermedades Respiratorias)[47] 2) "NOVEL antioxidant and oxidant biomarkers related to sarcopenia in COPD" (Published Heart and Lung)[48] and 3) "Is oxidative stress associated with disease severity, pulmonary function and metabolic syndrome in Chronic Obstructive Pulmonary Disease?" (published in Revista Clinica Española)[49]
3. *A new geriatric syndrome to study in individuals with COPD and in other population:* this topic will be presented as an article entitled "The join occurrence of osteoporosis and sarcopenia (osteosarcopenia): Definitions and Characteristics" (published Journal of the American Medical Directors Association)[50]
4. *A new exercise protocol, based on functional tasks, for older adults to prevent and treat patients with geriatrics syndromes:* this topic will be presented as two articles: 1) "Effects of an exercise model based on functional circuits in an older population with different levels of social participation" (published in the Journal Geriatric Gerontology International)[51] and 2) "Effect of dual-task training on clinical and biological factors related to sarcopenia in older adults" (formmated according to the reccommendations of the Journal Geriatric Gerontology International)

1.2 SARCOPENIA

In recent years, sarcopenia has been studied as one of the main geriatric syndromes that generates a great negative impact on functionality, which is associated with frailty, disability and mortality in the elderly population[52]. Although sarcopenia was initially a term used only to define the loss of muscle mass that occurs with aging[53], nowadays, sarcopenia has gained importance due to its serious consequences for health and functionality, even being considered a disease according to the International Statistical Classification of Diseases and Related Health Problems (ICD 10) of the World Health Organization[54].

Sarcopenia has been defined as the loss of muscle mass combined with loss of strength or physical performance by the European and Asian consensus on sarcopenia in older adults[14,25]. However, despite the fact that these definitions have been widely used by other researchers[55], there are currently different controversies, regarding diagnostic evaluations or criteria and cut-off points used to define low muscle mass, strength or physical performance[56]. In Latin America, most of the studies have used the European consensus to diagnose sarcopenia[57].

1.3 SARCOPENIA: PREVALENCE, IMPACT ON HEALTH AND FUNCTIONALITY

The prevalence of sarcopenia has great variability given by determinants such as geographical location, age, gender, education, social conditions, comorbidities and the measurement methods adopted [56,58,59]

According to an international report, the prevalence of sarcopenia is 1–29% in populations living in the community, 14–33% in chronic care populations, and 10% in the acute hospital care in individuals older than 50 years[60].

In Latin America, in a study with 5046 Mexican adults with an average age of 69.92 ± 7.73 years, the prevalence of pre-sarcopenia was 8.7% and 13.3% for sarcopenia, being higher in women[61]. While in Colombia the prevalence of sarcopenia can reach 11.5% in adults older than 60 years[62]. A little higher is the prevalence in Brazil where it can reach up to 16%, being also more prevalent in

women[57]. In Chile, the prevalence of sarcopenia is 19.1%, without great differences between men or women[57].

Sarcopenia influences the quality of life of older adults, bringing secondary consequences such as risk of falls, fractures, hospitalization and high rates of comorbidity, disability and mortality[63,64]. In addition, sarcopenia has been related to decreased functionality in the elderly population in aspects related to locomotion, balance, performance in basic and instrumental activities of daily life, as well as participation in social roles appropriate for their age and increased depressive symptoms[51,62,65–67]. Thus, the study, diagnosis, prevention and treatment of sarcopenia are essential to improve health and functionality of the elderly population.

1.4 SARCOPENIA: DIAGNOSTIC CRITERIA

Currently, there are 7 different groups of specialists who have defined sarcopenia[14,17,25,68–71], with different cut-off points or reference values (Table 1). This table was published in an article developed with our research group[59]. These consensuses represent populations from different parts of the world. The Asian Working Group for Sarcopenia (AWSG)[25], the European Working Group on Sarcopenia in Older People (EWGSOP)[14], and the Foundation for the National Institutes of Health (FNIH)[68] have defined sarcopenia in a similar way, considering it as a syndrome characterized by loss of muscle mass, muscle strength and physical performance. Likewise, the consensus of the Australian and New Zealand Society for Sarcopenia and Frailty Research[71] have chosen to use the criteria proposed by the European consensus, without new cut-off points for these populations.

On the other hand, there is the consensus of the International Working Group on Sarcopenia (IWGS)[69] that has defined sarcopenia as loss of muscle mass combined with low physical performance. Additionally, the Special Interest Group (SIG)[70] and the Society on Sarcopenia, Cachexia and Wasting Disorders (SCWD)[17], have also adopted this definition.

Despite the differences between the definitions and cut-off points that exist among the consensuses, all international groups agree that sarcopenia is no

longer the definition proposed by Rosenberg in 1997, as a loss of muscle mass that occurs with age[53]. Nowadays, sarcopenia is a combination of loss of muscle mass with decreased muscle function (strength and/or physical performance).

Table 1: Reference values to diagnose sarcopenia

Sarcopenia	***Low muscle mass***	***Low Muscle Strength***	***Low Physical Performance***
EWGSOP 2010	SMMI: <7.23 kg/m^2 for men and <5.67 kg/m^2 for woman	FPM: <30 Kg for men and < 20 Kg for woman	
EWGSOP 2018	ASM: ≤ 20 kg/m^2 for men and ≤ 15 kg/ m^2 for woman	FPM: <27 Kg for men and < 16 Kg for woman	
AWGS	ASMI: ≤ 7.0 Kg/m^2 for men and ≤ 5.4 Kg/m^2 for woman	HGS: <26 Kg for men and < 18 Kg for woman	VC ≤0.8 m/s
FNIH	ASM/BMI: <0.789 for men and < 0.512 for woman	HGS: <26 Kg for men and <16 Kg for woman	
SIG	≤ 2 standard deviations in a gender-specific mean for a young reference group.	No considered	
IWGS	SMMI: <7.23 kg/m^2 for men and <5.67 kg/ for woman	No considered	GS <1.0 m/s
SCWD	≤ 2 standard deviations from the mean ASM/height in a young reference population of the same sex and ethnicity.	No considered	6MWT < 400 m

AWGS: Asian Working Group for Sarcopenia; ASMI: appendicular skeletal muscle index; ASM: appendicular skeletal muscle mass; BMI: body mass index; EWGSOP: European Working Group on Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health Sarcopenia Project; GS: gait speed; IWGS: International Working Group on Sarcopenia ; SIG: Special Interest Group; SMI: skeletal muscle mass index; SCWD: Society on Sarcopenia, Cachexia and Wasting Disorders; 6MWT: 6 min walking test.

1.5 COPD AND SARCOPENIA

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of death worldwide[72]. It is a highly prevalent disease affecting up to 10% of adults over 40 years old, leading to disability and impairing quality of life[7,72]. COPD has been associated with different non-respiratory disorders, such as cardiovascular and metabolic problems[73], physiological alterations[74], balance impairment[75], systemic inflammation[10], oxidative stress[26] and sarcopenia[10,76].

The prevalence of sarcopenia in individuals over 65 years old is between 5%-13%[77], however, in subjects with COPD it is higher and may vary from 15%[22] to 55%[23]. In COPD, the presence of sarcopenia has been associated with changes in body composition, physical activity, exercise capacity, systemic inflammation and health status[9,24,27], being more prevalent among patients in quartile 3 or 4 of the BODE index (body-mass index, airflow obstruction, dyspnea, and exercise capacity index)[76]. Therefore, sarcopenia is related to disability and a poor prognosis in individuals with COPD[22,76]. Most of the information regarding the prevalence, etiology and clinical impact of the sarcopenia in individuals with COPD will be presented in Chapter II and III.

1.5 STATE OF THE ARTE ABOUT INTERVENTION IN INDIVIDUALS WITH COPD WHO HAVE SARCOPENIA

According to the American Thoracic Society and the European Respiratory Society Statement, rehabilitation programs in individuals with COPD have mainly focused on improving the aerobic capacity, peripheral and respiratory muscle strength, flexibility and physical activity levels [78]. Most of the literature in this field has followed this statetment[79]. Taking into account that individuals with COPD have secondary impairments beyond their respiratory symptoms (balance impairments, muscle weakness, etc.), other exercises modalities such as balance training and functional exercise have been proposed[80], however there is scarce evidence published so far[79–81].

The rehabilitation programs for older adults with sarcopenia included multicomponent exercises programs with resistance training, aerobic exercise, stretching, balance, dual tasks training and functional exercise[82]. However, most of these modalities have not been considered in the classical pulmonary rehabilitation programs for individuals with COPD with and without sarcopenia. One of the few studies to explore rehabilitation in individuals with COPD who have sarcopenia was conducted by Jones *et al.* [83]. This study demonstrated that multicomponent exercise-based intervention, improved a range of clinical variables associated with functional performance, exercise capacity, muscle strength and health status in patients with COPD. This exercise program included aerobic exercise, lower and upper limb resistance training and education classes

covered a variety of self-management topics (exercise, mediation, diet, increasing physical activity, etc.). However, this exercise program[83] did not include other important modalities such as balance, coordination, cognitive capacity or proprioceptive training, which are important to intervene in individuals with sarcopenia. Therefore, considering the functional impact of sarcopenia on different physical variables in individuals with COPD, it is necessary to incorporate other exercises modalities in the pulmonary rehabilitation which have not been fully considered by the international recommendations[78,84] (Figure 1).

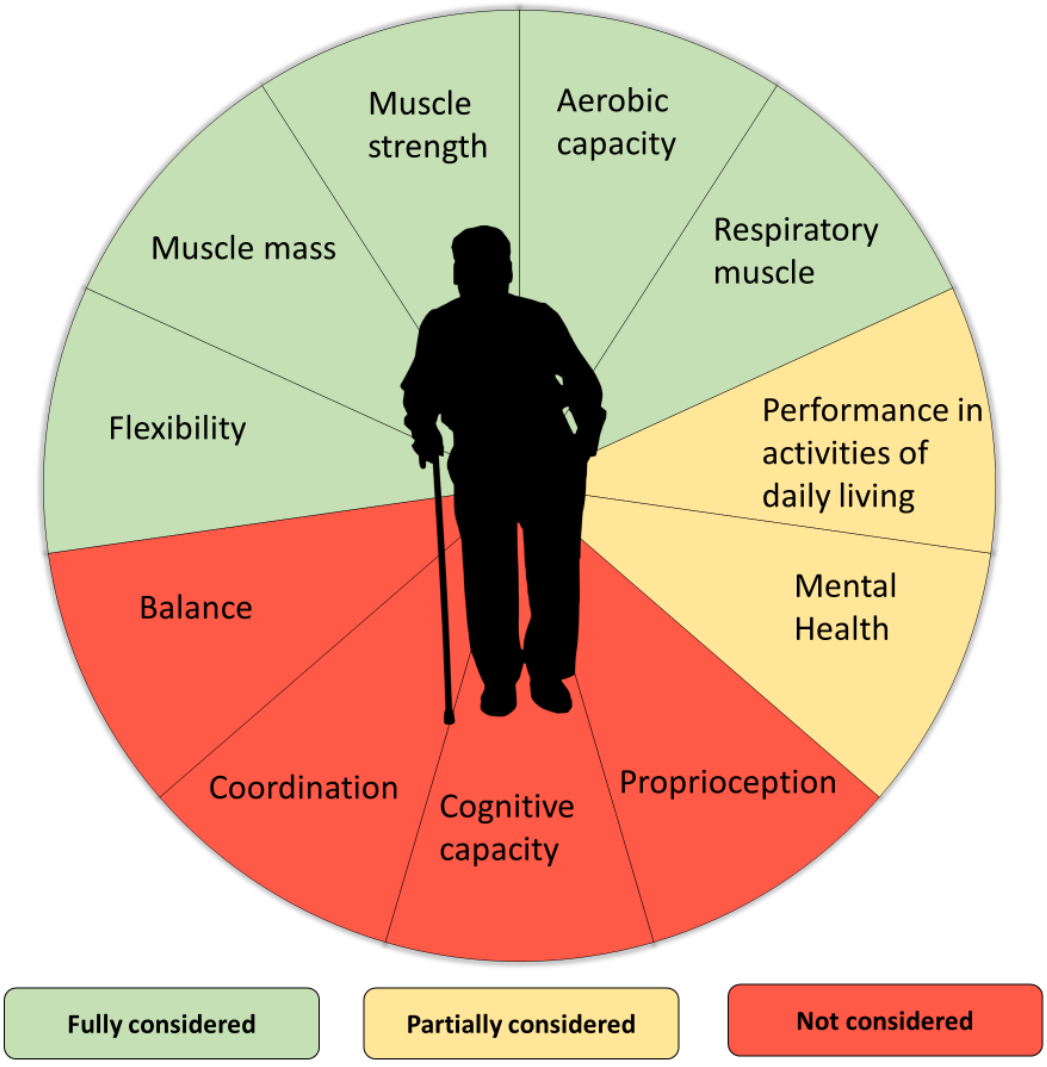

Figure 1: Clinical variables that sarcopenia negatively impacts in older adults. The different colors present those variables that are fully (green), partially (yellow), or not (red) considered in the rehabilitation program for individuals with COPD according to international statements.

According to an umbrella review of systematic review about the effectiveness of exercise in treating sarcopenia, there is lack of high quality research in this field[85]. Additionally, the best modality (resistance, aerobic, balance, etc) and dose (frequency, repetition, duration) have not been totally clarified, and there is a lack about specific exercises protocols for clinical variables of sarcopenia[85]. For this reason, we have proposed a protocol of multimodal exercise using functional tasks explaining the dose in each modality. This exercise protocol was initially tested in older adults from the community (Chapter IV) and we hope to be able to study, in the near future, the effects of this protocol in individuals with COPD and other populations with chronic conditions.

2.CHAPTER II. CLINICAL IMPACT, PREVALENCE AND CRITERIA TO DIAGNOSE SARCOPENIA IN INDIVIDUALS WITH COPD

PRESENTATION:

In this chapter the following article is presented: “Diagnosis, prevalence and clinical impact of sarcopenia in COPD: a systematic review and meta-analysis”. This article was developed in collaboration with researchers from Londrina State University (Brazil), Monash University (Australia) and University of Melbourne (Australia). This Article was published in the Journal of Cachexia, Sarcopenia and Muscle.

2.1: SYSTEMATIC REVIEW

Title: Diagnosis, prevalence and clinical impact of sarcopenia in COPD: a systematic review and meta-analysis.

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Article published in *Journal of Cachexia, Sarcopenia and Muscle*

(Q1/ Impact factor: 10.74)

Sepúlveda-Loyola W, Osadnik C, Phu S, Morita AA, Duque G, Probst VS. Diagnosis, prevalence, and clinical impact of sarcopenia in COPD: a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle. 2020;11(5):1164–76

ABSTRACT

Background: Sarcopenia prevalence and its clinical impact are reportedly variable in chronic obstructive pulmonary disease (COPD) due partly to definition criteria. This review aimed to identify the criteria used to diagnose sarcopenia and the prevalence and impact of sarcopenia on health outcomes in people with COPD.

Methods: This review was registered in PROSPERO (CRD42018092576). Five electronic databases were searched to August 2018 to identify studies related to sarcopenia and COPD. Study quality was assessed using validated instruments matched to study designs. Sarcopenia prevalence was determined using authors' definitions. Comparisons were made between people who did and did not have sarcopenia for pulmonary function, exercise capacity, quality of life, muscle strength, gait speed, physical activity levels, inflammation/oxidative stress and mortality.

Results: 23 studies (70% cross-sectional) from Europe (10), Asia (9), and North and South America (4) involving 9637 participants aged ≥40 years were included (69.5% male). Sarcopenia criteria were typically concordant with recommendations of European and Asian consensus bodies. Overall sarcopenia prevalence varied from 15.5% (95% confidence interval [CI] 11.8-19.1; combined muscle mass, strength and/or physical performance criteria) to 34% (95%CI 20.6-47.3; muscle mass criteria alone) (p=0.009 between-subgroups), and was greater in people with more severe (37.6% [95%CI 24.8-50.4]) versus less severe (19.1% [95%CI 10.2-28.0]) lung disease (p=0.020), but similar between males (41.0% [95%CI 26.2-55.9%]) and females (31.9% [95%CI 7.0-56.8%]) (p=0.538). People with sarcopenia had lower predicted forced expiratory volume in the first second (mean difference -7.1%; 95%CI -9.0 to -5.1%), and poorer exercise tolerance (standardized mean difference [SMD] -0.8; 95%CI -1.4 to -0.2) and quality of life (SMD 0.26; 95%CI 0.2-0.4) compared to those who did not (p<0.001 for all). No clear relationship was observed between sarcopenia and inflammatory or oxidative stress biomarkers. Incident mortality was unreported in the literature.

Conclusions: Sarcopenia is prevalent in a significant proportion of people with COPD and negatively impacts upon important clinical outcomes. Opportunities exist to optimize its early detection and management, and to evaluate its impact on mortality in this patient group.

Keywords: Sarcopenia, COPD, prevalence, diagnosis, aging.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a condition characterized by chronic inflammation[1] and extrapulmonary changes that negatively affect physical function (*e.g*. lower levels of physical activity[2], and reductions in muscle mass and strength[3,4]) and quality of life[5,6]. The presence of such factors is also closely related to the presence of sarcopenia[7], a syndrome characterized by lower muscle mass, muscle strength and physical performance[7]. Sarcopenia is a significant contributor to frailty in the elderly population, and is associated with increased rates of falls, hospitalization and mortality[8,9]. It has been estimated to occur in approximately 5%-13% of the 'healthy' older population[4,7].

People with COPD appear to have an increased risk of developing sarcopenia, with prevalence estimates ranging from 15%[10] to 55%[11]. In this patient group, sarcopenia appears to confer a negative impact upon clinical outcomes related to function and health[1,3,12–14] and its prevalence appears to increase with increasing COPD-related impairment. Although sarcopenia has also been shown to contribute towards poorer prognosis in people with COPD[3,10], the real clinical impact has not yet been analyzed. Additionally, the wide-ranging prevalence estimates of sarcopenia in COPD, however, makes its true impact somewhat difficult to accurately ascertain.

A significant factor contributing to this large variability appears to be choice of definition criteria[3,10,15]. International recommendations exist for the diagnosis of sarcopenia in older people such as those proposed by the European Working Group of Sarcopenia in Older People (EWGSOP)[7] and the Asian Group of Sarcopenia (AWGS)[16], yet these have not featured in published literature in the field of COPD. Considering the prevalence of both sarcopenia and COPD increase with increasing age, the impact of sarcopenia on a broader range of clinically important COPD-related outcomes is also not currently clear. This review therefore aimed to evaluate the literature pertaining

specifically to people with COPD to identify the criteria used to diagnose sarcopenia, estimate its prevalence and evaluate its impact upon health outcomes.

METHODOLOGY

Data sources and search strategy

The protocol for this review was registered in PROSPERO (CRD42018092576). Five electronic databases (PubMed, LILACS, EMBASE, The Cochrane Library and Scielo) were searched from inception until August 2018 using the following free-text and subject headings terms: 'COPD', 'pulmonary disease, chronic obstructive', 'chronic obstructive lung disease', 'COAD', 'chronic obstructive airway disease' and 'sarcopeni*' (Table S1). Hand-searching of reference lists from included articles was also conducted to identify additional potential studies. To be eligible for inclusion, studies must have been conducted on adults with COPD (aged ≥40 years), defined according to authors, irrespective of disease severity (GOLD criteria[17]) and reported upon a diagnosis of sarcopenia, defined according to any criteria provided it was stated in the methodology. Considering the nature of our research question, we included observational (*e.g.* cohort) and cross-sectional studies and clinical trials (whether randomized or not). Abstracts and publications published in languages other than English, Spanish or Portuguese were not eligible for inclusion.

The principal outcomes for this review were: 1) the criteria used to define sarcopenia and its prevalence; and 2) clinical data from studies that provided comparative data between people with COPD who did and did not have sarcopenia, as follows: a) quality of life, from either generic or respiratory-specific quality-of-life questionnaires; b) physical function, derived from common clinical tests of exercise capacity, muscle strength and balance; c) physical activity levels, measured by objective physical activity monitors; d) pulmonary function, measured by spirometry (*e.g.* FEV_1%

predicted); e) inflammatory or oxidative stress biomarkers (*e.g.* IL-6, TNF-α, protein-C reactive, catalase, paraxonase-1); and f) all-cause mortality.

Data management and quality appraisal

Database search yields were collated within a bibliographical reference manager software (State of the Art through Systematic Review [StArt] v.3.03[18]) and duplicates discarded. Citations were screened for eligibility upon title and abstract by two independent reviewers (W.S.L and A.A.M), and classified as either 'include', 'exclude' or 'maybe'. Those deemed 'include' or 'maybe' were reviewed in full text to derive a final yield, with any disagreements resolved via a third, independent assessor (V.S.P). This process was summarized in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations[19]. Data were extracted by two members of the team (W.S.L and A.A.M) using standardized templates appropriate for the study objectives.

Study quality was appraised using validated instruments tailored according to study design, as follows: (i) NIH Quality Assessment Tool for Observational Cohort and Cross-sectional studies, to assess the quality of cohort and cross-sectional studies; (ii) PEDro scale to assess the quality of randomized clinical trials; and (iii) JBI Critical Appraisal Checklist for Quasi-Experimental Studies to assess the quality of non-randomized controlled trials.

Statistical analysis

An overall estimate of sarcopenia prevalence was derived by pooling the proportion of patients with COPD who had detected sarcopenia in individual studies in a meta-analysis. For this purpose, only one prevalence estimate was used from each study. Where individual studies reported different types of sarcopenia (*e.g.* sarcopenia with normal body mass index, sarcopenic obesity, severe sarcopenia), an aggregated value, if able to be determined, or the most 'conventional' type was used. In order to

avoid double-counting, estimates from individual studies that evaluated sarcopenia via multiple diagnostic criteria (*e.g.* comparisons of different cut-off thresholds within a single cohort) were pooled using their primary stated method or that which most closely resembled the current EWGSOP recommendation[7,20]. Where able to be conducted, separate subgroups analysis were conducted to compare prevalence effect estimates between sarcopenia definitions (1 vs >1 diagnostic criteria), gender (male vs female) and disease severity (GOLD I-II vs III-IV), evaluated via Chi^2 test. This meta-analysis was performed via the 'metaprop' command in Stata SE 14.2 (Texas, USA) with 95% confidence intervals (95%CI) calculated using the score (Wilson) method, and a random-effects model (DerSimonian and Laird method) utilized due to the variability in sarcopenia definitions across studies.

Clinical outcome data from studies comparing people with COPD who did and did not have sarcopenia were meta-analysed via Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen 2014). Continuous outcome data evaluated using homogenous metrics (*e.g.* same test instrument) were summarized as mean differences (MD), while data arising from heterogenous metrics (*e.g.* same construct, different instrument) were summarized as standardized mean differences (SMD) and 95%CI. A random-effects model was used as the principal method of analysis, with statistical heterogeneity described via the I^2 statistic and interpreted according to Deeks and colleagues (values <25% considered low, 50%-75% moderate, and >75% high)[21].

RESULTS

A detailed summary of the literature search is provided in Figure 1. Two hundred and seventy-two unique records were identified through database searching, resulting in 23 articles involving 9637 participants included in the final review. Of these, 17 adopted a cross-sectional design, 5 were observational cohort studies and 1 was a non-randomized clinical trial. Most studies included patients with differing histories of smoking

(those who never smoked, former and current smokers). Comparative data between people with COPD who did and did not have sarcopenia were available from 17 studies. The overall quality of included studies was 'moderate' (full details in Table S2). Characteristics of included studies are presented in Table 1. The review sample spanned a diverse range of populations, including 10 studies from Europe, 9 from Asia and 4 from South America. Most participants were male (69.5%).

Methods used to assess sarcopenia

A summary of diagnostic criteria used to assess sarcopenia in the included studies is presented in Table 1. Measures of low muscle mass (LMM)[2,14,22–42], low muscle strength (LMS)[2,23,26–29,35] and low physical performance (LPP)[2,23,26,27,29,33–35] were used as the basis of diagnosis. Fourteen studies used LMM as the sole criteria to diagnose sarcopenia, while LMM was combined with LMS and/or LPP in nine studies[2,23,26–29,33–35]. Those studies utilized different cut-off points and methods to identify LMM, LMS and LPP. Muscle mass was measured by dual energy x-ray absorptiometry (DXA) (16 studies)[14,22,27,29–34,36,38–40,42], bio-electrical impedance analysis (BIA) (6 studies)[2,25,26,28,35,37,41] and calf circumference (1 study)[23]. Muscle strength was measured via handgrip dynamometry (7 studies)[2,23,26–29,35]. Physical performance was measured via gait speed (4 studies) [23,26,35,37] and six-minute walk test (6MWT) (4 studies) [27,29,33,34]. The different cut-off thresholds used to define 'positive' responses to each test are presented in Table 2. Muscle mass, muscle strength and physical performance were most commonly evaluated according to cut-off thresholds recommended by the EWGSOP[7] and the AWGS[16]. Comparisons between the main guidelines used to detect sarcopenia in individuals with COPD are available in Table S3.

Sarcopenia prevalence

Data were available for meta-analysis from 22 studies involving 9416 participants. The overall pooled prevalence estimate of sarcopenia in people with COPD

was 27.5% (95%CI: 18.4-36.5; Figure 2). These effect estimates were significantly higher in studies that used a single criterion (LMM; 34%, [95%CI 20.6-47.3]) than those that used > 1 criteria (LMM + LMS and/or LPP; 15.5% [95%CI 11.8-19.1]). The high statistical heterogeneity in this analysis (I^2 = 99.3%) meant individual study weighting was uniform (range 4.1-4.7%). In the studies that provided data specific to gender, sarcopenia was found to be higher in males (41.0% [95%CI 26.2-55.9) than in females (31.9% [95%CI 7.0-56.8]), however this difference was not statistically significant (p = 0.538) and gender did not predict effect size in meta-regression (Figures S1-S2). In the studies that provided data specific to disease severity, sarcopenia was found to be significantly higher in patients with more severe disease (GOLD stages III-IV; 37.6% [95%CI 24.8-50.4]) than those with less severe disease (GOLD stages I-II; 19.1% [95%CI 10.2-28.0]; test for between-group differences p = 0.020), with the proportion of participants having more severe disease being strongly predictive of effect sizes in meta-regression with high explanatory power (regression coefficient 0.715 [95%CI 0.342 to 1.088], p = 0.006; adjusted R^2 = 90.1%) (Figures S3-S4).

Impact of sarcopenia on clinical outcomes

Data from 11 studies involving 5367 participants were available for meta-analysis of pulmonary function, showing those with sarcopenia had, on average, poorer FEV_1% predicted than those without sarcopenia (MD -7.07% [95%CI -9.03 to -5.11]; I^2 = 83%, Figure 3a).

Data from 6 studies involving 2252 participants were available for outcomes related to exercise capacity. These were measured via the 6MWT[22,28,41], incremental shuttle walk test[2] and cardiopulmonary incremental cycle test[31,39]. Having sarcopenia was associated with poorer performance compared to those without sarcopenia (SMD -0.77 [95%CI -1.35 to -0.18]; I^2 = 96%, Figure 3b).

Four studies involving 1996 participants reported data on quality of life via the COPD Assessment Test[2,28] and St George's respiratory disease questionnaire[2,22,41] were included in the meta-analysis. Having sarcopenia was associated with poorer quality of life (SMD 0.42 [95%CI 0.07 to 0.77; I^2 = 85%, Figure 3c). Other studies not included in the meta-analysis reported similar findings[29,30] (Table 3).

A summary of findings related to the remaining review outcomes is presented in Table 3, however quantitative meta-analysis was not possible due to lack of sufficient data. Compared to non-sarcopenic individuals, those with sarcopenia had worse physical function (as measured by tests of balance, gait speed, strength and general daily function)[2,32,35], lower levels of daily physical activity[2,24,30,36], increased levels of dyspnoea during daily activities[2,28] and a heightened mortality risk, as measured via BODE[2,28,34]. Sarcopenia was more prevalent in the 4th quartile of BODE, ranging from 25% to 63.6%[2,28,34]. With respect to inflammatory biomarkers, CRP, IL-6 and TNF-α were reported to be higher[28,35,39] or not different[22,41] in subjects with sarcopenia compared to those without it. No differences were detected in levels of Fibrinogen[41] and IL-8[41]. No findings related to oxidative stress were reported in the included literature.

DISCUSSION

This systematic review and meta-analysis offers unique insight into the clinical relevance of sarcopenia for people with COPD. It describes the prevalence of the condition and how this is impacted by use of different criteria, cut-off thresholds and definitions, as well as rigorous examination of the effect of sarcopenia on important health outcomes related to pulmonary and physical function, quality of life, blood biomarkers, prognosis and risk of mortality.

Two predominant strategies appear to be in use to classify sarcopenia in COPD: definitions based upon independent assessment of LMM[22,24,30,32,36,38,41] , and definitions

that include both LMM and either LMS or LPP[2,23,26–29,33–35,37]. Use of LMM alone resulted in an estimated pooled prevalence of 34%, while LMM combined with LMS and/or physical function reduced this figure to 15.5%. Such variability has been previously reported in community-dwelling older adults[43]. Sarcopenia definition variability thus also likely explains some of the varied prevalence estimates in people with COPD. This relationship may not come as a surprise, as increasing the number of mandatory elements within a sarcopenia definition will inevitably reduce the incidence of detecting a 'positive' diagnosis. The trade-off of doing so, however, is a likely improvement in diagnostic accuracy. This is a significant premise underpinning current international recommendations[7,20,44,45] which sees sarcopenia defined as a geriatric syndrome[7,16,44,45] or disease[20] characterized by both LMM and LPP, not just LMM[46,47]. Only 9 of the included studies[2,23,26–29,33,35] implemented a definition of sarcopenia that would satisfy these new recommendations (Table 1). Our data suggest some of the variability in prevalence estimates is likely attributable to disease severity, with every 1% increase in study sample having GOLD stages III-IV increasing sarcopenia prevalence by 0.7%. While this relationship was not unexpected based on previous research[2,26], the high explanatory power (90.1%) in our meta-regression was striking. Detailed reporting and/or stratification by disease severity in this patient group appears advisable to ensure accurate conclusions are drawn from future studies seeking to advance our knowledge of the interplay between these two factors.

Recommendations advocate for DXA and BIA as the preferred methods to evaluate LMM for the purpose of detecting sarcopenia, including evaluation of muscles of both the lower limb and the chest wall[7,16,20,44,45,48–51]. These were commonly used within the studies included in this review (Tables 1-2). Despite this, we observed 12 different cut-off points used to classify test results as normal or abnormal. The most commonly used criteria were those of Newman *et al.*[52] and Baumgartner *et al.*[53], which are also considered by the EWGSOP[7]. Borda *et al.*[23] measured muscle mass with calf

circumference, which confers simplicity as a screening method for sarcopenia[54,55] but it is not recommended[7,16,20,44,45]. Similar advice is also available for the assessment of muscle strength (handgrip force) and physical performance (gait speed) [7,16], yet inconsistencies were again apparent. For example, gait speed was assessed using the 4-meter gait speed (4MGS)[23,26,35,37] and the 6MWT[27,29,33,34]. While the same cut-off was used to diagnose sarcopenia across both tests (<0.8m/sec), the two tests are vastly different. The 4MGS is typically performed at usual walking speed across a 4-metre distance (although variations also exist at different walk speeds and track lengths), while the 6MWT is typically performed on a 30-metre walking track with participants encouraged to walk as far as they can (often faster than normal speed) in order to assess exercise tolerance[56]. Deriving a measure of walking speed from the 6MWT (*i.e.* total distance [m] divided by 360 [secs]) poses a significant risk of inaccurate interpretation. For example, it could not distinguish between people walking slowly and fast but stopping to rest during the test. The prevalence of sarcopenia in the studies that used this approach[27,29,33,34] may therefore have been underestimated. It is thus crucial that future research not only implement consistent tests to diagnose sarcopenia, but also adopt standardized cut-off thresholds to facilitate accurate test interpretation.

Sarcopenia had a consistently negative impact on a range of COPD-related clinical outcomes, including exercise capacity, balance, quadriceps and hand grip strength, gait speed and physical activity levels[2,24,30,32,35]. It was also associated with increased symptom burden and poorer quality of life[2,24,28,30]. It is interesting that the two studies that measured dyspnoea (MRC scale)[2,28] classified sarcopenia according to physical function alone, as it raises the possibility that functional impairment may associate more strongly with dyspnoea than LMM[32]. This also raises some challenging issues related to clinical management strategies. As associations do not imply causation or directionality, should interventions targeting improvement in health outcomes for people with COPD who have sarcopenia be directed towards mitigating the defining

features of sarcopenia (*e.g.* muscle mass and physical performance) or their associated manifestations (*e.g.* low physical activity levels, poor balance, impaired lung function). To our knowledge, the precise impact of sarcopenia (and its severity) upon intervention effectiveness targeting these other areas has received scant attention to date in COPD. Sarcopenia has, however, been highlighted as an important 'treatable trait' in adult respiratory medicine[57]. One of the few studies to explore this area was conducted by Jones *et al.*[2] who demonstrated that pulmonary rehabilitation, a comprehensive, multicomponent exercise-based intervention, improved a range of clinical outcomes and reduced the incidence of sarcopenia in a cohort of patients with COPD. More research is clearly warranted to further validate the findings of Jones and colleagues, including the use of other recommended adjunctive therapies such as nutritional supplementation[7,58,59].

We were not able to investigate actual mortality in those who had sarcopenia due to a lack of available evidence. However, it is plausible that sarcopenia might associate with increased mortality in this population, considering it associated with poorer prognosis and a higher prevalence in patients with more severe lung disease (37.6% in GOLD stages III-IV compared to 19.1% in those with GOLD stages I-II). Leivseth *et al*.[60] reported that people with GOLD stages III and IV disease severity had a more than 6-fold increased risk of mortality in women and a more than double increased risk in men over 15 years of follow-up. Heightened mortality risk was also observed in individuals with COPD evaluated via BODE[2,28,34,38] which is a widely used, valid tool for predicting risk of death in COPD[61,62]. Costa et al[38] reported an increased prevalence of sarcopenia (OR: 3.89; 95% CI: 1.21-12.46) in those with GOLD stages III and IV, and these quartiles are related with lower 4-year survival (18%-57%)[61]. Sarcopenia also related to poorer quality of life, pulmonary and physical function, which are known factors associated with heightened mortality risk in COPD[63,64]. Sarcopenia has been associated with premature mortality in community-dwelling older adults in a cohort study with 4425 older adults

during a median 14.4 year follow up (HR: 1.32; 95% CI: 1.13-1.47)[65]. However, the lack of COPD-specific data suggests this remains an area in need of addressing in future research.

This systematic review has highlighted the clinical relevance of including measurements of muscle mass, muscle strength and physical performance in individuals with COPD, as these variables clearly associate with sarcopenia, exacerbations and poor prognosis[57,66]. The more widespread implementation of these measures in clinical practice could help identify patients with COPD at increased risk of future healthcare use related to exacerbations[66,67]. This is also an important priority from a public health economic perspective[68]. In Europe, on average, the healthcare system spends 6725€ per year per person (95%CI 6590–6863€) for each exacerbation of this disease[69]. In older people, sarcopenia is consistently associated with increased risk of incident disability, falls, hospitalization and mortality[65,70,71]. Sarcopenia has been associated with increased breathlessness, exacerbation frequency and frailty in individuals with COPD[66,72,73]. Hospitalizations also hasten deconditioning and muscle weakness, thereby worsening the sarcopenic state[66,74]. Earlier identification of sarcopenia may therefore help direct preventive health care to positively impact upon its healthcare burden.

We were unable to demonstrate a clear relationship between sarcopenia and inflammatory biomarkers across the included studies. Some authors[22,35,41] reported no differences between sarcopenic and non-sarcopenic patients with COPD, while Byun *et al.*[28] and Van de Bool *et al.*[39] observed higher levels of C-reactive protein, interlekin-6 and tumor necrosis factor-α. No studies evaluated the effect of sarcopenia on oxidative stress, despite convincing evidence of pathophysiological changes occurring in the COPD literature[75–77] and known associations between sarcopenia, oxidative stress[78,79], inflammation[1], and age-related alterations in muscle morphology[78,80–82]. This would appear a valuable area for future research.

As with all studies, the findings from the present review are not without some limitations. Due to the significant heterogeneity between studies in terms of factors such as sarcopenia definitions, participant characteristics and diagnostic cut-offs, the opportunity for meta-analysis was limited for some outcomes and clear interpretation of the clinical implications of some results was challenging. This review was unable to elucidate the direct relationship between sarcopenia and mortality due to a lack of data. This was not surprising due to the prolonged periods of follow-up required to observe such outcomes in cohorts of patients who would otherwise not typically have been at risk of imminent death. However, our observed association between sarcopenia and mortality risk (assessed via BODE) is noteworthy. Whilst not a pre-specified focus of our review, we also feel the lack of direct evidence highlighting the clinical impact of sarcopenia on healthcare expenditure represents an area to address in future studies. Additionally, despite this review including studies from four different continents (Asia, Europe, North and South America), data regarding participant race were not available which limits its potential applicability to specific patient subgroups. In addition, it was not considered the impact of differing sarcopenia sub-types (*e.g.* sarcopenic obesity, severe sarcopenia), despite their clinical relevance due to a lack of suitable data. This might have plausibly explained some of the observed variability in clinical outcome data. We also synthesized prevalence data via meta-analysis in contrast to our registered protocol. This was altered in light of access to appropriate statistical software to conduct this analysis while still allowing readers to identify the raw proportions of individual studies (as stated in the protocol) in Figure 2. The overall pooled effect from the present meta-analysis (27.5%) compared favorably against the protocol-based method utilizing median estimates from individual studies (26.1%).

In conclusion, sarcopenia is a clinically important condition that is prevalent within a substantial proportion of patients with COPD. Diagnostic accuracy appears sensitive to the criteria, test methods and cut-offs used to detect the individual components, as

well as markers of disease severity. Considering the negative impact of sarcopenia upon health outcomes, there may be merit in future strategies targeting early identification of sarcopenia in the clinical assessment of people with COPD to ultimately improve management strategies aiming to mitigate its impact upon individuals' lives.

ACKNOWLEDGMENTS

Author contributions: WSL and AAM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, CO and SP contributed substantially to statistical analysis and interpretation of the results, and GD and VSP contributed with the study design and writing of the manuscript. The authors of this manuscript certify that they comply with the ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle[83].

FINANCIAL/NONFINANCIAL DISCLOSURES

None declared.

ADDITIONAL INFORMATION

The supplementary Figures and Tables can be found in the Supplemental Materials section of the online article.

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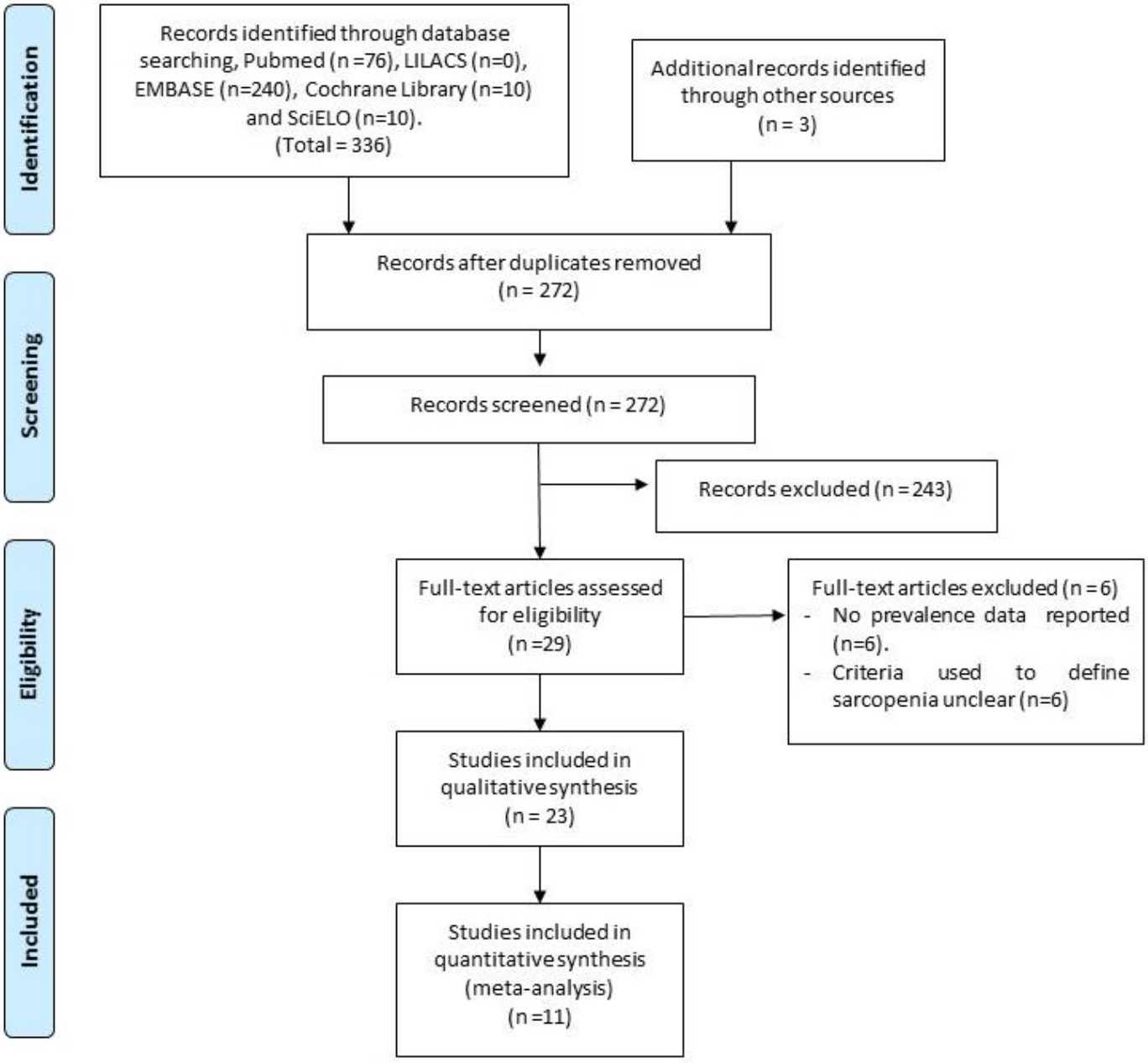
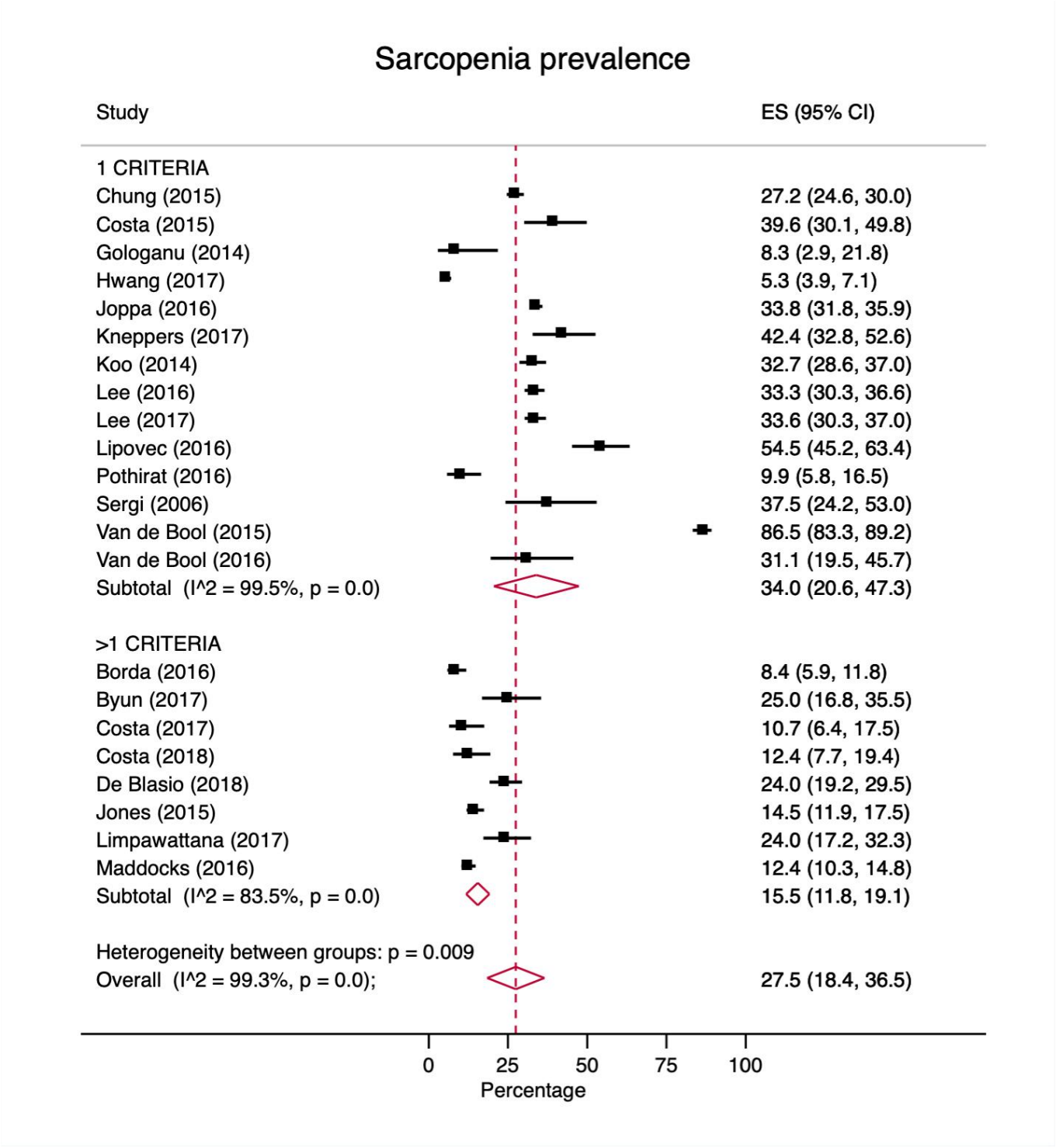
Figure 1: PRISMA flow diagram of article selection.



Legend: COPD: Chronic Obstructive Pulmonary Disease

Figure 2: Prevalence of sarcopenia in COPD according to different criteria



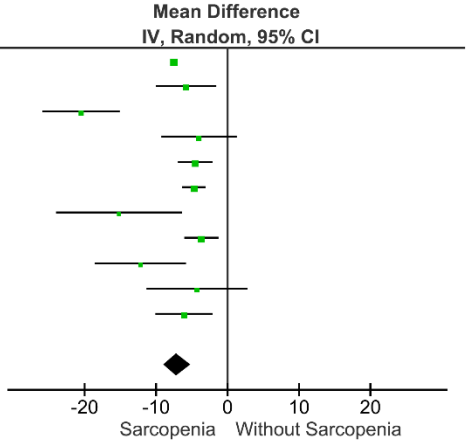
Legend: ES = effect size (prevalence %); I^2 = I^2 heterogeneity statistic. Random effects model used for analysis.

Figure 3: Clinical impact of sarcopenia in individuals with COPD.

a) Effect of sarcopenia on pulmonary function (FEV_1 % predicted value)

Study or Subgroup	Sarcopenia Mean	SD	Total	Without Sarcopenia Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
Koo et al. 2014	84.9	1.2	155	92.4	0.8	419	15.0%	-7.50 [-7.70, -7.30]	2014
Jones et al. 2015	40.5	18.9	90	46.3	18.9	532	8.8%	-5.80 [-10.02, -1.58]	2015
Van de Bool et al. 2015	43	19.6296	96	63.5	15.33333	68	7.0%	-20.50 [-25.86, -15.14]	2015
Lipovec et al. 2016	36	13	61	40	15	51	7.2%	-4.00 [-9.25, 1.25]	2016
Lee et al. 2016	74.15	17.45	286	78.68	15.17	572	12.3%	-4.53 [-6.90, -2.16]	2016
Joppa et al. 2016	45.6	15.7	485	50.3	15.5	1324	13.6%	-4.70 [-6.33, -3.07]	2016
Van de Bool et al. 2016	42.1	12	14	57.3	17.55555	31	3.7%	-15.20 [-24.01, -6.39]	2016
Lee et al. 2017	76.4	16.4	251	80.07	14.31	497	12.3%	-3.67 [-6.06, -1.28]	2017
Kneppers et al. 2017	36.4	12.9	39	48.6	18.3	53	5.8%	-12.20 [-18.58, -5.82]	2017
Byun et al. 2017	58	13.9	20	62.3	13.9	60	5.1%	-4.30 [-11.33, 2.73]	2017
de Blasio et al. 2018	39.6	12.5	63	45.7	18.2	200	9.2%	-6.10 [-10.09, -2.11]	2018
Total (95% CI)			**1560**			**3807**	**100.0%**	**-7.06 [-9.00, -5.11]**	

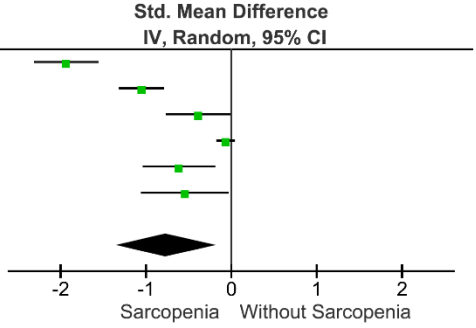
Heterogeneity: Tau² = 6.53; Chi² = 57.91, df = 10 (P < 0.00001); I² = 83%
Test for overall effect: Z = 7.11 (P < 0.00001)



b) Effect of sarcopenia on exercise tolerance in COPD

Study or Subgroup	Sarcopenia Mean	SD	Total	Without Sarcopenia Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Van de Bool et al. 2015	935.5	262.4	96	1,468	289.26	68	16.6%	-1.94 [-2.31, -1.56]	2015
Jones et al. 2015	157	118	90	309	153	220	17.2%	-1.05 [-1.31, -0.80]	2015
Lipovec et al. 2016	316	113	61	357	92	51	16.6%	-0.39 [-0.77, -0.02]	2016
Joppa et al. 2016	372	125	485	380	114	1009	17.7%	-0.07 [-0.18, 0.04]	2016
Kneppers et al. 2017	58.1	24.9	39	73.4	24.4	53	16.3%	-0.62 [-1.04, -0.19]	2017
Byun et al. 2017	350.8	78	20	389.3	66.9	60	15.7%	-0.55 [-1.06, -0.03]	2017
Total (95% CI)			**791**			**1461**	**100.0%**	**-0.77 [-1.35, -0.18]**	

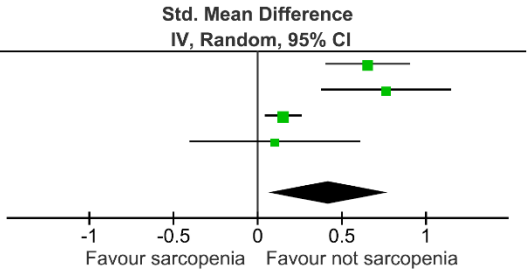
Heterogeneity: Tau² = 0.50; Chi² = 124.87, df = 5 (P < 0.00001); I² = 96%
Test for overall effect: Z = 2.56 (P = 0.01)



c) Effect of sarcopenia on quality of life in COPD

Study or Subgroup	Sarcopenia Mean	SD	Total	Without Sarcopenia Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Jones et al. 2015	24	9	90	19	7	220	27.2%	0.65 [0.40, 0.90]	2015
Lipovec et al. 2016	46	13	61	36	13	51	22.9%	0.76 [0.38, 1.15]	2016
Joppa et al. 2016	51.2	20.8	485	48.1	20.4	1009	30.7%	0.15 [0.04, 0.26]	2016
Byun et al. 2017	15	11	20	14	9.2	60	19.1%	0.10 [-0.40, 0.61]	2017
Total (95% CI)			**656**			**1340**	**100.0%**	**0.42 [0.07, 0.77]**	

Heterogeneity: Tau² = 0.10; Chi² = 20.29, df = 3 (P = 0.0001); I² = 85%
Test for overall effect: Z = 2.33 (P = 0.02)



Legend: I^2 = I^2 heterogeneity statistic. Random effects model used for analysis.

Table 1: Characteristics of the included studies regarding the prevalence of sarcopenia in subjects with COPD.

First author and year	Country	Study design	Sample size	Age (mean±SD)	Male n (%)	Smoking status (never/ former/ current) n	GOLD (%)	Prevalence of sarcopenia		Criteria (assessment method to detect sarcopenia)
								Total n (%)	Male n (%)	
Sergi *et al*. 2006 [32]	Italy	Cross-Sectional	40	75.7 ± 5.3	40 (100%)	-	-	15 (38%)	15 (100%)	LMM (DXA)
Koo *et al*. 2014 [36]	Korea	Cross-Sectional	574	64.0 ± 0.6	574 (100%)	103//231/240	I/II/III-IV (46/49/5)	155 (27%)	155 (100%)	LMM (DXA)
Gologanu *et al*. 2014 [37]	Romania	Cross-sectional	36	65.6 ± 7.5	12 (33%)	-	I/II/III/IV (0/39/42/19)	3 (8%)	-	LMM (BIA)
Jones *et al*. 2015 [2]	UK	Clinical non-randomized	622	-	354 (57%)	7/170/43	-	90 (14%)	57 (63%)	LMM (BIA) LMS (HGS) LPP (4MGS)
Costa *et al*. 2015 [38]	Brazil	Cross-Sectional	91	67.4 ± 8.7	41 (45%)	91 former smokers	I/II/III/IV (17/24/37/22	36 (40%)	20 (56%)	LMM (DXA)
Van de Bool *et al*. 2015 [39]	Netherlands	Retrospective	505	64 (median)	288 (57%)	13/360/132	I/II/III/IV (8/41/40/11)	437 (87%)	239 (55%)	LMM (DXA)
Chung *et al*. 2015 [40]	Korea	Retrospective	1039	64.5 ± 9.4 (male) 64.5 ± 10.2(female)	760 (73%)	129/136/771	I/II/III/IV (46/48/5/1)	283 (27%)	249 (88%)	LMM (DXA)
Joppa *et al*. 2016 [41]	ECLIPSE (12 countries and USA)	Cross-Sectional	2000	63.5 ± 7.1	1314(66%)	-	-	682 (34%)	509 (75%)	LMM (BIA)
Van de Bool *et al*. 2016 (van de Bool et al., 2016)	Netherlands	Cross-Sectional	45	42-77	29 (64%)	-	I/II/III/IV (6/36/49/9)	14 (31%)	13 (92%)	LMM (DXA)
Lipovec *et al*. 2016 [22]	Slovenia	Prospective observational	112	66 ± 8	74 (66%)	92 current smokers	I/II/III/IV (0/17/52/31)	61 (54%)	44 (72%)	LMM (DXA)
Borda *et al*. 2016 [23]	Colombia	Cross-sectional	334	71,1 ± 8,05	110 (33%)	-	-	28 (8%)	-	LMM (CC) LMS (HGS) LPP (3.4MGS)
Lee *et al*. 2016 [24]	Korea	Cross-sectional	858	-	-	-	-	286 (33%)	226 (79%)	LMM (DXA)
Pothirat *et al*. 2016 [25]	Thailand	Cross-sectional	121	-	-	121 former smokers	I/II/III/IV (26/25/10/39)	12 (10%)	-	LMM (BIA)
Maddock *et al*. 2016 [26]	UK	Prospective cohort	816	69.8 ± 9.7	484 (59%)	49/620/146	-	101 (12%)	-	LMM (BIA) LMS (HGS) LPP (4MGS)
Hwang *et al*. 2017[14]	Korea	Cross-Sectional	777	63.9 ± 10.6	777 (100%)	0/185/592	I/II/III-IV (43/50/7)	41 (5.3%)	41 (100%)	LMM (DXA)

Limpawattana *et al*. 2017 [29]	Thailand	Cross-Sectional	121	-	112 (92.6%)	7/104/10	-	29 (24%)	29 (100%)	LMM (DXA) LMS (HGS) LPP (6MWT)
Byun *et al*. 2017 [28]	Korea	Cross-Sectional	80	68.4 ± 8.9	67 (83.8%)	-	I/II/III/IV (30/39/6/25)	20 (25%)	17 (83%)	LMM (BIA) LMS (HGS)
Limpawattana *et al*. 2017 [27]	Thailand	Cross-sectional	121	70 ± 9	112 (92.6%)	7/104/10	I/II/III/IV (26/57/17/0)	29 (24%)	29 (100%)	LMM (DXA) LMS (HGS) LPP (6MWT)
Lee *et al*. 2017 [30]	Korea	Cross-sectional	748	-	-	-	-	251 (34%)	203 (81%)	LMM (DXA)
Kneppers *et al*. 2017 [31]	Slovenia	Prospective cohort	92	-	-	-	I/II/III/IV (3/24/50/23)	39 (42%)	29 (74%)	LMM (DXA)
Costa et al 2017[33]	Brazil	Cross-sectional	121	67.9 ± 8.6	56 (46%)	23 current smokers	-	13 (11%) 6 (5%) 11 (9%) 15 (12%)		LMM (DXA) LPP (6MWT)
Costa *et al*. 2018 [34]	Brazil	Cross-sectional	121	67.9 ± 8.6	56 (46%)	-	A/B/C/D (29/29/34/29)	15 (12%)	-	LMM (DXA) LPP (6MWT)
de Blasio *et al*. 2018 [35]	Italy	Cross-sectional	263	69.8 ± 8.0	185 (70%)	-	I-II/III/IV (11/18/31)	63 (24%)	-	MM (BIA) LMS (HGS) LPP (4MGS)

BIA: Bio-electrical Impedance Analysis; CC: Calf circumference; DXA: Dual Energy X-Ray Absorptiometry; HGS: Hand Grip Strength; LMM: Lower muscle mass; LMS: Lower muscle strength; LPP: Lower physical performance; 6MWT: 6-Minutes Walking Test; 4MGS: 4-Meter Gait Speed. 3.4 MGS: 3.4 -Meter Gait Speed; M: mean; SD: standard deviation; n: number of subjects.

Table 2: Criteria and cut-off points used to detect sarcopenia in individuals with COPD in the different studies.

Lower muscle mass			***References***
DXA	1.	EWGSOP[7] Newman *et al*. 2003[48] ASMI: <7.23 kg·m^2 for men and <5.67 kg·m^2 for women.	[22,31,39,42]
	2.	EWGSOP[7] Newman *et al*. 2003[48] Residuals of linear regression on appendicular lean mass adjusted for fat as well as height. Men: −2.29 Women: −1.73.	[33,38]
	3.	EWGSOP[7] Baumgartner *et al*. 1998[49] SMI: ≤ 7.26 kg/m^2 for men and ≤ 5.45 kg/ m^2 for women.	[33,38]
	4.	AWGS[16] ASMI: ≤ 7.0 Kg/m^2 for men and ≤ 5.4 Kg/m^2 for women.	[24,27,29,30]
	5.	FNIH[44] ALM/BMI: <0.789 for men and for < 0.512 women.	[33,34]
	6.	ASMMI: ≤ 2 standard deviations in a gender-specific mean for a young reference group.	[14,28,32,40,42]
	7.	SMI:< 1 standard deviations in a gender-specific mean for a young reference group.	[36]
	8.	Combination of criteria 2 and 3.	[33,38]
BIA	1-	EWGSOP[7] Janssen *et al*. 2002[63] SMI: ≤ 8.50 kg/m^2 for men and ≤5.75 kg/m^2 for women.	[2,26,35]
	2-	ATS[64] BMI >21 and FFMI ≤16 kg/m^2 for men or ≤15 kg/m^2 for women.	[25,37]
	3-	Franssen *et al*. 2014[65] Lower than the 10 percentile of the reference value for age, sex and BMI-specific.	[41]
	4-	ASMMI: ≤ 2 standard deviations in a gender-specific mean for a young reference group.	[28]
CC	1-	Calf circumference < 31 cm.	[23]
Lower muscle strength			
HGS	1-	EWGSOP[7] Laurentani *et al*. 2003[66] HGS: <30 Kg for men and < 20 Kg for women.	[2,26,28,35]
	2-	AWGS[16] HGS: <26 Kg for men and < 18 Kg for women.	[27,29]
	3-	Lower the last quintile in specific population.	[23]
Lower physical performance			
4MGS	1-	EWGSOP[7] Laurentani *et al*. 2003[66] GS: <0.8 m/s [both genders].	[2,26,35]
3.4MGS	1-	Lower the last quintile in specific population.	[23]
6MWT	1-	AWGS[16] Laurentani *et al*. 2003[66]	[27,29]

	GS: <0.8 m/s [both genders].	
2-	EWGSOP[7] Laurentani *et al*. 2003[66] GS: <0.8 m/s [both genders].	[33]
3-	FNIH[44] GS: <0.8 m/s [both genders].	[34]

AWGS: Asian Working Group for Sarcopenia; ATS: American Thoracic Society; ASMI: Appendicular skeletal muscle index; BIA: Bio-electrical Impedance Analysis; BMI: Body mass index; CC: Calf Circumference; EWGSOP: European Working Group on Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health Sarcopenia Project; DXA: Dual Energy X-Ray Absorptiometry; HGS: Hand Grip Strength; SMI: Skeletal muscle mass index; 6MWT: 6-Minutes Walking Test; 4MGS: 4-Meter Gait Speed. 3.4 MGS: 3.4 -Meter Gait Speed.

Table 3: Clinical impact of the sarcopenia in different variables in subjects with COPD.

Categories	Variables	Compared to individuals with COPD without sarcopenia	
		Sarcopenia (1 criterion)	**Sarcopenia (>1 criteria)**
Health–related quality of life	EQ-5D index (score)	Worse [24,30]	
Physical function	SPPB (score)		Worse [2]
	5STS (sec)		Worse [2]
	HGS (Kg)		Worse [2,35]
	QS (Kg)	Worse [32]	Worse [2]
	GS (m/sec)		Reduction [2,35]
Physical Activity level	Time in moderate and high activity (min/day)	Worse [36]	Worse [2]
	Total energy expenditure (kcal/week)		Worse [2]
	Daily Steps (steps/day)		N.d. [2]
	Prevalence of physical inactivity	Worse [24,30]	
Dyspnoea	MRC (score)	N.d. [32]	Worse [2,28]
Risk of mortality	Prevalence in BODE quartile 3 or 4	Higher [38]	Higher [2,28,34]
Inflammation	CRP (mg/L)	Augmented [39] / N.d [22,41]	Augmented [35]
	Fibrinogen (mg/L)	N.d. [41]	
	IL-6 (pg/mL)	N.d. [41]	Augmented [28]
	IL-8 (pg/mL)	N.d. [41]	
	TNF-α (pg/mL)	N.d. [41]	Augmented [28]

CAT: COPD Assessment Test; SGRQ: St George's respiratory disease questionnaire; EQ-5D index: EuroQol five-dimensional; 6MWT: 6-minutes walking test; ISWT: Incremental shuttle walk test; SPPB: Short physical performance battery; 5STS: five-repetition sit-to-stand test; HGS: Handgrip strength; QS: Quadriceps strength; GS: gait speed; MRC: Medical research council; BODE: Body mass index, Obstruction, Dyspnoea, Exercise tolerance index; CRP: C-reactive protein; IL: Interleukin; TNF- α: Tumor necrosis factor- alpha.
N.d.: no significant difference.

Supplemental Material

Table S1: Search strategy in each database (Supplementary data)

Pubmed	
Search #3 (74 articles)	#1 [All Fields] AND #2[All Fields]
Search #2 (5725 articles)	(sarcopenia[MeSH Terms]) OR Sarcope*[Title/Abstract]
Search #1 (62641 articles)	Pulmonary Disease, Chronic Obstructive [Mesh Terms] OR COPD [Text Word]
LILACS	
Search #3 (103 articles)	(tw:((tw:(DPOC)) OR (tw:(COPD)) OR (tw:(Pulmonary Disease, Chronic Obstructive)))) AND (tw:(Sarcopeni*))
Search #2 (5,935 articles)	(tw:(Sarcopeni*))
Search #1 (69,143 articles)	(tw:(DPOC)) OR (tw:(COPD)) OR (tw:(Pulmonary Disease, Chronic Obstructive))
EMBASE	
Search #6 (116 articles)	#4 AND #5
Search #5 (7,867 articles)	'sarcopeni*':ab,ti
Search #4 (73,473articles)	'copd':ab,ti OR 'chronic obstructive lung disease':ab,ti
Search #3 (228 articles)	#1 AND #2
Search #1 (10,753 articles)	'sarcopeni*'
Search #1 (109,757 articles)	'copd'/exp OR 'chronic obstructive lung disease'/exp
Cochrane Library	
Search #3 (10 articles)	#1 AND #2

Search #2 (456 articles)	"sarcopenia":ti,ab,kw or sarcopenic (Word variations have been searched)
Search #1 (14507 articles)	"COPD":ti,ab,kw or Pulmonary Disease, Chronic Obstructive (Word variations have been searched)
Scielo	
Search #8 (1 article)	#6 AND #7
Search #7 (125)	(ab:(Sarcopeni*))
Search 6 (1097)	#1 OR #2 OR #3 OR #4 OR #5
Search #5 (337)	(ab:(Enfermedad Pulmonar Obstructiva Crónica))
Search # 4 (320)	(ab:(Doença Pulmonar Obstrutiva Crônica))
Search # 3 (327)	(ab:(DPOC))
Search # 2 (707)	(ab:(Pulmonary Disease, Chronic Obstructive))
Search # 1 (702)	(ab:(COPD))

Table S2: Quality analysis (Supplementary data)

Instrument: NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Author: Sergi *et alet al*.2006

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?		x	
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			x
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?			x

11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Koo *et al.*2014

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and	x		

implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Gologanu *et al*. 2014

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			x

8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
10. Was the exposure(s) assessed more than once over time?				x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
12. Were the outcome assessors blinded to the exposure status of participants?				x
13. Was loss to follow-up after baseline 20% or less?				x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x			

Instrument: JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)

Author: Gologanu *et al*. 2014

Other (CD, NR, NA)*	**Other (CD, NR, NA)***	**Other (CD, NR, NA)***	**Other (CD, NR, NA)***
1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	x		
2. Were the participants included in any comparisons similar?	x		
3. Were the participants included in any comparisons receiving similar	x		

	treatment/care, other than the exposure or intervention of interest?		
4.	Was there a control group?	x	
5.	Were there multiple measurements of the outcome both pre and post the intervention/exposure?	x	
6.	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?		
7.	Were the outcomes of participants included in any comparisons measured in the same way?	x	
8.	Were outcomes measured in a reliable way?	x	
9.	Was appropriate statistical analysis used?	**x**	

Instrument: NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Author: Costa *et al*. 2015

	Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1.	Was the research question or objective in this paper clearly stated?	x		
2.	Was the study population clearly specified and defined?	x		
3.	Was the participation rate of eligible persons at least 50%?	x		
4.	Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5.	Was a sample size justification, power description, or variance and effect estimates provided?		x	

6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	x		
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?		x	
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?		x	

Author: Van de bool *et al*. 2015

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		

3. Was the participation rate of eligible persons at least 50%?				x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x			
5. Was a sample size justification, power description, or variance and effect estimates provided?		x		
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?				x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
10. Was the exposure(s) assessed more than once over time?				x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
12. Were the outcome assessors blinded to the exposure status of participants?				x
13. Was loss to follow-up after baseline 20% or less?				x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the	x			

relationship between exposure(s) and outcome(s)?

Author: Chung *et al* 2015.

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?		x	
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?			x

11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Joppa *et al* 2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?	x		
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	x		
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different	x		

levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?		x	
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Van de Bool *et al*. 2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		

5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?		x	
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?		x	

Instrument: JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)

Author: Lipovec *et al*.2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***

1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	x		
2. Were the participants included in any comparisons similar?	x		
3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	x		
4. Was there a control group?		x	
5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?	x		
6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?			
7. Were the outcomes of participants included in any comparisons measured in the same way?	x		
8. Were outcomes measured in a reliable way?	x		
9. Was appropriate statistical analysis used?	x		

Instrument: NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Author: Borda *et al*.2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?	x		
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study	x		

prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?			x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Lee *et al*. 2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***

1. Was the research question or objective in this paper clearly stated?	x			
2. Was the study population clearly specified and defined?	x			
3. Was the participation rate of eligible persons at least 50%?		x		
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x			
5. Was a sample size justification, power description, or variance and effect estimates provided?		x		
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?				x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
10. Was the exposure(s) assessed more than once over time?				x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x			
12. Were the outcome assessors blinded to the exposure status of participants?				x

13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Pothirat *et al*. 2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented	x		

consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Maddock *et al*. 2016

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	X		
3. Was the participation rate of eligible persons at least 50%?			X
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	X		
5. Was a sample size justification, power description, or variance and effect estimates provided?		X	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	X		

7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?		X	
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	X		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	X		
10. Was the exposure(s) assessed more than once over time?	X		
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	X		
12. Were the outcome assessors blinded to the exposure status of participants?		X	
13. Was loss to follow-up after baseline 20% or less?	X		
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	X		

Author: Hwang *et al*. 2017

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?		x	
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion	x		

	criteria for being in the study prespecified and applied uniformly to all participants?			
5.	Was a sample size justification, power description, or variance and effect estimates provided?		x	
6.	For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	x		
7.	Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8.	For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9.	Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10.	Was the exposure(s) assessed more than once over time?		x	
11.	Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12.	Were the outcome assessors blinded to the exposure status of participants?			x
13.	Was loss to follow-up after baseline 20% or less?			x
14.	Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Limpawattana *et al* 2017

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?	x		
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?	x		
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			x
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?		x	
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		

12. Were the outcome assessors blinded to the exposure status of participants?		x	
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Byun *et al*. 2017

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
5. Was a sample size justification, power description, or variance and effect estimates provided?		x	
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	x		
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	x		
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	x		

9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
10. Was the exposure(s) assessed more than once over time?			x
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	x		
12. Were the outcome assessors blinded to the exposure status of participants?			x
13. Was loss to follow-up after baseline 20% or less?			x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x		

Author: Limpawattana *et al* 2017.

Criteria	**Yes**	**No**	**Other (CD, NR, NA)***
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
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Author: Lee *et al*. 2017

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2. Was the study population clearly specified and defined?	x		

3. Was the participation rate of eligible persons at least 50%?				x
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13. Was loss to follow-up after baseline 20% or less?				x
14. Were key potential confounding variables measured and adjusted statistically for their impact on the	x			

relationship between exposure(s) and outcome(s)?

Author: Kneepers *et al*. 2017

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?	x		
2. Was the study population clearly specified and defined?	x		
3. Was the participation rate of eligible persons at least 50%?			x
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	x		
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Author: Costa *et al*. 2017

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Author: De Blasio *et al*. 2018

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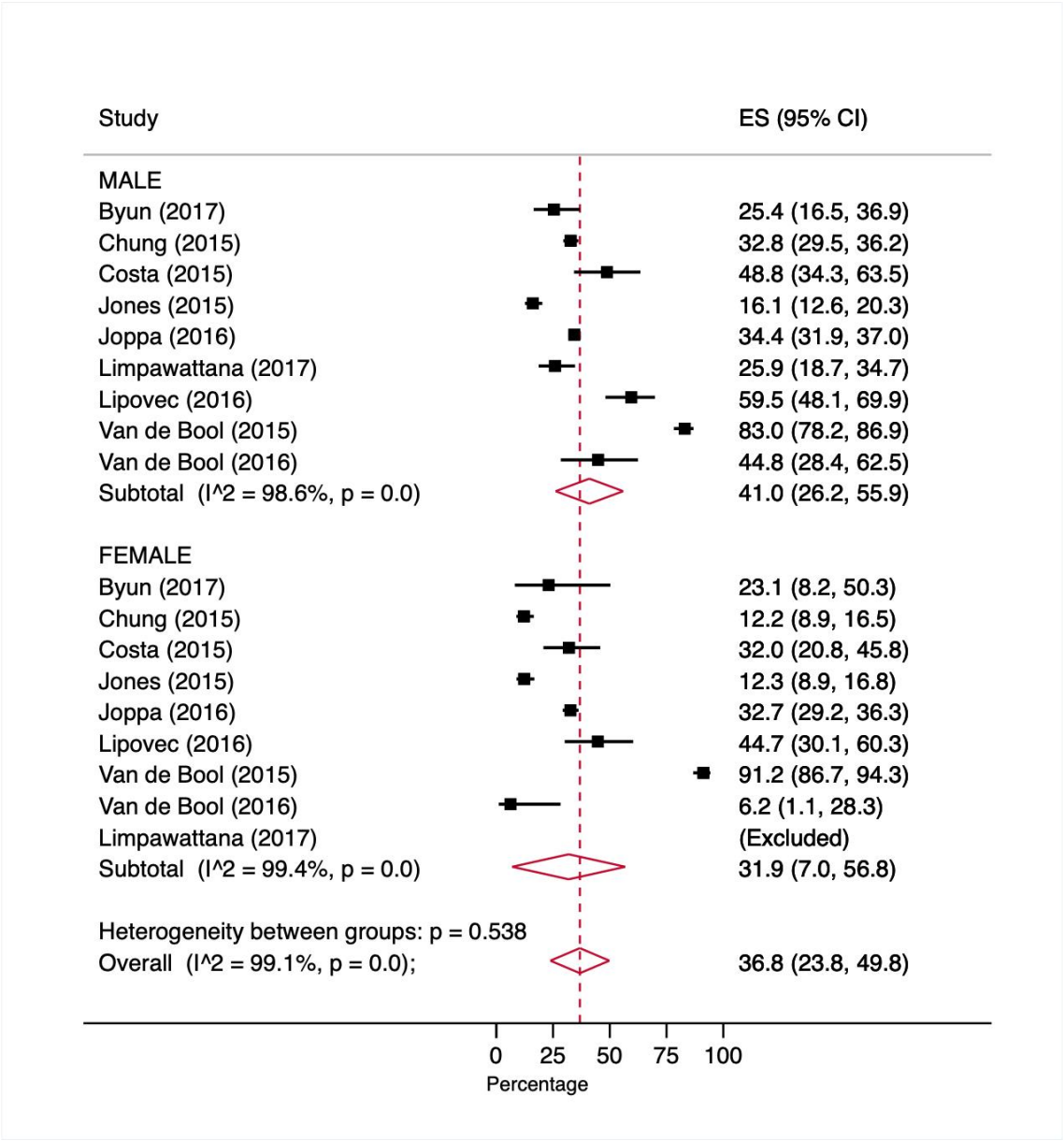
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	x

Table S3: Different cut-off points used to identify Sarcopenia.

Definition	*Lower MM*	*Lower MS*	*Lower PP*
EWGSOP 2010	ASMI: <7.23 kg·m−2 for men and <5.67 kg·m−2 for women.	HGS: <30 Kg for men and < 20 Kg for women.	GS ≤0.8 m/s
EWGSOP 2018	ASM: ≤ 20 kg/m^2 for men and ≤ 15 kg/ m^2 for women.	HGS: <27 Kg for men and < 16 Kg for women.	
AWGS	ASMI: ≤ 7.0 Kg/m^2 for men and ≤ 5.4 Kg/m^2 for women.	HGS: <26 Kg for men and < 18 Kg for women.	
FNIH	ALM/BMI: <0.789 for men and for < 0.512 women.	HGS: <26 Kg for men and < 16 Kg for women.	

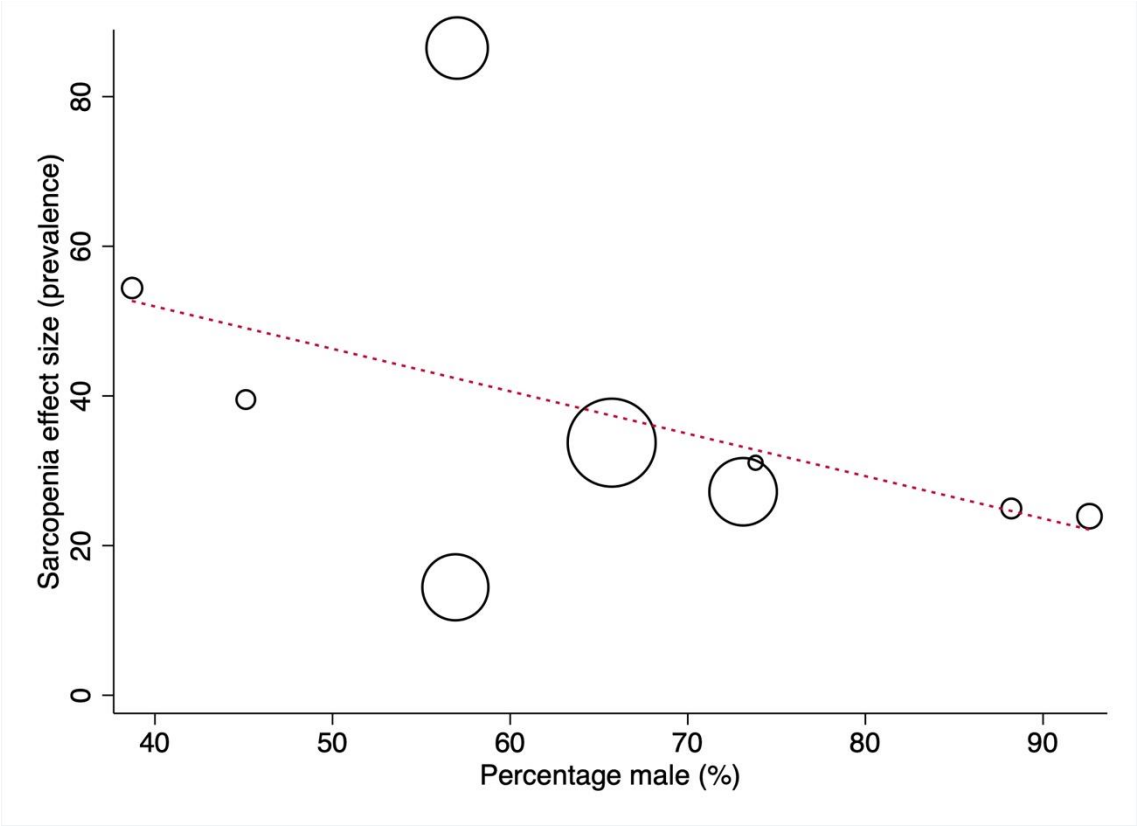
AWGS: Asian Working Group of Sarcopenia; EWGSOP: European Working Group of Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health; MM: muscle mass; MS: muscle strength; PP: physical performance.

Figure S1: Prevalence of sarcopenia by gender.



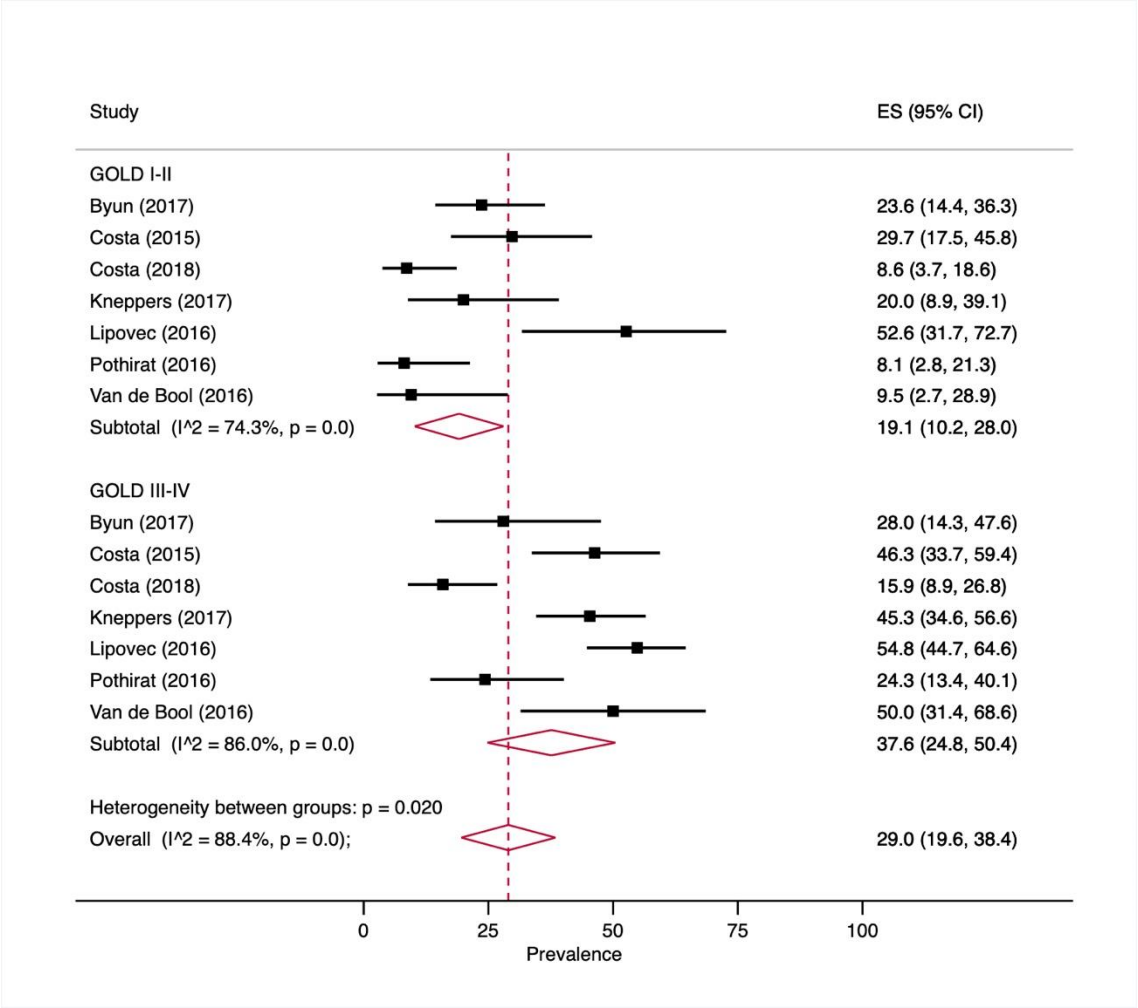
ES = effect size (prevalence); I^2 = I^2 heterogeneity statistic. Random effects model used for analysis, no significant difference between subgroups (p=0.5).

<u>Figure S2.</u> Meta-regression of effect of gender (percent male) on sarcopenia prevalence.



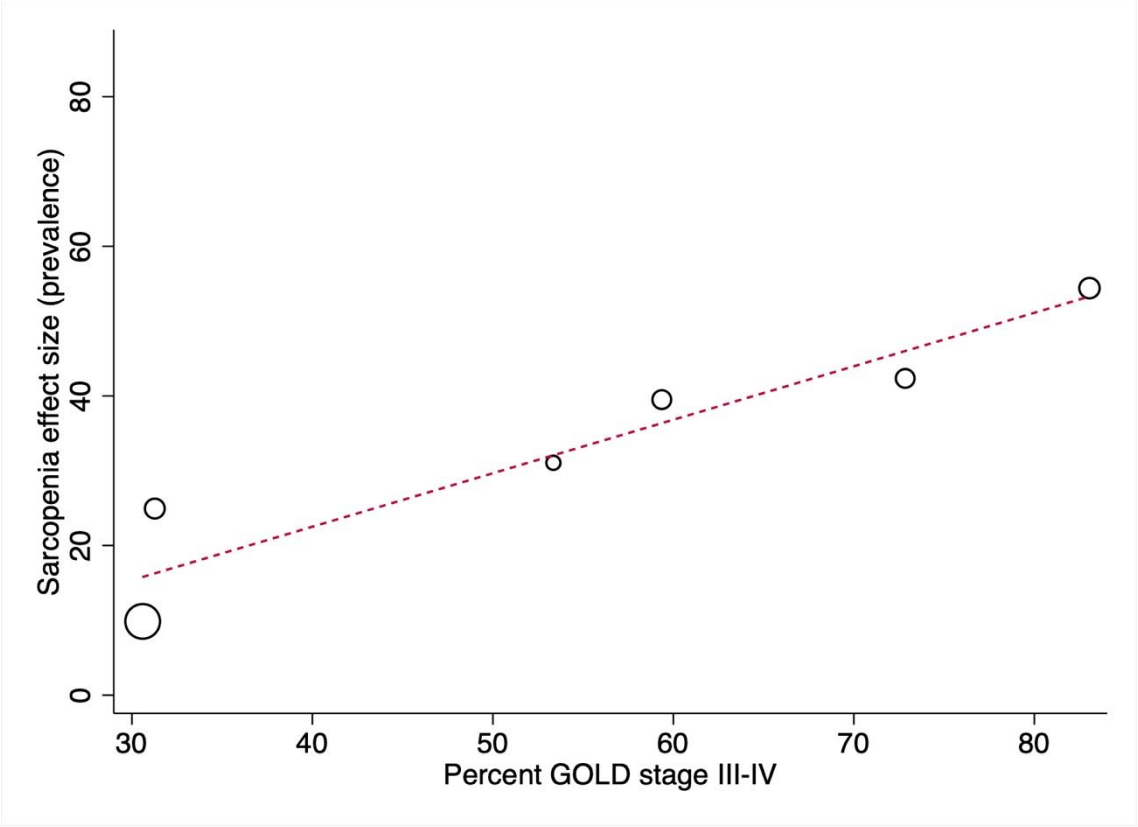
Regression coefficient -0.006 (95%CI -0.015 to 0.004), p = 0.206. Circle diameters reflective of proportional study sample size.

Figure S3. Prevalence of sarcopenia, by COPD disease severity.



ES = effect size (prevalence %); I^2 = I^2 heterogeneity statistic. Random effects model used for analysis.

<u>Figure S4.</u> Meta-regression of effect of disease severity (GOLD stages III-IV) on sarcopenia prevalence.



Regression coefficient 0.715 (95%CI 0.342 to 1.088), p = 0.006; adjusted R^2 = 90.1%.

Circle diameters reflective of proportional study sample size.

3.CHAPTER III. BIOLOGICAL MECHANISMS RELATED TO SARCOPENIA IN PATIENTS WITH COPD

PRESENTATION:

In this chapter the following articles are presented: 1) “Pathophysiological mechanisms of Sarcopenia in COPD” (published in the Revista Chilena de Enfermedades Respiratorias); 2) “NOVEL antioxidant and oxidant biomarkers related to sarcopenia in COPD” (Published in the Journal Heart and Lung) and 3) “Is oxidative stress associated with disease severity, pulmonary function and metabolic syndrome in Chronic Obstructive Pulmonary Disease?” (published in the Revista Clinica Española). These articles were developed in collaboration with researchers from different departments from the Londrina State University and University of Northern Parana (Brazil).

3.1: NARRATIVE REVIEW

Title: Pathophysiological mechanisms of Sarcopenia in COPD.

Walter Sepúlveda-Loyola[1,2]; Paulo Sergio[2]; Vanessa Suziane Probst[1,2].

[1]Program of Masters and Doctoral degree in Rehabilitation Sciences, Londrina State University (UEL) and University North of Paraná (UNOPAR), Londrina, Brazil.
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Article published in *Revista Chilena De Enfermedades Respiratorias*

(Q4/ Impact factor: 0.23)

Sepúlveda-loyola W, Sergio P, Probst S. Mecanismos fisiopatológicos de la sarcopenia en la EPOC. Rev Chil Enferm Respir. 2019;35(2):124–32

ABSTRACT

Sarcopenia is a disease characterized by loss of skeletal muscle, muscle strength and physical performance, being the major cause of frailty in the elderly. The sarcopenia is highly prevalent in individuals with Chronic obstructive pulmonary disease (COPD) leading to a poor prognosis and higher mortality in this population. The presence of sarcopenia in COPD is likely the result by the interaction between external and internal factors as systemic inflammation, oxidative stress and genetic polymorphisms, frequently observed in individuals with this respiratory disease. This review summarizes the current knowledge about the pathogenic mechanisms linking COPD with sarcopenia.

Keywords: Sarcopenia, COPD, oxidative stress, systemic inflammation, genetic polymorphisms.

Resumen:

La sarcopenia es una enfermedad caracterizada por la pérdida de masa muscular, fuerza muscular y rendimiento físico, siendo la principal causa de fragilidad en los adultos mayores. La sarcopenia es altamente prevalente en individuos con enfermedad pulmonar obstructiva crónica (EPOC) que conduce a un mal pronóstico y una mayor mortalidad en esta población. La presencia de sarcopenia en la EPOC es probablemente el resultado de la interacción entre factores externos e internos como la inflamación sistémica, el estrés oxidativo y los polimorfismos genéticos, frecuentemente observados en individuos con esta enfermedad respiratoria. Esta revisión resume el conocimiento sobre los mecanismos patogénicos asociados con la sarcopenia en la EPOC.

Palabras claves: Sarcopenia, EPOC, estrés oxidativo, inflamación sistémica, polimorfismos genéticos.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of death worldwide[1,2], it is a preventable and treatable pulmonary disease, which is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities caused by significant exposure to noxious particles

or gases[3]. Additionally, in this disease, there are extra pulmonary changes, such as declining in aerobic capacity, endurance, strength and balance, which affect the performing in activities of daily living (ADLs) impairing the quality of life[4–8]. Subjects with COPD have lower level of physical activity, which can decrease muscle mass and produce muscle weakness[9,10]. Muscle weakness increases in the presence of chronic diseases and is directly related to sarcopenia[11].

Sarcopenia has been defined by the European working group on sarcopenia in older people (EWSGOP) as a syndrome[12] or disease[13] characterized by loss of skeletal muscle, muscle strength and physical performance, being the major cause of frailty in the elderly[12]. This has a prevalence of approximately 5%-13% of all individuals over 65 years old[14]. Nevertheless, in subjects with COPD the prevalence of sarcopenia is even higher [15,16].

Sarcopenia is associated with metabolic change, immobility, mitochondrial dysfunction[11,17], oxidative stress[18,19] and systemic inflammation[11] which contribute to age-related deficits in muscle[20], through increased damage to the cell[18]. In COPD, there is strong evidence showing that pathophysiological changes are related to a systemic inflammation[21], oxidative stress (OS)[22] and genetic polymorphisms[23,24] which could be associated with the pronounced presence of sarcopenia in this disease, since those are the main etiological factors for this syndrome[11,18]. Therefore, this review aimed to know the main pathogenic mechanisms linked to subjects with COPD and sarcopenia.

METHODOLOGY

This review was conducted to identify the main pathologic mechanism related to sarcopenia in COPD. Five electronic databases were searched: PubMed, LILACS, EMBASE, Cochrane Library and Scielo from inception until December 2018 using the following search terms: "COPD", "pulmonary disease, chronic obstructive", “chronic obstructive lung disease”, “COAD”, “chronic obstructive airway disease” and

“sarcopeni*”. In studies that provide comparisons between people with COPD who do and do not have sarcopenia, we assessed inflammatory or oxidative stress biomarkers and genetic polymorphisms. Of 272 unique records identified through database searching, 24 articles studied the sarcopenia in this disease and only 5 of these performed blood biomarkers in sarcopenic COPD[25–2821]. Hand-searching of reference lists from each included article was also conducted to identify additional potential studies. Additionally, complementary references were used from the literature to create a possible explanation about the etiology of sarcopenia in COPD using the search terms: "COPD", "pulmonary disease, chronic obstructive", “chronic obstructive lung disease”, “COAD”, “chronic obstructive airway disease”, “aging” “inflammation”, “oxidative stress” and “genetic polymorphisms”. The information was organized in 5 different topics: 1) COPD and sarcopenia; 2) biological mechanisms of sarcopenia in COPD; 3) inflammation related to sarcopenia; 4) oxidative stress related to sarcopenia and 5) genetic polymorphisms related to sarcopenia.

COPD AND SARCOPENIA

The term sarcopenia is derived from the Greek words sarx (flesh) and penia (poverty)[29], although at the beginning the definition was only associated with lower muscle mass, nowadays is diagnosed as reduction in muscle strength and physical performance[13]. Sarcopenia was recognised as a musculoskeletal in the International Classification of Diseases 10th Revision (ICD-10) in 2016[30]. Additionally, the prevalence of sarcopenia in individuals over 65 years old is between 5%-13%[14], however, in subjects with COPD it is higher and may vary from 15%[15] to 55%[16].

In COPD, the presence of sarcopenia has been associated with changes in body composition, physical activity, exercise capacity, systemic inflammation and health status[9,24,27], being more prevalent among patients in BODE quartile 3 or 4 than between the other BODE quartiles[16]. The BODE index incorporates measurements of nutrition

(body mass index), airflow obstruction (forced expiratory volume in one second (FEV_1), dyspnea (Modified Medical Research Council) and exercise capacity (six minute walking test), which has been associated with mortality, severity and prognosis[31]. Therefore, sarcopenia is related to disability, severity and a poor prognosis in individuals with COPD[15,16].

BIOLOGICAL MECHANISMS OF SARCOPENIA IN COPD

In subjects with sarcopenia there is an increase in the degradation of myofibrillar proteins and decrease in the proteins synthesis, producing muscle atrophy and muscle weakness[32]. Although sarcopenia is a disease of the elderly, its development may be associated with other factors, which are not exclusively of older people, such as metabolic disorders, systemic inflammation, oxidative stress, decreasing in physical activity, mitochondrial dysfunction and cachexia[18,29]. These factors are also present in subjects with COPD and can potentially lead to sarcopenia in this disease (Figure 1)[21,22,33].

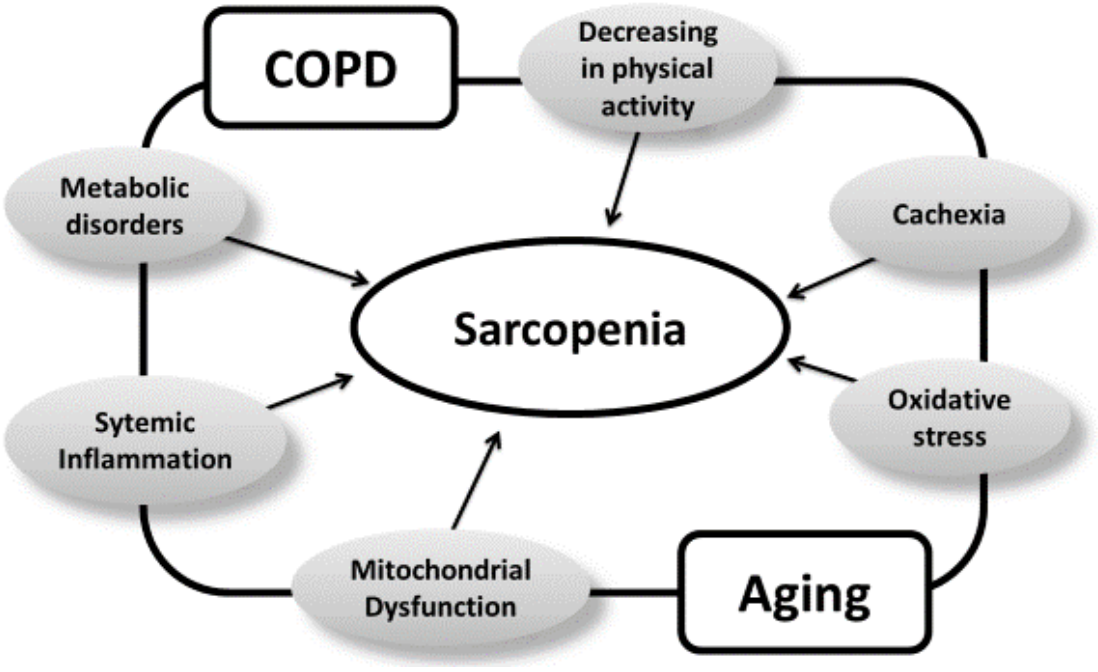

Figure 1. Features associated with sarcopenia among COPD and aging. COPD and Aging have been related to metabolic disorders, systemic inflammation, mitochondrial dysfunction, lower levels of physical activity, cachexia and oxidative stress. All these factors induce Sarcopenia.

The interaction between external and internal factors play an important role in the development of sarcopenia in subjects with COPD. The level of physical activity, the kind of diet, smoking and the use of steroids produce directly oxidative stress and systemic inflammation, which combined with genetic polymorphisms are associated with the origin of sarcopenia (figure 2)[11,18,22,34]. Oxidative stress, chronic inflammation and mitochondrial dysfunction play important roles in muscle atrophy, because these factors affect the balance among protein synthesis and breakdown[17,18]. Oxidative stress is an imbalance between oxidant and antioxidant species[35]. Reactive oxygen and nitrogen species (ROS/RNS) are second messengers for tumor necrosis factor alpha (TNF-α) in skeletal muscle, activating nuclear factor-Kb (NF-kB), directly and indirectly inducing systemic inflammation[18]. This systemic inflammation induces cellular apoptosis in the muscle, which is associated with muscle catabolism, declining both muscle mass and strength[36]. This muscle weakness is caused through two mechanisms: accelerated protein loss and contractile dysfunction[36].

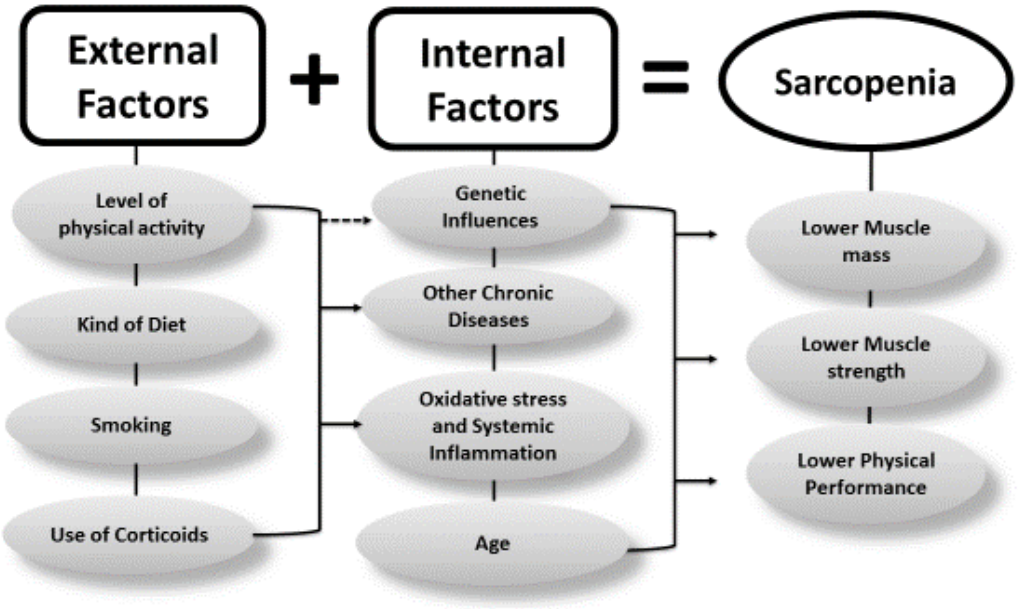

Figure 2. Factors associated with sarcopenia in COPD. Sarcopenia is induced by external and internal factors, which have direct association (continue line) or indirect association (dashed line) with each other and with sarcopenia.

INFLAMMATION RELATED TO SARCOPENIA

One of the important factors associated with sarcopenia in older people is the systemic inflammation[21,37]. Interleukin- 6 (IL-6), tumor necrosis factor-alpha and protein-C reactive (PCR) have been related to lower muscle mass, lower physical performance[38] and lower muscle strength[37] in older people. These biomarkers are increased in individuals with COPD compared to those without this disease[33,39]. Additionally, the inflammation has been associated with worse functional and health status in individuals with COPD[33,39,40], being one of the possible causes of sarcopenia in this disease[21]. Higher levels of IL-6[21], TNF-α[21] and PCR[25,28] have been reported in individuals with COPD and sarcopenia compared to those without sarcopenia. In this line, the systemic inflammation seems to affect the muscular mass and muscle strength in this population, since in the study

published by Byun *et al.* 2017[21], negative correlations were observed with inflammatory biomarkers[21].

There is a strong relation between the inflammation and oxidative stress, so these mechanisms are usually linked[22,41–43]. The systemic inflammation and the oxidative stress decrease the levels of insulin-like growth factor 1 (IGF-1) and can block the pathway of protein synthesis (figure 3), and this reduction is associated with sarcopenia, frailty and mortality[24,42]. The role of IGF-1 is to signal thought Akt pathway inducing protein synthesis by stimulation of mammalian target of rapamycin (mTOR)[32]. Signaling via IGF-1 begins with the IGF-1 ligand binding to its receptor, this induce a receptor phosphorylation and recruitment of insulin substrate 1 (IRS1). The phosphorylation of IRS1 activates the phosphoinositide-3-kinase (PI3K) pathway, activating protein kinase B (Akt) and mTOR inducing protein synthesis and muscle hypertrophy. IGF-1 can reduce with aging and chronic diseases, reducing the activity of Akt and mTOR[18]. These cascades signaled by inflammatory biomarkers and oxidative stress are produced during the aging process and it is exaggerated in the presence of chronic diseases, such as COPD, which is associated with sarcopenia (figure 3)[22,33].

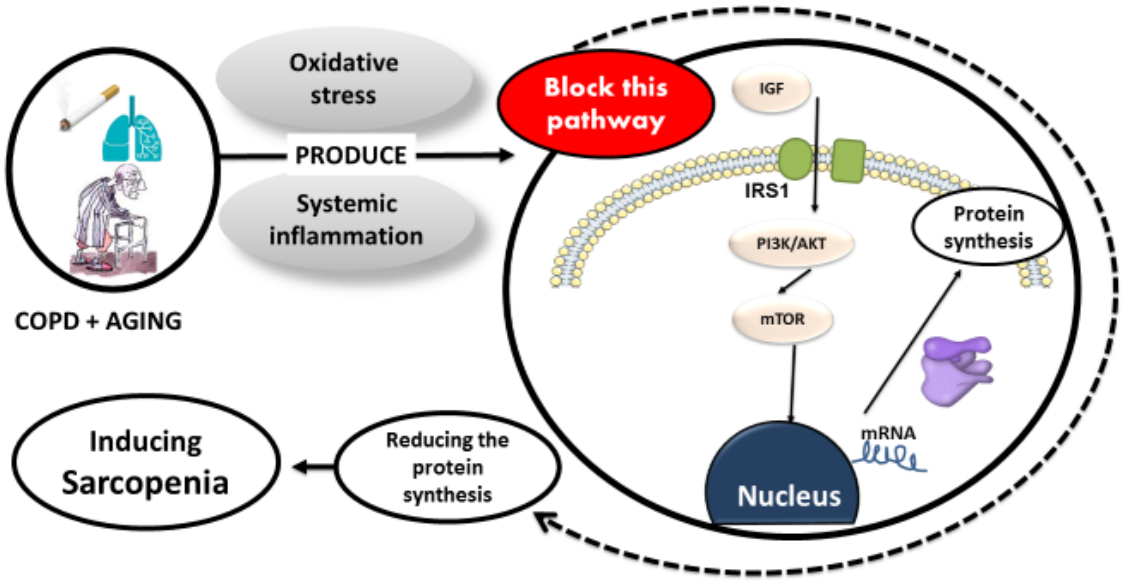

Figure 3. COPD and Aging induce Sarcopenia. COPD and Aging are associated with systemic inflammation and oxidative stress, they block the IGF/PI3K/AKT/mTOR pathway, reducing the protein synthesis, therefore, inducing Sarcopenia.

OXIDATIVE STRESS RELATED TO SARCOPENIA

Cesari *et al.* [44] demonstrated a positive correlation between antioxidant status with physical performance and muscle strength in elderly people. These authors showed that subjects with higher dietary intake of antioxidant such as vitamin C, was associated with higher skeletal muscle strength. In addition, Agler *et al.* [45] in a 10-year follow-up study with 38.597 women (≥45 years old) found that antioxidant supplementation reduced the risk of developing chronic lung disease by 10% (HR 0.90; 95% CI 0.81 to 0.99; p=0.029) and lowered carbonyl stress levels in the lung. Studies in individuals with COPD, showed a reduction in the antioxidant activity of superoxide dismutase (SOD), catalase (CAT), total protein sulfhydryls (SH), paraoxonase 1 (PON1), glutathione peroxidase and glutathione-S-transferase (GST)[46–49]. However, there are contradictory results with some antioxidant biomarkers, as SOD, CAT and SH, which have shown to be increased or not different, compared to individuals without COPD[50,51].

Subjects with COPD have an oxidant-antioxidant imbalance[46–48]. The levels of oxidative stress biomarkers are increased in individuals with COPD. [46–54]. Lipid peroxidation and protein oxidation are consequences of oxidative stress and cause of oxidative damage[48,50,55,56]. Thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) are the most commonly biomarkers of lipid peroxidation studied in subjects with COPD[48]. Niraj Dhakal *et al.* [48], Raut *et al.* [53] and Wozniak *et al.* [51] reported higher levels of MDA and TARBS in subjects with COPD compared to control group. In contrast with that, Syrine *et al.* [54] and Jammes *et al.* [57] found no differences in MDA and TBARS levels, respectively, among individuals with COPD and their counterparts. Additionally, aldehyde 4-hydroxy- 2-nonenal (4-HNE), the major compound of membrane lipid peroxidation, is elevated in individuals with COPD which is a key mediator in different mechanisms related to oxidative stress, inflammation and apoptosis[55,56].

With respect to protein oxidation, in individuals with COPD, the degree of oxidant-mediated protein damage has been investigated through the presence of advanced oxidation protein products (AOPP)[17]. Stanojkovic *et al.* [58] found higher levels of AOPP and MDA in subjects with COPD compared to apparently healthy, also, this study showed that elevated systemic inflammation is negatively correlated to antioxidant capacity and oxidant biomarkers. Therefore, these results emphasize the relationship between oxidative stress and inflammation.

There are few studies that reported associations between antioxidant markers and sarcopenia or physical activity in individuals with COPD[54]. The majority of the studies correlated oxidative stress biomarkers with body mass index, lung function, nutritional status, exacerbations or GOLD stages and inflammation[48,54,58–60]. The research of Byun and collaborators[21] is the only one which reported association among some inflammatory biomarkers with sarcopenia in subjects with COPD. Although there is no evidence reporting associations between sarcopenia and oxidative stress in this population, we can hypothesize that the oxidative stress plays an important role in the origin of

sarcopenia, since oxidative stress is linked to different biological mechanisms related to inflammation and muscle atrophy [11,48,50,55,56,58].

GENETIC POLYMORPHISMS RELATED TO SARCOPENIA

The influence of genetic in sarcopenia, have been currently studied[42]. Single nucleotide polymorphisms are related to systemic inflammation and decrease in protein synthesis, being associated with sarcopenia and frailty in older people[61]. Mutations in IGF-I and IGF-II genes in human reduce the synthesis of IGF, which are associated with muscle phenotypes, lower muscle strength, lower muscle mass, muscle damage and lower response to exercise training[62,63]. The C-1245T SNP (rs35767) is a genetic variation in the promoter region of the IGF-I, which has been studied in elderly, associated with decreases in muscle mass and muscle strength, since IGF-I gene regulates the protein synthesis and muscle hypertrophy[61,62]. In addition, genetic mutations can interfere in the response of the exercise.

Devaney et al[62] found that the polymorphism of IGF-II (rs3213221) produce greatest strength loss immediately after exercise, greatest soreness and highest post exercise serum creatine kinase, which is a signal of muscle damage[62]. Other polymorphism associated with sarcopenia and frailty is the mutation in ACTN-3 genes in humans (rs1815739), it can decrease the level of α-Actinin-3, which is a protein related with muscle contractions[64].

Pro-inflammatory biomarkers play a role in the muscle weakness[18]. The production of cytokines can be influenced by singe nucleotide polymorphisms (SNPs), usually in the promotor region of the genes[65]. Both sarcopenia and systemic inflammation can be influenced by genetic polymorphisms. In respect with that, Broekhuizen et al[66] found more prevalence of the IL-1β-511 polymorphism in COPD individuals with lower fat-free mass index in comparison with apparently healthy subjects. These findings suggest a genetic predisposition in the process of cachexia in subjects with COPD[66].

Finally, the genetic contribution to develop COPD has been well stablished in a systematic review developed by Bossé et al[67]. However, there are few studies showing correlation with sarcopenia in COPD with SNPs. Additionally, mutation of ILG-II and IGF-II genes have been little studied in individuals with COPD, since the majority of the SNPs associated with this disease have been related to systemic inflammation and pulmonary function[67].

CONCLUSIONS

The interaction between external and internal factors as systemic inflammation, oxidative stress and genetic polymorphisms are related with the presence of sarcopenia in subjects with COPD. However, new studies are necessary to identify some specific biomarkers and SPNs related with muscle strength, muscle mass and physical performance in this disease.

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3.2: ORIGINAL ARTICLE 1

Title: Novel antioxidant and oxidant biomarkers related to sarcopenia in COPD.

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Article published in *Heart and Lung*

(Q2/ Impact factor: 2.21)

Sepúlveda-Loyola W, de Castro LA, Matsumoto AK, Camillo CA, Barbosa DS, Galvan CCR, et al. NOVEL antioxidant and oxidant biomarkers related to sarcopenia in COPD. Hear Lung. 2020;000:1–8

ABSTRACT:

Background: The relation between oxidative stress (OS) and sarcopenia in COPD remains unknown.

Objective: To analyze OS levels and its association with sarcopenia in COPD.

Methods: Thirty-nine individuals with COPD (69±7years; 41%female) and thirty-five for the control group (69±7years; 43%female) were included. A*dvanced oxidation protein products* (AOPP), paraoxonase-1 (PON1), superoxide dismutase activity (SOD), catalase dismutase activity (CAT), sulfhydryl group (SH), nitric oxide metabolites (NOX), total radical trapping antioxidant parameter (TRAP) were analysed. OS markers were correlated with handgrip and quadriceps strength, gait speed, skeletal muscle mass index, fat-free mass index, maximum inspiratory and expiratory pressure. European criteria were used to identify sarcopenia.

Results: In COPD, antioxidant capacity was correlated with muscle mass and strength (r from 0.5 to 0.64) $P<0.05$ for all. TRAP≤ 850 µM/trolox and AOPP≤65 µM/l were associated with sarcopenia (OR:8.3; 95% CI: 1.4-49.6 and OR:14; 95%CI: 2.2-87.1, respectively; $P<0.05$ for both).

Conclusion: OS is associated with sarcopenia in COPD.

Keywords: Oxidative stress, Sarcopenia, COPD.

ABBREVIATIONS LIST

ACCI	Age-adjusted Charlson comorbidity index
AOPP	Advanced oxidation protein products
AUC	Area under curve
BMI	Body-mass index
CAT	Catalase activity
COPD	Chronic obstructive pulmonary disease
EWGSOP	European working group on sarcopenia in older people
FEV1	Forced expiratory volume in the first second
FFM	Fat free mass
FFMI	Fat free mass index
FVC	Forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GS	Gait speed
HDL	High-density lipoprotein cholesterol
HGF	Hand grip force
LDL	low-density lipoprotein cholesterol
NOX	Nitric oxide metabolites
MEP	Maximum expiratory pressure
MIP	Maximum inspiratory pressure
OS	Oxidative stress
PON1	Paraoxonase-1
QS	Quadriceps strength
ROS	Reactive oxygen species
ROC	Receiver-operating characteristic
SH	Sulfhydryl group
SMM	Skeletal muscle mass
SMMI	Skeletal muscle mass index
SOD	Superoxide dismutase activity
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TRAP	Total radical trapping antioxidant parameter

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a condition characterized by extrapulmonary consequences affecting functionality and quality of life [1,2]. The observed low levels of daily physical activity may induce a reduction in muscle mass and generate muscle weakness [3–6]. The latter increases in the presence of associated chronic diseases and is directly related to sarcopenia [7].

Sarcopenia is defined as a reduction in muscle strength, physical performance and muscle mass [7]. It is a major cause of frailty [5,7] and has a prevalence of roughly 5–13% in older individuals [8]. The prevalence of sarcopenia is yet higher in COPD varying from 15% [9] to 40% [4], depending on the classification's criteria [7] and has a negative impact on prognosis and physical function in individuals with COPD [4].

The pathogenesis of sarcopenia is multifactorial and is commonly attributed to metabolic changes, immobility, mitochondrial dysfunction [5,10], oxidative stress (OS) [11,12] and systemic inflammation [3], contributing to age-related alterations in the muscle [13], via increased damage to the cells [11,14].

There is evidence reporting that the pathophysiological response in individuals with COPD is related to the presence of OS produced by the oxidant / antioxidant imbalance [15–17]. However, literature is still inconclusive regarding what specific OS biomarkers are associated with these biological pathways. Different studies reported that a*dvanced oxidation protein products* (AOPP), sulfhydryl group (SH), superoxide dismutase activity (SOD) and catalase activity (CAT) are either increased, reduced or non-different between COPD and healthy subjects [15,18–21].

There is evidence that ROS (reactive oxygen species) may directly or indirectly modulate transcription factors and kinases inducing proteolytic pathways and cellular apoptosis in muscle. Since these mechanisms contribute to sarcopenia, it is reasonable to hypothesize that OS biomarkers are as well associated with clinical measurements used

to diagnose sarcopenia (muscle strength, muscle mass, and physical performance) [11,22,23]. The association (or lack thereof) between these outcomes, however, remains to be demonstrated in individuals with COPD. The aim of this study was, therefore, to analyze OS and its association with sarcopenia in COPD in comparison to apparently healthy individuals.

MATERIAL AND METHODS

Study design and sample

Patients with COPD (55 ≥ years old) were diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria[24] and recruited from the outpatient unit of the University Hospital of Londrina State University, Brazil. They would be excluded if presented an exacerbation episode in the two weeks before inclusion, diagnosis of bronchial asthma, presence of neurological or psychiatric diseases, arthritis, heart failure, alcohol dependence and use of antioxidant supplements. The control group included apparently healthy individuals recruited from the community who were matched for gender, age and ethnicity. The study was approved by the university ethics review board and all participants provided written informed consent (#1.830.048).

Procedures

Sarcopenia definition

Sarcopenia was defined following the European working group of sarcopenia in older people (EWGSOP). Individuals were deemed sarcopenic if both low muscle mass and low muscle function (handgrip strength and physical performance)[7].

Muscle mass

Muscle mass was quantified using a bioelectrical impedance (Biodynamics 310TM; Biodynamics Corp., USA) with measurements of fat-free mass (FFM), fat-free mass index (FFMI-FFM/ height^2), skeletal muscle mass (SMM) and skeletal muscle mass index (SMMI-SMM/ height^2). FFM was calculated using the formula of Kyle et at [26]. FFMI was considered reduced if <20.35kg/m^2 in men or < 14.65 kg/m^2 in women [27]. SMM was

estimated using the equation developed by Janssen et al. [28]. SMMI was considered reduced if SMMI ≤10.75 Kg/m^2 in men and ≤6.75 kg/m^2 in women [7].

Handgrip strength

Handgrip strength (HGS) was assessed using a hydraulic dynamometer (Jamar Plus + Digital 563213; Lafayette Instrument Company, USA). The highest value from tree attempts (1-minute rest between each attempt) was used. Low muscle strength for HGS was defined as ≤30 Kg in men and ≤20 Kg in women [7].

Physical performance

Physical performance was evaluated using the gait speed (GS) during the 4-meter gait speed test. Individuals were instructed to walk at their usual gait speed on a 4-meter marked track. The time was controlled by a stopwatch and the average speed to cover the distance of two attempts was used for analysis. Low physical performance was defined as GS ≤0.8 m/sec [7].

Quadriceps strength

Quadriceps strength (QS) was measured using a dynamometer (EMG System, Brazil) attached to a multi-station unit (CRW 1000; Embreex, Brazil) following previously standardized procedure [29]. In short, participants performed a minimum of four and a maximum of five maximal voluntary isometric contractions of the quadriceps for six seconds. The highest value was used for analysis [29].

Respiratory muscle strength

Respiratory muscle strength was measured as maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) using a digital manovacuometer (MVD 300, GlobalMed, Brazil) following previously standardized procedures [30]. Maneuvers were maintained for at least 2 seconds and the peak value was recorded. The best of 3 acceptable and reproducible maneuvers was used for analysis.

Pulmonary function

Pulmonary function was assessed with whole-body plethysmography (Vmax®, Carefusion, EUA). Measurements were performed according to the American Thoracic Society/European Respiratory Society guidelines [25], with reference values described for the Brazilian population [31].

Comorbidities

Presence of comorbidities was investigated using the Age-adjusted Charlson comorbidities index (ACCI) in both groups. ACCI includes 19 medical conditions and was scored using the algorithm proposed by Charlson et al. [32].

Oxidative stress biomarkers

Oxidative stress biomarkers were analyzed from blood samples. Approximately 40 mL of blood sample was obtained by venipuncture into vacuum tubes (Vacutainer®, Franklin Lakes, NJ USA) after 12 hours of fasting. Blood samples were centrifuged for 30 minutes at 3000 rpm (2100 xg) at 20°C, in order to obtain serum, plasma and concentrated of red blood cells to ensure the analysis of oxidative stress biomarkers, and then, frozen at -80°C. Most of the blood components were stored until all the subjects were evaluated (approximately 1 year).

Total radical-trapping antioxidant parameter (TRAP) [33], paraoxonase 1 activity (PON1) [34], superoxide dismutase activity (SOD) in erythrocytes [35], catalase activity (CAT) in erythrocytes [36], sulfhydryl groups (SH) [37], advanced oxidation protein products (AOPP) [38] and nitric oxide metabolites (NOx) [39]were used as OS biomarkers and whilst glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides were used as metabolic risk factors assayed following previous studies [33–39].

TRAP was evaluated on a microplate reader (Victor X-3, Perkin Elmer®, USA) and results were expressed in µM Trolox[33]. PON1 activity was determined by the rate of hydrolysis of chloromethyl phenyl acetate as well as by the rate of hydrolysis of phenyl

acetate under low salt condition. Analysis were conducted on a microplate reader (EnSpire, Perkin Elmer®, USA) [34]. SOD in erythrocytes was detected through a method based on the inhibition that this enzyme promotes in the auto-oxidation of pyrogallol in aqueous solution [35]. The amount of SOD that was able to inhibit 50% of pyrogallol oxidation was defined as a unit of enzymatic activity. The SOD reaction was read on a microplate reader (EnSpire, Perkin Elmer®, USA) with a wavelength of 420 nm. The results were expressed in U/min/gHb. CAT in erythrocytes was detected by measuring the decay in the concentration of H2O2 and oxygen generation, using the technique described by Aebi et al [36]. The CAT reaction was read on a microplate reader (EnSpire, Perkin Elmer®, USA) with a wavelength of 240 nm. The results were expressed in U/min/gHb. SH groups were evaluated in a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 412 nm and results were expressed in μM (Hu, 1994)[37]. AOPP was quantified on a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 340 nm and was expressed in mM of equivalent chloramine T[38]. NOx was evaluated on a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 540 nm by measuring the concentration of nitrite and nitrate and results were expressed as μM[39].

Statistical analysis

Statistical analysis was performed using SPSS (IBM Co., USA). The Kolmogorov–Smirnov test was used to analyze normality of data distribution. Comparisons between groups were done using unpaired t-tests. Frequencies were compared using the chi-squared test and odds ratio values were provided. Pearson's correlation coefficient was used to investigate the relation between OS biomarkers with muscle mass, muscle strength and gait speed. Linear regression analysis was performed to find association between antioxidants and oxidants with clinical measurements of sarcopenia (muscle mass, strength and gait speed). Logistic regression analysis was used to identify the factors associated with sarcopenia in COPD using different variables. Receiver-operating characteristic (ROC) curves, the area under the curve (AUC), Youden´s index,

sensitivity and specificity were used to identify OS best cut-offs identifying sarcopenia in individuals with COPD. Statistical significance was set at *P*<0.05.

RESULTS

Comparisons between individuals with COPD and apparently healthy individuals

Seventy-four subjects (thirty-nine patients with COPD and thirty-five apparently healthy subjects) were included. Socio-demographic characteristics, comorbidities and metabolic risk factors are reported in table 1. ACCI was significantly higher in subjects with COPD (*P*=0.004). There were no significant differences in metabolic risk factors between the groups.

Sarcopenia measurements are reported in table 2. Muscle mass and FFM were lower in COPD in comparison to their counterparts (*P*=0.009 and 0.035, respectively). More patients with COPD presented low muscle strength and sarcopenia than those in the CG (*P*=0.02 and 0.01, respectively). QS and MIP were worse in COPD than in CG (*P*<0.001 and <0.05, respectively). The prevalence of sarcopenia was 23% in individuals with COPD which is higher compared to the control group (*P*= 0.01). Additionally, 78% of the individuals with sarcopenia were in GOLD stage ≥3.

Antioxidant and oxidant biomarkers are reported in Figures 1 and 2. The following biomarkers were significantly higher in COPD in comparison to CG: sulfhydryl group (352±52 *versus* 313±44 Mm/mg protein), superoxide dismutase activity (43±14 *versus* 35±12 U/mgHb) and paraoxonase 1 (180±55 *versus* 152±48 U/ml), *p*<0.05 for all. Lower catalase activity was observed in COPD patients compared to CG (47±11 *versus* 54±11 U/mgHb; *P*=0.012). No differences between groups were observed for TRAP, AOPP and NOX.

Associations between oxidative stress biomarkers and clinical measurements of sarcopenia in subjects with COPD

TRAP was positively correlated with FFMI (r=0.5), SMMI (r=0.5), QS (r=0.64) and HGS (r=0.51); likewise, AOPP showed positive correlation with FFMI (r=0.43), SMMI (r=0.52), HGS (r=0.5), MIP (r=0.59) and MEP (r=0.46); and SOD showed negative correlation with SMMI (r= -0.36) and MIP (r= -0.45); $P<0.05$ for all.

Statistically significant correlations between biomarkers and clinical outcomes were included in the regression analysis models. Univariate analysis revealed significant associations for all clinical outcomes (FFMI, SMMI, QS, GS, S MIP and MEP). In the multivariate analysis models, TRAP and AOPP were the OS biomarkers that associated the most with clinical outcomes (R from 0.31 to 0.80). The complete list of univariate and multivariate analysis is provided in table 3.

Factors associated with prevalence of sarcopenia in subjects with COPD

Selection of presented cut-off points used in the logistic regression were done considering previously described values for the variables: ACCI (≥4); Body-mass index, BMI (<22 Kg/m^2); GOLD stage (≥3) and; FFMI (<14.65 Kg/m^2 for women and <20.35 Kg/m^2 for men)[4,27]. The selection of cut-offs for the outcomes without reference values were done via ROC analysis (Figure A.1 in appendices). Table 4 shows AUC, sensitivity and specificity of the cut-offs used to discriminate the presence of sarcopenia in COPD in the variables: AOPP, HDL, MEP, MIP, QS and TRAP. Other investigated biomarkers are not presented in the table 4 since AUC was lower than 0.5 with $P>0.05$.

Figure 3 describes the results of the logistic regression analysis. The prevalence and OR of all variables are reported as an orbital bubble chart. Age, gender (female), BMI, FFMI, MIP, MEP, QS, TRAP, AOPP and HDL were factors significantly associated with the development of sarcopenia in individuals with COPD ($p<0.05$ for all). Since SMMI, HGS and GS are criteria to diagnose sarcopenia, they were not considered in this analysis.

DISCUSSION

The present study confirms the increased prevalence of sarcopenia and higher levels of antioxidant activity in patients with COPD. Our findings add to the field that 1) OS biomarkers associate with clinical measurements of sarcopenia and; 2) the marked reduction of the TRAP and AOPP biomarkers are highly prevalent and are associated with sarcopenia.

The study showed that individuals with COPD present decreased muscle mass and quadriceps and inspiratory muscle weakness when compared with apparently healthy subjects. The prevalence of sarcopenia in individuals with COPD was 23% which is similar to previous studies reporting prevalence rates between 15% [9] to 40%[4] and significantly higher than the control group (3%). Additionally, and as observed in literature[4], sarcopenia was more prevalent in individuals with GOLD stage ≥3. Patients with COPD presented higher scores in the ACCI (more comorbidities with larger weights) and higher prevalence of smoking history. Of note, the abovementioned differences between COPD and the control group were expected and previously described[40].

COPD has been related to systemic inflammation and oxidative stress by different factors related to this disease as smoking history, medications, lower physical activity, hospitalization and other comorbidities associated with COPD [3,15,16]. In the present study, the SH was significantly increased in COPD. In line with our findings, Nadeem et al. [18] reported higher levels of glutathione (GSH), which is one of the organic components that contains SH. Higher levels of SH are related to GSH and this increase could be a compensatory response for the excess of oxidants in COPD [41]. PON1 is an enzyme with antioxidative and antiatherogenic properties, which is associated with high-density lipoprotein (HDL) [42]. Its activity was found reduced in different diseases associated with oxidative stress such as COPD [15,16]. The present study, however, reported higher levels of PON1 in the COPD group. PON1 is associated with HDL, and an increase in HDL levels can lead to an increase in PON1 activity [42]. We observed a non-significant trend (*P*=0.06) of larger HDL concentration in COPD than CG. In addition, 49% of the

individuals with COPD showed HDL≥57.5 mg/dl, which was significantly associated with prevalence of sarcopenia in COPD (OR: 13.8; 95% CI:1.519-125.7). Furthermore, 54% of the individuals were classified as GOLD ≥3, a group of subjects with known higher levels of HDL [43].

SOD is an important antioxidant enzyme that inhibits superoxide anion (O_2^-) and protects aerobic cells from oxidative stress [19]. We observed higher levels of superoxide dismutase in patients with COPD. Similar findings have been reported in other studies[18,19]. SOD is the only enzymatic system-decomposing O_2^- to H_2O_2 and it plays a significant role especially in the lungs and muscle cells [44]. In respect with that, we found that SOD was negatively correlated to SMMI (r: -0.36). Additionally, we found a moderate negative correlation between SOD and MIP (r= -0.45). Since a reduction in MIP induces respiratory overload, it can be hypothesized that respiratory muscle weakness might be related to an increase in SOD activity, as this enzyme is sensitive to the stress in the respiratory system [44]. Another important antioxidant enzyme is catalase. Literature, however, is still controversial about its levels in subjects with COPD [15,19,20]. Wozniak et al. evaluated 73 patients with COPD and observed no differences in CAT activity compared to the control group [19]. In contrast, Vibhuti et al. dosed the antioxidant capacity in 202 subjects with COPD and found a significant reduction in CAT activity compared to healthy controls [20]. The latter is in accordance with the present study. A decrease in CAT activity leads to ROS-induced cellular damage and limits the scavenging capacity for lipid peroxides and ROS, further impairing the pathogenesis of COPD [20]. Nitric oxide (NO) is an important element of vascular homeostasis [45]. OS reduces the synthesis of NO impairing the endothelial and pulmonary function [41,45]. Anes et al. [41] found a significant reduction in nitric oxide metabolites (NOx) levels in subjects with COPD compared to control group and observed an association between NOx and airflow obstruction. In the present study, albeit not statistically significant, NO levels were

reduced in individuals with COPD when compared to apparently healthy subjects (*P*=0.052).

AOPP is an oxidative biomarker used to estimate protein damage [38]. Although no differences were observed between COPD and control group in AOPP and NOx, it is not possible to confirm that the oxidants are not augmented in individuals with COPD. This can be said since the oxidative stress could be compensated by the higher antioxidant activity previously reported in the present study. Additionally, there are other oxidants related to other oxidative stress pathways which were unfortunately, not investigated.

This is the first study to investigate correlations between AOPP and sarcopenia in COPD. A likely explanation to the positive correlation between AOPP with muscle mass (FFMI: r=0.43; SMMI: r=0.51) and strength (HGS: r=0.5, MIP: r=0.6 and MEP: r=0.46) is that higher muscle mass and strength implies in more protein substrate to be oxidized [5,46] . In fact, higher levels of AOPP have been associated with oxidation of albumin, fibrinogen and lipoproteins [47]. On the other hand, low protein levels in blood (which is related to low protein oxidation) have been associated with worse muscle function[46]. This could explain at least in part why individuals with COPD who have sarcopenia presented decreased levels of AOPP.

In a multivariate analysis TRAP, AOPP and BMI were the variables explaining the increase in SMMI (R^2: 0.51). In the present study, AOPP and TRAP were the biomarkers with higher sensitivity and specificity to identify subjects with sarcopenia. Albeit AOPP presented association with sarcopenia measurements, TRAP was the most important biomarker associated with FFMI, QS and GS in subjects with COPD. This is a biomarker of antioxidant capacity of macromolecules which has been associated with increased muscle strength [47]. In this study, the positive association between TRAP and quadriceps strength reinforces the protective role of antioxidants on both muscle mass and muscle function reported by other authors[11,12]. In the present study, this biomarker combined with other outcomes explained 80% of FFMI which is a marker of muscle mass. Since

TRAP was highly associated with muscle mass, it is possible to hypothesize that the relation between TRAP and muscle mass is stronger in large muscle groups as quadriceps. In fact, this biomarker alone explained 43% of variation in quadriceps strength in our study. Considering that gait speed is associated with muscle power, (i.e. the combination of force and velocity), the significance of TRAP and QS explaining up to 51% of variability in GS could be anticipated. TRAP was associated with muscle mass and strength and physical performance in COPD, which are the main clinical measurements to identify sarcopenia according to EWGSOP[7]. Reduction in TRAP levels (i.e. ≤ 850 μM trolox) increases 8.3-fold the risk of having sarcopenia in subjects with COPD (*P*=0.043). This is a new finding and future investigations are encouraged to confirm these results since the knowledge of specific biomarkers and biological pathways would help to understand the responses to different treatment in this field. Additionally, the present results could be useful for developing future intervention with antioxidant medications or dietary supplements for sarcopenia in individuals with COPD. Sarcopenia has been highlighted as an important focus of treatment in adult respiratory medicine[48]. One of the few studies in this area was conducted by Jones *et al.*[49] who demonstrated that pulmonary rehabilitation, a multicomponent exercise-based intervention, improved a range of clinical outcomes and reduced the incidence of sarcopenia in patients with COPD. Not only exercise, but also nutritional support have been recommended as interventions for sarcopenia [50]. Current studies have reported that the combination of dietary intake of antioxidants with exercise is relevant for the treatment and prevention of sarcopenia in elderly people[51,52]. Therefore, more research is warranted to further validate these findings, including the use of other recommended therapies, such as antioxidant supplementation or medications combined with pulmonary rehabilitation in individuals with COPD[7,53,54]. This would help to identify the effects of these new approaches on clinical measurements of sarcopenia and on the levels of OS biomarkers.

It is important to highlight some potential limitations of the present study. Proteins quantity was not measured. Although it does not detract from the quality of our results, it could explain and reinforce the strength of the observed associations between AOPP and sarcopenia measurements. Additionally, only two oxidant biomarkers were dosed (NOX and AOPP), which do not explain all pathways related to oxidative stress reported in other studies. This is a cross-sectional study with a somewhat limited sample size. Therefore, causality of associations and the cut-off points reported should be interpreted with caution or cannot be postulated to other populations. In addition, some data presented wide confidence intervals which could explain the variability of the associations, regardless the statistical significance. Another important limitation was that a few cardiovascular diseases were not considered as exclusion criteria. It is known that apparent peripheral atherosclerosis, ischemic heart disease and hypertension, often present in patients with COPD have been also related to oxidative stress[55] and this could have interfered in the analysis. Importantly, the strengths of the study were that both groups were controlled for possible effects of confounding variables such as gender, age, alcohol intake and use of antioxidant supplements. Additionally, the diagnose of sarcopenia was established according to all criteria recommended by the EWGSOP, in contrast with other studies in COPD [3,4]. Finally, future studies in this field should include larger sample sizes, add more oxidant biomarkers and focus on sarcopenia stratification (presarcopenia, sarcopenia and severe sarcopenia) in order to support the findings of the present study.

CONCLUSIONS

Subjects with COPD showed higher levels of antioxidant activity, with exception of catalase activity. Additionally, patients with COPD presented a higher prevalence of sarcopenia compared to apparently healthy individuals. Finally, clinical measurements

of sarcopenia were correlated with OS biomarkers, being lower antioxidative capacity and protein oxidation associated with more prevalence of sarcopenia in COPD.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study was supported by the National Council for Scientific and Technological Development (CNPq), Brazil. Grant number 470853/2014; CNPq/MS/SCTIE/DECIT Nº34/2014.

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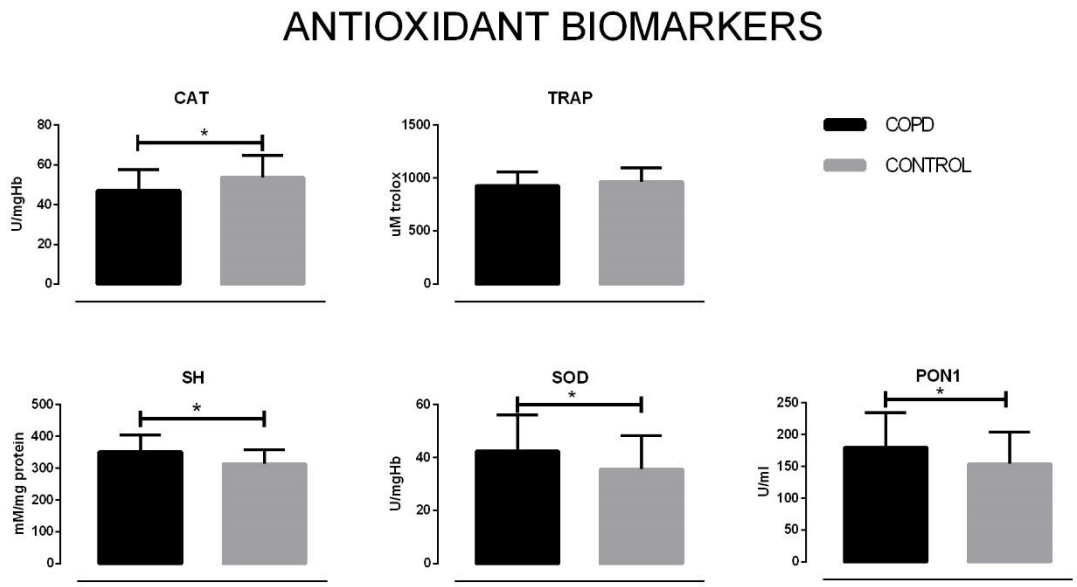
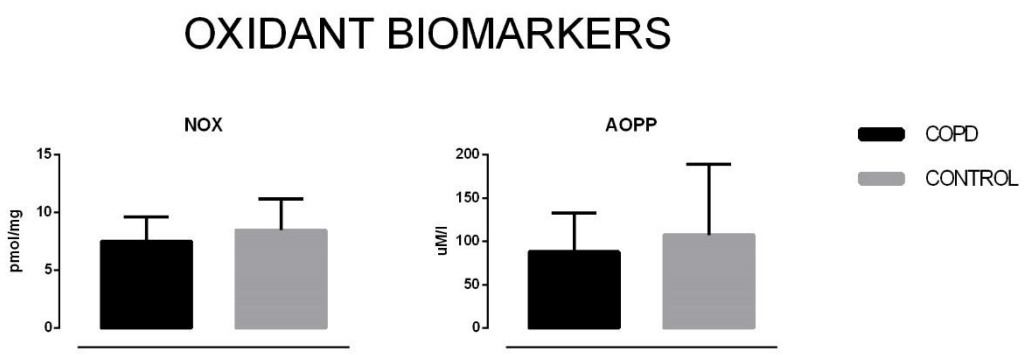
Figure 1



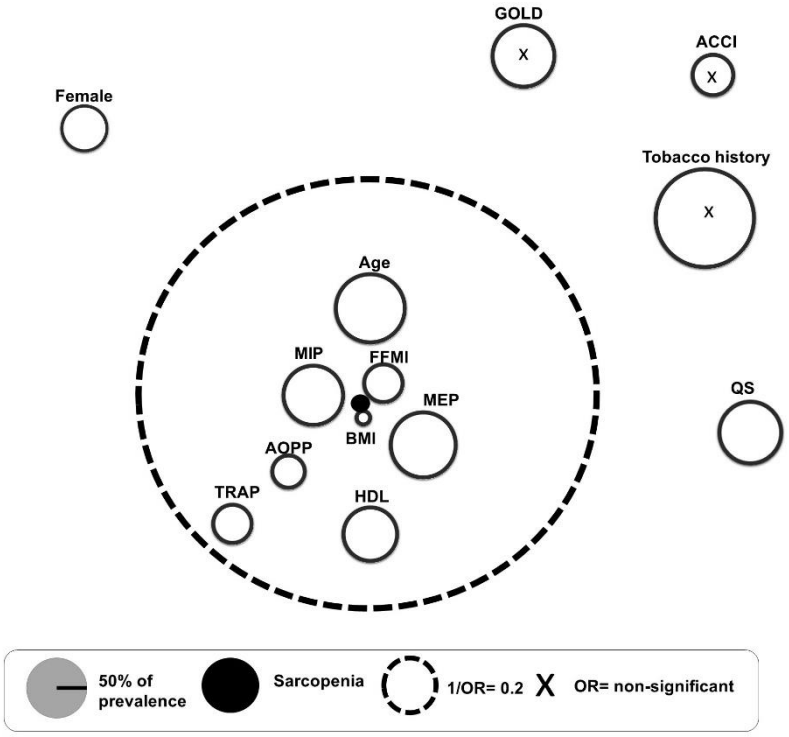
Legend: Data are expressed as mean ± standard deviation. COPD: chronic pulmonary obstructive disease; CAT: catalase activity; TRAP: total radical-trapping antioxidant parameter; SH: sulfhydryl group; SOD: superoxide dismutase activity; PON 1: paraoxonase 1 activity; *p≤0,05.

Figure 2



Legend: Data are expressed as mean ± standard deviation. COPD: chronic pulmonary obstructive disease; NOX: nitric oxide metabolites; AOPP: advanced oxidation protein products *p≤0,05.

Figure 3. Features associated with the odds to develop sarcopenia and the prevalence in individuals with COPD.



Legend: Factors with more than 10% of prevalence and stronger association with sarcopenia in individuals with COPD (Odds ratio [OR], 95% confidence interval, $P < 0.05$) are shown. The area of the circle shows the prevalence. The proximity to the center (sarcopenia) expresses the strength of the association among the factor and sarcopenia risk. All bubbles associated without statistical significant are marked with "X". Bubbles inside the dotted orbit have an OR ≥ 5 (1/OR=0.25). ACCI: age-adjusted Charlson comorbidity index (≥4); Age>67 years; AOPP: *advanced oxidation protein products (*≤65 μM/l*);* BMI: body mass index (<22 Kg/m^2); GOLD: global initiative for chronic obstructive lung disease (≥3); FFMI: fat-free mass index (<14.65 Kg/m^2 for women and <20.35 Kg/m^2 for men); HDL: high-density lipoprotein (≥57.5 mg/dl); MEP: maximal expiratory pressure (≤ 115 cmH_2O); MIP: maximal inspiratory pressure (≤ 77.5 cmH_2O); QS: quadriceps strength(≤26.15 Kg); TRAP: total radical trapping antioxidant parameter (≤ 850 μM trolox).

Table 1: Clinical characteristics of COPD and Control groups

Characteristics	COPD (n=39)	Control (n=35)
Age (years)	69 ± 6	69 ± 7
Female, n (%)	16 (41%)	15 (43%)
Height (m)	1.6 ± 9.7	1.6 ± 8.7
Weight (kg)	70 ± 17	73 ± 13
BMI (kg/m^2)	27 ± 6	28 ± 7
FVC %pred	86 ± 19	107 ±17*
FEV_1 %pred	49 ±13	98 ±16*
FEV_1/ FVC	46 ± 9	75 ± 5*
Smoking history, n (%)	34 (87%)	12 (34%)*
GOLD I-II, n (%)	18 (46%)	-
GOLD III-IV, n (%)	21 (54%)	-
Racial classification		
White	24 (61%)	27 (77%)
Black	5 (13%)	1 (3%)
Mulatto	5 (13%)	6 (17%)
Yellow	5 (13%)	1 (3%)
Charlson Comorbidity index		
Heart Failure history	4 (10%)	0 (0%)
Diabetes	5 (12%)	7 (20%)
Vascular peripheral Disease	7(18%)	6 (17%)
Age- Adjusted Charlson Comorbidity index		
Mean scores	4.4 ± 1.2	3 ± 1.2 *
0-1 (n, % of total)	0 (0%)	0 (0%)
2-3 (n, % of total)	11 (28%)	18 (51%) *
4-5 (n, % of total)	21(54%)	14 (40%)
≥6 (n, % of total)	7 (18%)	3 (9%)
Metabolic Risk Factors Biomarkers		
Glucose (mg/dl)	116 ± 38	109 ± 23†
Cholesterol (mg/dl)	201 ± 43	191 ± 43
LDL (mg/dl)	115 ± 38	110 ± 35
HDL (mg/dl)	65 ± 34	54 ± 12
Triglycerides (mg/dl)	130 ± 88	132 ± 96

Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; BMI: body mass index; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; *P≤0.05; **P≤0.001. †statistical analysis n=33.

Table 2: Sarcopenia measurements in COPD and Control groups

Measurements of Sarcopenia		COPD (n=39)	Control (n=35)
QS (Kg)		22 ± 9	39 ± 13**
MIP (cmH_2O)		79 ± 25	92 ± 31*
MEP (cmH_2O)		115 ± 36	124 ± 38
FFM (Kg)		37 ± 18	46 ± 7*
FFMI (Kg/m^2)		17 ± 3	18 ± 5
SMM (Kg)		21 ± 10	25 ± 6*
Criteria to diagnose Sarcopenia			
MM	SMMI (Kg/m^2)	8 ± 4	9 ± 2
MS	HGS (Kg)	33 ± 9	33 ± 7
PP	GS (m/sec)	1.1 ± 0.14	1.17 ± 0.18
Subjects with lower MM, n (%)		11 (28%)	8 (23%)
Subjects with lower MS, n (%)		10 (27%)	2 (6%)*
Subjects with lower PP, n (%)		0 (0%)	0 (0%)
Subjects with sarcopenia, n (%)		9 (23%)	1 (3%)*

Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; QS: quadriceps strength; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; FFM: fat-free mass; FFMI: fat-free mass index; SM: skeletal muscle mass; SMMI: skeletal muscle mass index; HGS: handgrip strength; GS: gait speed; MM: muscle mass; MS: muscle strength; PP: physical performance; *P≤0.05; **P≤0.001.

Table 3: Predictors of clinical measurements of sarcopenia in individuals with COPD

Dependent Variables	Univariate Analysis			Multivariate analysis		
	Explanatory variables	R^2	p	Explanatory variables	R^2	p
FFMI	TRAP	0.25	0.005	TRAP + BMI	0.80	0.0001
	Age	0.02	0.43			
	BMI	0.69	0.0004	Excluded: age		
SMMI	TRAP	0.10	0.07	TRAP+AOPP+BMI	0.51	0.0004
	SOD	0.13	0.046			
	AOPP	0.26	0.004			
	Age	0.02	0.823	Excluded: SOD and age		
	BMI	0.42	0.0007			
QS	TRAP	0.38	0.001	TRAP	0.43	0.004
	Age	0.11	0.085	Excluded: age and BMI		
	BMI	0.01	0.56			
GS	TRAP	0.26	0.008	TRAP + BMI	0.51	0.038
	Age	0.04	0.08			
	BMI	0.13	0.07	Excluded: age		
HGS	AOPP	0.20	0.015	AOPP + BMI	0.31	0.009
	Age	0.04	0.26			
	BMI	0.21	0.011	Excluded: age		
MEP	AOPP	0.10	0.07	AOPP + BMI	0.17	0.063
	Age	0.03	0.3			
	BMI	0.12	0.038	Excluded: age		
MIP	AOPP	0.26	0.002	AOPP	0.31	0.008
	SOD	0.12	0.048			
	Age	0.04	0.256	Excluded: SOD, age and BMI		
	BMI	0.12	0.046			

COPD: Chronic pulmonary obstructive disease; FFMI: fat-free mass index; SMMI: skeletal muscle mass index; QS: quadriceps strength; GS: gait speed; HGS: handgrip strength; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; TRAP: total radical trapping antioxidant parameter; AOPP: a*dvanced oxidation protein products*; SOD: superoxide dismutase activity.

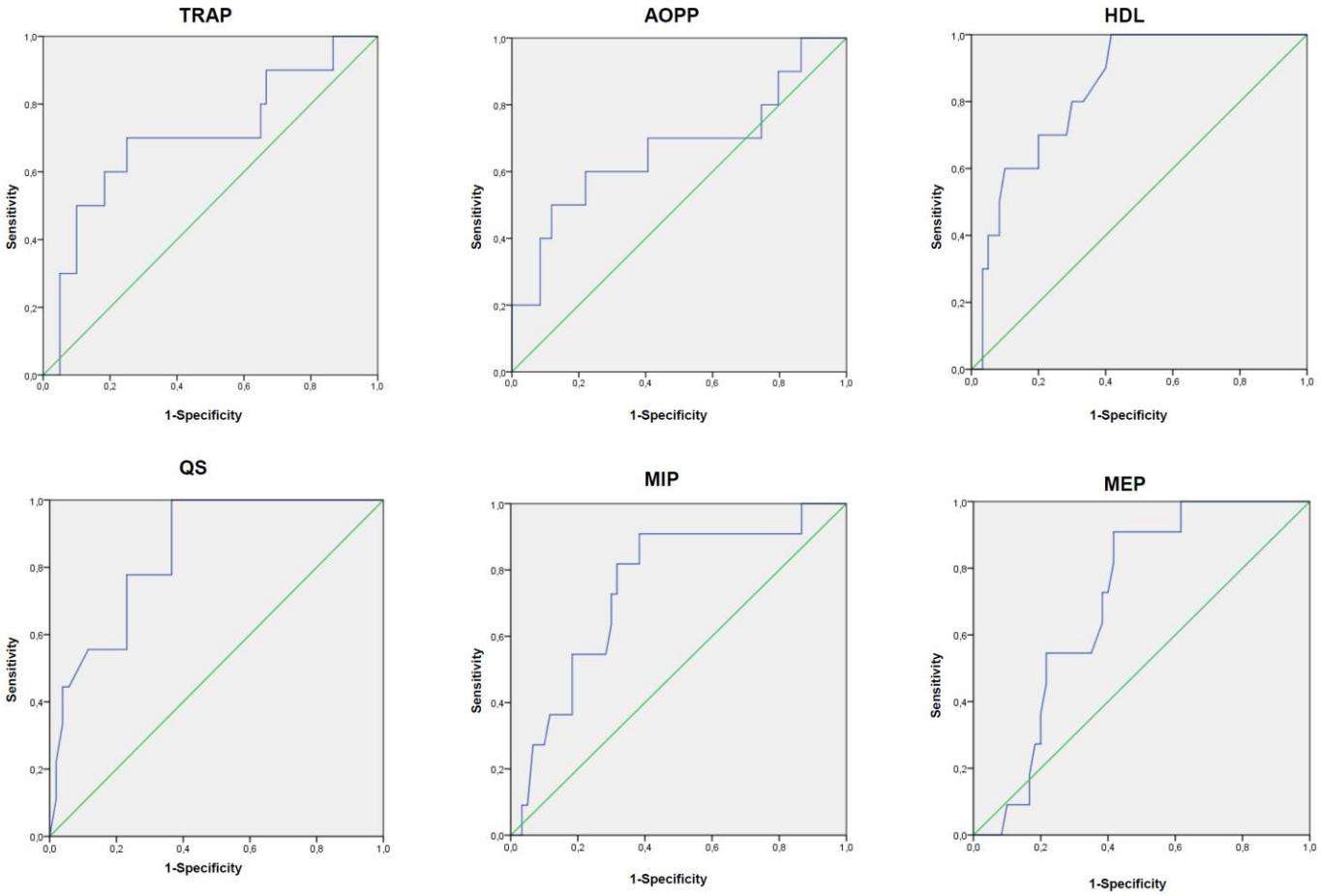
Table 4: ROC curve analysis to detect individuals with sarcopenia

	TRAP	AOPP	HDL	QS	MIP	MEP
Cut off	≤ 885 µM trolox	≤ 65 µM/l	≥57.5 mg/dl	≤ 26.15 Kg	≤ 77.5 cmH_2O	≤115 cmH_2O
Sensitivity	78%	68%	89%	100%	90%	70%
Specificity	74%	75%	66%	63%	63%	69%
Younden´s index	0.52	0.43	0.55	0.63	0.53	0.39
AUC	0.78	0.70	0.86	0.86	0.74	0.72
95% CI	0.48-0.945	0.58-0.965	0.76-0.963	0.74-0.976	0.58-0.904	0.585-0.85
P	0.0024*	0.007*	0.00046**	0.001**	0.014*	0.03*

TRAP: Total radical-trapping antioxidant parameter; AOPP: advanced oxidation protein products; HDL: high-density lipoprotein; QS: quadriceps strength; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure. *****P≤0.05; ***P*≤0.001.

Supplemental Material

Figure A.1. Receiver-operating characteristic (ROC) curves of factors associated with sarcopenia



Legend: AOPP: *advanced oxidation protein products;* HDL: high-density lipoprotein; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; QS: quadriceps strength; TRAP: total radical trapping antioxidant parameter.

3.3: ORIGINAL ARTICLE 2

Title: Is oxidative stress associated with disease severity, pulmonary function and metabolic syndrome in Chronic Obstructive Pulmonary Disease?

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Article published in *Revista Clinica Española*

(Q3/ Impact factor: 1.556)

Sepúlveda Loyola WA, Vilaça Cavallari Machado F, Araújo de Castro L, Hissnauer Leal Baltus T, Rampazzo Morelli N, Landucci Bonifácio K, et al. Is oxidative stress associated with disease severity, pulmonary function and metabolic syndrome in chronic obstructive pulmonary disease? Rev Clin Esp. 2019;219(9):477–84.

RESUMEN

Objetivo: Investigar las asociaciones entre los biomarcadores oxidantes/antioxidantes con estado de gravedad, función pulmonar y la presencia de síndrome metabólico (SM) en pacientes con EPOC.

Métodos: Se incluyeron 74 sujetos, 39 con EPOC (edad: 69 ± 7 años; mujeres: 41%) y 35 para el grupo control (edad: 69 ± 7 años; mujeres: 43%). Fueron diagnosticados con SM y asignados en uno de los cuatro subgrupos, EPOC y control (con y sin SM, respectivamente). Se analizaron productos de oxidación avanzada de proteína (AOPP), paraoxonasa-1 (PON1), actividad de catalasa (CAT), grupo sulfhidrilo (SH) e hidroperóxido de lípidos totales (LOOH). La función pulmonar fue analizada por medio de un pletismógrafo.

Resultados: El estado de gravedad de la EPOC (GOLD≥3) y la función pulmonar fueron asociados con SH y AOPP (p≤0.03 para todos). La prevalencia de SM se asoció con AOPP en la EPOC (p= 0.04). Los individuos con EPOC y SM mostraron niveles de AOPP más altos en comparación con los sujetos con EPOC sin SM (p<0,0001).

Conclusión: La gravedad de la EPOC, deterioro de la función pulmonar y la presencia de síndrome metabólico están asociados con el estrés oxidativo en individuos con EPOC.

Palabra clave: Enfermedad Pulmonar Obstructiva Crónica; Síndrome Metabólico; **Estrés** Oxidativo.

ABSTRACT

Objective: To investigate associations between oxidant/antioxidant biomarkers with the disease severity, pulmonary function and diagnosis of metabolic syndrome (MetS) in patients with COPD.

Methods: 74 subjects were included, 39 with COPD (age: 69±7years; female: 41%) and 35 for control group (age: 69±7years; female: 43%). They were diagnosed with MetS and allocated in one of four subgroups, COPD and control (with and without MetS, respectively). Advanced oxidation protein products (AOPP), paraoxonase-1 (PON1), catalase activity (CAT), sulfhydryl group (SH) and total lipid hydroperoxide (LOOH) were assayed. Pulmonary function was performed with a plethysmograph.

Results: COPD severity (GOLD≥3) and pulmonary function were associated with SH and AOPP ($p \leq 0.03$ for all). The prevalence of MetS was associated with AOPP in COPD ($p=0.04$). Individuals with COPD and MetS showed higher AOPP compared to COPD without MetS ($p<0.0001$).

Conclusion: COPD severity, worse pulmonary function and presence of metabolic syndrome are associated with oxidative stress in individuals with COPD.

Keywords: Pulmonary Disease, Chronic Obstructive; Metabolic Syndrome; Oxidative Stress.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is defined as a common, preventable and treatable disease characterized by persistent respiratory symptoms and airflow limitation frequently caused by significant exposure to noxious particles or gases[1]. Besides these abnormalities in the respiratory tract of COPD patients, chronic inflammation may be present and could play a role in the multiple comorbidities and symptoms observed in patients with COPD[2,3], such as metabolic syndrome (MetS) and oxidative stress (OS).

MetS is a “clustering” of risk factors for cardiovascular disease, associated with insulin resistance, dyslipidemia, central obesity, hypertension, and impaired glucose tolerance[4]. A recent systematic review showed that the prevalence of MetS in patients with COPD is approximately 34%, being higher compared with controls[5]. Both MetS, as well as COPD, have been associated with increase in reactive oxygen species (ROS).

As ROS can induce cellular damage, cells rely on an antioxidant defensive system based mainly on enzymatic components, such as paraoxonase-1 (PON1) and catalase (CAT). When antioxidant defensive system is impaired and/or overwhelmed by the presence of ROS, it may lead to an imbalance between oxidative/antioxidative biomarkers which is termed OS[6]. OS has already been studied in stable[7,8] and exacerbated[9,10] patients with COPD, and higher levels of ROS in patients with COPD compared to healthy controls has been reported.

OS may be a link in many comorbidities in subjects with COPD, including MetS[11], but until now, there are no studies comparing OS biomarkers and the antioxidant capacity in patients with COPD with and without MetS. This is of key importance since OS has been negatively correlated to pulmonary function in

patients with COPD[7]. Therefore, the aim of this study was to investigate associations between oxidant/antioxidant biomarkers with the disease severity, pulmonary function and diagnosis of MetS in patients with COPD.

MATERIAL AND METHODS

Study design and sample

A cross-sectional observational study was conducted with COPD patients (55 ≥ years old) recruited from pneumology ambulatory unity at the University Hospital of Londrina State University, Brazil. COPD was diagnosed according to GOLD criteria[1]. The exclusion criteria were: subjects with an exacerbation during the last four weeks, diagnosis of bronchial asthma, arthritis, presence of neurological or psychiatric diseases, heart failure, alcohol dependence and use of antioxidant supplements, since these factors could affect the levels of oxidant/antioxidant biomarkers. In addition, subjects without COPD or any other chronic disease which could interfere in the performance of the tests, matched for gender, age and ethnicity to the patients were recruited from the community in order to compose a control group. All individuals were allocated to one of four subgroups: 1) COPD (COPD without Mets), 2) COPD-MetS (COPD with MetS), 3) Control (control without MetS) and 4) Control-MetS (Control with MetS). All subjects signed an informed consent before being evaluated. The study was approved by the Ethics Committee on Research Involving Human Subjects of the Londrina State University (1.830.048).

Pulmonary function tests

Forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio, were performed with a plethysmograph (Elite Series™ Plethysmograph – MedGraphics). Measurements were assessed according to the American Thoracic Society/European Respiratory Society guidelines[12]. The reference values used were those for the Brazilian population[13].

Measurement of Comorbidities

The age-adjusted Charlson comorbidities index (ACCI) was used to quantify the overall burden of comorbidities. ACCI includes 19 medical conditions with corresponding weights. Comorbidities were weighted and scored using an algorithm proposed by Charlson et al[14].

Measurement of biomarkers

Peripheral venous blood was collected from the antecubital vein after 10 hours overnight fasting. The blood was contained in tubes and centrifuged at 3000 rpm for 10 minutes to separate the blood components (plasma, serum and hematocrit). The level of metabolic risk factor biomarkers (glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides) were assayed according to standard laboratory techniques at maximum four hours after withdrawing.

The tubes for OS biomarkers and antioxidative defence parameters were stored in a freezer at -80 Celsius degrees until blood collection were completed. The following biomarkers were dosed, advanced oxidation protein products (AOPP) [15] and total lipid hydroperoxide (LOOH) [16], as oxidative biomarkers and CAT[17]; sulfhydryl groups (SH) [18] and PON1[19], as antioxidant biomarkers. All

biomarkers were measured in triplicate assays at the Post graduation Laboratory at the University Hospital, Londrina State University, Brazil, following protocols described in previous studies[15-19]. All the chosen biomarkers have already been studied in patients with COPD[20], but not their association with the diagnosis of MetS and pulmonary function.

Metabolic Syndrome Criteria

MetS was diagnosed according to the American Heart Association/National Heart, Lung, and Blood Scientific Statement criteria[21]: elevated fasting glucose (GLU) ≥100mg/dL, reduced high-density lipoprotein cholesterol (HDL-C) <40 mg/dL for men and <50 mg/dL for women, higher triglycerides levels (TG) ≥150mg/dL, elevated blood pressure ≥130 mmHg systolic blood pressure or ≥85 mmHg diastolic blood pressure and waist circumference ≥102 cm in men and ≥88 cm in women.

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 (IBM Co., Armonk, NY, USA). All data were expressed as mean ± SD. The normality of the data was analysed with Shapiro-Wilk test. Comparison among the four studied groups were performed using the one-way analysis of variance (ANOVA), with post-hoc of Bonferroni for parametric data and Kruskal-Wallis test for non-parametric, with 95% confidence intervals (CIs). Pearson's correlation coefficient was used to assess relationships among pulmonary function variables and OS biomarkers. Logistic regression was used to evaluate associations between OS biomarkers with the presence of MetS and with COPD severity (GOLD ≥3). Statistical

significance was set as $p<0.05$. Finally, since it was a convenience sampling, the post hoc power analysis was checked using the software GPower 3.1 (Franz Faul, Universität Kiel, Germany).

RESULTS

Seventy-four individuals were included in this study, thirty-nine with COPD allocated in COPD with MetS (COPD-MetS: n=11) and COPD without MetS (COPD: n=28) and thirty-five individuals for control group allocated in control group with MetS (Control-MetS: n=9) and control group without MetS (Control: n=26). Socio-demographic characteristics, age-adjusted Charlson comorbidity index and metabolic risk factors are reported in Table 1. There were no differences in age, gender and BMI between groups. Age-adjusted Charlson comorbidity index was significantly higher in COPD and COPD-MetS in comparison with Control ($p=0.025$ and $p=0.006$, respectively). FVC %predicted was reduced in COPD and COPD-MetS in comparison with Control ($p<0.001$, for both). As expected, FEV_1 %predicted and FEV_1/FVC were lower in subjects with COPD in comparison to their counterparts ($p<0.05$ for all). HDL-C levels were higher in individuals with COPD compared to subjects belonging to the groups COPD-Mets and Control-MetS ($p<0.05$ for all).

Antioxidant biomarkers are reported in Figure 1. CAT levels were lower in COPD in comparison with Control ($p=0.003$). SH levels were higher in COPD and COPD-MetS in comparison to Control ($p<0.05$ for all). Oxidant biomarkers are reported in Figure 2. AOPP levels were lower in COPD compared to COPD-MetS and Control-MetS ($p<0.05$ for all).

OS biomarkers and pulmonary function variables were only correlated in individuals with COPD-MetS. AOPP was negatively correlated with FEV_1 %predicted and FEV_1/FVC (r=-0.68 and -0.56, respectively; p<0.05 for both) in this group. SH was positively correlated with FEV_1 %predicted and FEV_1/FVC (r=0.52 and 0.77, respectively; p<0.05 for both). CAT was negatively correlated with FEV_1/FVC (r=-0.61; p<0.05).

Table 2 shows the logistic regression analysis between factors associated with COPD severity (GOLD 3 and 4) in subjects with COPD. In a univariate analysis, age, BMI, AOPP, CAT, and SH were associated with COPD severity (p<0.10), which were considered for the multivariate analysis. SH (p=0.036) and BMI (p=0.008) and AOPP (p=0.037) were associated with COPD severity.

Table 3 shows the logistic regression analysis between factors associated with the presence of MetS in subjects with COPD. In a univariate analysis, AOPP was associated with the presence of metabolic syndrome in individuals with COPD (p<0.05). For the multivariate analysis, other variables associated with metabolic syndrome (age, BMI and CAT; p<0.10) were considered. This multivariate analysis, which considered age, BMI, AOPP and catalase as explanatory variables of the prevalence of MetS in COPD, revealed that AOPP was the only one that showed a positive and significant association with MetS in this group. The chance to have MetS increases 5% for each increment AOPP (μM/l) in individuals with COPD (p=0.04). Similar statistical analysis was completed for the control groups, however, no significant associations were found.

DISCUSSION

The present study adds to the field that 1) Antioxidant response is increased in COPD with and without MetS; 2) Antioxidants are correlated to pulmonary function only in COPD-MetS 3) Protein oxidation is higher in COPD-MetS compared to COPD without MetS and 4) OS is associated with worse pulmonary function, COPD severity and the presence of MetS.

Our study showed that the prevalence of metabolic syndrome was 28% in subjects with COPD and 25% in the control group. These results are in agreement with other studies[21,22]. A systematic review showed that the prevalence of MetS vary from 21% to 58% in individuals with COPD versus 17% to 54% for individuals without COPD[5]. In the present study, higher prevalence of metabolic syndrome in individuals with severe and very severe COPD was observed, we found that 36% of the COPD with MetS were classified as GOLD I-II and 64% as GOLD III-IV. Similar results were found by Diez-manglano et al.[24] which observed 60% of subjects classified as severe and very severe COPD were diagnosed with MetS. Additionally, in our study, more comorbidities were found in individuals with COPD-MetS and COPD without MetS in comparison with the control group. These findings are in accordance with other studies which have reported prevalence of other comorbidities in individuals with COPD, comorbidities have been associated with worse quality of life and prognosis and higher frequency of exacerbations in this population[23-26].

Regarding the antioxidant biomarkers, PON1, CAT and SH were investigated. PON1 is an antioxidant enzyme linked to HDL-C in the peripheral blood, that in individuals with COPD its enzymatic action has been reported reduced[27]. Although no differences in PON1 activity were detected, we observed lower levels of HDL-C in COPD-MetS compared to COPD, which are similar with

other study[28]. In this line, reductions on the levels of HDL-C have a clinical impact related to inflammation and OS[29], since HDL plays an important role as anti-inflammatory and antioxidant biomarker[30].

CAT is an important antioxidant which play a role in removing H_2O_2 and its action prevents the injurious effects of OS[31]. As observed in the present study, lower levels of CAT were also found in a study with 202 COPD patients compared to 136 healthy controls[32]. In addition, CAT was negatively correlated to FEV1/FVC in COPD-MetS (r=-0.61; $p<0.05$) and these values were even greater than the findings reported by Anes et al.32 (r=-0.39). A possible explanation for this correlation is that subjects with MetS have higher levels of reactive oxygen species[29], which can induce epithelial damage on the lung and reduce the pulmonary function and this can result in an increase in the catalase activity as a compensatory response[31-32].

Another important antioxidant biomarker is the SH[34]. We found that SH is a protective factor to COPD severity, reducing the prevalence of GOLD 3 and 4 in 2% for each unit of SH (OR 0.98; 95%CI: 0.96-1.001; p=0.036). Also, correlations between SH with FEV_1 %predicted (r=0.52) and with FEV_1/FVC (r= 0.77) in individuals with COPD-MetS were found. In this context, Zinellu et al.[34] observed that protein sulfhydryl group has been positively correlated to pulmonary function in subjects with COPD, however, they did not compare individuals with and without MetS. Furthermore, there is no evidence reporting the association between SH or other antioxidant biomarker with pulmonary function in COPD with MetS. Since this syndrome is associated with inflammation and OS, it can be hypothesized that it may interfere in the antioxidant capacity and in the pulmonary function of these individuals.

Another key point with respect to the antioxidant capacity was that higher levels of SH in subjects with COPD were observed in comparison with control group. This result has been described by previous studies[33,35] as a compensatory response for excess of oxidants, leading to augmented levels of glutathione in individuals with COPD. In this regard, glutathione is one of the organic components that contain proteins thiols, which are composed by SH[36]. Proteins thiols reduce the H_2O_2 and LOOH[37]. Although the levels of SH were higher in individuals with COPD compared to control group, no difference were observed among LOOH levels in our study.

Augmented levels of proteins oxidation, measured through AOPP were observed in COPD with metabolic syndrome compared to COPD without MetS[15]. Ben Anes et al.[33] reported similar results in this disease, however this study did not compare individuals with and without MetS. Furthermore, OS biomarkers have been little investigated in COPD with Mets. Therefore, there is no evidence exploring the AOPP levels in this population with MetS, in contrast with apparently healthy subjects[38]. In our study, control subjects with MetS presented higher levels of this biomarkers compared to control group without MetS. Regarding that, evidence showed that AOPP levels is considered the most important determinant of metabolic syndrome in elderly individuals[38]. Correspondingly, in individuals with COPD we also found this association with AOPP, which was the most important factor related to the prevalence of MetS in COPD, incrementing its prevalence in 5% for each unit of AOPP (R^2= 0.57; OR:1.05; 95%CI: 1.004-1.100; *p*=0.04). Therefore, it can provide support that advanced oxidation proteins products are the main factors associated with metabolic syndrome in COPD, and it was also correlated with worse pulmonary function in COPD-MetS (FEV_1%pred:

r=-0.68 and FEV_1/FVC: r= -0.56) and COPD severity (OR 1.03; 95%CI: 1.002-1.05; *p*=0.037). Hence, considering that MetS in COPD has been associated with worse pulmonary function[26] and prognosis in individuals with COPD [23-25], these are new and important findings for the literature and clinical practice, since they showed that pulmonary function, COPD severity and the prevalence of metabolic syndrome can be related with antioxidant and oxidative biomarkers in patients with COPD. In short, our study explored that the etiology of several clinical implications may be associated with antioxidant and oxidative biomarkers.

Finally, the present study has some limitations. First, the sample in each group was small, not equal and it was a convenience sampling, however, our sample size was even larger in comparison to other studies[32,38] Additionally, strong correlations were found between the studied variables, and a retrospectively power calculation revealed that the study´s sample size had 95% power (alpha= 0.05) to detect the difference of oxidative stress biomarkers among the groups (effect size= 0.49). Second, it was a cross-sectional observational study, therefore causality of associations should be interpreted with caution. In spite of that, the strengths of the study were that it adds new information to the field of OS in COPD with metabolic syndrome. Additionally, the groups were controlled for possible effects of confounding variables which can interfere in OS biomarkers, such as alcohol intake and use of antioxidant supplements. Also, the diagnosis of MetS was established according to international criteria[21].

CONCLUSION

In conclusion, oxidative stress is associated with COPD severity, worse pulmonary function and the presence of MetS in this disease. In addition,

augmented antioxidant activity was observed in subjects with COPD in comparison to control group. In COPD with MetS, OS biomarkers were only correlated to pulmonary function. Finally, COPD subjects with MetS showed higher levels of proteins oxidations in comparison with COPD without MetS.

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Table 1: Clinical characteristics of COPD and Control groups with and without MetS.				
Variable	COPD-MetS (n=11) Group a	COPD (n=28) Group b	CONTROL-MetS (n=9) Group c	CONTROL (n=26) Group d
Age (year)	66.5±6	69.8±6.6	68.5±8	68.6±5.9
Female, n(%)	4 (37%)	12 (60%)	3(34%)	12 (46%)
BMI (kg/m^2)	30.2±3.5	26±5.7	29.2±6.2	26.9±7.2
ACCI (score)	4.55 ± 1.2 *d	4.3±1.3 *d	4.3±1.3 *d	3.9±1.3
FVC %predicted	82±16 *d	88±21*d	98±18	111±15
FEV_1 %predicted	46±13 *c,d	49±15 *c,d	90±17	102±15
FEV_1/FVC	46±9 *c,d	47±10 *c,d	74±5	76±5
GOLD I-II, n(%)	4 (36%)	14 (50%)	-	-
GOLD III-IV, n(%)	7 (64%)	14 (50%)	-	-
Metabolic Risk Factors Biomarkers				
Glucose (mg/dl)	127.7±23.2	111.3±41.8	144.6±88.2	119.6±43
Cholesterol (mg/dl)	203.8±39.9	200.5±45	189±58.2	193.8±37.9
LDL-C (mg/dl)	123.4±42.8	112.1±36.7	99.3±39.4	114.2±34.2
HDL-C (mg/dl)	42.7±9.7	67.5±20 *a,c	52.3±9.11	54.3±13.3
Triglycerides (mg/dl)	186.1±75.6	109.6±84.4	155.7±84.4	124.3±72

Data are expressed as mean ± standard deviation. COPD: Chronic obstructive pulmonary disease; MetS: Metabolic syndrome; ACCI: Age-adjusted Charlson comorbidity index; FEV_1: Forced expiratory volume in the first second; FVC: Forced vital capacity; BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; *Statistically significant (p≤0.05); Statistically significant between groups (*p*<0.05) expressed in letters a; b; c and d (COPD-MetS; COPD; Control-MetS and Control; respectively).

Table 2: Features associated with COPD Severity (GOLD ≥ 3)		
Variable	**Univariate analysis OR (95% CI)**	**Multivariate analysis OR (95% CI)**
Age (year)	1.07 (0.97-1.18) †	
Gender (female)	1.18 (0.32-4.250)	-
BMI (kg/m^2)	0.90 (0.79-1.02) †	0.82 (0.66-0.97) *
Tobacco history	1.9 (0.28-12.89)	-
MetS	1.75 (0.41-7.345)	-
ACCI (score)	1.12 (0.67-1.8)	-
AOPP (µM/l)	1.01 (0.993-1.03) †	1.03 (1.002-1.05) *
LOOH (Mm/L)	1 (1.0-1001)	**-**
CAT (U/mgHb)	1.04 (0.97-1.15) †	-
SH (mM/mg protein)	0.99 (0.98-1.005) †	0.98 (0.96-1.001) *
PON1(U/ml)	0.99 (0.99 -1.01)	-

Data are expressed as odds ratio (OR) and 95% of confidence interval (95%CI). ACCI: Age-adjusted Charlson comorbidity index; AOPP: Advanced oxidation protein products; BMI: Body mass index; CAT: Catalase; GOLD: Global initiative for chronic obstructive lung disease; LOOH: Total lipid-hydroperoxide; MetS: Metabolic syndrome; SH: Sulfhydryl group; PON1: Paraoxonase 1; Statistically significant *($p<0.05$) and † ($p<0.10$) respectively).

Table 3: Features associated with metabolic syndrome in COPD

Variable	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)
Age (year)	0.913 (0.809-1.03) †	-
Gender (female)	3.7 (0.674-20.69)	-
BMI (kg/m^2)	1.166 (0.99-1.37) †	-
Tobacco history	1.67(0.165-16.8)	-
GOLD (≥3)	1.4 (0.325-6.027)	-
ACCI (score)	1.165 (0.65-2.09)	-
AOPP (µM/l)	1.04 (1.006-1.07) *	1.05 (1.004-1.100) *
LOOH (Mm/L)	1 (1.0-1001)	**-**
CAT (U/mgHb)	1.075 (0.99-1.17) †	-
SH (mM/mg protein)	1.007 (0.99-1.022)	-
PON1(U/ml)	1.004 (0.99-1.018)	-

Data are expressed as odds ratio (OR) and 95% of confidence interval (95%CI). ACCI: Age-adjusted Charlson comorbidity index; AOPP: Advanced oxidation protein products; BMI: Body mass index; CAT: Catalase; GOLD: Global initiative for chronic obstructive lung disease; LOOH: Total lipid-hydroperoxide; SH: Sulfhydryl group; PON1: Paraoxonase 1; Statistically significant *($p<0.05$) and † ($p<0.10$) respectively).

4.CHAPTER IV. A NEW GERIATRIC SYNDROME TO BE STUDIED IN INDIVIDUALS WITH COPD AND OTHER CHRONIC CONDITIONS

PRESENTATION:

In this chapter the following article is presented: “The join occurrence of osteoporosis and sarcopenia (osteosarcopenia): Definitions and Characteristics”. This article was developed in older adults from Australia, in collaboration with researchers from Londrina State University (Brazil) and University of Melbourne (Australia). This article was published in the Journal of the American Medical Directors Association**.**

4.1 ORIGINAL ARTICLE

Title: The Joint Occurrence of Osteoporosis and Sarcopenia (Osteosarcopenia): Definitions and Characteristics.

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Article published in the Journal of the American Association of Medical Directors

(Q1/ Impact factor: 4.669)

Sepúlveda-Loyola W, Phu S, Bani Hassan E, Brennan-Olsen SL, Zanker J, Vogrin S, et al. The Joint Occurrence of Osteoporosis and Sarcopenia (Osteosarcopenia): Definitions and Characteristics. J Am Med Dir Assoc [Internet]. 2020 Feb;21(2):220–5. Available from: https://doi.org/10.1016/j.jamda.2019.09.005

ABSTRACT

Objectives: We sought to examine the associations of osteosarcopenia with physical performance, balance and falls and fractures in community-dwelling older adults. Additionally, we aimed to determine which clinical outcomes are dependent on specific components of osteosarcopenia.

Design: Cross-sectional study

Setting and Participants: 253 participants (77% women; aged 77.9 ± 0.42 years) who presented for a falls and fractures risk assessment in Melbourne, Australia

Methods: Participants were mobile, community-dwelling older adults (≥65 years) free of cognitive impairment. Body composition [via dual energy X-ray absorptiometry], physical performance [via Timed Up and Go (TUG) and Short Physical Performance Battery (SPPB)] and balance [via Four-Square Step test (FSS) and posturography] were examined. Falls in the past year and fractures in the past 5 years were also self-reported. Osteosarcopenia was defined as (i) low bone mineral density (BMD) (T-score <-1 SD) combined with sarcopenia, and (ii) osteoporosis (BMD T-score ≤-2.5 SD) combined with severe sarcopenia. For sarcopenia, we employed the European Working Group on Sarcopenia in Older People (EWGSOP1), the revised criteria (EWGSOP2), and the Foundation for the National Institutes for Health (FNIH). Kruskal-Wallis and logistic regression were used for statistical analysis.

Results: Osteosarcopenia was associated with worse SPPB, TUG, FSS, limit of stability, falls and fractures history. Additionally, osteosarcopenia (using the severe sarcopenia classification) conferred an increased rate of falls (OR from 2.83 to 3.63: $p< 0.05$ for all) and fractures (OR from 3.86 to 4.38; $p< 0.05$ for all) when employing the EWGSOP2 and FNIH definitions, respectively.

Conclusions and Implications: Osteosarcopenia was associated with a greater impairment of physical performance and balance. The EWGSOP2 and FNIH criteria resulted in the strongest associations with physical performance, and self-reported falls and fractures.

INTRODUCTION

Osteosarcopenia is defined as the concomitant presence of osteoporosis or osteopenia combined with sarcopenia.[1,2] This new geriatric syndrome associates with higher disability, fracture and falls in older people[3,4] compared to either disease alone.[5]

Sarcopenia has recently been recognized as a disease in the International Disease Classification-10th Revision-Clinical Modification (ICD-10-CM) in 2016,[6] emphasizing the importance of this debilitating musculoskeletal condition which can increase the risk of disability and mortality in older people.[7] Severe sarcopenia in particular, defined as the presence of low muscle mass, physical performance and strength[8] may result in additional adverse outcomes including an increased risk of falls.[9] Furthermore, there is increasing evidence of crosstalk between muscle and bone. In the case of sarcopenia, greater systemic inflammation and oxidative stress have been found, both of which are related to reduced bone mineral density (BMD).[10–12] With regards to osteopenia and osteoporosis, both are strongly associated with both fracture risk and frailty,[13] resulting in declines in quality of life and increased mortality, particularly in those who experience hip fractures.[14]

Given the clinical outcomes associated with each of the two components of osteosarcopenia, the diagnosis of osteosarcopenia is imperative to enable clinical care.[15,16] While the diagnostic criteria of osteopenia/osteoporosis are well established;[13,17] sarcopenic lacks an international consensus.[8,18–20] In addition, existing definitions of sarcopenia have shown minimal agreement.[21,22] This may impact the diagnosis and prevalence of osteosarcopenia, and the prioritization of interventions and maintenance of health outcomes.

At present, there is a paucity of data investigating the impact of osteosarcopenia (or severe sarcopenia alone) on physical performance in a population of high-risk community-dwelling older adults. As such, the aim of this study was to (i) determine the associations between osteosarcopenia and clinical outcomes, and (ii) examine the impacts of severity of bone (osteopenia/osteoporosis) and muscle (sarcopenia vs severe sarcopenia) on these clinical

outcomes in community-dwelling older adults. We hypothesized osteosarcopenia is associated with worse physical function, balance, falls and fractures, and its impact increases when osteosarcopenia is diagnosed using osteoporosis combined with severe sarcopenia.

METHODS

Study Population

A cross-sectional study of older adults (≥65 years old) who attended an assessment for falls and fracture risk at a clinic in Melbourne (Victoria, Australia). Inclusion criteria were as follows: ability to mobilize independently or with the use of gait aids, no cognitive impairment (mini-mental state examination score >18/30), risk or history of falls and/or fractures (determined by general practitioner), and willingness to attend the clinic. This study was approved by the Western Health local Human Ethics Research Committee (DB2017.13 and QA2018.80-46205).

Physical Performance

Physical performance was evaluated by handgrip strength (HGS), gait speed (GS), Timed Up and Go (TUG), five times sit-to-stand test (5STS) test and Short Physical Performance Battery (SPPB). HGS was assessed in a seated position using a Jamar hydraulic dynamometer (Sammons Preston Inc.) and the forearm and wrist resting on the chair arms. Participants performed 3 trials on each hand, alternating between each hand, with a rest of 30 seconds between tests. The highest value was recorded and used for analysis.[24]

Gait speed was measured using the GAITRite® system (CIR Systems Inc, Havertown, PA) with the mat measuring 580cm x 89cm x 0.625cm, and a sample rate of 120 Hz. Participants were instructed to walk at their usual gait speed, as previously reported,[25] with the fastest of two attempts used for analysis.

The TUG evaluated the time it takes to stand up from a chair, walk 3 meters, turn, walk back to the chair, and sit down again at normal pace.[26]

Standardized operating procedures were followed for the SPPB,[27] which consisted of three timed components: standing balance, 4-m gait speed, and time to complete five chair-stands. Participant scores for each component were totaled between 0 and 12 used for analysis. Poor performance was defined as TUG ≥20sec, SPPB ≤8 points and 5STS >15sec.[18]

Dynamic and Static Balance

Dynamic balance was measured by four-square step test (FSS) following validated guidelines.[28] Poor dynamic balance was defined as FSS >15sec.[25,28]

Static balance was assessed using the Balance Rehabilitation Unit (BRU; Medicaa™, Uruguay), which provided results for limits of stability (LOS), center of pressure (CoP) area and sway velocity for six different conditions: 1) eyes opened on hard surface; 2) eyes closed on hard surface; 3) eyes closed on foam; 4) eyes opened with saccadic stimulation; 5) eyes opened with horizontal vestibular stimulation and 6) eyes opened with vertical vestibular stimulation. Visual stimulus was displayed using 3D virtual-reality goggles with moving objects (conditions 4 to 6). Each condition lasted for 1 minute. To measure LOS, participants were instructed to shift their weight in anteroposterior and lateral directions using the ankle strategy for balance (without moving the feet or bending at the hip). Poor static balance was considered as LOS <170cm^2.[25]

Self-reported falls (in the past year) and fractures (in the past 5 years), body mass index (BMI) and Charlson age-comorbidity index (CACI) were also assessed.[29]

Diagnosis of Osteosarcopenia

Cut-points used to diagnose osteosarcopenia are presented in Table 1. Several osteosarcopenia categories were created depending on the definition of sarcopenia employed. Osteosarcopenia was detected with the combination of low BMD and sarcopenia.[2] Low BMD was defined as T-score <-1SD according to World Health Organization criteria for osteopenia.[17] As a result, osteoporotic participants were included in this category. Sarcopenia was defined as low appendicular lean mass (LALM) plus low muscle strength (LMS) or low

physical performance (LPP).[8,18–20] The following definitions of sarcopenia were used: European Working Group on Sarcopenia in Older People (EWGSOP1), and its revised criteria (EWGSOP2), the Foundation for the National Institutes for Health (FNIH).[8,18,19]

For the second objective of this study, we selected a subgroup of osteoporotic participants using a T-score of ≤-2.5SD and combined this with a severe sarcopenia group who fulfilled all 3 criteria for sarcopenia (LALM, LMS and LPP). Finally, we performed an analysis excluding measurements of lean mass (i.e. those presenting with low BMD, LMS and LPP) to examine the effect of lean mass measures on clinical outcomes. Appendicular lean mass and areal bone mineral density (BMD) at three sites (lumbar spine, total hip, and femoral neck) were assessed using a Hologic Horizon dual energy X-ray absorptiometry (DXA) machine (Bedford, USA).

Statistical Analysis

Statistical analysis was performed using SPPSS version 25 (SPSS, IBM Co., Armonk, NY, USA). Data are expressed as frequency and percentage, mean ± SD and/or median and interquartile range. The Kolmogorov–Smirnov test was used to analyze normality of data distribution. McNemar's test was used to compare the prevalence among the definitions of osteosarcopenia. Kruskal-Wallis test and Dunn-Bonferroni post-hoc was used to compare the clinical outcomes between the groups (non-osteosarcopenia, osteosarcopenia, and osteosarcopenia with severe sarcopenia component) in participants with BMD T-score <-1SD and ≤-2.5SD separately, using the different cut-points for sarcopenia (EWGSOP1; EWGSOP2 and FNIH). Logistic regression was used to determine associations between the different groups: 1) BMD <-1 + sarcopenia; 2) BMD ≤-2.5 + sarcopenia; 3) BMD <-1 + severe sarcopenia; 4) BMD ≤-2.5 + sarcopenia; 5) BMD <-1 + LPP and LMS; 6) BMD ≤-2.5 + LPP and LMS) with worse physical performance, dynamic and static balance, and falls and fracture history. The logistic regression was adjusted for age, gender and Charlson Age-Comorbidity Index (CACI). Statistical significance was set as $p<0.05$.

RESULTS

253 individuals were included in this study (age: 77.9 ± 0.42 years old; women 77%; BMI 28.3 ± 6 kg/m^2). For BMD, 14 (6%), 69 (27%) and 158 (62%) were classified as having normal BMD, osteopenia and osteoporosis, respectively (Table 2). Mean CACI score was 4.6±1.8 points with 45% presenting with a score ≥ 5 points. When assessing fall and fracture history, 130 (54%) participants presented with ≥2 falls in the past year and 37 (13%) reported ≥2 fractures.

Prevalence of Osteosarcopenia

Prevalence of osteosarcopenia according to each definition varied between 11% and 21% and is reported in Figure 1. We observed significant differences among EWGSOP2 (21%) and FNIH (20%) compared to EWGSOP1 (11%) with divergences due to ALM (p<0.05 for all). When using T-score of ≤-2.5 combined with sarcopenia, the prevalence of osteosarcopenia was reduced and ranged from 8% to 19%. We observed difference among EWGSOP2 (19%) and FNIH (14%) compared to EWGSOP1 (8%) (p<0.05 for all).

Prevalence of Osteosarcopenia in those with Severe Sarcopenia

The prevalence of osteosarcopenia with a severe sarcopenia component for various definitions is reported in Figure 1. When using a BMD T-score of <-1 and severe sarcopenia, prevalence varied from 9% to 11%. For a BMD T-score of ≤-2.5 combined with severe sarcopenia, the prevalence ranged from 5% to 9%. No significant differences between the prevalence were observed.

Physical Performance in Osteosarcopenia Groups

A comparison between physical performance, balance and falls history for the osteosarcopenia groups is presented in Figure 2.

Osteosarcopenic participants with severe sarcopenia presented with lower physical performance (SPPB, TUG) compared to non-osteosarcopenic participants regardless of definitions. Similarly, only the osteosarcopenic participants with severe sarcopenia based on

EWGSOP1 and 2 showed lower physical performance (SPPB) compared to osteosarcopenic participants. For the 5STS test, only those with osteosarcopenia (FNIH) and osteosarcopenia with osteoporosis and severe sarcopenia (EWGSOP2) presented lower performance compared to non-osteosarcopenic. Results for physical performance are shown in Figures 2A-C.

Static and Dynamic Balance in Osteosarcopenia Groups

Only participants with osteosarcopenia with a severe sarcopenia component presented with poor dynamic balance (FSS) compared with non-osteosarcopenia regardless of definition used (Figure 2D). For static balance, a significant difference in LOS was found when using the EWGSOP2 and FNIH criteria, to diagnose osteosarcopenia, irrespective of T-score (Figure 2E). For those who presented with osteosarcopenia with severe sarcopenia, only the FNIH criteria showed poor dynamic and static balance compared to the non-osteosarcopenic group (Figure 2D-E).

Adverse Events in Osteosarcopenia

No difference in falls and fracture history was observed in individuals with osteosarcopenia with and without severe component compared to the other groups. Number of fractures were similar in all groups and definitions (1, IQR 1,2). Higher rates of falls in the past year were seen in individuals presenting with osteosarcopenia with severe sarcopenia using EWGSOP2 of 2 falls (1, 5.5) compared to individuals with osteosarcopenia who reported 1 fall (1-2), p= 0.039 (Figure 2F).

Associations Between Osteosarcopenia and Outcome Measures

Osteosarcopenia diagnosed using EWGSOP2 was associated with worse physical performance and falls, regardless of T-score (OR 0.49-3.07; p<0.05 for all). When based on the FNIH criteria, osteosarcopenia was associated with worse physical performance and dynamic balance only when it was combined with a T-score of <-1 (OR 3.59-4.84; p<0.05 for all). No significant associations were found with static balance. Individuals diagnosed with

sarcopenia (EWGSOP1 and EWGSOP2) presented with a lower association with multiple falls when it was combined with a T-score <-1 (Table 3).

Larger Associations Between Osteosarcopenia with a Severe Sarcopenia and Outcome Measures

Regardless of definition, there was a significant association or trend towards association between osteosarcopenia with severe sarcopenia, SPPB and TUG performance (OR 2.56-7.19; $p<0.05$ for all). No significant associations were evident for 5STS time. When using EWGSOP2 criteria, there was an association with multiple falls irrespective of T-score (OR 2.83-3.63; $p<0.05$ for all). Osteosarcopenia with severe sarcopenia according to FNIH was significantly associated with multiple fractures regardless of T-score (OR 3.86-4.38; $p<0.05$ for all) (Table 3).

Exclusion of Lean mass Further Increases Associations with Outcome Measures

Irrespective of the definition used, when we excluded LALM and combined LPP and LMS with low BMD, there was an association with worse physical performance (SPPB and TUG) and multiple falls (OR 2.18-7.89; $p<0.05$ for all). When using the cut-points for LPP and LMS according to EWGSOP1 combined with low BMD, there was an addition association with balance measures (OR 2.20; 95% IC: 1.3-6.0; $p<0.05$ for all) (Table 3).

DISCUSSION

We aimed to determine the clinical implications of combined osteopenia/osteoporosis and sarcopenia (osteosarcopenia) in older adults. In agreement with current literature, we found that individuals diagnosed with osteosarcopenia not only presented with greater impairments in strength, lower limb performance and balance, but were also associated with higher rates of falls and fractures. With respect to these outcomes, our findings suggest the definition of the “osteo” component of osteosarcopenia (BMD <-1 or ≤-2.5) may not significantly affect clinical outcomes. However, the sarcopenia definition may affect results on clinical outcomes

in individuals with osteosarcopenia. Therefore, presence of a severe sarcopenia component was associated with adverse events such as falls and fractures.

Minimal Differences Between 'Osteo' Definitions

The diagnosis of osteopenia/osteoporosis has been well established over the past few decades,[13,17] with a T-score of <-1 indicative of osteopenia and ≤-2.5 osteoporosis. The consistent and routine use of these cut-points in clinical practice enable effective diagnosis and implementation of interventions. Despite the links between osteoporosis, fracture and poor clinical outcomes,[30] we did not find differences in fracture rates in osteopenic compared to osteoporotic classifications. In corroboration, studies have reported discrepancies in reported fractures and BMD, with osteopenic older adults experiencing fracture rates similar to, and in some cases greater than those diagnosed with osteoporosis.[31] Given these findings, it appears the use of osteopenia to diagnose osteosarcopenic participants may be sufficient to capture those with greater prevalence of fractures. Interestingly, we report greater associations with multiple fractures when using the FNIH criteria to diagnose osteosarcopenia irrespective of the 'osteo' component combined with severe sarcopenia. However, no associations were observed when employing EWGSOP 1 or 2, which may be due to the limited number of participants (17%) reporting multiple fractures. Finally, similarities were evident in the physical performance of osteosarcopenic older adults regardless of BMD.

Osteosarcopenia with a Severe Sarcopenia Component

Several definitions exist for diagnosis of sarcopenia[8,18–20,32] which have resulted in differing prevalence estimates, in line with previous studies.[21] These differences in prevalence may be attributed to variation in cut-points used for muscle strength and ALM, including the methods of correcting ALM for h^2 and BMI. Despite this, we noted an overall increase in OR for all outcome measures assessed, which was more notable and somewhat expected for physical performance measures. Static and dynamic balance also displayed further declines in those presenting with osteosarcopenia with a severe sarcopenia component, whilst we failed to

report the OR for dynamic balance due to large proportion (53% of participants) who were unable to complete the FSS test. While the impact of sarcopenia and severe sarcopenia on static balance has not been well studied, there is well-established evidence demonstrating that resistance training can improve both postural control[33] and muscle mass in older adults.[34] In regards to falls, those diagnosed as osteosarcopenic with severe sarcopenia displayed a higher likelihood of falls (OR 1.93-3.63), but only those diagnosed using the EWGSOP2 definition provided significant results. These results are in agreement with other studies which have showed up to three-fold increase in falls risk in sarcopenic older adults.[35] Although the limitations in assessing risk for multiple falls has been previously discussed, this study found participants diagnosed as osteosarcopenic according to the FNIH criteria presented with a significantly greater fracture rates. In this sense, considering the evidence of the increased fracture risk in overweight/obese individuals,[36,37] this finding may in part be explained by the greater distribution of overweight/obese older adults using the FNIH criteria (76%, compared to 18% and 38% using EWGSOP1 and EWGSOP2, respectively), which has been reported previously.[38] Our findings were also in agreement with another study under the term 'dysmobility syndrome' (combination of osteosarcopenia and overweight/obesity), where a greater percentage of falls and fracture history have been reported.[39]

Further Differences with Removal of Appendicular Lean Mass Measures

The use of DXA to determine appendicular lean mass involves the direct measurement of bone and fat mass, followed by calculations to quantify lean mass.[40] This method is influenced by body cell mass and vasculature,[40] which may account for the lack of effect of muscle mass on clinical outcomes reported in various studies.[41,42] Given this evidence, we decided to exclude ALM measures from sarcopenia definitions and combine low BMD, LMS and LPP to assess whether associations with physical performance, balance and outcomes change. In doing so, we found even stronger associations with performance, balance and falls when compared to severe sarcopenia. In particular, the OR for falls showed a significant association for all measures. These findings highlight the lack of an accurate "gold standard" for assessing

muscle mass and need for further research in this area, with several new methods recently proposed including the use of D3-creatine intramuscular and marrow fat.[43,44]

Strength and Limitations

This study provides relevant information for clinical practice by reporting the impact of different definitions of osteosarcopenia on clinical outcomes and their associations with physical performance, balance, falls and fracture history. Compared to previous studies,[1,5,23] we combined different criteria to diagnose osteosarcopenia, including a severe component. Finally, the assessments utilized to measure physical performance,[24,26,27] static and dynamic balance[18,25] are validated in older adults, and we also used a gold standard to measure osteopenia/osteoporosis,[17,40] which is also commonly accepted to define sarcopenia.[40] However, there were several limitations in this study. First, given the cross-sectional study design, we are unable to comment on causality. Secondly, as our study sample consisted primarily of women, this may skew results given that women have shown increased falls risk, however, results remained significant after adjustment. Finally, the majority of participants presented with a fall and/or fracture history, with 95% reporting a fall in the past year and 81% experiencing a fracture. Given a lack of participants experiencing no falls or fractures, associations reported in this study may have been increased.

CONCLUSIONS AND IMPLICATIONS

In conclusion, osteosarcopenia was strongly associated with worse physical performance, falls and fractures history in community-dwelling older adults. Additionally, osteosarcopenia with a severe sarcopenia component was associated with increased falls when based on EWGSOP2 and fractures when using the FNIH definition. In this study, the assessment of lean mass and definition use of osteopenia/osteoporosis did not significantly affect results, with severe sarcopenia being the driving factor. Future longitudinal studies should be developed to monitor an individual's progression from a healthy state to the individual and/or combined components of osteosarcopenia to establish the best definition of this syndrome,

and enhance assessment of the clinical impacts of osteosarcopenia on functionality, falls and fracture risk.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose

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FIGURES AND TABLES

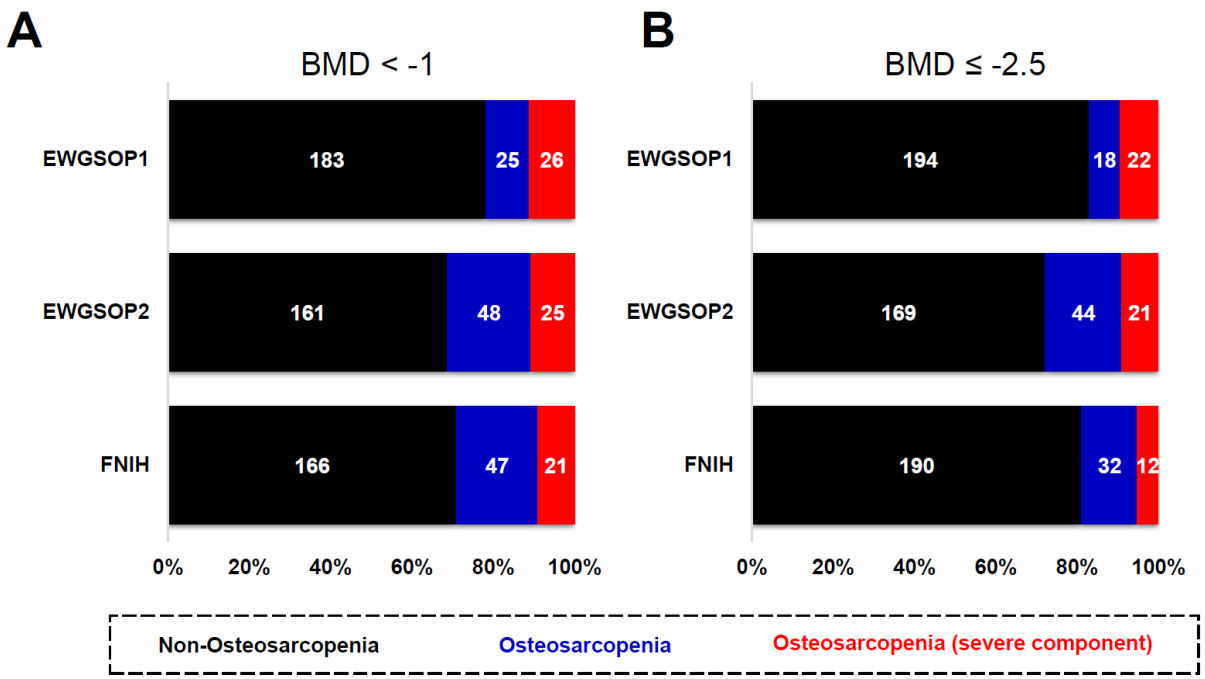

Figure 1: Prevalence of Osteosarcopenia and Osteosarcopenia (with severe sarcopenia component) using different definitions and comparing with (A) BMD T-score <1 SD, and (B) BMD T-score ≤2.5 SD.

Abbreviations: EWGSOP = European Working Group of Sarcopenia in Older People; FNIH = The Foundation for the National Institutes of Health; BMD: Bone mineral density.

Table 1: Different cut-points used to identify osteosarcopenia.

Definition	*Low BMD*	*Low ALM*		*Low MS*		*Low PP*
Osteosarcopenia (EWGSOP1)	T-score <-1	ASMI: <7.23 kg·m^2 for men and <5.67 kg·m^2 for women	**AND**	HGS: <30 Kg for men and <20 Kg for women	**OR**	GS ≤0.8 m/s
Osteosarcopenia (EWGSOP2)		ASM: ≤ 20 kg for men and ≤ 15 kg for women		HGS: <27 Kg for men and <16 Kg for women		
Osteosarcopenia (FNIH)		ASM/BMI: <0.789 for men and for <0.512 women		HGS: <26 Kg for men and <16 Kg for women		

Abbreviations: ALM: appendicular lean mass; ASM: Appendicular skeletal mass; ASMI: Appendicular skeletal mass index; BMD: Bone mineral density; BMI: Body mass index; EWGSOP: European Working Group of Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health; GS: Gait speed; HGS: Hand grip strength; MS: Muscle strength; PP: Physical performance.

Table 2: Clinical characteristics of study participants.

Variables	Sample Size	Mean ± SD/ N (%)
Age, years	253	77.9 ± 0.42
Women, n (%)	253	196 (77%)
ASM, Kg/m^2	242	16.8 ± 4.2
ASMI, Kg/m^2	242	6.6 ±1.2
Femoral T-score	223	-2.11 ± 1.03
Lumbar T-score	231	-1.19 ± 1.6
Arm T-score	134	-2.23 ± 1.58
CACI total, score	234	4.6 ± 1.8
Number of falls in the last year	250	
0		10 (4%)
1		106 (42%)
>1		134 (54%)
Number of fractures	246	
0		42 (17%)
1		162 (66%)
>1		42 (17%)
BMI classifications	253	
<18.5		7 (3%)
18.5-25		76 (30%)
25-30		84 (33%)
≥30		86 (34%)
BMI, kg/m^2		28.3 ± 6
Physical Performance		
Handgrip Strength, Kg	251	22.39 ± 7.9
Sit to Stand time, s	248	16.6 ± 6.8
Gait speed, m/s	247	0.73 ± 0.29
Timed up go, s	240	17.9 ± 8.1
Short Physical Performance Battery score	247	7 ± 2.7
Dynamic Balance		
Four Square Step test, s	120	19.1 ± 7.9
Static Balance		
Limits of stability, cm^2	222	113.2 ± 65
Eyes open on hard surface		
CoP area, cm^2	222	6.9 ± 5.9
Oscillation, cm/s	222	1.7 ± 0.92
Eyes closed on hard surface		
CoP area, cm^2	199	7.6 ± 7
Oscillation, cm/s	199	2.3 ± 1.4
Eyes closed on foam		
CoP area, cm^2	110	25.4 ± 19.6
Oscillation, cm/s	110	4.6 ± 1.9
Saccadic dual task		
CoP area, cm^2	189	7.3 ±8.2*
Oscillation, cm/s	189	2.6 ± 1.5
Horizontal vestibular interaction		
CoP area, cm^2	173	13.3 ±12.9
Oscillation, cm/s	173	3.7 ± 2.2
Vertical vestibular interaction		
CoP area, cm^2	166	11.3 ± 10.4
Oscillation, cm/s	166	3.6 ± 2.0

Abbreviations: CACI: Charlson age-comorbidity index ; ASM: Appendicular skeletal muscle mass; ASMI: Appendicular skeletal muscle mass index; BMI: Body mass index; CoP: Center of pressure.

Table 3: Associations among different criteria to detect osteosarcopenia with physical performance, dynamic and static balance.

Classification of osteosarcopenia	***Worse Physical Performance***			***Worse dynamic and static balance***		***Multiple Falls***	***Multiple Fractures***
	SPPB ≤ 8 score *OR (95%IC)*	TUG ≥ 20 s *OR (95%IC)*	5STS > 15 s *OR (95%IC)*	FSS > 15 s *OR (95%IC)*	LOS < 120 cm *OR (95%IC)*	≥2 falls *OR (95%IC)*	≥2 fractures *OR (95%IC)*
BMD <-1 + Sarcopenia according to							
EWGSOP1	1.29 (0.5-3.6)	0.84 (0.3-2.3)	0.85 (0.3-2.7)	1.32 (0.3-5.3)	2.64 (0.7-9.6)	0.28 (0.1-0.7) *	0.48 (0.1-2.2)
EWGSOP2	3.06 (1.2-7.6) *	3.07 (1.5-6.2) *	1.95 (0.7-5.7)	2.81 (0.6-12.6)	1.96 (0.8-4.8)	0.49 (0.2-0.9) *	0.74 (0.3-1.9)
FNIH	3.59 (1.4-9.3) *	1.29 (0.7-2.6)	3.70 (1.1-13.3) *	4.84 (1.1-21.6) *	1.87 (0.7-4.5)	0.69 (0.3-1.4)	0.45 (0.2-1.4)
BMD ≤-2.5 + Sarcopenia according to							
EWGSOP1	1.63 (0.5-5.4)	0.75 (0.2-2.5)	0.75 (0.2-2.7)	0.93 (0.2-4.3)	2.62 (0.6-12.2)	0.71 (0.1-0.6) *	0.66 (0.14-3.1)
EWGSOP2	3.45 (1.3-9.0) *	3.06 (1.5-6.3) *	2.26 (0.7-7.2)	2.30 (0.5-10.9)	2.11 (0.8-5.4)	0.49 (0.2-1) ǂ	0.85 (0.32-2.2)
FNIH	2.41 (0.8-6.8) ǂ	1.35 (0.6-3.3)	3.47 (0.8-15.9)	2.27 (0.4-12.6)	1.51 (0.6-4.1)	0.88 (0.4-1.9)	0.52 (0.1-1.8)
BMD <-1 + Severe Sarcopenia according to							
EWGSOP1	7.19 (1.6-33.2) *	2.32 (0.9-5.6) ǂ	1.7 (0.3-8.6)	-	2.37 (0.7-7.6)	2.48 (0.9-6.4)	1.61 (0.5-4.8)
EWGSOP2	5.71 (1.3-25.8) *	2.36 (0.9-5.7) ǂ	0.85 (0.2-3.5)	-	4.36 (0.9-19.8) ǂ	2.83 (1.1-7.6) *	0.82 (0.2-2.9)
FNIH	3.66 (0.9-13.5) ǂ	3.83 (1.4-10.2) *	0.88 (0.21-3.68)	-	2.73 (0.7-10.2)	1.93 (0.7-5.2)	3.86 (1.3-11.3) *
BMD ≤-2.5 + Severe Sarcopenia according to							
EWGSOP1	6.22 (1.3-29.2) *	2.56 (1.0-6.5) *	1.72 (0.3-8.6)	-	1.97 (0.6-6.4)	2.61 (0.9-7.2) ǂ	1.39 (0.4-4.5)
EWGSOP2	3.99 (0.9-18.3) ǂ	3.38 (1.2-9.2) *	0.85 (0.2-3.5)	-	3.21 (0.7-14.9)	3.63 (1.1-11.6) *	0.69 (0.1-3.2)
FNIH	2.39 (0.5-11.9)	3.39 (0.9-12.1) ǂ	0.91 (0.2-4.9)	-	2.08 (0.4-10.5)	2.75 (0.7-10.9)	4.38 (1.1-17.4) *
BMD <-1 + LPP and LMS according to							
EWGSOP1	7.89 (3.3- 18.7) **	5.14 (2.7-9.7) **	2.10 (0.8-5.7)	-	2.20 (1.3-6.0) *	2.60 (1.4-4.8) *	1.92 (0.9-4.1) ǂ
EWGSOP2	7.85 (2.3-27.1) **	3.60 (1.7-7.5) **	1.25 (0.4-4.1)	-	2.35 (0.9-6.1) ǂ	2.89 (1.3-6.3) *	1.75 (0.7-4.1)
FNIH	6.97 (2.0-24.12) *	3.46 (1.6-7.3) **	1.10 (0.3-3.7)	-	2.54 (9.1-7.1) ǂ	2.92 (1.3-6.5) *	1.91 (0.8-4.6)
BMD ≤-2.5 + LPP and LMS according to							
EWGSOP1	5.63 (2.2-14.2) **	3.45 (1.8-6.7) **	2.12 (0.7-6.1)	-	2.17 (0.9-4.9) ǂ	2.18 (1.4-4.2) *	1.82 (0.8-3.9)
EWGSOP2	6.02 (1.3-26.9) *	3.27 (1.4-7.9) *	1.25 (0.3-4.9)	-	1.90 (0.6-6.0)	3.23 (1.2-8.6) *	1.95 (0.7-5.4)
FNIH	5.53 (1.2-24.8) *	2.87 (1.2-6.9) *	1.06 (0.3-4.2)	-	1.69 (0.5-5.4)	2.92 (1.1-7.8) *	2.06 (0.7-5.8)

Adjusted for age, gender and Charlson age-comorbidity index.
Abbreviations: BMD: bone mineral density; EWGSOP: European Working Group of Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health; FSS Four-square step test; LMS: muscle strength; LOS: Limit of stability; LPP: physical performance; SPPB: Short physical performance battery; 5STS: 5 times Sit-to-stand test; TUG: Timed Up and Go; **. S**tatistical significance ** $p \leq 0.001$; * $p < 0.05$; ǂ $p < 0.09$.
Note: OR for FSS could not be performed for several definitions due to small sample size.

5.CHAPTER V. A NEW EXERCISE PROTOCOL, BASED ON FUNCTIONAL TASKS, FOR OLDER ADULTS TO PREVENT AND TREAT PATIENTS WITH GERIATRIC SYNDROMES.

PRESENTATION:

In this chapter the following articles are presented: 1) “Effects of an exercise model based on functional circuits in an older population with different levels of social participation” (published in the Journal Geriatric Gerontology International) and 2) “Effect of dual-task training on clinical and biological factors related to sarcopenia in older adults” (formmated according to the reccommendations of the Journal Geriatric Gerontology International)**.** The first article included older adults from Chile and was developed in collaboration with researchers from Londrina State University (Brazil) and the Primary Care Center from Requinoa (Chile). In this article, we proposed a new exercise protocol for older adults. In the second article, we used the same protocol now in a group of Brazilians elderly from the community and we assessed the effects of this exercise protocol on clinical variables of sarcopenia and on the oxidative stress biomarkers previously studied, as presented on chapter III of this thesis.

5.1 ORIGINAL ARTICLE

Title: Effects of an exercise model based on functional circuits in an older population with different levels of social participation.

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Article published in Geriatrics & Gerontology International

(Q1/ Impact factor: 2.73)

Loyola WS, Camillo CA, Torres CV, Probst VS. Effects of an exercise model based on functional circuits in an older population with different levels of social participation. Geriatr Gerontol Int. 2017

Abstract

Aim: To investigate whether being part of a community organization interfered on subjects' overall functionality. The magnitude of responses to a community exercise program based on functional circuits in socially active and socially non-active older adults was also investigated.

Methods: A total of 200 older adults with age ≥60 years old from Requinoa, Chile participated in the study. Subjects were separated into two groups according to the level of social participation: socially active (SA) and socially non-active (SNA). During an evaluation, data regarding the presence of comorbidities (Charlson comorbidity index, CCI), disability level (Modified Health Assessment Questionnaire), dynamic balance (timed up-and-go test, TUG), muscle force (handgrip dynamometry) and sociodemographic conditions were collected. Participants followed an exercise program consisting of a functional exercise circuit including balance, resistance and aerobic exercises, twice a week, for 12 weeks.

Results: Both CCI and disability were higher in SNA compared with SA upon inclusion. Both groups improved disability (∆-0.25 [-0.5 – -0.0625]pts for SNA and (∆-0.125 [-0.5 – 0]pts for SA) and balance (∆-2[-3 – 0]sec for SNA and (∆-1 [-3 – 0]sec for SA) after training ($p<0.05$ for all). Changes from baseline were statistically higher in the SNA group. Handgrip force improved only in subjects in the SNA group (∆1.7[0.6 – 2.8] kg, $p=0.0001$). No differences, however, were observed between the magnitude of improvements of handgrip between groups.

Conclusions: Although socially non-active older adults had more comorbidity and disability than their socially active counterparts, they showed a higher response to a community exercise program.

Key words: Aging, exercise, health status, physical function, social participation.

Introduction:

Ageing leads to a reduction in aerobic capacity, strength, balance and flexibility.(1) These alterations have direct impact on functionality, potentially causing sarcopenia and frailty specially when comorbidities and sedentarism are present.(2) In addition, factors such as socioeconomic status, education, social participation, race and ethnicity may contribute to variation in biological and health outcomes.(3,4) It has been estimated that by 2030, 25% of the world population will be composed of adults with age superior to 70 years old.(5) This predicted shift of life expectation will force governments to change policies of public health. Further than the resources to pharmacological treatments, the new policies will need to include the development of prevention strategies and health promotion such as community programs.(6)

In order to subjects to reach a health ageing, two widely known protective factors must be incorporated in the daily routine: social relationship (SR) and physical activity (PA).(7) Evidence shows that SR affect a range of health outcomes, including mental and physical health, health habits, and mortality risk.(7–9) A recent meta-analysis found that individuals with inadequate social relationships had a 50% greater risk of mortality than their counterpart.(10)

Family support, marital status and social participation in community organizations (COs) have been identified to potentially influence physical activity.(7) Commonly, older adults participate in religious groups, sports, senior centers and different community organizations.(11) The participation in physical activity programs is translated into health benefits by increasing aerobic capacity, strength, balance and preventing the risk of falls.(3,12–14) Benefits are also observed in reducing comorbidities,(3) promoting bone mineralization, improving memory and learning.(15) A bottleneck is the rarely offered individualized exercise prescription in community exercise programs. The combination of limited availability of health professionals and increased demand (a typical scenario in Latin America, with limited economical resources) hinders a more tailored exercise program to participants.(16) Furthermore, community programs deal with a very

heterogeneous population with different comorbidities, social status and even participation in COs. Therefore, it is reasonable to hypothesize that differences in functionality among older adults with different levels of social participation exist.

The aim of this study was to investigate whether being part of a COs interfered on subjects' functionality. Additionally, the magnitude of responses to a community exercise program based on functional circuits in socially active and socially non-active older adults was investigated.

Materials and Methods

A clinical study was conducted with older adults from the commune of Requinoa, Chile. Subjects would be included if were older than 60 years old and were classified as independent according to the functional scale for the older people designed by Chilean Ministry of Health,(17) and were registered in the primary care system of their city commune. Individuals were ineligible for the study if were unable to walk, had a history of recent hip fracture or stroke or had any of the absolute contraindications to exercising from the American College of Sports Medicine (ACSM) guidelines for exercise testing and prescription.(18) The study was approved by the ethics committee of the Primary Care Center of the commune and all participants provided a written informed consent to the participation in the program. Participants in this study were volunteers and were separated into two groups according to the level of social participation: socially active (SA) and socially non-active (SNA). A socially active subject was defined as a person who attend the COs at least once a week. A socially non active was defined as a person who not attend the COs any time a week. The COs considered were: religious groups, sport, cultural, neighborhood association and senior centers.

Measurements

Handgrip force was assessed with individuals on a standing position using a digital hand dynamometer (Jamar Dynamometer). The shoulder was positioned at adducted and

neutrally rotated position with the elbow at 90° and the forearm and wrist at neutral position.(19) Three repetitions were performed on the right hand with 30-second rest between tests. The highest value (in kilograms) from the three attempts was used as the maximal force.

Dynamic balance and agility was assessed with timed up-and-go test (TUG). Subjects stood up from a seated position, walked 3m, turned around, and returned to the seated position on a chair. After a practice trial, the shortest time (in seconds) of two trials was recorded and used as the TUG score.(20)

The index of disability was measured by the Modified Health Assessment Questionnaire (MHAQ). The MHAQ assess degree of difficulty, satisfaction with function and changes over the past 6 months in 8 different items (dressing, arising, eating, walking, hygiene, reaching, gripping, and getting in and out of car).(21) Subjects choose between four options in each of the items (without any difficulty, with some difficulty, with much difficulty, unable to do). The questionnaire was administered during a face-to-face interview always by the same professional.

The Charlson comorbidity index (CCI) was used to quantify the overall burden of comorbidities. The index includes 19 medical conditions with corresponding weights. Comorbidities were weighted and scored using an algorithm proposed by Charlson et al.(22) Afterwards, the total score was adjusted by age (age-adjusted Charlson comorbidity index, ACCI) (25)

Interventions

The exercise program consisted of 35-45 min sessions on non-consecutive days, two times per week, for 12 weeks (24 sessions). Each session started with a warm-up (e. g; jogging, walking, flexibility exercise, etc.) or group dynamics and followed a standardized functional exercise circuit (FEC) consisted of aerobic, resistance, balance and coordination exercises applied as functional tasks in 15 stations (1 minute of duration

each). All the activities were performed in training groups with a limit of 15 participants (including individuals belonging to SNA and SA). Subjects had to complete the FEC twice. Details of all included activities of the program are provided in table 1.

For the exercise prescription, a subject was evaluated for one minute in each station, where she/he was requested to perform either the maximum number of repetitions (MNR) or the maximum level of difficulty according to the characteristic of the task. The percentage of requirement for each task was calculated according to the initial evaluation. The sequence was: 1st week 50% of MNR, 3rd week 60% of MNR, 5th week 70% of MNR, 7th week 90% of MNR, 9th week 110% of MNR and 11th week 150% of MNR. Exercise stations including balance exercises were not evaluated with repetitions but instead by level of difficulty and progression of these tasks were done by reducing stability. Exercise sessions were always supervised by the same professionals.

Statistical Analysis

The software used for the statistical analysis was Statistical Package for the Social Sciences (SPSS) ver. 19.0 (IBM Co., Armonk, NY, USA) GraphPad PRISM 5.00 (GraphPad Software, Inc., San Diego, CA, USA). Data normality was evaluated using the Kolmogorov-Smirnov test, and expressed as mean and standard deviation or median and interquartile range according to the data distribution. Paired comparisons (baseline versus post program) were done by the paired t-test or Wilcoxon test according to the distribution of data. The comparison between SA and SNA was done by unpaired t-test or Mann-Whitney, also following data distribution. Categorical data was compared using Chi square test. The level of significance was established as $p<0,05$

Results

Two hundred subjects were initially included in the exercise program separated in 15 training groups. One hundred sixty-four patients (73±6 years, body mass index 28±4 Kg/m^2) completed all the 24 sessions of the program (66% of SNA and 82% of SA).

There were no significant difference of the dropout rate between SNA and SA (p=0.091). Socio-demographic characteristic of the subjects are reported in table 2. There were no significant differences in body weight or body composition between the groups. There were more women (p=0.031) and subjects with higher education (p=0.019) in the SA group. The age-adjusted Charlson comorbidity index (ACCI) was higher in SNA compared with SA (p=0.04). Amongst socially non-active subjects, 51% had an ACCI score of 2–3, and 49% had an ACCI score of 4–5. Amongst socially active subjects, 64% had an ACCI score 2-3, and 36% had an ACCI score of 4–5 (Table 2). At the initial evaluation, the index of disability was higher in the socially non-active group compared with the socially active group ($p < 0.05$) (Table 3).

Table 3 shows the changes of assessed outcomes after the exercise program. There was a statistically significant reduction in disability measured with MHAQ as well as an improvement of dynamic balance and agility measured by the TUG ($p<0.05$ for all outcomes in both groups). In comparison to the SA, SNA showed statistically higher improvement in both MHAQ (Δ-0.25 [-0.5 – -0.0625] pts for SNA and (Δ-0.125 [-0.5 – 0] pts for SA, p=0.0002) and TUG (Δ-2[-3 – 0] sec for SNA and (Δ-1 [-3 – 0] sec for SA, p=0.0093). Handgrip force improved in SNA (Δ1.7[0.6 – 2.8] kg, p=0.0001) whereas no significant improvement was observed in SA (Δ1,7 [-4.0 – 6.1]kg, $p>0.05$). No differences, however, were observed between the magnitude of improvements of handgrip between groups.

Discussion

This study found that SNA individuals presented higher levels of disability and comorbidities in comparison to SA individuals. Despite this, subjects in SNA were able to improve muscle force after a specific training program, which was not the case in the SA subjects. Similar improvements in disability and dynamic balance were observed in both SA and SNA individuals. However, more pronounce improvements were observed in SNA subjects.

Social ties has been significantly associated with cardiovascular diseases, high blood pleasure, cancer and changes in the immune and endocrine function.(7,24) Brummett et al. 2001,(8) found that adults with coronary artery disease socially isolated had a relative risk of 2.43 (95%CI 1.39–3.19, p=0.001) for cardiac mortality. Furthermore, Kiecolt-Glaser et al. 2002(4) described that low quality or quantity of social relationships is associated with increase of inflammatory biomarkers and impaired immune function, through negative emotion. Biomarkers of inflammation are a triggering factor to developed different chronic disease, such as diabetes mellitus, hypertension, metabolic syndrome, pulmonary and cardiovascular disease.(25) In the present study, socially non-active subjects had more comorbidities and disability. Although it is not possible to verify causality of activity status on the onset of allied diseases/comorbidities, it can be hypothesized that the higher prevalence of comorbidities in SNA can be somehow linked with social isolation, since there is evidence that the social interaction benefits the immune, endocrine, and cardiovascular functions.(7,26)

Other important finding is that the functional exercise circuit induced improvements in both groups for the gait speed and dynamic balance evaluated by the TUG and decreased the disability evaluated by MHAQ. This is consistent with previous studies about positive effects of the exercise in elderly people, such as improvement in force, gait speed, dynamic balance and preventing a disability new comorbidities.(3,13–15,27) However, it is worth to point that exercise generated greater benefits in socially non-active subjects than in the socially active ones, and that handgrip force post training only improved in SNA subjects. A possible explanation could be that the SNA had a higher disability than SA at the initial evaluation leaving more room for improvement in SNA than in SA. Importantly, the training program allowed SNA subjects to perform physical activities and interact with other people twice a week, since people from both groups were included in the exercise sessions (SNA and SA). It can be hypothesized that the participation of SNA subjects together with SA individuals could have induced the

adherence to two protective factors for a healthy ageing: physical activity and social relationship.(28)

Participation in group activities and social support has a positive impact on people's health. (10,28) In fact, the World Health Organization has recognized that the prevention of social isolation and loneliness is considered necessary to maintain functionality, to prevent disability and frailty in older people.(29) It can be assumed that the elderly from the SNA group were people without social participation who probably experienced loneliness. Hence, other positive effect of the program was to integrate non-active people into groups which allowed them to interact with other individuals, making them feel as part of the community.

Commonly, the participants of the exercise program in community are socially active and have more access to the information about the different programs and benefits. In the present study, even though it was not statistically significant, there was a trend of higher dropout rates in the SNA group ($p=0.091$). This could be related to several issues, such as lower level of information or access to such programs,(30) lower educational level and marital status (fewer subjects married). Nevertheless, subjects who decided to participate, improved their functionality even more than the ones from the SA group.

Many scientific evidences show that social participation affects the health and mortality.(9,10,24,26) However, there is little evidence about the effects of social participation on functional capacity in older people. Although it is not possible to imply causality, this study is among the very few that provide evidence of the association of the social participation and functional capacity in elderly people, and the different response of the exercise program in this population.

A potential limitation of the present study is the lack of investigation of training effects on a broader scope of outcomes. Holt-Lunstad et al, suggested that other factors could influence social status (e.g., family support, economical status and emotional function) (10). Another limitation was a lower number of men attending COs. Commonly, members

of COs in Chile are women, because men generally continue working after retirement or do not show interest in belonging to any organization, for causes not yet completely elucidated and should be studied in future researches. Therefore, future studies should address new health strategies that could be developed in order to increase the participation of men and also socially non-active subjects.

In conclusion, the present findings show that despite the fact that socially non-active older adults are more disabled and with more comorbidity than their socially active counterparts, they had a higher response in terms of balance and disability to a community exercise program based on functional circuits. In fact, socially non-active subjects showed more prominent improvements in terms of muscle force.

Acknowledgments

This work was supported by Primary Health Care from the Commune of Requinoa, and by the Program *"Adultos mayores mas autovalentes"* from the Ministry of Health, Chile. The authors want to acknowledge Directive from Community Organization for Older People, Municipality of Requinoa, Health Service O'Higgins and University of Aconcagua, Chile for its collaboration and physiotherapist Gloria Luna from Del Rosario University, Colombia, for having inspired this kind of exercise.

Disclosure statement.

The authors declare no conflict of interest.

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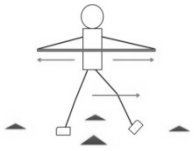
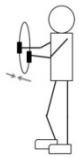
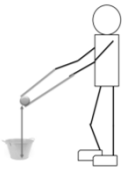
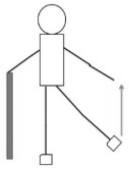
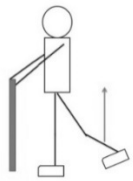
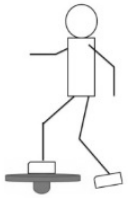
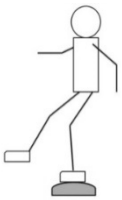
neighbourhood influence social and physical functioning in older age? Findings from a British survey of ageing. Soc Sci Med. 2007;64(12):2533–49.

Table 1 Functional Exercise Circuit

Exercise	Aim	Description of task	Progression from initial test (week)
	Agility Coordination Aerobic Capacity	Walking over 5 obstacles, 50 cm apart each other, forth and backwards. Instructed to repeat this according to the percentage of MNR. The maximum number of laps at the initial evaluation was used as MNR.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Coordination	Standing: Keeping one foot on the centre, moving the other foot in the direction of the cones (forward, back, left and right). Simultaneously to the lower limb exercises, arms must perform abduction and adduction when the lower limb returns to the centre. Instructed to repeat with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetition in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Coordination	Walking over 5 marked lines on the floor, 50 cm apart each other, in a lateral gait, left and right. Repeat this according to the percentage of MNR. MNR was calculated from the maximum number of laps in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Strength	Standing: Grip the resistance wheel, keeping your back straight at all time. Repeat it according to the percentage of MNR.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR

		MNR was calculated from the maximum number of repetition in the initial evaluation.	9th -10th 110 % of MNR 11th -12th 150% of MNR
	Strength	Sitting on a chair: Biceps pulley from 90º to 120º of elbow flexion. Repeat it according to the percentage of MNR. MNR was calculated with the moderate resistance chosen by the patient at baseline.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Aerobic capacity	Standing: Stationary gait with the elevation of each lower limbs. Repeat with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetition between both lowers limbs in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Strength	Sitting on a recumbent chair: Abdominal crunches ranging from 45º to 90º position. Repeat it according to the percentage of MNR. MNR was calculated from the maximum number of repetition in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Coordination Accuracy	Standing: Take the ball with the poles and hold it, then leave it in the plastic pot and repeat it. The level of difficulty and progression were done adding other tasks.	1st-3rd keep the ball for 10 seconds in the initial position. 4-9th keep the ball and walk for 15 seconds

			10-12th keep the ball and walk with obstacles as long as possible.
	Strength Power	Standing: Rest your hands on the back of the chair for stability if necessary and perform a Squat. Repeat it according to the percentage of MNR. MNR was calculated from the maximum number of repetition in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Strength	Standing: Rest your hands on the back of a chair for stability. Raise one leg to the side as far as is comfortable, keeping your back and hips straight. Avoid tilting to the other leg. Repeat it with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetition in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Strength	Standing: Rest your hands on the back of a chair for stability. Standing upright, raise your leg backwards, keeping it straight. Keep the back straight as you take your leg back. Repeat it with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetition in the initial evaluation.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR
	Balance	Standing: Put one foot over the balance boar and keep your back straight and the other foot on the floor.	1st-3rd keep the position for 30 seconds.

		Hold the balance for 30 seconds. In the beginning you can rest your hands on the back of the chair for more stability. The level of difficulty and progression were done adding other tasks.	4-9th keep the position for 30 seconds, with an object in both hand 10-12th keep the position for 30 seconds, and move your other leg forward and backward.
	Aerobic Capacity	Sitting on a chair: Run a static bike at a speed of at least 60 RPM, at a light load. The progression was done adding a progressive load during the training.	1st -2nd 1 3rd -4th 2 5th -6th 3 7th -8th 4 9th -10th 5 11th -12th 6
	Balance	Standing: Put one foot over the balance-disc and keep your back straight and rise the other foot; keeping the balance for 15 seconds. And change your leg. In the beginning you can rest your hands on the back of the chair for more stability. The level of difficulty and progression were done adding other tasks.	1st-3rd keep the position for 15 seconds. 4-9th keep the position for 15 seconds, with an object in a hand 10-12th keep the position for 15 seconds, and move your other leg forward and backward.
	Strength Power Coordination	Pass behind your back the elastic band and stretch forward with your arms, doing lunges, one leg at a time. Keeping your back straight. . Repeat it with each leg according to the percentage of MNR.	1st -2nd 50% of MNR 3rd -4th 60% of MNR 5th -6th 70% of MNR 7th -8th 90% of MNR 9th -10th 110 % of MNR 11th -12th 150% of MNR

	MNR was calculated from the maximum number of repetition in the initial evaluation.	

MNR: maximum number of repetition

Table 2 Socio-demographic characteristics of SNA and SA

Variable	Group	
	SNA (n= 41)	SA (n= 123)
Age (years)	74 ± 6	71 ± 5
Female, n (%)	26 (63%)	101(82%)*
BMI (kg/m^2)	28 ± 4	27 ± 5
Marital Status		
Married	19 (46%)	68 (55%)
Widowed	3 (8%)	11 (9%)
Single	19 (46%)	42 (34%)
Educational Level		
Less than primary education	27 (66%)	80 (65%)
Primary education	13 (32%)	24 (20%)
Secondary Education	1 (2%)	4(3%)
Higher Education	0 (0%)	15(12%)*
ACCI		
Mean scores	3,5 ± 1	3 ± 1*
0-1 (n, % of total)	0 (0%)	0 (0%)
2-3 (n, % of total)	21 (51%)	79 (64%)
4-5 (n, % of total)	20 (49%)	44 (36%)
≥ 6 (n, % of total)	0 (0%)	0 (0%)

Data are expressed as mean ± standard deviation or absolute number and frequency. NAS: Non-active socially group; AS: Active socially group; BMI: Body mass index; ACCI: Age-adjusted Charlson comorbidity index; *Statistically significant (p≤0,05)

Table 3: Baseline (Week 0) Characteristics of SNA and SA

Measures	SNA (n= 41)		SA (n= 123)	
	Pre	Post	Pre	Post
Handgrip force (kg)	23 [19-33]	24 [20-36]*	23 [19-27]	24 [20-28]
Disability MHAQ	0.375 [0.125-0.582]	0 [0-0.125]*	0 [0-0.125]**	0 [0.125-33]*
Timed Up and Go (sec)	10 [8-11]	8 [7-9]*	9 [8-10]	8[7-9]*

Data are expressed as median [interquartile range]. NAS: Non-active socially group; AS: Active socially group; MHAQ: Modified Health Assessment Questionnaire; *: statistically significant inter group;**: statistically significant intragroup (p≤0,05).

5.2 ORIGINAL ARTICLE

Title: Effect of dual-task training on clinical and biological factors related to sarcopenia in older adults.

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Article formatted to be submitted to the Journal Geriatrics & Gerontology International

(Q1/ Impact factor: 2.73)

Abstract

Aim: the objective was to analyze the effects of community-based dual-task training on clinical measurements of sarcopenia (muscle mass, strength and physical performance) and oxidative stress biomarkers in non-sarcopenic older adults.

Methods: 48 older adults who had not practiced physical exercises for at least 3 months were included in this study. Fat-free mass (FFM), fat mass (FM), calf circunference (CC), handgrip strenght (HGS), 5-repetition sit-to stand (5STS), 4-meter gait speed (4MGS), six-minute walking test (6MWT) and oxidative stress blood biomakers were evaluated. Individuals were allocated into one of these two groups: dual-task training (experimental group) and simple-task training (control) for 12 weeks, 3 times a week, 50 minutes each session.

Results: Dual-task training increased the FFM, CC, and reduced the FM (Cohen's effect size from 0.4 to 1.0; $p< 0.05$ for all). Both groups showed improvement in 4MGS, 5STS and 6MWT (Cohen's effect size from 0.5 to 1.0; $p< 0.05$ for all). All the antioxidant biomakers (glutathione transferase, catalase, total plasma antioxidant potential and sulfhydryl grouping) increased after dual-task training (Cohen's effect size from 0.4 to 1.0; $p< 0.05$ for all).

Conclusion: Community-based dual-task training improved muscle mass, muscle strenght and physical performance, which are the cllinical measurements of sarcopenia. Additionally, this intervention also increased the antioxidant capacity in healthy older adults.

Key words: Aging; Sarcopenia; Oxidative stress; Physical exercise; Aged.

INTRODUCTION

Sarcopenia is a disease defined as a reduction in muscle strength, physical performance and muscle mass[1]. This is associated with metabolic changes, immobility, systemic inflammation and oxidative stress[2–4]. Sarcopenia has a prevalence of approximately 5–13% in older adults[5] and is the major cause of frailty[2], being related to worse physical function, quality of life, history of falls and fractures, hospitalization and mortality[6].

The clinical impact of sarcopenia also implies in extremely high health costs. In terms of financial burden, a 10% reduction in the prevalence of sarcopenia would save $1.1 billion per year[7], so its prevention remains imperative. Exercise is undoubtedly the most studied intervention to avoid and treat sarcopenia[8], demonstrating positive effects on muscle mass, muscle strength and physical performance, which are the clinical measurements used to diagnose sarcopenia[9]. Different exercise programs have been proposed to prevent sarcopenia[1], however, there is a lack of high-quality studies according to the last systematic review published in this field[10].

Most of the exercise interventions for sarcopenia have focused on strength training and nutritional supplementation, targeting the intervention for sarcopenia as a problem related only to muscle mass and muscle strength[10]. However, sarcopenia is a multidimensional condition, characterized by reduction in gait speed, balance, coordination and even cognitive status[11]. Merchant *et al.* have recently shown that dual-task exercise is effective to increase gait speed, handgrip strength and reduce falls[12], which may possibly diminish the prevalence of sarcopenia. However, regarding dual-task training, there are few studies available in this field[12] and with low methodological quality[13–16]. Additionally, the effects on muscle mass, an important component of sarcopenia, have not been previously addressed[15] and there are only few studies that simultaneously investigated the effects of dual-task training on physical domains and biological biomarkers related to sarcopenia[17]. For this reason, the objective of the present study was to analyze the effects of community-based dual-task training on

clinical measurements of sarcopenia (muscle mass, strength and physical performance) and oxidative stress biomarkers in non-sarcopenic older adults.

METHODS

It was a non-randomized controled trial with 58 older adults, recruited via social media, social networks and community health care centers from Londrina, Brazil. It was a convenience sampling. The inclusion criteria were individuals with age ≥ than 60 years, with physical independence, and who had not practiced exercise in the previous 3 months. Those older adults who presented history of recent hip fracture or knee injury, any absolute contraindications to perform exercise, some apparent degree of cognitive impairment that would hinder exercise performance and assessment tests with clinical suspicion of sarcopenia according to the EWGSOP2 algorithm[1] were excluded. The study was approved by the Ethics Committee from the University and all participants provided written informed consent to participate in this study (registration number: 2.771.022).

Procedures

Those older adults who met the inclusion criteria were assessed for body composition, muscle strength, physical performance, and oxidative stress levels, which are factors related to sarcopenia.

Body composition

Body composition was assessed by bioelectrical impedance (Biodynamics 310TM, Biodynamics Corp. USA). The fat mass and fat-free mass values were obtained directly from the device. Muscle mass was also evaluated through the calf circumference. For this assessment, we used an inelastic measuring tape and the measurement was

performed at the maximum circumference in the plane perpendicular to the longitudinal line of the calf.

Muscle strength

Muscle strength was assessed by the handgrip strength test, which measures the maximum isometric strength of the hand and forearm muscles. Force was measured by the right hand using a portable dynamometer (hydraulic dynamometer SH 5001, Saehan Medical, USA). The test was performed in a sitting position, with the arm parallel to the trunk, the elbow flexed at 90 degrees and the forearm and hand in neutral position. The test was repeated three times and the highest value was considered for the analysis. To assess lower limb quadriceps strength and functionality, the 5-repetition sit to stand test (5STS) was used[1]. It was performed using a chair with 43 cm height and, with a chronometer, the time was counted while the individual raised five times from the sitting position without using the arms.

Physical performance

Physical performance was assessed by the 4-meter walking speed test (4MGS)[1]. Subjects were instructed to walk at their usual pace and walk the distance of 4 meters. Two tests were performed and the best result was selected for the analysis.

The 6-minute walk test (6MWT) was performed to measure exercise capacity. Subjects were instructed to cover the greatest distance as possible within 6 minutes in a 30-meter-long flat corridor. Two tests were performed and the best result was selected for analysis.

Oxidative stress biomarkers

A qualified professional collected 10 ml of blood from each individual. Subjects needed to fast for 10 hours to perform the exam. For the oxidative stress analyzes, the following markers were assessed: Total Plasma Antioxidant Potential (TRAP), Total Plasma Sulfhydryl (SH), Catalase (CAT), Glutathione Transferase (GT), Nitric Oxide (NO),

Advanced Protein Oxidation Products (AOPP) according to methodlogy previously publised[18].

Other measurements

Charlson's comorbidity index[19] and Motreal CognItive asstement (MoCA)[20] were also analysed in order to describe the comorbidities and cognitive status of the studied population.

Interventions

After the initial evaluation, subjects were allocated into two intervention groups (experimental and control group). Both interventions were developed in two community centers, including different exercise protocols: a dual-task protocol was included as the experimental group and a simple task protocol was considered as the control group. The allocation of the older adults was matched for gender, age, body mass index (BMI) and presence of comorbidities (Charlson index), however, it was according to their proximity to the location where the program took place (non-randomized). Blinding of physiotherapists involved in the treatment groups and patients was not possible, given the nature of the interventions. However, evaluators involved in the assessment tests and the statistician were blinded to which group subjects belong. Upon completion of the training regimen, all assessment tests were repeated.

Experimental Group

For the experimental group we used a dual-task protocol called functional exercise circuit (FEC)[21], which include a battery of exercises of various modalities, being aerobic, resistance, balance and coordination, applied as functional tasks and divided into 15 stations, as shown in figure 1. The older adult overcome obstacles, perform walking tasks with objects in both hands, maintain balance positions with different unstable platforms

and perform tasks with both arms simultaneously. The activities were carried out in 2 training groups, each with a maximum of 15 participants, distributed in a circuit. One minute was counted at each station for participants to move on to the next exercise. The session ended when all participants completed 2 laps of the circuit. For exercise prescription, each participant was evaluated for 1 minute at each station, where they were asked to perform the maximum number of repetitions (MNR) or to reach the maximum level of difficulty according to the characteristics of each task. The intensity was calculated according to the initial assessment, from 50% of MNR (1st week) to 150% of MNR (11th week). Stations of balance, coordination and accuracy were evaluated by level of difficulty adding the performance of a task using both hands, such as holding objects and balls. The progression of these tasks was accomplished by reducing stability and increasing speed. Each session started with a 10-minute warm-up, alternating between running, walking, stretching and recreation, followed by the standardized functional exercise circuit. The sessions lasted 40 to 50 minutes on non-consecutive days, three times a week for 12 weeks (36 sessions).

Control Group

The control group received a simple task exercise program that include stretching, walking in an electric treadmill (EMBREEX 566 BX 3.0/ 566 BX 1.8/ 566 BXI, Embreex, Brazil) and relaxion exercises. Subjects were allocated into two subgroups for better monitoring. The first 10 minutes were used for stretching of the trunk, upper and lower limbs. Then, they were placed on the treadmill and told to walk as fast as possible for 30 consecutive minutes, maintaining a self-selected speed corresponded to levels 4 to 6 from the Borg scale. The configuration of the treadmills was without inclination. For those who needed help, qualified professionals assisted in setting the equipment. The walking speed and the subjective perception of effort, using the Borg scale, were recorded after the first 10 minutes and at the end of the training session. Finally, stretching and

relaxation exercises were performed for 10 minutes, totaling 50 minutes of each training session, 3 times a week, for 12 weeks (36 sessions).

Statistical analysis

The analyzes were performed using the following statistical software: SPSS version 22.0 (IBM, Armonk, USA) and JAPS version 0.16.1 (JASP Team, The Netherlands). The normality of the variables was verified by the Shapiro-Wilk test and described as mean and standard deviation or median and interquartile range. Intragroup comparisons (pre and post) were performed using the paired Student's t-test or Wilcoxon test according to data distribution. The intergroup comparisons were assessed using the unpaired Student's t test or the Mann-Whitney test, also according to the data distribution. Categorical data were compared using the $\chi 2$ test and $p \leq 0.05$ was defined as statistically significant. For the comparisons between groups, we used analysis of covariance (ANCOVA) for differences in primary and secondary outcomes using continuous scales, with adjustment for baseline levels of outcomes, using group differences in the mean change from baseline to post-interventions as the dependent variable. This analysis was performed to avoid imbalances between groups, since participants with low scores at baseline tend to improve more than those with high scores, a phenomenon known as regression to the mean. Finally, we calculated Cohen's effect size (ES) for the effect of dual-task intervention, considering the effect to be trivial (<0.2), small (0.2–0.5), medium (0.5–0.8) or large (>0.8).

Results

Forty eight older adults were included in the study, 27 for the intervention group and 21 for the control group). Table 1 describes the baseline characteristics of the older adults in both groups. Since both groups were matched for gender, age, BMI and presence of comorbidities, there were no differences between groups (Table 1). The cognitive status

was not different beteween the groups (p= 0.08).

Body composition

Table 2 shows the comparisons between pre and post intervention and the effect on body composition, functional capacity and oxidative stress variables. The experimental group showed increase in fat-free mass (ES: 0.4 [95%CI: 0.002-0.8]; p= 0.049) and calf circumference (ES: 1 [95%CI: 0.5-1.3]; p< 0.001) and reduction in fat mass (ES: 0.5 [95%CI: 0.1- 0.9]; p= 0.02). No changes were observed in fat mass and fat-free mass in the control group. The control group presented a significant decrease in calf circumference (ES: 0.6 [95%CI: 0.1-1.1]; p= 0.011). The effect of the dual-task training compared to the simple-task training on body composition variables was higher (ES varied from 0.5 to 0.6).

Muscle Strength

Regarding muscle strength, the performance in the 5STS improved in both groups after training (Experimental group ES: 0.7 [95%CI: 0.3-1.01]; p=0.002 and Control group ES: 1.0 [95%CI: 0.4-1.5]; p<0.001). The Cohen's effect size in the intergoup comparison was 0.2. No differences were observed in hand grip strenght in both groups.

Physical performance

In relation to physical performance, speed in the 4MGS test increased in the experimental (ES: 0.5 [95%CI: 0.2-0.9]; p=0.012) and control group (ES: 0.5 [95%CI: 0.1-1.01]; p=0.02). The Cohen's effect size in integroup comparison was 0.1. The distance walked in the 6MWT increased aftert training in the experimental group (ES: 0.6 [95%CI: 0.2-1]; p=0.003) and in the control (ES: 0.7 [95%CI: 0.2-1.2]; p=0.004). The Cohen's effect size in the intergroup comparison was 0.1.

Oxidative Stress

Levels of NO increased significatly only in the experimental group (ES: 0.4 [95%CI: 0.03-

0.9]; p=0.035) and compared to the control group the Cohen's effect size was 0.7. All the antioxidant biomakers increased after training in the experimental group, GT (ES: 0.6 [95%CI: 0.2-1.01]; p=0.004), SH (ES: 1 [95%CI: 0.5-1.5]; p<0.001), TRAP (ES: 0.7 [95%CI: 0.2-1.1]; p=0.002), and CAT (ES: 0.4 [95%CI: 0.03-0.8]; p=0.034). Only SH (ES: 0.6 [95%CI: 0.1-1.1]; p=0.019) and CAT (ES: 0.9 [95%CI: 0.7-0.9]; p<0.001) increased in the control group. the Cohen's effect size for intergroup comparison varied from 0.08 to 0.6. The effect of the dual-task training compared to the simple-task training on the antioxidant markers was higher (ES varied from 0.08 to 0.6).

Discussion

In the present study, it was observed that dual-task training compared to simple-task training produce improvements in all clinical measuremnts of sarcopenia (muscle mass, muscle strenght and physical performance) and in the antioxidant capacity of physically independent elderly from the community.

Exercise is the intervention that has been more studied in the prevention and treatment of sarcopenia[8]. There is evidence demonstrating the positive effects of exercise on the specific measurements of sarcopenia such as muscle strength and mass as well as physical performance[9]. Different interventions with exercise programs have been proposed to prevent sarcopenia[22–25], however, the vast majority of interventions have not included dual-task training. On the other hand, this intervention has a main focus on training functional tasks that older adults frequently perform such as walking over obstacles or going up and down stairs, performing activities with the hands simultaneously [15]. For this reason, most of the evidence in dual-task training have been focused on outcomes such as balance and cognition[15,26] and not on clinical variables related to sarcopenia such as body composition, strength or physical performance.

In the present study, we have observed that dual-task training generated a positive effect on body composition, increasing muscle mass and decreasing fat mass. These effects

have not been previously studied in this exercise modality[15]. In addition to these effects, there was also an increase in the calf circumference, which is an important clinical measurement related to the risk of sarcopenia, frailty, hospitalization and mortality[27]. These findings on body composition can be explained because the protocol that we used in the dual-task training, included functional task prescribed through a test of maximum number of repetitions. Therefore, given that the execution of the task was prescribed at a moderate to high intensity, considering the MNR, the dual-task training generated a similar effect on body composition than other exercise modalities such as aerobic or strength training that have used this range of intensity[28]. Additionally, FEC was the dual-task protocol included in this study, which incorporate a battery of exercises of various modalities including aerobic and resistance exercises. It is well established that aerobic and strength exercises from moderate to high intensity generate effects on body composition, increasing muscle mass and decreasing the fat percentage[28,29], which generate important benefits in the prevention and treatment of other metabolic comorbidities. Although the objective of the dual-task training is not to change body composition, a previous study with this protocol showed improvements in the lipid profile[30], which could be associated with the reduction in fat mass observed in the present study. This indicates that regardless the type of motor task performed by the older adult, if it is prescribed at adequate intensity, it can produce physiological effects. The physiological effects of dual-task training have been previously reported[17,30] and it was also observed in our study with the increase in the antioxidant capacity.

In the literature, although there are controversial results about the effect of dual-task exercises on physical function, most of the evidence has reported positive effects of this intervention for physical and cognitive health in older adults[13–16]. One of the few studies that has not reported benefits for physical function was published by Morita *et al.*[31]. However, this was a non-randomized clinical study with a 2-year intervention follow-up, so other confounding factors such as aging process or diseases may have an impact on

functional decline that were not considered in the final analysis. On the other hand, two randomized control trials[13,14] have reported important improvements on gait speed, physical function, and balance in older adults after dual task training. In our study we found benefits in physical function in both single task and dual-task training. Similar findings were observed in the randomized controlled trial published by *Konak et al.*[16]. Therefore, considering our results and previous research[13–16], despite the fact that both dual and simple task training are recommended to improve physical function, dual-task training is clearly a more complex modality, and it is closer to what the individual does on a daily basis, because most of the activities involve simultaneous performance of different tasks[32,33]. In addition, impairment in gait and balance performance under dual-task testing are associated with future risk of falls[33]. For this reason, the more older adults are trained with the dual-task, the better they will perform in daily activities, resulting in lower risk of fall and disability[33].

In our study we have also observed that dual-task training generates an increase in all the antioxidant biomarkers compared to the simple-task training. SH and CAT, important antioxidants biomarkers, increased in both exercise protocols. SH considered to be the largest and most frequent antioxidants in plasma and CAT the largest primary antioxidant defense component of H_2O_2, both biomakers have protective effect on muscle mass and function according to a current laboratory study with mice[34]. Moreover, GT and TRAP increased only in the dual-task training. These biomarkers have been related to improvements in muscle mass and strength and physical performance in studies with older adults[18], which can be related to the increase in fat-free mass and calf circunference observed in this group in our study. On the other hand, regarding AOPP, a biomarker of protein oxidation related to the presence of sarcopenia[18], no changes were observed, probably because increasing in TRAP, an antioxidant of protein was found. Although an excessive production of reactive oxygen species can be harmful, contributing to early cell death, the increase of some biomarkers such as NO, observed

in the dual-task group, has a positive effect for cardiovascular health[35]. NO has been associated with angiogenesis, restoring blood flow to limb tissues, improving cardiopulmonar capacity, which can be related to the increase in the 6MWT observed in the dual- task group. It is also important to highlight that few studies have investigated the influence of dual-task training on physical domains and biological biomarkers simultaneously[17]. The positive results found in the present study open a new field of knowledge about the benefits generated by this type of exercise modality in different physical and biological variables in older adults.

A potential limitation of the present study was the lack of randomization which increases the risk of bias. However, we allow the older adults to choose the exercise intervention close to their homes in order to increase adherence to the training regimens. Additionally, the allocation of the older adults was matched for gender, age, body mass index (BMI) and presence of comorbidities, for that reason we have not observed differences at baseline between the groups. On the other hand, evaluators involved in the assessment tests and the statistician were blinded to which group subjects belonged. Another limitation could be the fact that we studied the effects of exercise on clinical variables of sarcopenia in older adults who have not been diagnosed with sarcopenia. Therefore, future studies should investigate the effects of this exercise modality in inviduals with sarcopenia, analysing the biological makers included in the present and previous resarch[17].

In conclusion, community-based dual-task training improved muscle mass, muscle strenght and physical performance, which are the cllinical measurements of sarcopenia. Additionally, this intervention also increased the antioxidant capacity in healthy older adults. From this perspective, this type of community-based training could be used as an intervention for the prevention and treatment of sarcopenia. Future randomized controlled trials are needed to assess the effectiveness of dual-task interventions in this population.

Acknowledgements

This study was financed in part by the *Programa de Apoio à Extensão Universitária do Ministério da Educação (PROEXT)* and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES).* WSL and RPT received scholarships from CAPES, Brazil. VSP is supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

Disclosure statement

The authors declare no conflict of interest.

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Table 1 – Baseline characteristics

Variables	**Intervention (n=27)**	**Control (n=21)**	***P***
Female, n (%)	21 (78%)	17 (81%)	0,602
Age (years)	69 ± 1	70 ± 1	0.6368
Height (m)	1.554 ± 0.015	1.545 ± 0.011	0.6563
Weight (kg)	70.16 ± 2.75	67.90 ± 2.24	0.5418
BMI (kg/m²)	28.88 ± 0.88	28.49 ± 0.98	0.7634
Charlson's comorbidity index (points)	0 [0-1]	0 [0-1]	0.1672
MoCA, score	21.3 ± 4	19.4 ± 4	0.09

Data presented as mean ± standard deviation or median [interquartile range]; BMI: Body mass index, MoCA: Montreal Cognitive Assestment.

Intervention (dual-task training)

Control (simple-task training)

Table 2 – Changes in body composition, muscle strenght, physical function and oxidative stress biomarkers

	Intervention group			Control group			Difference between group			
Variables	Baseline	Post-intervention	*p*	Baseline	Post-intervention	*p*	*Mean change*	*Effect Size*	*CI 95 %*	*P value*
Group							*between group*	*Cohen's d*	*For Cohen's d*	*between groups*
Body Composition										
FFM (kg)	41.07±6.94	41.66±7.33	0.273	40.41±4.27	40.06±3.71	0.384	1.54	0.2	-3.5 to 6.3	0.10
FFM (%)	59.21±6.36	60.64±6.77	0.049*	60.24±7.08	60.18±6.01	0.933	0.42	0.1	-5.5 to 7.3	0.10
FM (kg)	29.10±9.41	27.85±9.57	0.017*	27.20±8.44	29.18±11.55	0.385	-1.3	0.6	-8.3 to 0.9	0.03**
FM (%)	40.79±6.36	39.36±6.77	0.049*	39.35±7.16	42.54±14.47	0.353	-3.2	0.4	0.3 to 7.0	0.04**
Calf circ. right (cm)	35.5 [34.0-36.5]	36.5 [35.0-39.0] ‡	< 0.001*	36.45±2.59	34.93±3.07‡	0.011*	1.5	0.5	0.07 to 3.1	0.04**
Muscle strength										
HGS right (kg)	28 [24-36]	30 [24-35]	0.680	28±6	28±6	0.872	2	.09	-0.03 to 0.02	0.79
5STS (s)	11.82 [9.98-13.70]	9.80 [8.29-10.76]	0.002*	13.14±2.90	10.32±2.75	< 0.001*	-0.52	0.2	1.2 to 2.4	0.10
Physical performance										
4MGST (s)	3.71±0.56	3.45±0.52	0.012*	4.11±0.98	3.55±0.54	0.020*	-0.1	0.1	-0.7 to 0.5	0.10
6MWT predict (%)	109.5±18.25	123.9±14.99	0.003*	111.9±17.79	122.7±17.56	0.004*	1.2	0.1	-0.7 to 0.2	0.10
Oxidative markers										
NO (pmol/mg)	4.93 [3.78-6.87] †	9.01 [6.93-12.22]	0.035*	7.66 [6.77-13.01] †	8.80 [7.29-10.36]	0.899	0.21	.07	-3.0 to 4.0	0.10
AOPP (uM/l)	97.56 [63.9-125.3]	104.5 [85.1-126.2]	0.387	89.46±22.59	94.53±30.35	0.477	10	.05	-2.9 to .04	0.10
Antioxidant markers										
GT (U/mgHb)	6.20 [5.89-6.77]†	7.22 [6.51-7.52]	0.004*	7.10±1.07†	7.19±0.93	0.780	0.03	.005	-1.9 to 1.9	0.10
TRAP (uM trolox)	856.6±181.9	949.0±168.2	0.002*	933.4±145.3	971.7±156.2	0.350	-22.7	0.2	-2.1 to -3.4	0.69
SH (mM/mg)	230.1±37.9†	307.7±74.6‡	< 0.001*	195.5 [181.9-221.2] †	235.4 [200.9-278.6] ‡	0.020*	72.3	0.6	3.4 to 9.6	0.03**
CAT (U/mgHb)	63.11±23.40†	72.89±16.75‡	0.034*	81.73±20.30†	107.10±18.73‡	< 0.001*	-34.21	.02	-5.8 to -5.0	0.22

Data presented as mean ± standard deviation or median [interquartile range]; 4MGST: 4 meter gait speed test; 6MWT: 6-minute walk test; 5STS: 5-repetitions si-to-stand; HGS: handgrip strength; FFM: fat-free mass, FM: fat mass; NO: nitric oxide; AOPP: advanced oxidation protein products; GT: glutathione transferase; TRAP: total plasma antioxidant potential; SH: sulfhydryl

grouping; CAT: catalase.

† Baseline characteristics of the studie sample that *p* value ≤ 0.05

‡ The change of the post results inter-group that *p* value ≤ 0.05.

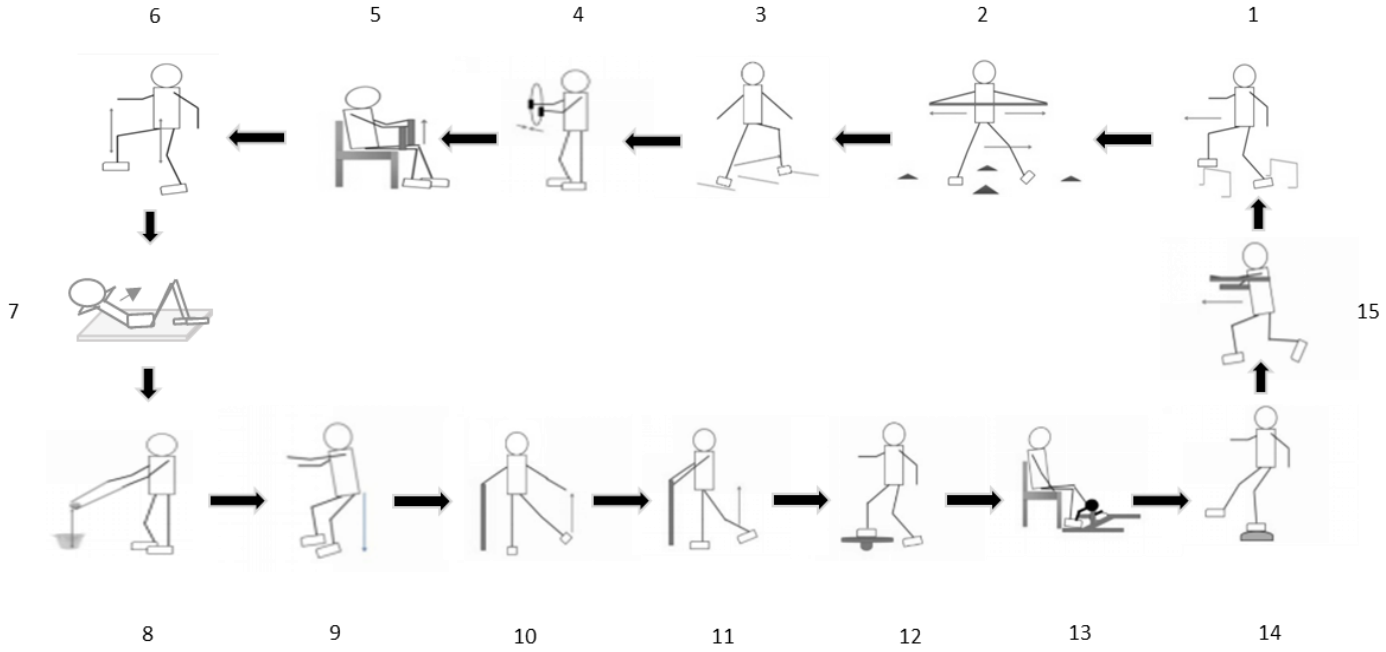
* $p \leq 0.05$

** Difference between groups adjusted for baseline scores analyzed with ANCOVA ($p < .05$).

Intervention (dual-task training)

Control (simple-task training)

Figure 1 – Functional exercise circuit (FEC).



1 (Agility, Coordination, and Aerobic Capacity) - Obstacle crossing.
2 (Coordination) - Lead the foot towards the cones and abduction and adduction of arms.
3 (Coordination) - Obstacle side walking.
4 (Strength) - Upper limb adduction movement with arch.
5 (Strength) - Elbow flexion
6 (Aerobic Capacity) - Stationary walk.
7 (Strength) - Trunk flexion.
8 (Coordination and Precision) - Put a ball in the bucket.
9 (Strength) - Squatt.
10 (Strength) - Hip abduction.
11 (Strength) - Hip extension.
12 (Balance) - Maintain balance with one foot in front and one in back.
13 (Aerobic Capacity) - Portable exercise bike.
14 (Balance) - Maintain unipodal balance in the bubble.
15 (Strength and Coordination) - Hip and knee flexion and upper limb extension.

FINAL CONCLUSION

Sarcopenia is a disease highly prevalent in individuals with COPD, generating a negative impact on important clinical and functional outcomes. Its etiology in individuals with COPD is due to the simultaneous presence of various biological factors such as oxidative stress and systemic inflammation. In addition, the reduction in physical activity levels and exercise limitation are other causes related to the presence of sarcopenia in individuals with COPD. Sarcopenia may also be associated with low bone mineral mass, a syndrome called osteosarcopenia, which has a greater impact than sarcopenia alone on physical function, risk of falls and fractures.

The prevention and treatment of sarcopenia are essential to improve health status and functionality in the elderly population. Exercise is arguably the intervention that has been more studied in the prevention and treatment of sarcopenia. Our exercise protocol, using functional exercise circuit (FEC), is a new modality of dual-task training that can be recommended as a treatment for older adults in public health services due to: 1) its benefits in different clinical variables related to sarcopenia (physical function, muscle mass and antioxidant biomarkers); 2) the low-cost equipment required; and 3) the possibility to treat 15 patients per session. Future research should study the effects of this protocol in older adults with COPD and other chronic conditions or geriatric syndromes, such as frailty or osteosarcopenia and in other clinical contexts (nursing homes, hospitals, etc.).

FUTURE PERSPECTIVES

In this PhD thesis we have answered six different gaps related to prevalence, diagnosis, clinical impact, and etiology of sarcopenia in COPD. In addition, we have studied a new geriatric syndrome called osteosarcopenia and we proposed a new exercise intervention. Despite having produced seven different articles, the gaps in the literature were not fully resolved, even new fields of knowledge are opened in many of these topics, which could be addressed in the future with new research studies (table 2).

Table 1: Research question, results from the present thesis, and topics for future studies.

Research question	Main Results	Future studies
What is the prevalence of sarcopenia in individuals with COPD?	The prevalence of sarcopenia varied from 15.5% to 34% in individuals with COPD.	Futures studies could investigate the prevalence of pre sarcopenia and sarcopenic obesity in individuals with COPD.
What is the clinical impact of sarcopenia in individuals with COPD?	Sarcopenia has a negative impact on pulmonary function, exercise tolerance, quality of life, physical function, physical activity level, dyspnea, prognosis, and risk of mortality.	Future studies could investigate the longitudinal effect of sarcopenia on theses and other important clinical variables (hospitalization, disability, frailty, etc.).

What are the criteria to diagnose sarcopenia in individuals with COPD?	Muscle mass, muscle strength and physical performance from the Asian and European Working Group for Sarcopenia were the main criteria used to diagnose sarcopenia in this population.	Futures studies could analyze specific cut-off points for muscle mass, muscle strength and physical performance in individuals with COPD that can be used to diagnose sarcopenia.
What are the biological factors associated with the presence of sarcopenia in individuals with COPD?	The oxidative stress is one of the main biological factors related to the presence of sarcopenia in individuals with COPD.	Futures studies could analyze the longitudinal association between oxidative stress biomarkers with the presence of sarcopenia in individuals with COPD, and also, to include other biological factors related to sarcopenia, such as genetic or inflammatory biomarkers.
What is the clinical impact of the joint occurrence of Osteoporosis and Sarcopenia (Osteosarcopenia) in older adults?	Osteosarcopenia is strongly associated with worse physical performance, falls and fractures history in community-dwelling older adults (using the FNIH and EWGSOP definition for sarcopenia).	Futures studies could analyze the impact and prevalence of osteosarcopenia in individuals with COPD and other chronic diseases.
What is the effect of a new exercise protocol (based on	We have proposed an exercise intervention program	Futures studies could analyze the effects of this intervention

functional task) on clinical variables related to sarcopenia in older adults?	based on functional tasks called functional exercise circuit (FEC), which has improved muscle mass, physical function and antioxidant capacity in community older adults.	on clinical measurements of sarcopenia in individuals with COPD and other chronic diseases, through randomized clinical trials.

EWGSOP: European Working Group on Sarcopenia in Older People; FNIH: The Foundation for the National Institutes of Health Sarcopenia Project.

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APPENDING

REVIEW

Journal of Cachexia, Sarcopenia and Muscle (2020)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12600

Diagnosis, prevalence, and clinical impact of sarcopenia in COPD: a systematic review and meta-analysis

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Abstract

Sarcopenia prevalence and its clinical impact are reportedly variable in chronic obstructive pulmonary disease (COPD) due partly to definition criteria. This review aimed to identify the criteria used to diagnose sarcopenia and the prevalence and impact of sarcopenia on health outcomes in people with COPD. This review was registered in PROSPERO (CRD42018092576). Five electronic databases were searched to August 2018 to identify studies related to sarcopenia and COPD. Study quality was assessed using validated instruments matched to study designs. Sarcopenia prevalence was determined using authors' definitions. Comparisons were made between people who did and did not have sarcopenia for pulmonary function, exercise capacity, quality of life, muscle strength, gait speed, physical activity levels, inflammation/oxidative stress, and mortality. Twenty-three studies (70% cross-sectional) from Europe (10), Asia (9), and North and South America (4) involving 9637 participants aged ≥40 years were included (69.5% men). Sarcopenia criteria were typically concordant with recommendations of hEuropean and Asian consensus bodies. Overall sarcopenia prevalence varied from 15.5% [95% confidence interval (CI) 11.8–19.1; combined muscle mass, strength, and/or physical performance criteria] to 34% (95%CI 20.6–47.3; muscle mass criteria alone) (P = 0.009 between subgroups) and was greater in people with more severe [37.6% (95%CI 24.8–50.4)] versus less severe [19.1% (95%CI 10.2–28.0)] lung disease (P = 0.020), but similar between men [41.0% (95%CI 26.2–55.9%)] and women [31.9% (95%CI 7.0–56.8%)] (P = 0.538). People with sarcopenia had lower predicted forced expiratory volume in the first second (mean difference −7.1%; 95%CI −9.0 to −5.1%) and poorer exercise tolerance (standardized mean difference −0.8; 95%CI −1.4 to −0.2) and quality of life (standardized mean difference 0.26; 95%CI 0.2–0.4) compared with those who did not (P < 0.001 for all). No clear relationship was observed between sarcopenia and inflammatory or oxidative stress biomarkers. Incident mortality was unreported in the literature. Sarcopenia is prevalent in a significant proportion of people with COPD and negatively impacts upon important clinical outcomes. Opportunities exist to optimize its early detection and management and to evaluate its impact on mortality in this patient group.

Keywords Sarcopenia; COPD; Prevalence; Diagnosis; Aging

Received: 20 December 2019; Revised: 6 June 2020; Accepted: 10 June 2020
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Introduction

Chronic obstructive pulmonary disease (COPD) is a condition characterized by chronic inflammation[1] and extrapulmonary changes that negatively affect physical function (e.g. lower levels of physical activity[2] and reductions in muscle mass and strength[3,4]) and quality of life.[5,6] The presence of such factors is also closely related to the presence of sarcopenia,[7] a syndrome characterized by lower muscle mass, muscle strength, and physical performance.[7] Sarcopenia is a significant contributor to frailty in the elderly population and is associated with increased rates of falls, hospitalization, and mortality.[8,9] It has been estimated to occur in approximately 5–13% of the 'healthy' older population.[4,7]

People with COPD appear to have an increased risk of developing sarcopenia, with prevalence estimates ranging from 15%[2] to 55%.[10] In this patient group, sarcopenia appears to confer a negative impact upon clinical outcomes related to function and health[1,3,11–13] and its prevalence appears to increase with increasing COPD-related impairment. Although sarcopenia has also been shown to contribute towards poorer prognosis in people with COPD,[2,3] the real clinical impact has not yet been analysed. Additionally, the wide-ranging prevalence estimates of sarcopenia in COPD, however, make its true impact somewhat difficult to accurately ascertain.

A significant factor contributing to this large variability appears to be choice of definition criteria.[2,3,14] International recommendations exist for the diagnosis of sarcopenia in older people such as those proposed by the European Working Group of Sarcopenia in Older People (EWGSOP)[7] and the Asian Group of Sarcopenia,[15] yet these have not been featured in published literature in the field of COPD. Considering the prevalence of both sarcopenia and COPD increase with increasing age, the impact of sarcopenia on a broader range of clinically important COPD-related outcomes is also not currently clear. This review therefore aimed to evaluate the literature pertaining specifically to people with COPD to identify the criteria used to diagnose sarcopenia, estimate its prevalence, and evaluate its impact upon health outcomes.

Methodology

Data sources and search strategy

The protocol for this review was registered in PROSPERO (CRD42018092576). Five electronic databases (i.e. PubMed, LILACS, EMBASE, The Cochrane Library, and Scielo) were searched from inception until August 2018 using the following free-text and subject heading terms: 'COPD', 'pulmonary disease, chronic obstructive', 'chronic obstructive lung disease', 'COAD', 'chronic obstructive airway disease', and 'sarcopeni*' (Supporting Information, *Table* S1). Hand searching of reference lists from included articles was also conducted to identify additional potential studies. To be eligible for inclusion, studies must have been conducted on adults with COPD (aged ≥40 years), defined according to authors, irrespective of disease severity (GOLD: Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease[16] and reported upon a diagnosis of sarcopenia, defined according to any criteria provided it was stated in the methodology. Considering the nature of our research question, we included observational (e.g. cohort) and cross-sectional studies and clinical trials (whether randomized or not). Abstracts and publications published in languages other than English, Spanish, or Portuguese were not eligible for inclusion.

The principal outcomes for this review were (i) the criteria used to define sarcopenia and its prevalence and (ii) clinical data from studies that provided comparative data between people with COPD who did and did not have sarcopenia, as follows: (a) quality of life, from either generic or respiratory-specific quality of life questionnaires; (b) physical function, derived from common clinical tests of exercise capacity, muscle strength, and balance; (c) physical activity levels, measured by objective physical activity monitors; (d) pulmonary function, measured by spirometry (e.g. FEV_1% predicted); (e) inflammatory or oxidative stress biomarkers [e.g. interleukin (IL)-6, tumour necrosis factor-alpha, C-reactive protein, catalase, paraxonase-1]; and (f) all-cause mortality.

Data management and quality appraisal

Database search yields were collated within a bibliographical reference manager software (StArt v.3.03[17]), and duplicates were discarded. Citations were screened for eligibility upon title and abstract by two independent reviewers (W.S.L and A.A.M) and classified as either 'include', 'exclude', or 'maybe'. Those deemed 'include' or 'maybe' were reviewed in full text to derive a final yield, with any disagreements resolved via a third, independent assessor (V.S.P). This process was summarized in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations.[18] Data were extracted by two members of the team (W.S.L and A.A.M) using standardized templates appropriate for the study objectives.

Study quality was appraised using validated instruments tailored according to study design, as follows: (i) National Institutes of Health Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies, to assess the quality of cohort and cross-sectional studies; (ii) PEDro scale to assess the quality of randomized clinical trials; and (iii) Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies to assess the quality of non-randomized controlled trials.

DOI: 10.1002/jcsm.12600

Statistical analysis

An overall estimate of sarcopenia prevalence was derived by pooling the proportion of patients with COPD who had detected sarcopenia in individual studies in a meta-analysis. For this purpose, only one prevalence estimate was used from each study. Where individual studies reported different types of sarcopenia (e.g. sarcopenia with normal body mass index, sarcopenic obesity, severe sarcopenia), an aggregated value, if able to be determined, or the most 'conventional' type was used. In order to avoid double counting, estimates from individual studies that evaluated sarcopenia via multiple diagnostic criteria (e.g. comparisons of different cut-off thresholds within a single cohort) were pooled using their primary stated method or that which most closely resembled the current EWGSOP recommendation.[7,19] Where able to be conducted, separate subgroup analyses were conducted to compare prevalence effect estimates between sarcopenia definitions (1 vs >1 diagnostic criteria), gender (male versus female), and disease severity (GOLD I–II versus III–IV), evaluated via χ^2 test. This meta-analysis was performed via the 'metaprop' command in Stata SE 14.2 (Texas, USA) with 95% confidence intervals (CIs) calculated using the score (Wilson) method and a random-effects model (DerSimonian and Laird method) utilized due to the variability in sarcopenia definitions across studies.

Clinical outcome data from studies comparing people with COPD who did and did not have sarcopenia were meta-analysed via Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen 2014). Continuous outcome data evaluated using homogenous metrics (e.g. same test instrument) were summarized as mean differences, while data arising from heterogenous metrics (e.g. same construct, different instrument) were summarized as standardized mean differences (SMDs) and 95%CI. A random-effects model was used as the principal method of analysis, with statistical heterogeneity described via the I^2 statistic and interpreted according to Deeks and colleagues (values <25% considered low, 50–75% moderate, and >75% high).[20]

Results

A detailed summary of the literature search is provided in *Figure* 1. Two hundred and seventy-two unique records were identified through database searching, resulting in 23

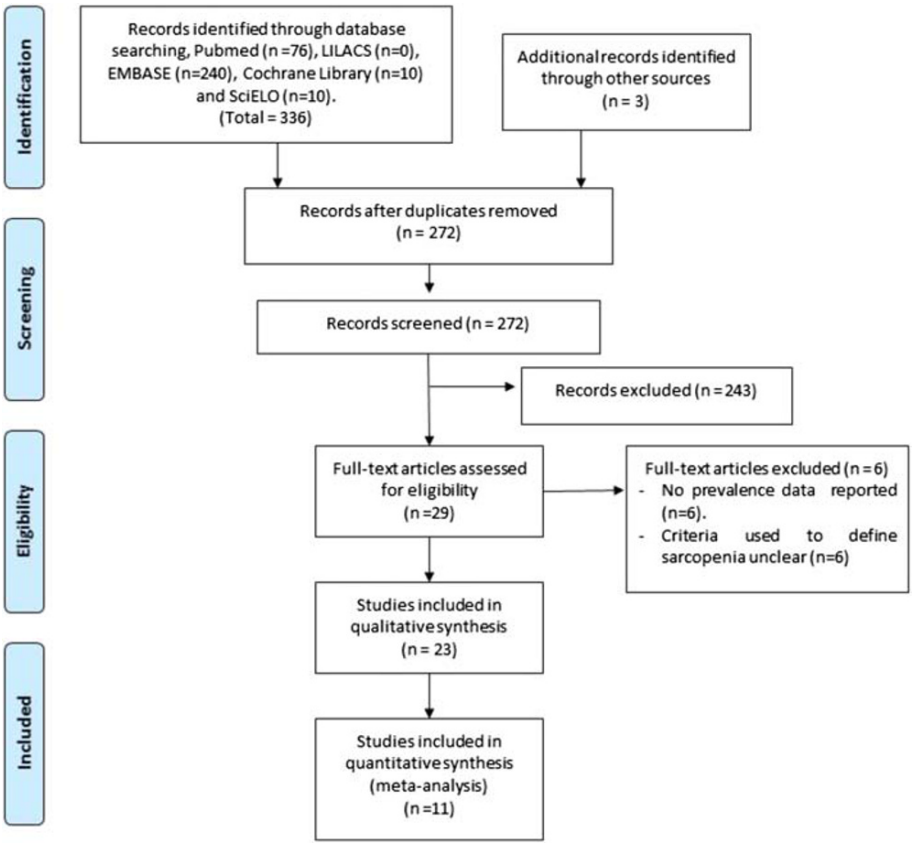

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of article selection.

DOI: 10.1002/jcsm.12600

articles involving 9637 participants included in the final review. Of these, seventeen adopted a cross-sectional design, five were observational cohort studies, and one was a non-randomized clinical trial. Most studies included patients with differing histories of smoking (those who never smoked and former and current smokers). Comparative data between people with COPD who did and did not have sarcopenia were available from 17 studies. The overall quality of included studies was 'moderate' (full details in *Table* S2). Characteristics of included studies are presented in *Table* 1. The review sample spanned a diverse range of populations, including ten studies from Europe, nine from Asia, and four from South America. Most participants were men (69.5%).

Methods used to assess sarcopenia

A summary of diagnostic criteria used to assess sarcopenia in the included studies is presented in *Table* 1. Measures of low muscle mass (LMM),[1,2,13,21–40] low muscle strength (LMS),[1,2,29,32–34,39] and low physical performance (LPP)[2,29,32–34,37–39] were used as the basis of diagnosis. Fourteen studies used LMM as the sole criteria to diagnose sarcopenia, while LMM was combined with LMS and/or LPP in nine studies.[1,2,29,32–34,37–39] Those studies utilized different cut-off points and methods to identify LMM, LMS, and LPP. Muscle mass was measured by dual-energy X-ray absorptiometry (sixteen studies),[13,21,22,24–26,28,33–38,40] bioelectrical impedance analysis (six studies),[1,2,23,27,31,32,39] and calf circumference (one study).[29] Muscle strength was measured via handgrip dynamometry (seven studies).[1,2,29,32–34,39] Physical performance was measured via gait speed (four studies)[23,29,32,39] and 6 min walk test (6MWT) (four studies).[33,34,37,38] The different cut-off thresholds used to define 'positive' responses to each test are presented in *Table* 2. Muscle mass, muscle strength, and physical performance were most commonly evaluated according to cut-off thresholds recommended by the EWGSOP[7] and the Asian Group of Sarcopenia.[15] Comparisons between the main guidelines used to detect sarcopenia in individuals with COPD are available in *Table* S3.

Sarcopenia prevalence

Data were available for meta-analysis from 22 studies involving 9416 participants. The overall pooled prevalence estimate of sarcopenia in people with COPD was 27.5% (95%CI 18.4–36.5; *Figure* 2). These effect estimates were significantly higher in studies that used a single criterion [LMM; 34%, (95%CI 20.6–47.3)] than those that used >1 criteria [LMM + LMS and/or LPP; 15.5% (95%CI 11.8–19.1)]. The high statistical heterogeneity in this analysis (I^2 = 99.3%) meant that individual study weighting was uniform (range 4.1–4.7%). In the studies that provided data specific to gender, sarcopenia was found to be higher in men [41.0% (95%CI 26.2–55.9)] than in women [31.9% (95%CI 7.0–56.8)]; however, this difference was not statistically significant (P = 0.538) and gender did not predict effect size in meta-regression (*Figures* S1–S2). In the studies that provided data specific to disease severity, sarcopenia was found to be significantly higher in patients with more severe disease [GOLD stages III–IV; 37.6% (95%CI 24.8–50.4)] than those with less severe disease [GOLD stages I–II; 19.1% (95%CI 10.2–28.0)], with test for between-group differences (P = 0.020) with the proportion of participants having more severe disease being strongly predictive of effect sizes in meta-regression with high explanatory power [regression coefficient 0.715 (95%CI 0.342–1.088), P = 0.006; adjusted R^2 = 90.1%] (*Figures* S3–S4).

Impact of sarcopenia on clinical outcomes

Data from 11 studies involving 5367 participants were available for meta-analysis of pulmonary function, showing that those with sarcopenia had, on average, poorer FEV_1% predicted than those without sarcopenia [mean difference −7.07% (95%CI −9.03 to −5.11); I^2 = 83%, *Figure* 3A].

Data from six studies involving 2252 participants were available for outcomes related to exercise capacity. These were measured via the 6MWT,[1,27,28] incremental shuttle walk test,[2] and cardiopulmonary incremental cycle test.[25,36] Having sarcopenia was associated with poorer performance compared with those without sarcopenia [SMD −0.77 (95%CI −1.35 to −0.18); I^2 = 96%, *Figure* 3B].

Four studies involving 1996 participants reported data on quality of life via the COPD Assessment Test,[1,2] and St George's Respiratory Disease Questionnaire[2,27,28] was included in the meta-analysis. Having sarcopenia was associated with poorer quality of life [SMD 0.42 (95%CI 0.07–0.77); I^2 = 85%, *Figure* 3C]. Other studies not included in the meta-analysis reported similar findings[33,35] (*Table* 3).

A summary of findings related to the remaining review outcomes is presented in *Table* 3; however, quantitative meta-analysis was not possible due to lack of sufficient data. Compared with non-sarcopenic individuals, those with sarcopenia had worse physical function (as measured by tests of balance, gait speed, strength, and general daily function),[2,21,39] lower levels of daily physical activity,[2,22,30,35] increased levels of dyspnoea during daily activities,[1,2] and a heightened mortality risk, as measured via body mass index, obstruction, dyspnoea, and exercise tolerance (BODE) index.[1,2,38] Sarcopenia was more prevalent in the fourth quartile of BODE, ranging from 25% to 63.6%.[1,2,38] With respect to inflammatory biomarkers, C-reactive protein, IL-6, and tumour necrosis factor-alpha were reported to be higher[1,25,39] or not different[27,28] in subjects with sarcopenia compared with those without it. No differences were

Table 1 Characteristics of the included studies regarding the prevalence of sarcopenia in subjects with chronic obstructive pulmonary disease

First author and year	Country	Study design	Sample size	Age (mean ± SD)	Male, *n* (%)	Smoking status (never/former/current), *n*	GOLD (%)	Prevalence of sarcopenia		Criteria (assessment method to detect sarcopenia)
								Total, *n* (%)	Male, *n* (%)	
Sergi *et al*. 2006[21]	Italy	Cross-sectional	40	75.7 ± 5.3	40 (100%)	—	—	15 (38%)	15 (100%)	LMM (DXA)
Koo *et al*. 2014[22]	Korea	Cross-sectional	574	64.0 ± 0.6	574 (100%)	103/231/240	I/II/III–IV (46/49/5)	155 (27%)	155 (100%)	LMM (DXA)
Gologanu *et al*. 2014[23]	Romania	Cross-sectional	36	65.6 ± 7.5	12 (33%)	—	I/II/III/IV (0/39/42/19)	3 (8%)	—	LMM (BIA)
Jones *et al*. 2015[2]	UK	Clinical non-randomized	622	—	354 (57%)	7/170/43	—	90 (14%)	57 (63%)	LMM (BIA) LMS (HGS) LPP (4MGS)
Costa *et al*. 2015[24]	Brazil	Cross-sectional	91	67.4 ± 8.7	41 (45%)	91 former smokers	I/II/III/IV (17/24/37/22)	36 (40%)	20 (56%)	LMM (DXA)
Van de Bool *et al*. 2015 [25]	Netherlands	Retrospective	505	64 (median)	288 (57%)	13/360/132	I/II/III/IV (8/41/40/11)	437 (87%)	239 (55%)	LMM (DXA)
Chung *et al*. 2015[26]	Korea	Retrospective	1039	64.5 ± 9.4 (male) 64.5 ± 10.2 (female)	760 (73%)	129/136/771	I/II/III/IV (46/48/5/1)	283 (27%)	249 (88%)	LMM (DXA)
Joppa *et al*. 2016[27]	ECLIPSE (12 countries and USA)	Cross-sectional	2000	63.5 ± 7.1	1314(66%)	—	—	682 (34%)	509 (75%)	LMM (BIA)
Van de Bool *et al*. 2016 (van de Bool *et al*. 2016)	Netherlands	Cross-sectional	45	42–77	29 (64%)	—	I/II/III/IV (6/36/49/9)	14 (31%)	13 (92%)	LMM (DXA)
Lipovec *et al*. 2016[28]	Slovenia	Prospective observational	112	66 ± 8	74 (66%)	92 current smokers	I/II/III/IV (0/17/52/31)	61 (54%)	44 (72%)	LMM (DXA)
Borda *et al*. 2016[29]	Colombia	Cross-sectional	334	71.1 ± 8.05	110 (33%)	—	—	28 (8%)	—	LMM (CC) LMS (HGS) LPP (3.4MGS)
Lee *et al*. 2016[30]	Korea	Cross-sectional	858	—	—	—	—	286 (33%)	226 (79%)	LMM (DXA)
Pothirat *et al*. 2016[31]	Thailand	Cross-sectional	121	—	—	121 former smokers	I/II/III/IV (26/25/10/39)	12 (10%)	—	LMM (BIA)
Maddock *et al*. 2016[32]	UK	Prospective cohort	816	69.8 ± 9.7	484 (59%)	49/620/146	—	101 (12%)	—	LMM (BIA) LMS (HGS) LPP (4MGS)
Hwang *et al*. 2017[13]	Korea	Cross-sectional	777	63.9 ± 10.6	777 (100%)	0/185/592	I/II/III–IV (43/50/7)	41 (5.3%)	41 (100%)	LMM (DXA)
Limpawattana *et al*. 2017[33]	Thailand	Cross-sectional	121	—	112 (92.6%)	7/104/10	—	29 (24%)	29 (100%)	LMM (DXA) LMS (HGS) LPP (6MWT)
Byun *et al*. 2017[1]	Korea	Cross-sectional	80	68.4 ± 8.9	67 (83.8%)	—	I/II/III/IV (30/39/6/25)	20 (25%)	17 (83%)	LMM (BIA) LMS (HGS)
Limpawattana *et al*. 2017[34]	Thailand	Cross-sectional	121	70 ± 9	112 (92.6%)	7/104/10	I/II/III/IV (26/57/17/0)	29 (24%)	29 (100%)	LMM (DXA) LMS (HGS) LPP (6MWT)
Lee *et al*. 2017[35]	Korea	Cross-sectional	748	—	—	—	—	251 (34%)	203 (81%)	LMM (DXA)

(*Continues*)

Table 1 (continued)

First author and year	Country	Study design	Sample size	Age (mean ± SD)	Male, *n* (%)	Smoking status (never/former/current), *n*	GOLD (%)	Prevalence of sarcopenia		Criteria (assessment method to detect sarcopenia)
								Total, *n* (%)	Male, *n* (%)	
Kneppers *et al.* 2017[36]	Slovenia	Prospective cohort	92	—	—	—	I/II/III/IV (3/24/50/23)	39 (42%)	29 (74%)	LMM (DXA)
Costa *et al.* 2017[37]	Brazil	Cross-sectional	121	67.9 ± 8.6	56 (46%)	23 current smokers	—	13 (11%) 6 (5%) 11 (9%) 15 (12%)		LMM (DXA) LPP (6MWT)
Costa *et al.* 2018[38]	Brazil	Cross-sectional	121	67.9 ± 8.6	56 (46%)	—	A/B/C/D (29/29/34/29)	15 (12%)	—	LMM (DXA) LPP (6MWT) LMS (HGS) LPP (4MGS)

3.4 MGS, 3.4 m gait speed; 4MGS, 4 m gait speed; 6MWT, 6 min walking test; BIA, bioelectrical impedance analysis; CC, calf circumference; DXA, dual-energy X-ray absorptiometry; HGS, handgrip strength; LMM, lower muscle mass; LMS, lower muscle strength; LPP, lower physical performance; SD, standard deviation.

detected in levels of fibrinogen[27] and IL-8.[27] No findings related to oxidative stress were reported in the included literature.

Discussion

This systematic review and meta-analysis offers unique insight into the clinical relevance of sarcopenia for people with COPD. It describes the prevalence of the condition and how this is impacted by use of different criteria, cut-off thresholds and definitions, as well as rigorous examination of the effect of sarcopenia on important health outcomes related to pulmonary and physical function, quality of life, blood biomarkers, prognosis, and risk of mortality.

Two predominant strategies appear to be in use to classify sarcopenia in COPD: definitions based upon independent assessment of LMM[21,22,24,27,28,30,35] and definitions that include both LMM and either LMS or LPP.[1,2,23,29,32–34,37–39] Use of LMM alone resulted in an estimated pooled prevalence of 34%, while LMM combined with LMS and/or physical function reduced this figure to 15.5%. Such variability has been previously reported in community-dwelling older adults.[48] Sarcopenia definition variability thus also likely explains some of the varied prevalence estimates in people with COPD. This relationship may not come as a surprise, as increasing the number of mandatory elements within a sarcopenia definition will inevitably reduce the incidence of detecting a 'positive' diagnosis. The trade-off of doing so, however, is a likely improvement in diagnostic accuracy. This is a significant premise underpinning current international recommendations,[7,19,43,49] which sees sarcopenia defined as a geriatric syndrome[7,15,43,49] or disease[19] characterized by both LMM and LPP, not just LMM.[50,51] Only nine of the included studies[1,2,29,32–34,37,39] implemented a definition of sarcopenia that would satisfy these new recommendations (*Table* 1). Our data suggest that some of the variability in prevalence estimates is likely attributable to disease severity, with every 1% increase in study sample having GOLD stages III–IV increasing sarcopenia prevalence by 0.7%. While this relationship was not unexpected based on previous research,[2,32] the high explanatory power (90.1%) in our meta-regression was striking. Detailed reporting and/or stratification by disease severity in this patient group appears advisable to ensure that accurate conclusions are drawn from future studies seeking to advance our knowledge of the interplay between these two factors.

Recommendations advocate for dual-energy X-ray absorptiometry and bioelectrical impedance analysis as the preferred methods to evaluate LMM for the purpose of detecting sarcopenia, including evaluation of muscles of both the lower limb and the chest wall.[7,15,19,41–43,49,52,53] These were commonly used within the studies included in this

Table 2 Criteria and cut-off points used to detect sarcopenia in individuals with chronic obstructive pulmonary disease in the different studies

Lower muscle mass		References
DXA	1. EWGSOP[7] Newman *et al*. 2003[41] ASMI: <7.23 kg/m^2 for men and <5.67 kg/m^2 for women.	Van de Bool *et al*. 2015,[25] Lipovec *et al*. 2016,[28] Kneppers *et al*. 2017,[36] and van de Bool *et al*.[40]
	2. EWGSOP[7] Newman *et al*. 2003[41] Residuals of linear regression on appendicular lean mass adjusted for fat as well as height. Men: −2.29, women: −1.73.	Costa *et al*. 2015[24] and Costa *et al*. 2017[37]
	3. EWGSOP[7] Baumgartner *et al*. 1998[42] SMI: ≤7.26 kg/m^2 for men and ≤5.45 kg/m^2 for women.	Costa *et al*. 2015[24] and Costa *et al*. 2017[37]
	4. AWGS[15] ASMI: ≤7.0 kg/m^2 for men and ≤5.4 kg/m^2 for women.	Lee and Choi,[30] Limpawattana *et al*.,[33,34] and Lee *et al*.[35]
	5. FNIH[43] ALM/BMI: <0.789 for men and for < 0.512 women.	Costa *et al*. 2017[37] and Costa *et al*. 2018[38]
	6. ASMMI: ≤ 2 standard deviations in a gender-specific mean for a young reference group.	Byun *et al*.,[1] Hwang *et al*.,[13] Sergi *et al*.,[21] Chung *et al*.,[26] and van de Bool *et al*.[40]
	7. SMI:<1 standard deviations in a gender-specific mean for a young reference group.	Koo *et al*.[22]
	8. Combination of criteria 2 and 3.	Costa *et al*. 2015[24] and Costa *et al*. 2017[37]
BIA	1. EWGSOP[7] Janssen *et al*. 2002[44] SMI: ≤8.50 kg/m^2 for men and ≤5.75 kg/m^2 for women.	Jones *et al*.,[2] Maddocks *et al*.,[32] and de Blasio *et al*.[39]
	2. ATS[45] BMI >21 and FFMI ≤16 kg/m^2 for men or ≤15 kg/m^2 for women.	Gologanu *et al*.[23] and Pothirat *et al*.[31]
	3. Franssen *et al*. 2014[46] Lower than the 10 percentile of the reference value for age, sex, and BMI specific.	Joppa *et al*.[27]
	4. ASMMI: ≤2 standard deviations in a gender-specific mean for a young reference group.	Byun *et al*.[1]
CC	1. Calf circumference <31 cm.	Borda *et al*.[29]
Lower muscle strength		
HGS	1. EWGSOP[7] Laurentani *et al*. 2003[47] HGS: <30 kg for men and <20 kg for women.	Byun *et al*.,[1] Jones *et al*.,[2] Maddocks *et al*.,[32] and de Blasio *et al*.[39]
	2. AWGS[15] HGS: <26 kg for men and <18 kg for women.	Limpawattana *et al*.[33,34]
	3. Lower the last quintile in specific population.	Borda *et al*.[29]
Lower physical performance		
4MGS	1. EWGSOP[7] Laurentani *et al*. 2003[47] GS: <0.8 m/s (both genders).	Jones *et al*.,[2] Maddocks *et al*.,[32] and de Blasio *et al*.[39]
3.4MGS	1. Lower the last quintile in specific population.	Borda *et al*.[29]
6MWT	1. AWGS[15] Laurentani *et al*. 2003[47] GS: <0.8 m/s (both genders).	Limpawattana *et al*.[33,34]
	2. EWGSOP[7] Laurentani *et al*. 2003[47] GS: <0.8 m/s (both genders).	Costa *et al*. 2017[37]
	3. FNIH[43] GS: <0.8 m/s (both genders).	Costa *et al*. 2018[38]

3.4 MGS, 3.4 m gait speed; 4MGS, 4 m gait speed; 6MWT, 6 min walking test; ASMI, appendicular skeletal muscle index; ATS, American Thoracic Society; AWGS, Asian Working Group for Sarcopenia; BIA, bioelectrical impedance analysis; BMI, body mass index; CC, calf circumference; DXA, dual-energy X-ray absorptiometry; EWGSOP, European Working Group on Sarcopenia in Older People; FNIH, The Foundation for the National Institutes of Health Sarcopenia Project; HGS, handgrip strength; SMI, skeletal muscle mass index.

review (*Tables* 1 and 2). Despite this, we observed 12 different cut-off points used to classify test results as normal or abnormal. The most commonly used criteria were those of Newman *et al*.[54] and Baumgartner *et al*.,[55] which are also considered by the EWGSOP.[7] Borda *et al*.[29] measured muscle mass with calf circumference, which confers simplicity as a screening method for sarcopenia,[56,57] but it is not recommended.[7,15,19,43,49] Similar advice is also available for the assessment of muscle strength (handgrip force) and physical performance (gait speed),[7,15] yet inconsistencies were again apparent. For example, gait speed was assessed using the 4 m gait speed[23,29,32,39] and the 6MWT.[33,34,37,38] While the same cut-off was used to diagnose sarcopenia across both tests (<0.8 m/s), the two tests are vastly different. The 4 m gait speed is typically performed at usual walking speed across a 4 m distance (although variations also exist at different walk speeds and track lengths), while the 6MWT is typically performed on a 30 m walking track with participants encouraged to walk as far as they can (often faster than normal speed) in order to assess exercise tolerance.[58] Deriving a measure of walking speed from the 6MWT [i.e. total distance (m) divided by 360 (s)] poses a significant risk of inaccurate interpretation. For example, it could not distinguish between people walking slowly and fast but stopping to rest during the test. The prevalence of sarcopenia in the studies that used this approach[33,34,37,38] may therefore have been underestimated. It is thus crucial that future research not only implement consistent tests to diagnose sarcopenia, but also adopt standardized cut-off thresholds to facilitate accurate test interpretation.

Sarcopenia had a consistently negative impact on a range of COPD-related clinical outcomes, including exercise capacity, balance, quadriceps, and handgrip strength, gait speed, and physical activity levels.[2,21,30,35,39] It was also associated with increased symptom burden and poorer quality of life.[1,2,30,35] It is interesting that the two studies that measured dyspnoea (Medical Research Council scale)[1,2] classified sarcopenia according to physical function alone, as it raises the possibility that functional impairment may associate more strongly with dyspnoea than LMM.[21] This also raises

DOI: 10.1002/jcsm.12600

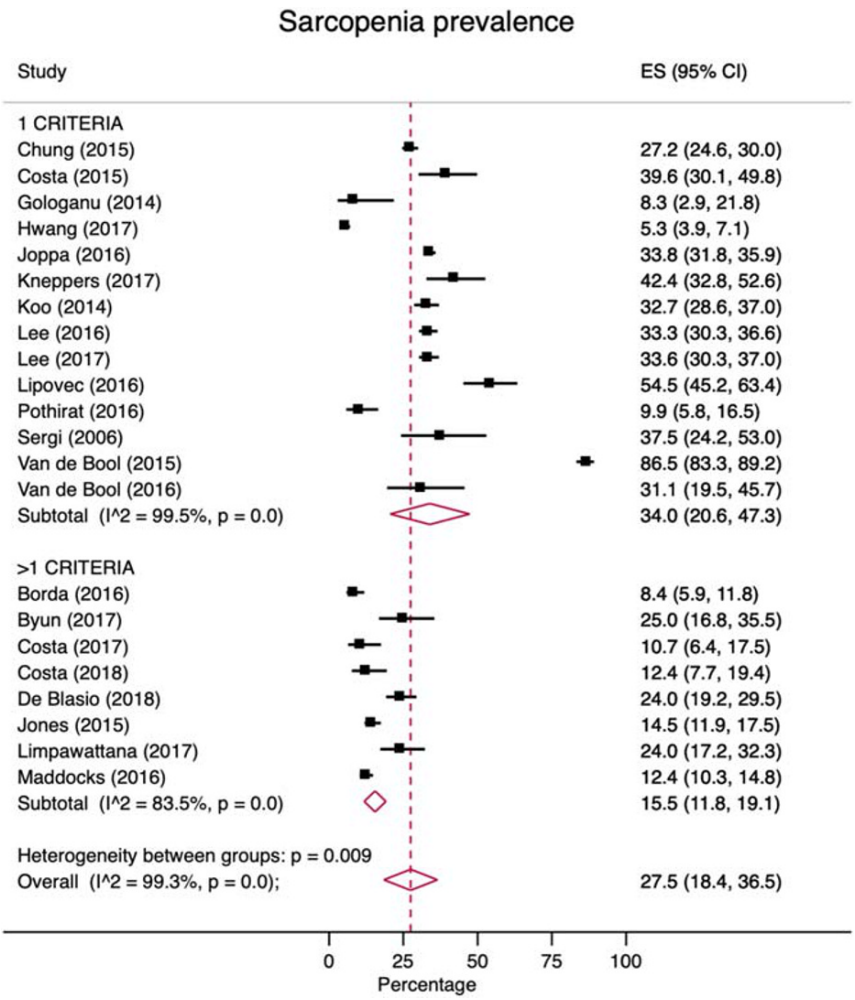

Figure 2 Prevalence of sarcopenia in chronic obstructive pulmonary disease according to different criteria. CI, confidence interval; ES, effect size (prevalence %); I^2, I^2 heterogeneity statistic. Random effects model used for analysis.

some challenging issues related to clinical management strategies. As associations do not imply causation or directionality, should interventions targeting improvement in health outcomes for people with COPD who have sarcopenia be directed towards mitigating the defining features of sarcopenia (e.g. muscle mass and physical performance) or their associated manifestations (e.g. low physical activity levels, poor balance, impaired lung function)? To our knowledge, the precise impact of sarcopenia (and its severity) upon intervention effectiveness targeting these other areas has received scant attention to date in COPD. Sarcopenia has, however, been highlighted as an important 'treatable trait' in adult respiratory medicine.[59] One of the few studies to explore this area was conducted by Jones *et al.*[2] who demonstrated that pulmonary rehabilitation, a comprehensive, multicomponent exercise-based intervention, improved a range of clinical outcomes and reduced the incidence of sarcopenia in a cohort of patients with COPD. More research is clearly warranted to further validate the findings of Jones and colleagues, including the use of other recommended adjunctive therapies such as nutritional supplementation.[7,60,61]

We were not able to investigate actual mortality in those who had sarcopenia due to a lack of available evidence. However, it is plausible that sarcopenia might associate with increased mortality in this population, considering that it associated with poorer prognosis and a higher prevalence in patients with more severe lung disease (37.6% in GOLD stages III–IV compared with 19.1% in those with GOLD stages I–II). Leivseth *et al.*[62] reported that people with GOLD stages III and IV disease severity had a more than sixfold increased risk of mortality in women and a more than double increased risk in men over 15 years of follow-up. Heightened mortality risk was also observed in individuals with COPD evaluated via BODE,[1,2,24,38] which is a widely used, valid tool for predicting risk of death in COPD.[63,64] Costa *et al.*[24] reported an increased prevalence of sarcopenia (odds ratio 3.89; 95%CI 1.21–12.46) in those with GOLD stages III and IV, and these quartiles are related with lower 4 year survival (18–57%).[63]

DOI: 10.1002/jcsm.12600

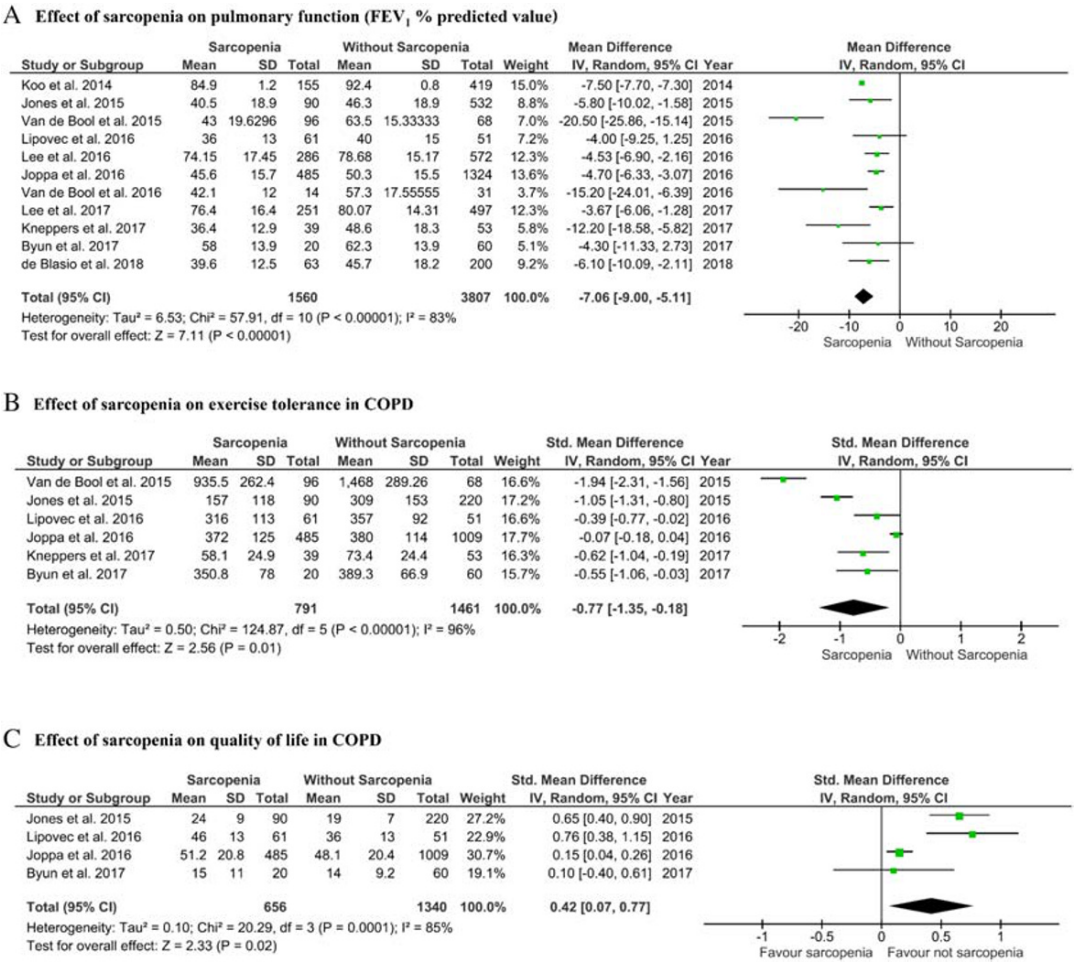

Figure 3 Clinical impact of sarcopenia in individuals with COPD. COPD, chronic obstructive pulmonary disease; I^2, I^2 heterogeneity statistic. Random effects model used for analysis.

Sarcopenia also related to poorer quality of life and pulmonary and physical function, which are known factors associated with heightened mortality risk in COPD.[44,45] Sarcopenia has been associated with premature mortality in community-dwelling older adults in a cohort study with 4425 older adults during a median 14.4 year follow-up (hazard ratio 1.32; 95%CI 1.13–1.47).[46] However, the lack of COPD-specific data suggests that this remains an area in need of addressing in future research.

This systematic review has highlighted the clinical relevance of including measurements of muscle mass, muscle strength, and physical performance in individuals with COPD, as these variables clearly associate with sarcopenia, exacerbations, and poor prognosis.[47,59] The more widespread implementation of these measures in clinical practice could help identify patients with COPD at increased risk of future healthcare use related to exacerbations.[47,65] This is also an important priority from a public health economic perspective.[66] In Europe, on average, the healthcare system spends €6725 per year per person (95%CI €6590–€6863) for each exacerbation of this disease.[67] In older people, sarcopenia is consistently associated with increased risk of incident disability, falls, hospitalization, and mortality.[46,68,69] Sarcopenia has been associated with increased breathlessness, exacerbation frequency, and frailty in individuals with COPD.[47,70,71] Hospitalizations also hasten deconditioning and muscle weakness, thereby worsening the sarcopenic state.[47,72] Earlier identification of sarcopenia may therefore help direct preventive healthcare to positively impact upon its healthcare burden.

We were unable to demonstrate a clear relationship between sarcopenia and inflammatory biomarkers across the included studies. Some authors[27,28,39] reported no differences between sarcopenic and non-sarcopenic patients with COPD, while Byun *et al.*[1] and Van de Bool *et al.*[25] observed higher levels of C-reactive protein, IL-6, and tumour necrosis factor-alpha. No studies evaluated the effect of sarcopenia on oxidative stress, despite convincing evidence of pathophysiological changes occurring in the COPD literature[73–75] and known associations between sarcopenia, oxidative stress,[76,77]

DOI: 10.1002/jcsm.12600

Table 3 Clinical impact of the sarcopenia in different variables in subjects with chronic obstructive pulmonary disease

Categories	Variables	Compared with individuals with COPD without sarcopenia	
		Sarcopenia (1 criterion)	Sarcopenia (>1 criterion)
Health-related quality of life	EQ-5D index (score)	Worse[30,35]	
Physical function	SPPB (score)		Worse[2]
	5STS (s)		Worse[2]
	HGS (kg)		Worse[2,39]
	QS (kg)	Worse [21]	Worse[2]
	GS (m/s)		Reduction[2,39]
Physical activity level	Time in moderate and high activity (min/day)	Worse[22]	Worse[2]
	Total energy expenditure (kcal/week)		Worse[2]
	Daily Steps (steps/day)		N.d.[2]
	Prevalence of physical inactivity	Worse[30,35]	
Dyspnoea	MRC (score)	N.d.[21]	Worse[1,2]
Risk of mortality	Prevalence in BODE quartile 3 or 4	Higher[24]	Higher[1,2,38]
Inflammation	CRP (mg/L)	Augmented[25] /N.d[27,28]	Augmented[39]
	Fibrinogen (mg/L)	N.d.[27]	
	IL-6 (pg/mL)	N.d.[27]	Augmented[1]
	IL-8 (pg/mL)	N.d.[27]	
	TNF-α (pg/mL)	N.d.[27]	Augmented[1]

5STS, five-repetition sit-to-stand test; 6MWT, 6 min walking test; BODE, body mass index, obstruction, dyspnoea, and exercise tolerance index; CAT, COPD Assessment Test; CRP, C-reactive protein; EQ-5D index, EuroQol five-dimensional; GS, gait speed; HGS, handgrip strength; IL, interleukin; ISWT, incremental shuttle walk test; MRC, Medical Research Council; N.d., no significant difference; QS, quadriceps strength; SGRQ, St George's respiratory disease questionnaire; SPPB, short physical performance battery; TNF-α, tumour necrosis factor-alpha.

inflammation,[1] and age-related alterations in muscle morphology.[76,78–80] This would appear a valuable area for future research.

As with all studies, the findings from the present review are not without some limitations. Due to the significant heterogeneity between studies in terms of factors such as sarcopenia definitions, participant characteristics, and diagnostic cut-offs, the opportunity for meta-analysis was limited for some outcomes and clear interpretation of the clinical implications of some results was challenging. This review was unable to elucidate the direct relationship between sarcopenia and mortality due to a lack of data. This was not surprising due to the prolonged periods of follow-up required to observe such outcomes in cohorts of patients who would otherwise not typically have been at risk of imminent death. However, our observed association between sarcopenia and mortality risk (assessed via BODE) is noteworthy. While not a pre-specified focus of our review, we also feel that the lack of direct evidence highlighting the clinical impact of sarcopenia on healthcare expenditure represents an area to address in future studies. Additionally, despite this review including studies from four different continents (Asia, Europe, North America, and South America), data regarding participant race were not available, which limits its potential applicability to specific patient subgroups. In addition, it was not considered the impact of differing sarcopenia subtypes (e.g. sarcopenic obesity, severe sarcopenia), despite their clinical relevance due to a lack of suitable data. This might have plausibly explained some of the observed variability in clinical outcome data. We also synthesized prevalence data via meta-analysis in contrast to our registered protocol. This was altered in light of access to appropriate statistical software to conduct this analysis while still allowing readers to identify the raw proportions of individual studies (as stated in the protocol) in *Figure* 2. The overall pooled effect from the present meta-analysis (27.5%) compared favourably against the protocol-based method utilizing median estimates from individual studies (26.1%).

In conclusion, sarcopenia is a clinically important condition that is prevalent within a substantial proportion of patients with COPD. Diagnostic accuracy appears sensitive to the criteria, test methods, and cut-offs used to detect the individual components, as well as markers of disease severity. Considering the negative impact of sarcopenia upon health outcomes, there may be merit in future strategies targeting early identification of sarcopenia in the clinical assessment of people with COPD to ultimately improve management strategies aiming to mitigate its impact upon individuals' lives.

Author contributions

W.S.L. and A.A.M. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, C.O. and S.P. contributed substantially to statistical analysis and interpretation of the results, and G.D. and V.S.P. contributed with the study design and writing of the manuscript. The authors of this manuscript certify that they comply with the ethical guidelines for publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[81]

DOI: 10.1002/jcsm.12600

Conflict of interest

The authors have disclosed no conflicts of interest. We declare no financial support or relationships that may pose conflict of interest. This work has not been published anywhere.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. WASL is supported by CAPES - Brasil. VSP is supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Brasil.

Online supplementary material

The supplementary figures and tables can be found in the Supporting Information section of the online article.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Search strategy in each database (Supplementary data)
Table S2: Quality analysis (Supplementary data)
Table S3: Different cut-off points used to identify Sarcopenia.
Figure S1: Prevalence of sarcopenia by gender.
Figure S2. Meta-regression of effect of gender (percent male) on sarcopenia prevalence.
Figure S3. Prevalence of sarcopenia, by COPD disease severity.
Figure S4. Meta-regression of effect of disease severity (GOLD stages III-IV) on sarcopenia prevalence.

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Mecanismos fisiopatológicos de la sarcopenia en la EPOC

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Pathophysiological mechanism of sarcopenia in COPD

Sarcopenia is a disease characterized by loss of skeletal muscle, muscle strength and physical performance, being the major cause of frailty in the elderly. The sarcopenia is highly prevalent in individuals with Chronic obstructive pulmonary disease (COPD) leading to a poor prognosis and higher mortality in this population. The presence of sarcopenia in COPD is likely the result by the interaction between external and internal factors as systemic inflammation, oxidative stress and genetic polymorphisms, frequently observed in individuals with this respiratory disease. This review summarizes the current knowledge about the pathogenic mechanisms linking COPD with sarcopenia.

***Key words**: Sarcopenia; pulmonary disease chronic obstructive; oxidative stress; inflammation; polymorphisms genetic.*

Resumen

La sarcopenia es una enfermedad caracterizada por la pérdida de masa muscular, fuerza muscular y rendimiento físico, siendo la principal causa de fragilidad en los adultos mayores. La sarcopenia es altamente prevalente en individuos con enfermedad pulmonar obstructiva crónica (EPOC) que conduce a un mal pronóstico y una mayor mortalidad en esta población. La presencia de sarcopenia en la EPOC es probablemente el resultado de la interacción entre factores externos e internos como la inflamación sistémica, el estrés oxidativo y los polimorfismos genéticos, frecuentemente observados en individuos con esta enfermedad respiratoria. Esta revisión resume el conocimiento sobre los mecanismos patogénicos asociados con la sarcopenia en la EPOC.

***Palabras clave**: Sarcopenia; enfermedad pulmonar obstructiva crónica; estrés oxidativo; inflamación; polimorfismos genéticos.*

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Introducción

La enfermedad pulmonar obstructiva crónica (EPOC) es una de las principales causas de muerte en el mundo[1,2], es una enfermedad prevenible y tratable, que se caracteriza por síntomas respiratorios persistentes y una limitación del flujo aéreo debido a anomalías de las vías respiratorias y/o alveolares causadas por una exposición significativa a partículas nocivas o gases[3]. Esta enfermedad es caracterizada por cambios extrapulmonares, como una disminución de la capacidad aeróbica, resistencia, fuerza y equilibrio, que afectan el rendimiento en las actividades de la vida diaria y la calidad de vida[4-8]. Además, los sujetos con EPOC tienen un nivel más bajo de actividad física, lo que puede disminuir la masa muscular y producir debilidad muscular[9,10]. La debilidad muscular aumenta en presencia de enfermedades crónicas y está directamente relacionada con la sarcopenia[11].

La sarcopenia ha sido definida por el grupo europeo de trabajo sobre sarcopenia en adultos mayores (EWSGOP) como un síndrome[12] o una enfermedad[13] caracterizada por la pérdida de masa muscular, fuerza muscular y rendimiento físico, siendo la principal causa de fragilidad entre los ancianos[12]. Este síndrome tiene una prevalencia de aproximadamente 5% a 13% de todos los individuos mayores de 65 años[14]. Sin embargo, en sujetos con EPOC la prevalencia de sarcopenia es aún mayor[15,16].

La sarcopenia ha sido asociada a cambios metabólicos, inmovilidad, disfunción mitocondrial[11,17], estrés oxidativo[18,19] e inflamación sistémica[11], que contribuyen a déficits musculares relacionados con la edad[20], a través del aumento del daño celular[18]. En la EPOC, hay pruebas sólidas que demuestran que los cambios fisiopatológicos están relacionados con una inflamación sistémica[21], el estrés oxidativo (OS)[22,23] y los polimorfismos genéticos[24,25], que podrían estar asociados con la presencia acentuada de sarcopenia en esta enfermedad, ya que son los principales factores etiológicos para este síndrome[11,18]. Por lo tanto, esta revisión tuvo como objetivo conocer los principales mecanismos patógenos relacionados con la sarcopenia en individuos con la EPOC.

Metodología

Se realizaron búsquedas en cinco bases de datos electrónicas: PubMed, LILACS, EMBASE, Cochrane Library y SciELO desde su inicio hasta diciembre de 2018 utilizando los siguientes términos de búsqueda en inglés: "COPD", "pulmonary disease, chronic obstructive", "chronic obstructive lung disease", "COAD", "chronic obstructive airway disease" y "sarcopeni*". En estudios que proporcionaron comparaciones entre personas con EPOC que tienen y no tienen sarcopenia, evaluamos los biomarcadores de estrés oxidativo, inflamación sistémica y polimorfismos genéticos. De los 272 registros únicos identificados a través de la búsqueda en la base de datos, 24 artículos estudiaron la sarcopenia en esta enfermedad y solo 5 de ellos determinaron biomarcadores sanguíneos en sujetos con EPOC y sarcopenia[21,26-29]. También fue realizada una búsqueda manual de las referencias de cada artículo incluido, para identificar otros estudios del área. Además, se utilizaron referencias complementarias de la literatura para crear una posible explicación sobre la etiología de la sarcopenia en la EPOC mediante los términos de búsqueda en inglés: "COPD", "pulmonary disease, chronic obstructive", "chronic obstructive lung disease", "COAD", "chronic obstructive airway disease", "aging" "inflammation", "oxidative stress" y "genetic polymorphisms". La información obtenida se organizó en 5 temas diferentes: 1) EPOC y sarcopenia; 2) mecanismos biológicos de la sarcopenia en la EPOC; 3) relación entre la inflamación y la sarcopenia; 4) relación entre el estrés oxidativo y la sarcopenia y 5) relación entre los polimorfismos genéticos y la sarcopenia.

EPOC y sarcopenia

El origen de la palabra sarcopenia deriva del griego *sarx* (carne) y *penia* (pobreza)[30], por lo que al principio fue solamente relacionada a una reducción de la masa muscular. Sin embargo, hoy en día este término se ha ampliado un poco más, siendo también relacionado con la reducción de la fuerza muscular y el rendimiento físico[13]. Tanto ha evolucionado este concepto hoy en día, que la sarcopenia ha sido reconocida como una enfermedad musculoesquelética por la décima Revisión de la Clasificación Internacional de Enfermedades (CIE-10) en el año 2016[31]. Influenciado por la gran prevalencia de la sarcopenia en personas mayores de 65 años, que puede variar entre 5% y al 13%[14]. También, un gran interés ha nacido por el estudio de la sarcopenia en individuos con EPOC, debido a su impacto negativo para la funcionalidad y la calidad de vida[15,32,33], y su alta prevalencia que puede variar desde 15% a un 55%[16].

Mecanismos biológicos de la sarcopenia en la EPOC

En sujetos con sarcopenia, hay un aumento en la degradación de las proteínas miofibrilares y una disminución en la síntesis de proteínas, produciendo atrofia muscular y debilidad muscular[35]. Aunque la sarcopenia es una enfermedad de los ancianos, su desarrollo puede estar asociado con otros factores, que no son exclusivamente de personas adultas mayores, como trastornos metabólicos, inflamación sistémica, estrés oxidativo, disminución de la actividad física, disfunción mitocondrial y caquexia[18,30]. Estos factores también están presentes en sujetos con EPOC y pueden llevar a la aparición de la sarcopenia en esta enfermedad (Figura 1) [21,22,35].

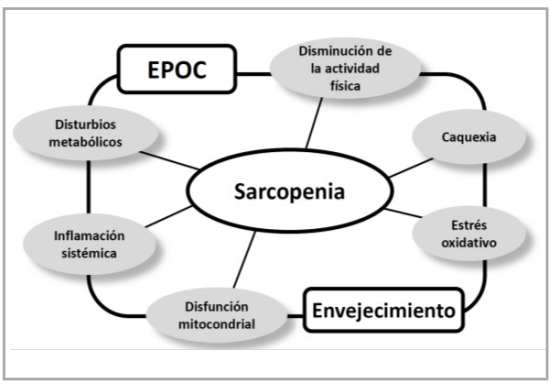

Figura 1. Características asociadas a la sarcopenia en la EPOC y el envejecimiento.

La EPOC y el envejecimiento se han relacionado con trastornos metabólicos, inflamación sistémica, disfunción mitocondrial, niveles más bajos de actividad física, caquexia y estrés oxidativo. Todos estos factores inducen la aparición de sarcopenia.

La interacción entre factores externos e internos de un individuo juegan un papel importante en el desarrollo de la sarcopenia en pacientes con EPOC. El nivel de actividad física, el tipo de dieta, el tabaquismo y el uso de corticoides producen directamente estrés oxidativo e inflamación sistémica, que combinados con polimorfismos genéticos son los responsables del origen de la sarcopenia (Figura 2)[11,18,22,36]. El estrés oxidativo, la inflamación crónica y la disfunción mitocondrial juegan un papel importante en la atrofia muscular, porque estos factores afectan el equilibrio entre la síntesis de proteínas y su degradación[17,18]. El estrés oxidativo es un desequilibrio entre las especies oxidantes y antioxidantes[37]. Las especies reactivas de oxígeno y nitrógeno (ROS / RNS) son segundos mensajeros para el factor de necrosis tumoral alfa (TNF-α) en el músculo esquelético, activando el factor nuclear-KB (NF-kB), que induce directa e indirectamente la inflamación sistémica[18]. Esta inflamación sistémica induce la apoptosis celular en el músculo, que se asocia con el catabolismo muscular, disminuyendo tanto la masa muscular como la fuerza[38]. Esta debilidad muscular se produce a través de dos mecanismos principales: pérdida acelerada de proteínas y disfunción contráctil[38].

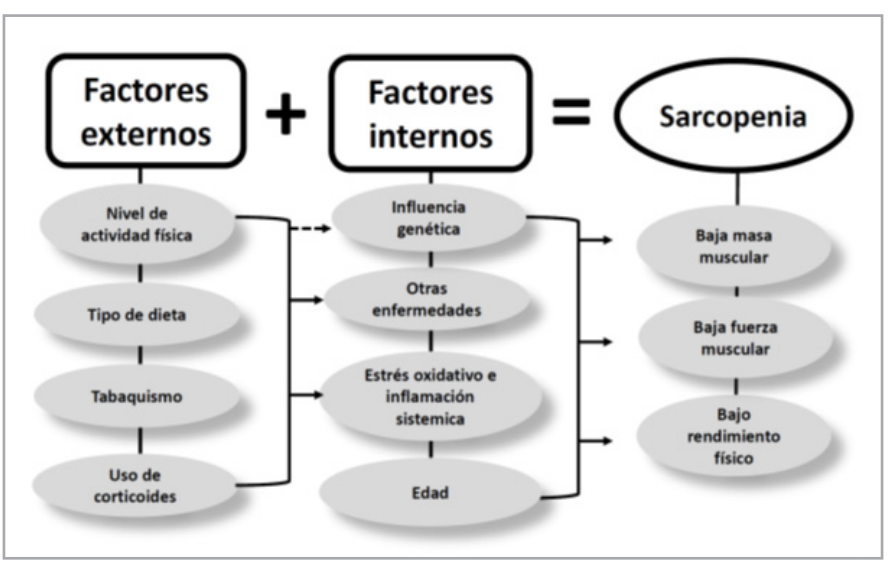

Figura 2. Factores asociados con la sarcopenia en la EPOC. La sarcopenia es inducida por factores externos e internos, que tienen asociación directa (línea continua) o asociación indirecta (línea discontinua) entre sí y con la sarcopenia.

Relación entre la inflamación y la sarcopenia

Uno de los factores importantes asociados con la sarcopenia en las personas mayores es la inflamación sistémica[21,39]. La interleucina-6 (IL-6), el factor de necrosis tumoral alfa y la proteína C reactiva (PCR) se relacionaron con una menor masa muscular, un menor rendimiento físico[40] y una menor fuerza muscular[39] en adultos mayores. Estos biomarcadores están aumentados en individuos con EPOC comparado con aquellos sin esta enfermedad[35,41]. Adicionalmente, la inflamación se ha asociado con un peor estado funcional y de salud en individuos con EPOC[35,41,42], siendo una de las posibles causas de sarcopenia en esta enfermedad[21]. Se han observado niveles más altos de IL-6[21], TNF-α[21] y PCR[26,29] en individuos con EPOC con sarcopenia en comparación con aquellos sin sarcopenia. En este contexto, la inflamación sistémica parece afectar la masa y la fuerza muscular en esta población, ya que en el estudio publicado por Byun et al. 2017[21], se observaron correlaciones negativas entre estas variables con los biomarcadores inflamatorios[21].

Existe una fuerte relación entre la inflamación y el estrés oxidativo, por lo que estos mecanismos suelen estar vinculados[22,43,45]. La inflamación sistémica y el estrés oxidativo disminuyen los niveles del factor de crecimiento insulínico de tipo 1 (IGF-1) y pueden bloquear la vía de la síntesis de proteínas (Figura 3), esta reducción está asociada con sarcopenia, fragilidad y mortalidad[25,44]. El papel del IGF-1 es señalar la vía de proteína quinasa B (Akt) que induce la síntesis de proteínas mediante la estimulación de la diana de rapamicina en los mamíferos (mTOR)[34]. La señalización a través de IGF-1 comienza con la unión del ligando IGF-1 a su receptor, lo que induce una fosforilación del receptor y el reclutamiento del sustrato de insulina 1 (IRS1). La fosforilación de IRS1 activa la ruta del fosfoinositol-3-quinasa (PI3K), activando la Akt, la síntesis de proteínas inductora de mTOR y la hipertrofia muscular. IGF-1 es reducida durante el proceso de envejecimiento y la presencia enfermedades crónicas, disminuyendo la actividad de Akt y mTOR[18].

Estas cascadas señalizadas por biomarcadores inflamatorios y estrés oxidativo son activadas por el proceso de envejecimiento y son estimuladas en presencia de enfermedades crónicas, como la EPOC, lo cual está asociado con sarcopenia (Figura 3)[22,35].

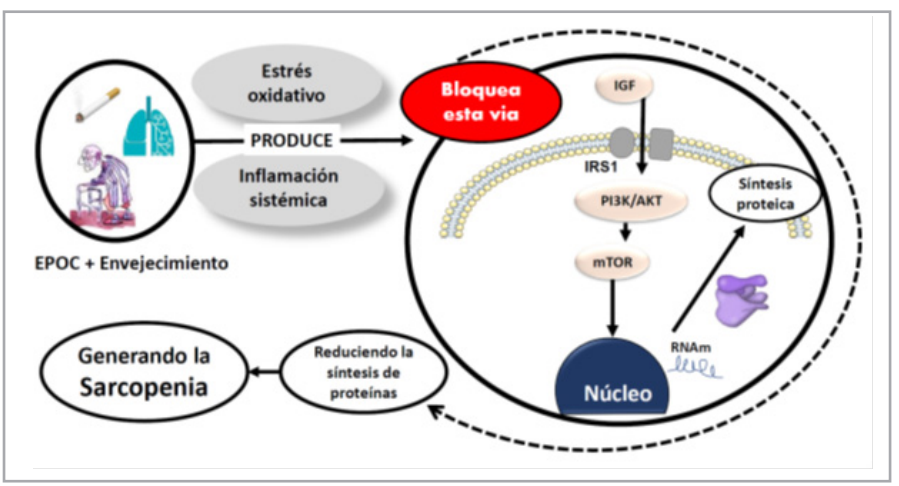

Figura 3. La EPOC combinada con el envejecimiento generan la sarcopenia. La EPOC y el envejecimiento están asociados con la inflamación sistémica y el estrés oxidativo, los cuales bloquean la vía IGF-1 / PI3K / AKT / mTOR, lo que reduce la síntesis de proteínas musculares, por lo tanto, induce la sarcopenia.

Relación entre el estrés oxidativo y la sarcopenia

Cesari et al.[46], demostraron una correlación positiva entre la capacidad antioxidante con el rendimiento físico y la fuerza muscular en ancianos. Estos autores mostraron que los sujetos con mayor ingesta de antioxidantes en la dieta, como la vitamina C, son los que tienen mayor fuerza muscular. Además, Agler et al.[47], en un estudio de seguimiento de 10 años con 38.597 mujeres (≥45 años) encontraron que la suplementación con antioxidantes redujo el riesgo de desarrollar enfermedad pulmonar crónica en un 10% (HR 0,90; IC del 95%: 0,81 a 0,99; p = 0,029), adicionalmente se asoció con una disminución de los niveles de estrés carbonilo en el pulmón. Los estudios en individuos con EPOC demostraron una reducción en la actividad antioxidante de la

superóxido dismutasa (SOD), catalasa (CAT), proteínas sulfhidrilas totales (SH), paraoxonasa 1 (PON1), glutatión peroxidasa y glutatión-S-transferasa (GST)[48–51]. Sin embargo, hay resultados contradictorios con algunos biomarcadores antioxidantes, como SH, superóxido dismutasa y catalasa, que han demostrado un aumento o no diferencias, en comparación con los individuos sin la EPOC[52,53].

Los sujetos con EPOC tienen un desequilibrio entre los niveles de oxidantes y antioxidantes[48-50]. Los niveles de biomarcadores oxidantes están aumentados en individuos con EPOC[48–56]. La peroxidación lipídica y la oxidación de proteínas son consecuencias del estrés oxidativo y causan daño oxidativo[50,52,57,58]. Se ha demostrado, que este daño oxidativo estaría relacionado con un deterioro en la función pulmonar, gravedad de la enfermedad y la presencia de síndrome metabólico en individuos con EPOC[23].

Las sustancias reactivas al ácido tiobarbitúrico (TBARS) y el malondialdehído (MDA) son los biomarcadores más comunes de la peroxidación de lípidos estudiados en sujetos con EPOC[50]. Niraj Dhakal et al.[50], Raut et al.[55] y Wozniak et al.[53], informaron niveles más altos de MDA y TARBS en sujetos con EPOC en comparación con el grupo control. Por otro lado, Syrine et al.[56] y Jammes et al.[59], no observaron diferencias en los niveles de MDA y TBARS, respectivamente, entre los individuos con EPOC y aquellos sin esta enfermedad. Además, el aldehído 4-hidroxi-2-nonenal (4-HNE), el principal compuesto de la peroxidación lipídica de la membrana, está elevado en individuos con EPOC, que es un mediador clave en diferentes mecanismos relacionados con el estrés oxidativo, la inflamación y la apoptosis celular[57,58].

Con respecto a la oxidación de proteínas, en individuos con EPOC, el grado del daño oxidativo en las proteínas se ha investigado a través de la presencia de productos de oxidación avanzada de proteínas (AOPP)[17]. Stanojkovic et al.[60], encontraron niveles más altos de AOPP y MDA en sujetos con EPOC en comparación con individuos aparentemente sanos, además, este estudio mostró que la inflamación sistémica elevada está correlacionada negativamente con la capacidad antioxidante. Por lo tanto, estos resultados enfatizan la relación entre el estrés oxidativo y la inflamación.

Existe escasa evidencia sobre los marcadores de estrés oxidativo y la sarcopenia en individuos con EPOC[56]. La mayoría de los estudios correlacionaron los biomarcadores del estrés oxidativo con el índice de masa corporal, la función pulmonar, el estado nutricional, las exacerbaciones, estadios de GOLD y la inflamación sistémica[23,50,56,60–62.] La investigación de Byun et al[21] es la única evidencia que ha informado una asociación entre algunos biomarcadores inflamatorios con sarcopenia en sujetos con EPOC. Aunque no hay evidencia de asociación entre la sarcopenia y el estrés oxidativo en esta población, podemos suponer que el estrés oxidativo desempeña un papel importante en el origen de la sarcopenia, ya que el estrés oxidativo está vinculado a diferentes mecanismos biológicos relacionados con la inflamación y la atrofia muscular [50,52,57,58,60].

Relación entre los polimorfismos genéticos y la sarcopenia

La influencia de la genética en la sarcopenia, ha sido estudiada recientemente[44]. Los polimorfismos de un solo nucleótido (SNP) están relacionados con la inflamación sistémica y la disminución de la síntesis de proteínas, por lo que se han asociado con la sarcopenia y la fragilidad en los adultos mayores[63]. Las mutaciones en los genes IGF-I e IGF-II en seres humanos reducen la síntesis de IGF, esto se ha relacionado a fenotipos caracterizados por menor fuerza muscular, menor masa muscular y una menor respuesta al entrenamiento con ejercicios[64,65]. El C-1245T SNP (rs35767) es una variación genética en la región promotora del IGF-I, que se ha estudiado en ancianos, asociada con disminuciones de la masa muscular y la fuerza muscular, ya que el gen IGF-I regula la síntesis de proteínas del músculo y la hipertrofia[65,66]. Además, las mutaciones genéticas pueden interferir en la respuesta del ejercicio.

Devaney et al[64], encontraron que el polimorfismo de IGF-II (rs3213221) produce una mayor pérdida de fuerza muscular, mayor dolor y aumento en los niveles de la creatina quinasa sérica posterior al ejercicio, por lo que este gen se ha relacionado con mayor riesgo de daño muscular[66]. Otro polimorfismo asociado con sarcopenia y fragilidad es la mutación en el gene ACTN-3 en humanos (rs1815739), lo que puede disminuir el nivel de α-Actinina-3, alterando la función contráctil del músculo, debido a que es una proteína fundamental en la contracción muscular[66].

Como fue explicando anteriormente, los biomarcadores proinflamatorios desempeñan un papel en la debilidad muscular[18]. La producción de citoquinas inflamatorias puede estar influenciada por polimorfismos genéticos, generalmente en la región promotora de los genes[67]. De este modo, tanto la sarcopenia como la inflamación sistémica pueden verse influidas por polimorfismos genéticos. En este sentido, Broekhuizen et al.[68],

encontraron una mayor prevalencia del polimorfismo IL-1β-511 en individuos con EPOC con menor índice de masa magra en comparación con sujetos aparentemente sanos. Estos hallazgos sugieren una predisposición genética en el proceso de caquexia en sujetos con EPOC[68].

Finalmente, la contribución genética al desarrollo de la EPOC ha sido bien establecida en una revisión sistemática desarrollada por Bossé et al[71]. Sin embargo, hay pocos estudios que muestren correlación entre la presencia de sarcopenia en la EPOC con factores genéticos. Además, la mutación de los genes ILG-II e IGF-II ha sido poco estudiada en individuos con EPOC, ya que la mayoría de los polimorfismos de un solo nucleótido (SNP) asociados con esta enfermedad se han relacionado con la inflamación sistémica y la función pulmonar[69].

Conclusiones

La interacción entre factores externos e internos como la inflamación sistémica, el estrés oxidativo y los polimorfismos genéticos están relacionados con la prevalencia de la sarcopenia en individuos con la EPOC. Sin embargo, son necesarios nuevos estudios para identificar algunos biomarcadores sanguíneos y genéticos específicos relacionados con la fuerza muscular, la masa muscular y el rendimiento físico en esta enfermedad.

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Heart & Lung 000 (2020) 1–8

Contents lists available at ScienceDirect

Heart & Lung

journal homepage: www.heartandlung.com

NOVEL antioxidant and oxidant biomarkers related to sarcopenia in COPD.

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ARTICLE INFO

Article History:
Received 17 September 2019
Revised 15 May 2020
Accepted 1 June 2020
Available online xxx

Keywords:
Oxidative stress
Sarcopenia
COPD

ABSTRACT

Background: The relation between oxidative stress (OS) and sarcopenia in COPD remains unknown.
Objective: To analyze OS levels and its association with sarcopenia in COPD.
Methods: Thirty-nine individuals with COPD (69±7years; 41%female) and thirty-five for the control group (69±7years; 43%female) were included. Advanced oxidation protein products (AOPP), paraoxonase-1 (PON1), superoxide dismutase activity (SOD), catalase dismutase activity (CAT), sulfhydryl group (SH), nitric oxide metabolites (NOX), total radical trapping antioxidant parameter (TRAP) were analysed. OS markers were correlated with handgrip and quadriceps strength, gait speed, skeletal muscle mass index, fat-free mass index, maximum inspiratory and expiratory pressure. European criteria were used to identify sarcopenia.
Results: In COPD, antioxidant capacity was correlated with muscle mass and strength (r from 0.5 to 0.64) $P<0.05$ for all. TRAP≤ 850 μM/trolox and AOPP≤65 μM/l were associated with sarcopenia (OR:8.3; 95% CI: 1.4–49.6 and OR:14; 95%CI: 2.2–87.1, respectively; $P<0.05$ for both).
Conclusion: OS is associated with sarcopenia in COPD.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a condition characterized by extrapulmonary consequences affecting functionality and quality of life.[1, 2] The observed low levels of daily physical activity may induce a reduction in muscle mass and generate muscle weakness.[3–6] The latter increases in the presence of associated chronic diseases and is directly related to sarcopenia.[7]

Sarcopenia is defined as a reduction in muscle strength, physical performance and muscle mass.[7] It is a major cause of frailty[5, 7] and has a prevalence of roughly 5–13% in older individuals.[8] The prevalence of sarcopenia is yet higher in COPD varying from 15%[9] to 40%,[4] depending on the classification's criteria[7] and has a negative impact on prognosis and physical function in individuals with COPD.[4]

The pathogenesis of sarcopenia is multifactorial and is commonly attributed to metabolic changes, immobility, mitochondrial dysfunction,[5, 10] oxidative stress (OS)[11, 12] and systemic inflammation,[3] contributing to age-related alterations in the muscle,[13] via increased damage to the cells.[11, 14]

There is evidence reporting that the pathophysiological response in individuals with COPD is related to the presence of OS produced by the oxidant / antioxidant imbalance.[15–17] However, literature is still inconclusive regarding what specific OS biomarkers are associated with these biological pathways. Different studies reported that advanced oxidation protein products (AOPP), sulfhydryl group (SH), superoxide dismutase activity (SOD) and catalase activity (CAT) are either increased, reduced or non-different between COPD and healthy subjects.[15, 18–21]

There is evidence that ROS (reactive oxygen species) may directly or indirectly modulate transcription factors and kinases inducing proteolytic pathways and cellular apoptosis in muscle. Since these

Abbreviations: ACCI, Age-adjusted Charlson comorbidity index; AOPP, Advanced oxidation protein products; AUC, Area under curve; BMI, Body-mass index; CAT, Catalase activity; COPD, Chronic obstructive pulmonary disease; EWGSOP, European working group on sarcopenia in older people; FEV1, Forced expiratory volume in the first second; FFM, Fat free mass; FFMI, Fat free mass index; FVC, Forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; GS, Gait speed; HDL, High-density lipoprotein cholesterol; HGF, Hand grip force; LDL, low-density lipoprotein cholesterol; NOX, Nitric oxide metabolites; MEP, Maximum expiratory pressure; MIP, Maximum inspiratory pressure; OS, Oxidative stress; PON1, Paraoxonase-1; QS, Quadriceps strength; ROS, Reactive oxygen species; ROC, Receiver-operating characteristic; SH, Sulfhydryl group; SMM, Skeletal muscle mass; SMMI, Skeletal muscle mass index; SOD, Superoxide dismutase activity; SD, Standard deviation; SPSS, Statistical Package for the Social Sciences; TRAP, Total radical trapping antioxidant parameter

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https://doi.org/10.1016/j.hrtlng.2020.06.001
0147-9563/

mechanisms contribute to sarcopenia, it is reasonable to hypothesize that OS biomarkers are as well associated with clinical measurements used to diagnose sarcopenia (muscle strength, muscle mass, and physical performance).[11, 22, 23] The association (or lack thereof) between these outcomes, however, remains to be demonstrated in individuals with COPD. The aim of this study was, therefore, to analyze OS and its association with sarcopenia in COPD in comparison to apparently healthy individuals.

Material and methods

Study design and sample

Patients with COPD (55 ≥ years old) were diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria[24] and recruited from the outpatient unit of the University Hospital of Londrina State University, Brazil. They would be excluded if presented an exacerbation episode in the two weeks before inclusion, diagnosis of bronchial asthma, presence of neurological or psychiatric diseases, arthritis, heart failure, alcohol dependence and use of antioxidant supplements. The control group included apparently healthy individuals recruited from the community who were matched for gender, age and ethnicity. The study was approved by the university ethics review board and all participants provided written informed consent (#1.830.048).

Procedures

Sarcopenia definition

Sarcopenia was defined following the European working group of sarcopenia in older people (EWGSOP). Individuals were deemed sarcopenic if both low muscle mass and low muscle function (handgrip strength and physical performance).[7]

Muscle mass

Muscle mass was quantified using a bioelectrical impedance (Biodynamics 310TM; Biodynamics Corp., USA) with measurements of fat-free mass (FFM), fat-free mass index (FFMI-FFM/ height^2), skeletal muscle mass (SMM) and skeletal muscle mass index (SMMI-SMM/ height^2). FFM was calculated using the formula of Kyle et at.[26] FFMI was considered reduced if <20.35 kg/m^2 in men or < 14.65 kg/m^2 in women.[27] SMM was estimated using the equation developed by Janssen et al.[28] SMMI was considered reduced if SMMI ≤10.75 Kg/m^2 in men and ≤6.75 kg/m^2 in women.[7]

Handgrip strength

Handgrip strength (HGS) was assessed using a hydraulic dynamometer (Jamar Plus + Digital 563,213; Lafayette Instrument Company, USA). The highest value from tree attempts (1-minute rest between each attempt) was used. Low muscle strength for HGS was defined as ≤30 Kg in men and ≤20 Kg in women.[7]

Physical performance

Physical performance was evaluated using the gait speed (GS) during the 4-meter gait speed test. Individuals were instructed to walk at their usual gait speed on a 4-meter marked track. The time was controlled by a stopwatch and the average speed to cover the distance of two attempts was used for analysis. Low physical performance was defined as GS ≤0.8 m/*sec*.[7]

Quadriceps strength

Quadriceps strength (QS) was measured using a dynamometer (EMG System, Brazil) attached to a multi-station unit (CRW 1000; Embreex, Brazil) following previously standardized procedure.[29] In short, participants performed a minimum of four and a maximum of five maximal voluntary isometric contractions of the quadriceps for six seconds. The highest value was used for analysis.[29]

Respiratory muscle strength

Respiratory muscle strength was measured as maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) using a digital manovacuometer (MVD 300, GlobalMed, Brazil) following previously standardized procedures.[30] Maneuvers were maintained for at least 2 s and the peak value was recorded. The best of 3 acceptable and reproducible maneuvers was used for analysis.

Pulmonary function

Pulmonary function was assessed with whole-body plethysmography (Vmax®, Carefusion, EUA). Measurements were performed according to the American Thoracic Society/European Respiratory Society guidelines,[25] with reference values described for the Brazilian population.[31]

Comorbidities

Presence of comorbidities was investigated using the Age-adjusted Charlson comorbidities index (ACCI) in both groups. ACCI includes 19 medical conditions and was scored using the algorithm proposed by Charlson et al.[32]

Oxidative stress biomarkers

Oxidative stress biomarkers were analyzed from blood samples. Approximately 40 mL of blood sample was obtained by venipuncture into vacuum tubes (Vacutainer®, Franklin Lakes, NJ USA) after 12 h of fasting. Blood samples were centrifuged for 30 min at 3000 rpm (2100 xg) at 20 °C, in order to obtain serum, plasma and concentrated of red blood cells to ensure the analysis of oxidative stress biomarkers, and then, frozen at −80 °C. Most of the blood components were stored until all the subjects were evaluated (approximately 1 year).

Total radical-trapping antioxidant parameter (TRAP),[33] paraoxonase 1 activity (PON1),[34] superoxide dismutase activity (SOD) in erythrocytes,[35] catalase activity (CAT) in erythrocytes,[36] sulfhydryl groups (SH),[37] advanced oxidation protein products (AOPP)[38] and nitric oxide metabolites (NOx)[39] were used as OS biomarkers and whilst glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides were used as metabolic risk factors assayed following previous studies.[33–39]

TRAP was evaluated on a microplate reader (Victor X-3, Perkin Elmer®, USA) and results were expressed in μM Trolox.[33] PON1 activity was determined by the rate of hydrolysis of chloromethyl phenyl acetate as well as by the rate of hydrolysis of phenyl acetate under low salt condition. Analysis were conducted on a microplate reader (EnSpire, Perkin Elmer®, USA).[34] SOD in erythrocytes was detected through a method based on the inhibition that this enzyme promotes in the auto-oxidation of pyrogallol in aqueous solution.[35] The amount of SOD that was able to inhibit 50% of pyrogallol oxidation was defined as a unit of enzymatic activity. The SOD reaction was read on a microplate reader (EnSpire, Perkin Elmer®, USA) with a wavelength of 420 nm. The results were expressed in U/min/gHb. CAT in erythrocytes was detected by measuring the decay in the concentration of H2O2 and oxygen generation, using the technique described by Aebi et al.[36] The CAT reaction was read on a microplate reader (EnSpire, Perkin Elmer®, USA) with a wavelength of 240 nm. The results were expressed in U/min/gHb. SH groups were evaluated in a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 412 nm and results were expressed in μM (Hu, 1994).[37] AOPP was quantified on a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 340 nm and was expressed in mM of equivalent chloramine T.[38] NOx was evaluated on a microplate reader (EnSpire, Perkin Elmer®, USA) at a wavelength of 540 nm by measuring the concentration of nitrite and nitrate and results were expressed as μM.[39]

Statistical analysis

Statistical analysis was performed using SPSS (IBM Co., USA). The Kolmogorov–Smirnov test was used to analyze normality of data distribution. Comparisons between groups were done using unpaired t-tests. Frequencies were compared using the chi-squared test and odds ratio values were provided. Pearson's correlation coefficient was used to investigate the relation between OS biomarkers with muscle mass, muscle strength and gait speed. Linear regression analysis was performed to find association between antioxidants and oxidants with clinical measurements of sarcopenia (muscle mass, strength and gait speed). Logistic regression analysis was used to identify the factors associated with sarcopenia in COPD using different variables. Receiver-operating characteristic (ROC) curves, the area under the curve (AUC), Youden's index, sensitivity and specificity were used to identify OS best cut-offs identifying sarcopenia in individuals with COPD. Statistical significance was set at $P<0.05$.

Results

Comparisons between individuals with COPD and apparently healthy individuals

Seventy-four subjects (thirty-nine patients with COPD and thirty-five apparently healthy subjects) were included. Socio-demographic characteristics, comorbidities and metabolic risk factors are reported in table 1. ACCI was significantly higher in subjects with COPD (P = 0.004). There were no significant differences in metabolic risk factors between the groups.

Sarcopenia measurements are reported in table 2. Muscle mass and FFM were lower in COPD in comparison to their counterparts (P = 0.009 and 0.035, respectively). More patients with COPD presented low muscle strength and sarcopenia than those in the CG (P = 0.02 and 0.01, respectively). QS and MIP were worse in COPD than in CG (P<0.001 and <0.05, respectively). The prevalence of sarcopenia was 23% in individuals with COPD which is higher compared to the control group (P = 0.01). Additionally, 78% of the individuals with sarcopenia were in GOLD stage ≥3.

Antioxidant and oxidant biomarkers are reported in Figs. 1 and 2. The following biomarkers were significantly higher in COPD in comparison to CG: sulfhydryl group (352±52 *versus* 313±44 Mm/mg protein), superoxide dismutase activity (43±14 *versus* 35±12 U/mgHb) and paraoxonase 1 (180±55 *versus* 152±48 U/ml), p<0.05 for all. Lower catalase activity was observed in COPD patients compared to CG (47±11 *versus* 54±11 U/mgHb; P = 0.012). No differences between groups were observed for TRAP, AOPP and NOX.

Associations between oxidative stress biomarkers and clinical measurements of sarcopenia in subjects with COPD

TRAP was positively correlated with FFMI (r = 0.5), SMMI (r = 0.5), QS (r = 0.64) and HGS (r = 0.51); likewise, AOPP showed positive correlation with FFMI (r = 0.43), SMMI (r = 0.52), HGS (r = 0.5), MIP (r = 0.59) and MEP (r = 0.46); and SOD showed negative correlation with SMMI (r= −0.36) and MIP (r= −0.45); P<0.05 for all.

Statistically significant correlations between biomarkers and clinical outcomes were included in the regression analysis models. Univariate analysis revealed significant associations for all clinical outcomes (FFMI, SMMI, QS, GS, S MIP and MEP). In the multivariate analysis models, TRAP and AOPP were the OS biomarkers that associated the most with clinical outcomes (R from 0.31 to 0.80). The complete list of univariate and multivariate analysis is provided in table 3.

Table 1
Clinical characteristics of COPD and Control groups.

Characteristics	COPD (n = 39)	Control (n = 35)
Age (years)	69 ± 6	69 ± 7
Female, n (%)	23 (41%)	15 (43%)
Height (m)	1.6 ± 9.7	1.6 ± 8.7
Weight (kg)	70 ± 17	73 ± 13
BMI (kg/m^2)	27 ± 6	28 ± 7
FVC%pred	86 ± 19	107 ± 17*
FEV_1%pred	49 ± 13	98 ± 16*
FEV_1/ FVC	46 ± 9	75 ± 5*
Smoking history, n (%)	34 (87%)	12 (34%)*
GOLD I-II, n (%)	18 (46%)	–
GOLD III-IV, n (%)	21 (54%)	–
Racial classification		
White	24 (61%)	27 (77%)
Black	5 (13%)	1 (3%)
Mulatto	5 (13%)	6 (17%)
Yellow	5 (13%)	1 (3%)
Charlson Comorbidity index		
Heart Failure history	4 (10%)	0 (0%)
Diabetes	5 (12%)	7 (20%)
Vascular peripheral Disease	7(18%)	6 (17%)
Age- Adjusted Charlson Comorbidity index		
Mean scores	4.4 ± 1.2	3 ± 1.2 *
0–1 (n,% of total)	0 (0%)	0 (0%)
2–3 (n,% of total)	11 (28%)	18 (51%) *
4–5 (n,% of total)	21(54%)	14 (40%)
≥6 (n,% of total)	7 (18%)	3 (9%)
Metabolic Risk Factors Biomarkers		
Glucose (mg/dl)	116 ± 38	109 ± 23†
Cholesterol (mg/dl)	201 ± 43	191 ± 43
LDL (mg/dl)	115 ± 38	110 ± 35
HDL (mg/dl)	65 ± 34	54 ± 12
Triglycerides (mg/dl)	130 ± 88	132 ± 96

Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; BMI: body mass index; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; *$P \leq 0.05$; **$P \leq 0.001$. †statistical analysis n = 33.

Factors associated with prevalence of sarcopenia in subjects with COPD

Selection of presented cut-off points used in the logistic regression were done considering previously described values for the variables: ACCI (≥4); Body-mass index, BMI (<22 Kg/m^2); GOLD stage

Table 2
Sarcopenia measurements in COPD and Control groups.

Measurements of Sarcopenia		COPD (n = 39)	Control (n = 35)
QS (Kg)		22 ± 9	39 ± 13**
MIP (cmH_2O)		79 ± 25	92 ± 31*
MEP (cmH_2O)		115 ± 36	124 ± 38
FFM (Kg)		37 ± 18	46 ± 7*
FFMI (Kg/m^2)		17 ± 3	18 ± 5
SMM (Kg)		21 ± 10	25 ± 6*
Criteria to diagnose Sarcopenia			
MM	SMMI (Kg/m^2)	8 ± 4	9 ± 2
MS	HGS (Kg)	33 ± 9	33 ± 7
PP	GS (m/*sec*)	1.1 ± 0.14	1.17 ± 0.18
Subjects with lower MM, n (%)		11 (28%)	8 (23%)
Subjects with lower MS, n (%)		10 (27%)	2 (6%)*
Subjects with lower PP, n (%)		0 (0%)	0 (0%)
Subjects with sarcopenia, n (%)		9 (23%)	1 (3%)*

Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; QS: quadriceps strength; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; FFM: fat-free mass; FFMI: fat-free mass index; SM: skeletal muscle mass; SMMI: skeletal muscle mass index; HGS: handgrip strength; GS: gait speed; MM: muscle mass; MS: muscle strength; PP: physical performance; *$P \leq 0.05$; **$P \leq 0.001$.

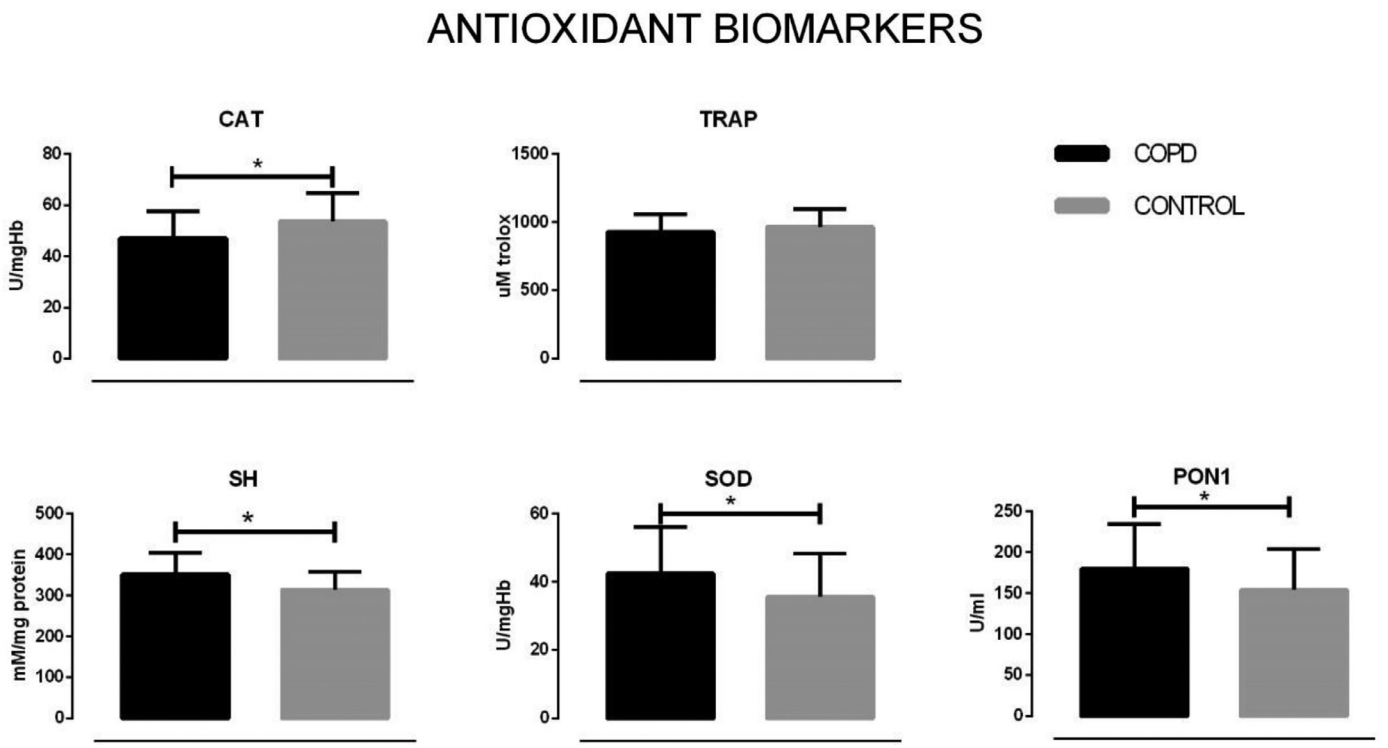

Fig. 1. Data are expressed as mean ± standard deviation. COPD: chronic pulmonary obstructive disease; CAT: catalase activity; TRAP: total radical-trapping antioxidant parameter; SH: sulfhydryl group; SOD: superoxide dismutase activity; PON 1: paraoxonase 1 activity; $^{*}p \leq 0{,}05$.

(≥3) and; FFMI (<14.65 Kg/m^2 for women and <20.35 Kg/m^2 for men).[4, 27] The selection of cut-offs for the outcomes without reference values were done via ROC analysis (Fig. A.1 in appendices). Table 4 shows AUC, sensitivity and specificity of the cut-offs used to discriminate the presence of sarcopenia in COPD in the variables: AOPP, HDL, MEP, MIP, QS and TRAP. Other investigated biomarkers are not presented in the table 4 since AUC was lower than 0.5 with $P>0.05$.

Fig. 3 describes the results of the logistic regression analysis. The prevalence and OR of all variables are reported as an orbital bubble chart. Age, gender (female), BMI, FFMI, MIP, MEP, QS, TRAP, AOPP and HDL were factors significantly associated with the development of sarcopenia in individuals with COPD ($p<0.05$ for all). Since SMMI, HGS and GS are criteria to diagnose sarcopenia, they were not considered in this analysis.

Discussion

The present study confirms the increased prevalence of sarcopenia and higher levels of antioxidant activity in patients with COPD. Our findings add to the field that 1) OS biomarkers associate with clinical measurements of sarcopenia and; 2) the marked reduction of the TRAP and AOPP biomarkers are highly prevalent and are associated with sarcopenia.

The study showed that individuals with COPD present decreased muscle mass and quadriceps and inspiratory muscle weakness when compared with apparently healthy subjects. The prevalence of sarcopenia in individuals with COPD was 23% which is similar to previous studies reporting prevalence rates between 15%[9] to 40%[4] and significantly higher than the control group (3%). Additionally, and as observed in literature,[4] sarcopenia was more prevalent in individuals

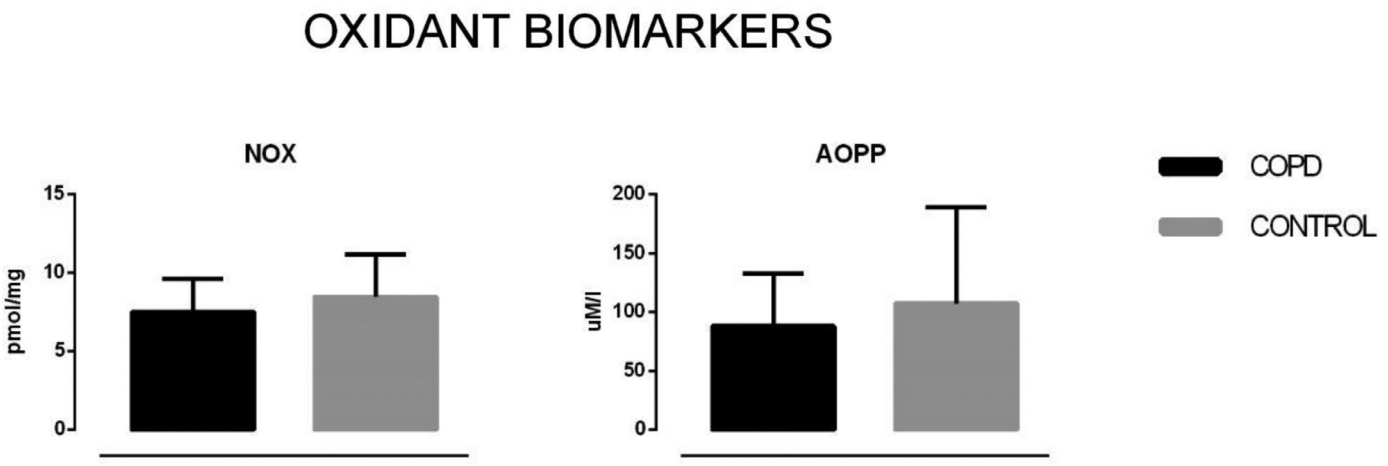

Fig. 2. Data are expressed as mean ± standard deviation. COPD: chronic pulmonary obstructive disease; NOX: nitric oxide metabolites; AOPP: advanced oxidation protein products $^{*}p \leq 0{,}05$.

Table 3
Predictors of clinical measurements of sarcopenia in individuals with COPD.

Dependent Variables	Univariate Analysis			Multivariate analysis		
	Explanatory variables	R^2	P	Explanatory variables	R^2	p
FFMI	TRAP	0.25	0.005	TRAP + BMI	0.80	0.0001
	Age	0.02	0.43			
	BMI	0.69	0.0004	Excluded: age		
SMMI	TRAP	0.10	0.07	TRAP+AOPP+BMI	0.51	0.0004
	SOD	0.13	0.046			
	AOPP	0.26	0.004			
	Age	0.02	0.823	Excluded: SOD and age		
	BMI	0.42	0.0007			
QS	TRAP	0.38	0.001	TRAP	0.43	0.004
	Age	0.11	0.085	Excluded: age and BMI		
	BMI	0.01	0.56			
GS	TRAP	0.26	0.008	TRAP + BMI	0.51	0.038
	Age	0.04	0.08			
	BMI	0.13	0.07	Excluded: age		
HGS	AOPP	0.20	0.015	AOPP + BMI	0.31	0.009
	Age	0.04	0.26			
	BMI	0.21	0.011	Excluded: age		
MEP	AOPP	0.10	0.07	AOPP + BMI	0.17	0.063
	Age	0.03	0.3			
	BMI	0.12	0.038	Excluded: age		
MIP	AOPP	0.26	0.002	AOPP	0.31	0.008
	SOD	0.12	0.048			
	Age	0.04	0.256	Excluded: SOD, age and BMI		
	BMI	0.12	0.046			

COPD: Chronic pulmonary obstructive disease; FFMI: fat-free mass index; SMMI: skeletal muscle mass index; QS: quadriceps strength; GS: gait speed; HGS: handgrip strength; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; TRAP: total radical trapping antioxidant parameter; AOPP: advanced oxidation protein products; SOD: superoxide dismutase activity.

with GOLD stage ≥3. Patients with COPD presented higher scores in the ACCI (more comorbidities with larger weights) and higher prevalence of smoking history. Of note, the abovementioned differences between COPD and the control group were expected and previously described.[40]

COPD has been related to systemic inflammation and oxidative stress by different factors related to this disease as smoking history, medications, lower physical activity, hospitalization and other comorbidities associated with COPD.[3, 15, 16] In the present study, the SH was significantly increased in COPD. In line with our findings, Nadeem et al.[18] reported higher levels of glutathione (GSH), which is one of the organic components that contains SH. Higher levels of SH are related to GSH and this increase could be a compensatory response for the excess of oxidants in COPD.[41] PON1 is an enzyme with antioxidative and antiatherogenic properties, which is associated with high-density lipoprotein (HDL).[42] Its activity was found reduced in different diseases associated with oxidative stress such as COPD.[15, 16] The present study, however, reported higher levels of PON1 in the COPD group. PON1 is associated with HDL, and an increase in HDL levels can lead to an increase in PON1 activity.[42] We observed a non-significant trend (P = 0.06) of larger HDL concentration in COPD than CG. In addition, 49% of the individuals with COPD showed HDL≥57.5 mg/dl, which was significantly associated with prevalence of sarcopenia in COPD (OR: 13.8; 95% CI:1.519–125.7). Furthermore, 54% of the individuals were classified as GOLD ≥3, a group of subjects with known higher levels of HDL.[43]

SOD is an important antioxidant enzyme that inhibits superoxide anion (O_2^-) and protects aerobic cells from oxidative stress.[19] We observed higher levels of superoxide dismutase in patients with COPD. Similar findings have been reported in other studies.[18, 19] SOD is the only enzymatic system-decomposing O_2^- to H_2O_2 and it plays a significant role especially in the lungs and muscle cells.[44] In respect with that, we found that SOD was negatively correlated to SMMI (r: −0.36). Additionally, we found a moderate negative correlation between SOD and MIP (r= −0.45). Since a reduction in MIP induces respiratory overload, it can be hypothesized that respiratory muscle weakness might be related to an increase in SOD activity, as this enzyme is sensitive to the stress in the respiratory system.[44] Another important antioxidant enzyme is catalase. Literature, however, is still controversial about its levels in subjects with COPD.[15, 19, 20] Wozniak et al. evaluated 73 patients with COPD and observed no differences in CAT activity compared to the control group.[19] In contrast, Vibhuti et al. dosed the antioxidant capacity in 202 subjects with COPD and found a significant reduction in CAT activity compared to healthy

Table 4
ROC curve analysis to detect individuals with sarcopenia.

	TRAP	AOPP	HDL	QS	MIP	MEP
Cut off	≤ 885 μM trolox	≤ 65 μM/l	≥57.5 mg/dl	≤ 26.15 Kg	≤ 77.5 cmH_2O	≤115 cmH_2O
Sensitivity	78%	68%	89%	100%	90%	70%
Specificity	74%	75%	66%	63%	63%	69%
Younden's index	0.52	0.43	0.55	0.63	0.53	0.39
AUC	0.78	0.70	0.86	0.86	0.74	0.72
95% CI	0.48–0.945	0.58–0.965	0.76–0.963	0.74–0.976	0.58–0.904	0.585–0.85
P	0.0024*	0.007*	0.00046**	0.001**	0.014*	0.03*

TRAP: Total radical-trapping antioxidant parameter; AOPP: advanced oxidation protein products; HDL: high-density lipoprotein; QS: quadriceps strength; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure. *$P \leq 0.05$; **$P \leq 0.001$.

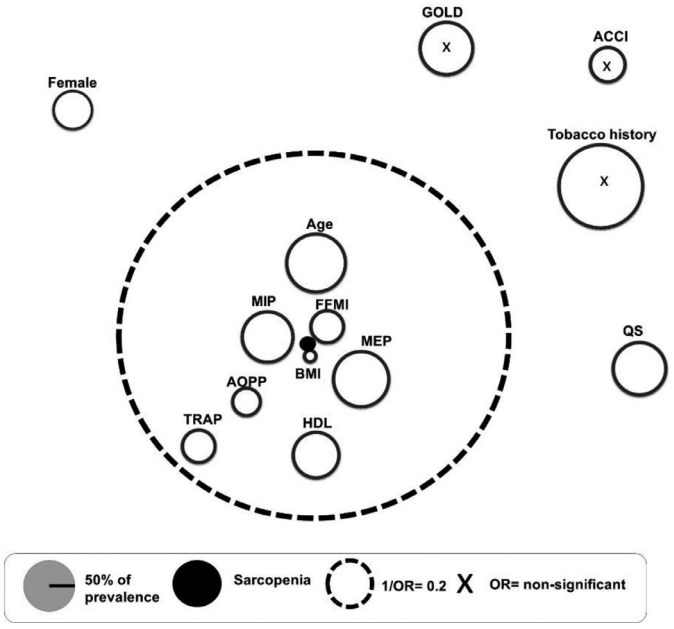

Fig. 3. Features associated with the odds to develop sarcopenia and the prevalence in individuals with COPD.

Factors with more than 10% of prevalence and stronger association with sarcopenia in individuals with COPD (Odds ratio [OR], 95% confidence interval, $P < 0.05$) are shown. The area of the circle shows the prevalence. The proximity to the center (sarcopenia) expresses the strength of the association among the factor and sarcopenia risk. All bubbles associated without statistical significant are marked with "X". Bubbles inside the dotted orbit have an OR $\geq$ 5 (1/OR=0.25). ACCI: age-adjusted Charlson comorbidity index ($\geq$4); Age>67 years; AOPP: advanced oxidation protein products ($\leq$65 μM/l); BMI: body mass index (<22 Kg/m^2); GOLD: global initiative for chronic obstructive lung disease ($\geq$3); FFMI: fat-free mass index (<14.65 Kg/m^2 for women and <20.35 Kg/m^2 for men); HDL: high-density lipoprotein ($\geq$57.5 mg/dl); MEP: maximal expiratory pressure ($\leq$ 115 cmH_2O); MIP: maximal inspiratory pressure ($\leq$ 77.5 cmH_2O); QS: quadriceps strength($\leq$26.15 Kg); TRAP: total radical trapping antioxidant parameter ($\leq$ 850 μM trolox).

controls.[20] The latter is in accordance with the present study. A decrease in CAT activity leads to ROS-induced cellular damage and limits the scavenging capacity for lipid peroxides and ROS, further impairing the pathogenesis of COPD.[20] Nitric oxide (NO) is an important element of vascular homeostasis.[45] OS reduces the synthesis of NO impairing the endothelial and pulmonary function.[41, 45] Anes et al.[41] found a significant reduction in nitric oxide metabolites (NOx) levels in subjects with COPD compared to control group and observed an association between NOx and airflow obstruction. In the present study, albeit not statistically significant, NO levels were reduced in individuals with COPD when compared to apparently healthy subjects ($P = 0.052$).

AOPP is an oxidative biomarker used to estimate protein damage.[38] Although no differences were observed between COPD and control group in AOPP and NOx, it is not possible to confirm that the oxidants are not augmented in individuals with COPD. This can be said since the oxidative stress could be compensated by the higher antioxidant activity previously reported in the present study. Additionally, there are other oxidants related to other oxidative stress pathways which were unfortunately, not investigated.

This is the first study to investigate correlations between AOPP and sarcopenia in COPD. A likely explanation to the positive correlation between AOPP with muscle mass (FFMI: $r = 0.43$; SMMI: $r = 0.51$) and strength (HGS: $r = 0.5$, MIP: $r = 0.6$ and MEP: $r = 0.46$) is that higher muscle mass and strength implies in more protein substrate to be oxidized.[5, 46] In fact, higher levels of AOPP have been associated with oxidation of albumin, fibrinogen and lipoproteins.[47] On the other hand, low protein levels in blood (which is related to low protein oxidation) have been associated with worse muscle function.[46] This could explain at least in part why individuals with COPD who have sarcopenia presented decreased levels of AOPP.

In a multivariate analysis TRAP, AOPP and BMI were the variables explaining the increase in SMMI (R^2: 0.51). In the present study, AOPP and TRAP were the biomarkers with higher sensitivity and specificity to identify subjects with sarcopenia. Albeit AOPP presented association with sarcopenia measurements, TRAP was the most important biomarker associated with FFMI, QS and GS in subjects with COPD. This is a biomarker of antioxidant capacity of macromolecules which has been associated with increased muscle strength.[47] In this study, the positive association between TRAP and quadriceps strength reinforces the protective role of antioxidants on both muscle mass and muscle function reported by other authors.[11, 12] In the present study, this biomarker combined with other outcomes explained 80% of FFMI which is a marker of muscle mass. Since TRAP was highly associated with muscle mass, it is possible to hypothesize that the relation between TRAP and muscle mass is stronger in large muscle groups as quadriceps. In fact, this biomarker alone explained 43% of variation in quadriceps strength in our study. Considering that gait speed is associated with muscle power, (*i.e.* the combination of force and velocity), the significance of TRAP and QS explaining up to 51% of variability in GS could be anticipated. TRAP was associated with muscle mass and strength and physical performance in COPD, which are the main clinical measurements to identify sarcopenia according to EWGSOP.[7] Reduction in TRAP levels (*i.e.* $\leq$ 850 μM trolox) increases 8.3-fold the risk of having sarcopenia in subjects with COPD ($P = 0.043$). This is a new finding and future investigations are encouraged to confirm these results since the knowledge of specific biomarkers and biological pathways would help to understand the responses to different treatment in this field. Additionally, the present results could be useful for developing future intervention with antioxidant medications or dietary supplements for sarcopenia in individuals with COPD. Sarcopenia has been highlighted as an important focus of treatment in adult respiratory medicine.[48] One of the few studies in this area was conducted by Jones et al.[49] who demonstrated that pulmonary rehabilitation, a multicomponent exercise-based intervention, improved a range of clinical outcomes and reduced the incidence of sarcopenia in patients with COPD. Not only exercise, but also nutritional support have been recommended as interventions for sarcopenia.[50] Current studies have reported that the combination of dietary intake of antioxidants with exercise is relevant for the treatment and prevention of sarcopenia in elderly people.[51, 52] Therefore, more research is warranted to further validate these findings, including the use of other recommended therapies, such as antioxidant supplementation or medications combined with pulmonary rehabilitation in individuals with COPD.[7, 53, 54] This would help to identify the effects of these new approaches on clinical measurements of sarcopenia and on the levels of OS biomarkers.

It is important to highlight some potential limitations of the present study. Proteins quantity was not measured. Although it does not detract from the quality of our results, it could explain and reinforce the strength of the observed associations between AOPP and sarcopenia measurements. Additionally, only two oxidant biomarkers were dosed (NOX and AOPP), which do not explain all pathways related to oxidative stress reported in other studies. This is a cross-sectional study with a somewhat limited sample size. Therefore, causality of associations and the cut-off points reported should be interpreted with caution or cannot be postulated to other populations. In addition, some data presented wide confidence intervals which could explain the variability of the associations, regardless the statistical significance. Another important limitation was that a few cardiovascular diseases

were not considered as exclusion criteria. It is known that apparent peripheral atherosclerosis, ischemic heart disease and hypertension, often present in patients with COPD have been also related to oxidative stress[55] and this could have interfered in the analysis. Importantly, the strengths of the study were that both groups were controlled for possible effects of confounding variables such as gender, age, alcohol intake and use of antioxidant supplements. Additionally, the diagnose of sarcopenia was established according to all criteria recommended by the EWGSOP, in contrast with other studies in COPD.[3, 4] Finally, future studies in this field should include larger sample sizes, add more oxidant biomarkers and focus on sarcopenia stratification (pre-sarcopenia, sarcopenia and severe sarcopenia) in order to support the findings of the present study.

Conclusions

Subjects with COPD showed higher levels of antioxidant activity, with exception of catalase activity. Additionally, patients with COPD presented a higher prevalence of sarcopenia compared to apparently healthy individuals. Finally, clinical measurements of sarcopenia were correlated with OS biomarkers, being lower antioxidative capacity and protein oxidation associated with more prevalence of sarcopenia in COPD.

Declaration of Conflicts of Interest

The authors declare no conflict of interest.

Funding

This study was supported by the National Council for Scientific and Technological Development (CNPq), Brazil. Grant number 470853/2014; CNPq/MS/SCTIE/DECIT N°34/2014. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Acknowledgments

The authors acknowledge National Council for Scientific and Technological Development (CNPq), Brazil and the professionals from the clinical hospital, Londrina State University, Brazil; patients and volunteers from the community organizations from Londrina for its collaboration.

Appendix

Fig. A.1.

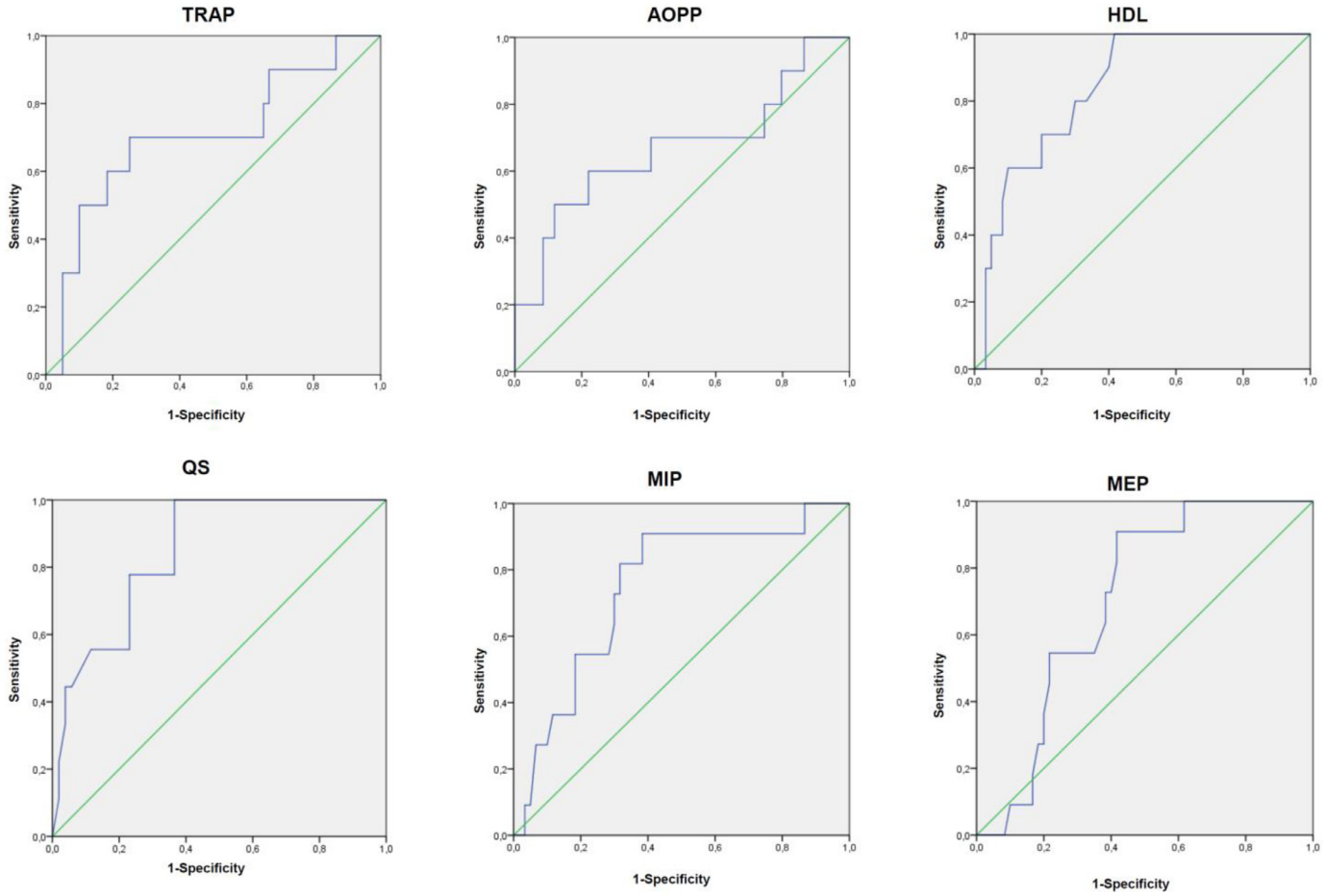

Fig. A.1. Receiver-operating characteristic (ROC) curves of factors associated with sarcopenia. AOPP: advanced oxidation protein products; HDL: high-density lipoprotein; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; QS: quadriceps strength; TRAP: total radical trapping antioxidant parameter.

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Rev Clin Esp. 2019;xxx(xx):xxx–xxx

Revista Clínica Española

www.elsevier.es/rce

ORIGINAL

¿Está el estrés oxidativo asociado a la gravedad de la enfermedad, a la función pulmonar y al síndrome metabólico en la enfermedad pulmonar obstructiva crónica?

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Recibido el 21 de marzo de 2019; aceptado el 26 de abril de 2019

PALABRAS CLAVE
Enfermedad pulmonar obstructiva crónica;
Síndrome metabólico;
Estrés oxidativo

Resumen
Objetivo: Investigar las asociaciones entre los biomarcadores oxidantes/antioxidantes y el estado de gravedad, la función pulmonar y la presencia de síndrome metabólico (SM) en pacientes con EPOC.
Métodos: Se incluyeron 74 sujetos, 39 con EPOC (edad 69 ± 7 años; mujeres 41%) y 35 para el grupo control (edad 69 ± 7 años; mujeres: 43%). Fueron diagnosticados con SM y asignados a uno de los 4 subgrupos: EPOC y control, con y sin SM, respectivamente. Se analizaron los productos de oxidación avanzada de proteína (AOPP), la paraoxonasa-1, la actividad de catalasa, el grupo sulfhidrilo y el hidroperóxido de lípidos totales. La función pulmonar fue analizada por medio de un pletismógrafo.
Resultados: El estado de gravedad de la EPOC (GOLD ≥ 3) y la función pulmonar fueron asociados con el grupo sulfhidrilo y AOPP ($p \leq 0{,}03$ para todos). La prevalencia de SM se asoció con AOPP en la EPOC ($p = 0{,}04$). Los individuos con EPOC y SM mostraron niveles de AOPP más altos en comparación con los sujetos con EPOC sin SM ($p < 0{,}0001$).
Conclusión: La gravedad de la EPOC, el deterioro de la función pulmonar y la presencia de síndrome metabólico están asociados con el estrés oxidativo en individuos con EPOC.

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https://doi.org/10.1016/j.rce.2019.04.007

Cómo citar este artículo: Sepúlveda Loyola WA, et al. ¿Está el estrés oxidativo asociado a la gravedad de la enfermedad, a la función pulmonar y al síndrome metabólico en la enfermedad pulmonar obstructiva crónica? Rev Clin Esp. 2019. https://doi.org/10.1016/j.rce.2019.04.007

KEYWORDS
Chronic obstructive pulmonary disease;
Metabolic syndrome;
Oxidative stress

Is oxidative stress associated with disease severity, pulmonary function and metabolic syndrome in chronic obstructive pulmonary disease?

Abstract
Objective: To investigate associations between oxidant/antioxidant biomarkers with the disease severity, pulmonary function and diagnosis of metabolic syndrome (MetS) in patients with COPD.
Methods: Seventy-four subjects were included, 39 with COPD (age 69 ± 7 years; female 41%) and 35 for control group (age 69 ± 7 years; female 43%). They were diagnosed with MetS and allocated in one of 4 subgroups: COPD and control, with and without MetS, respectively. Advanced oxidation protein products (AOPP), paraoxonase-1, catalase activity, sulfhydryl group and total lipid hydroperoxide were assayed. Pulmonary function was performed with a plethysmograph.
Results: COPD severity (GOLD ≥ 3) and pulmonary function were associated with sulfhydryl group and AOPP ($P \leq .03$ for all). The prevalence of MetS was associated with AOPP in COPD ($P = .04$). Individuals with COPD and MetS showed higher AOPP compared to COPD without MetS ($P < .0001$).
Conclusion: COPD severity, worse pulmonary function and presence of metabolic syndrome are associated with oxidative stress in individuals with COPD.

Introducción

Se define la enfermedad pulmonar obstructiva crónica (EPOC) como una dolencia común, prevenible y tratable, caracterizada por síntomas respiratorios persistentes y limitación del flujo del aire debida frecuentemente a una exposición significativa a partículas o gases nocivos[1]. Acompañando a dichas anomalías del tracto respiratorio de los pacientes afectados por la EPOC, se puede presentar inflamación crónica, la cual podría jugar un papel en las múltiples comorbilidades y síntomas observados en los pacientes con EPOC[2,3], tales como el síndrome metabólico (SM) y el estrés oxidativo (EO).

El SM es un conjunto de factores de riesgo de enfermedad cardiovascular, relacionado con resistencia a la insulina, dislipidemia, obesidad central, hipertensión e intolerancia a la glucosa[4]. Una revisión sistemática reciente puso de manifiesto que la prevalencia del SM en pacientes con EPOC es de aproximadamente el 34%, siendo mayor en comparación con los controles[5]. Tanto el SM como la EPOC se han asociado con un aumento de las especies reactivas de oxígeno (ROS).

Al ser las ROS capaces de inducir daño celular, las células dependen de un sistema antioxidante de defensa basado principalmente en componentes enzimáticos, tales como la paraoxonasa-1 (PON1) y la catalasa (CAT). Cuando el sistema defensivo antioxidante falla o es sobrepasado por la presencia de ROS, se puede llegar a un desequilibrio entre los biomarcadores oxidantes/antioxidantes, conocido como EO[6]. Ya se ha estudiado el EO en pacientes con EPOC estable[7,8] y exacerbada[9,10], y se ha publicado que pacientes con EPOC mostraban unos niveles de ROS más altos que los controles.

En individuos con EPOC, el EO puede asociarse a muchas comorbilidades, incluido el SM[11], si bien hasta la fecha no se han realizado estudios en los que se comparen los marcadores de EO y la capacidad antioxidante en pacientes con EPOC aquejados o no de SM. La importancia de este hecho es clave, ya que el EO, en pacientes con EPOC, se ha relacionado de manera negativa con la función pulmonar[7]. Por ello, el objeto de este estudio fue investigar las relaciones existentes entre los biomarcadores oxidantes/antioxidantes y la gravedad de la enfermedad, la función pulmonar y el diagnóstico de SM en pacientes con EPOC.

Material y métodos

Diseño del estudio y muestra

Se realizó un estudio observacional de corte transversal con pacientes con EPOC (≥ 55 años) procedentes de la unidad ambulatoria de neumología del Hospital Universitario de la Universidad Estatal de Londrina, Brasil. Los pacientes fueron diagnosticados de EPOC de acuerdo con los criterios GOLD[1]. Los criterios de exclusión fueron: sujetos con una exacerbación en las últimas 4 semanas, diagnóstico de asma bronquial, artritis, presencia de enfermedades neurológicas o psiquiátricas, insuficiencia cardiaca, alcoholismo y empleo de suplementos antioxidantes, ya que todos estos factores podrían afectar los niveles de los biomarcadores oxidantes/antioxidantes. Además, con el fin de crear un grupo control, se seleccionaron de la comunidad sujetos sin EPOC o cualquier otra enfermedad crónica que pudiera interferir en el rendimiento de las pruebas, y que coincidían por sexo, edad y origen étnico con los pacientes. Los participantes se repartieron en uno de los siguientes 4 subgrupos: 1) EPOC (EPOC sin SM); 2) EPOC-SM (EPOC con SM); 3) control (control sin SM), y 4) control-SM (control con SM). Todos ellos firmaron un consentimiento informado antes de ser evaluados. El estudio fue aprobado por el Comité de Ética sobre

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Investigación con Sujetos Humanos de la Universidad Estatal de Londrina (1.830.048).

Pruebas de función pulmonar

Con un pletismógrafo (Elite Series™ Plethysmograph, MedGraphics) se llevaron a cabo las pruebas siguientes: volumen espiratorio forzado en el primer segundo (FEV_1), la capacidad vital forzada (FVC) y la ratio FEV_1/FVC. La valoración de las pruebas se hizo conforme a las directrices de la American Thoracic Society/European Respiratory Society[12]. Los valores de referencia empleados fueron los de la población brasileña[13].

Medición de las comorbilidades

Se usó el índice de comorbilidades de Charlson ajustado por edad para calcular la carga total de comorbilidades. Dicho índice incluye 19 enfermedades médicas con sus pesos correspondientes. La ponderación y la puntuación de las comorbilidades se llevó a cabo empleando un algoritmo propuesto por Charlson et al.[14].

Medición de los biomarcadores

La sangre venosa periférica se extrajo de la vena antecubital tras 10 h de ayuno nocturno. La sangre se recogió en tubos y se centrifugó a 3.000 rpm durante 10 min para separar los componentes sanguíneos (plasma, suero y hematocrito). Los niveles de biomarcadores de factor riesgo metabólico (glucosa, colesterol total, lipoproteínas de alta densidad [c-HDL], lipoproteínas de baja densidad [c-LDL], triglicéridos) fueron analizados según las técnicas de laboratorio estándar en un máximo de 4 h tras la extracción.

Los tubos para los biomarcadores de EO y los parámetros de defensa antioxidante se almacenaron en un congelador a −80 °C hasta completar toda la recogida de sangre. Se midieron como biomarcadores oxidativos los siguientes: productos de oxidación avanzada a proteínas (AOPP)[15] e hidroperóxido lipídico total (LOOH)[16], además de la CAT[17]. Como marcadores antioxidantes se analizaron los grupos sulfhidrilos (SH)[18] y la PON1[19]. Todos los biomarcadores se midieron en ensayos por triplicado en el Laboratorio de Postgrado del Hospital Universitario de la Universidad Estatal de Londrina, Brasil, siguiendo los protocolos descritos en estudios anteriores[15–19]. Todos los biomarcadores seleccionados habían sido estudiados previamente en pacientes con EPOC[20], pero no su relación con el diagnóstico de SM y la función pulmonar.

Criterios de síndrome metabólico

Se diagnosticó el SM conforme a los criterios de la American Heart Association/National Heart, Lung, and Blood Scientific Statement[21]: glucosa en ayunas alta ≥ 100 mg/dl, c-HDL < 40 mg/dl para hombres y < 50 mg/ml para mujeres, niveles de triglicéridos ≥ 150 mg/dl, presión sanguínea sistólica y diastólica ≥ 130 y ≥ 85 mmHg, respectivamente, y circunferencia de la cintura ≥ 102 cm en hombres y ≥ 88 cm en mujeres.

Análisis estadístico

Para el análisis estadístico se utilizó el SPSS® 19.0 (IBM Co., Armonk, NY, EE. UU.). Todos los datos se expresaron como media ± DE. Para analizar la normalidad de los datos se utilizó la prueba de Shapiro-Wilk. La comparación entre los 4 grupos estudiados se llevó a cabo mediante el análisis de varianza de una sola vía (ANOVA), con post-hoc de Bonferroni para los datos paramétricos y prueba de Kruskal-Wallis para los no paramétricos, con intervalos de confianza del 95% (IC 95%). Se usó el coeficiente de correlación de Pearson para evaluar las relaciones entre las variables de la función pulmonar y los biomarcadores de EO. Para evaluar las asociaciones entre los biomarcadores de EO y la presencia de SM y la gravedad de la EPOC (GOLD ≥ 3) se usó una curva de regresión logística. La significación estadística se fijó en un valor de $p < 0,05$. Por último, como era un muestreo de conveniencia, el análisis de potencia post-hoc se verificó utilizando el software GPower 3.1 (Franz Faul, Universität Kiel, Alemania).

Resultados

En este estudio se incluyeron 74 individuos, de los cuales 39 tenían EPOC, repartiéndose entre los grupos EPOC con SM (EPOC-SM, n = 11) y EPOC sin SM (EPOC, n = 28); los 35 individuos restantes se incluyeron en el grupo control, distribuyéndose en los grupos control con SM (control-SM, n = 9) y control sin SM (control, n = 26). En la tabla 1 se detallan las características sociodemográficas, el índice de comorbilidades de Charlson ajustado por edad y los factores de riesgo metabólico. No hubo diferencias entre grupos en cuanto a edad, sexo e IMC. El índice de comorbilidades de Charlson ajustado por edad fue significativamente superior en los subgrupos EPOC y EPOC-SM en comparación con los control ($p = 0,025$ y $p = 0,006$, respectivamente). El FVC% previsto fue menor en EPOC y EPOC-SM que en los controles ($p < 0,001$ para ambos). Como se esperaba, el FEV_1% previsto fue inferior en los sujetos con EPOC en comparación con sus equivalentes ($p < 0,05$ para todos). Los niveles de c-HDL fueron más altos en los individuos con EPOC que los de los pertenecientes a los grupos EPOC-SM y control-SM ($p < 0,05$ para todos).

En la figura 1 se reflejan los biomarcadores antioxidantes. Los niveles de CAT fueron menores en EPOC que en control ($p = 0,003$). Los niveles de SH fueron superiores en EPOC y EPOC-SM que en control ($p < 0,05$ para todos). Los biomarcadores oxidantes se muestran en la figura 2. Los niveles de AOPP fueron menores en EPOC que en EPOC-SM y control-SM ($p < 0,05$ para todos).

Los biomarcadores de EO y las variables de función pulmonar se correlacionaron únicamente en los sujetos con EPOC-SM. En este grupo, los AOPP se relacionaron negativamente con el FEV_1% previsto y FEV_1/FVC ($r = -0,68$ y $-0,56$, respectivamente; $p < 0,05$ para ambos). La correlación SH con FEV_1% previsto y FEV_1/FVC fue positiva ($r = -0,52$ y $0,77$, respectivamente; $p < 0,05$ para ambos). La CAT se correlacionó negativamente con FEV_1/FVC ($r = -0,61$; $p < 0,05$).

La tabla 2 muestra el análisis de regresión logística entre los factores asociados con la gravedad de la EPOC (GOLD 3 y 4) en los sujetos con EPOC. En un análisis univariante, la

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Tabla 1 Características clínicas de los grupos EPOC y control con y sin SM

Variable	EPOC-SM (n = 11) Grupo a	EPOC (n = 28) Grupo b	Control-SM (n = 9) Grupo c	Control (n = 26) Grupo d
Edad (años)	66,5 ± 6	69,8 ± 6,6	68,5 ± 8	68,6 ± 5,9
Mujer, n (%)	4 (37)	12 (60)	3 (34)	12 (46)
IMC (kg/m²)	30,2 ± 3,5	26 ± 5,7	29,2 ± 6,2	26,9 ± 7,2
ACCI (puntuación)	4,55 ± 1,2*d	4,3 ± 1,3*d	4,3 ± 1,3*d	3,9 ± 1,3*a,b,c
FVC% previsto	82 ± 16*d	88 ± 21*d	98 ± 18	111 ± 15*a,b
FEV_1% previsto	46 ± 13*c,d	49 ± 15*c,d	90 ± 17*a,b	102 ± 15*a,b
FEV_1/FVC	46 ± 9*c,d	47 ± 10*c,d	74 ± 5*a,b	76 ± 5*a,b
GOLD 1-2, n (%)	4 (36)	14 (50)	-	-
GOLD 3-4, n (%)	7 (64)	14 (50)	-	-
Biomarcadores de los factores de riesgo metabólico				
Glucosa (mg/dl)	127,7 ± 23,2	111,3 ± 41,8	144,6 ± 88,2	119,6 ± 43
Colesterol (mg/dl)	203,8 ± 39,9	200,5 ± 45	189 ± 58,2	193,8 ± 37,9
c-LDL (mg/dl)	123,4 ± 42,8	112,1 ± 36,7	99,3 ± 39,4	114,2 ± 34,2
c-HDL (mg/dl)	42,7 ± 9,7*b	67,5 ± 20*a,c	52,3 ± 9,11*b	54,3 ± 13,3
Triglicéridos (mg/dl)	186,1 ± 75,6	109,6 ± 84,4	155,7 ± 84,4	124,3 ± 72

ACCI: índice de comorbilidades de Charlson ajustado por edad; c-HDL: colesterol unido a lipoproteínas de alta densidad; c-LDL: colesterol unido a lipoproteínas de baja densidad; EPOC: Enfermedad pulmonar obstructiva crónica; FEV_1: volumen espiratorio forzado en el primer segundo; FVC: capacidad vital forzada; GOLD: Iniciativa Global para la Enfermedad Pulmonar Obstructiva Crónica; IMC: índice de masa corporal; SM: síndrome metabólico.
Los datos se expresan como media ± desviación estándar, excepto donde se indica.
* $p \leq 0,05$.
a,b,c,d Estadísticamente significativo entre grupos ($p < 0,05$) expresada con las letras a b c y d (EPOC-SM, EPOC, control-SM y control, respectivamente).

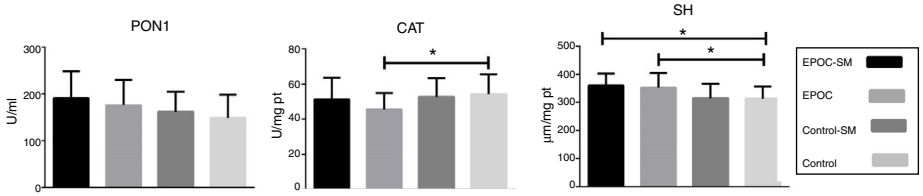

Figura 1 Comparación de biomarcadores antioxidantes entre EPOC y grupo control con y sin SM. Los datos se expresan como media ± desviación estándar.
CAT: catalasa; EPOC: enfermedad pulmonar obstructiva crónica; PON1: paraoxonasa 1; SH: grupo sulfhidrilo; SM: síndrome metabólico. *Estadísticamente significativo ($p \leq 0,05$).

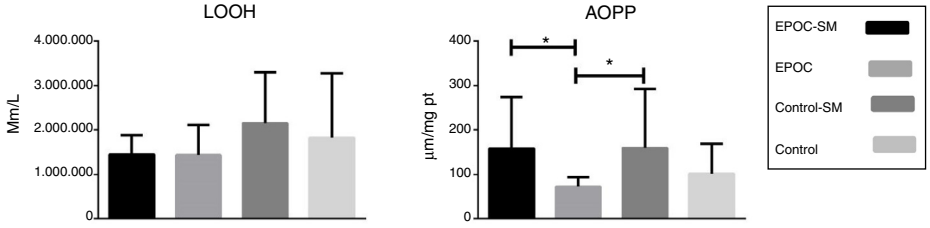

Figura 2 Comparación de los biomarcadores oxidantes entre EPOC y grupo control con y sin SM. Los datos se expresan como media ± desviación estándar.
AOPP: productos de la oxidación avanzada de las proteínas; EPOC: enfermedad pulmonar obstructiva crónica; LOOH: hidroperóxidos lipídicos totales; SM: síndrome metabólico. *Estadísticamente significativo ($p \leq 0,05$).

edad, el IMC, los AOPP, la CAT y el SH se asociaron con la gravedad de la EPOC ($p < 0,10$), y fueron tenidos en cuenta para el análisis multivariante. El SH ($p = 0,036$), el IMC ($p = 0,008$) y el AOPP ($p = 0,037$) se asociaron con la gravedad de la EPOC.

La tabla 3 muestra el análisis de regresión entre factores asociados con la presencia de SM en sujetos con EPOC. En un análisis univariante, los AOPP se asociaron con la presencia de SM en individuos con EPOC ($p < 0,05$). Para el análisis multivariante, se tuvieron en cuenta otras variables asociadas

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Tabla 2 Características asociadas a la gravedad de la EPOC (GOLD ≥ 3)

Variable	Análisis univariante OR (IC 95%)	Análisis multivariante OR (IC 95%)
Edad (años)	1,07 (0,97-1,18)**	
Sexo (mujer)	1,18 (0,32-4,250)	-
IMC (kg/m^2)	0,90 (0,79-1,02)**	0,82 (0,66-0,97)*
Historial de tabaquismo	1,9 (0,28-12,89)	-
SM	1,75 (0,41-7,345)	-
ACCI (puntuación)	1,12 (0,67-1,8)	-
AOPP (μM/l)	1,01 (0,993-1,03)**	1,03 (1,002-1,05)*
LOOH (Mm/l)	1 (1,0-1,001)	-
CAT (U/mgHb)	1,04 (0,97-1,15)**	-
SH (mM/mg proteína)	0,99 (0,98-1,005)**	0,98 (0,96-1,001)*
PON1 (U/ml)	0,99 (0,99-1,01)	-

ACCI: índice de comorbilidades de Charlson ajustado por edad; AOPP: productos de oxidación avanzada de proteína; CAT: catalasa; GOLD: Iniciativa Global para la Enfermedad Pulmonar Obstructiva Crónica; IC 95%: intervalo de confianza del 95%; IMC: índice de masa corporal; LOOH: hidroperóxido de lípidos totales; OR: odds ratio; PON1: paraoxonasa-1; SH: grupo sulfhidrilo; SM: síndrome metabólico.
* $p < 0,05$.
** $p < 0,10$.

Tabla 3 Características asociadas con el síndrome metabólico en la EPOC

Variable	Análisis univariante OR (IC 95%)	Análisis multivariante OR (IC 95%)
Edad (años)	0,913 (0,809-1,03)**	-
Sexo (mujer)	3,7 (0,674-20,69)	-
IMC (kg/m^2)	1,166 (0,99-1,37)**	-
Historial de tabaquismo	1,67(0,165-16,8)	-
GOLD (≥ 3)	1,4 (0,325-6,027)	-
ACCI (puntuación)	1,165 (0,65-2,09)	-
AOPP (μM/l)	1,04 (1,006-1,07)*	1,05 (1,004-1,100)*
LOOH (Mm/l)	1 (1,0-1001)	-
CAT (U/mgHb)	1,075 (0,99-1,17)**	-
SH (mM/mg proteína)	1,007 (0,99-1,022)	-
PON1 (U/ml)	1,004 (0,99-1,018)	-

ACCI: índice de comorbilidades de Charlson ajustado por edad; AOPP: productos de oxidación avanzada de proteína; CAT: catalasa; GOLD: Iniciativa Global para la Enfermedad Pulmonar Obstructiva Crónica; IC 95%: intervalo de confianza del 95%; IMC: índice de masa corporal; LOOH: hidroperóxido de lípidos totales; OR: odds ratio; PON1: paraoxonasa-1; SH: grupo sulfhidrilo.
* $p < 0,05$.
** $p < 0,10$.

con el SM (edad, IMC y CAT; $p < 0,10$). Este análisis multivariante, que tuvo en cuenta la edad, el IMC, los AOPP y la CAT como variables explicativas de la prevalencia del SM en la EPOC, reveló que los AOPP fueron los únicos que mostraron una asociación positiva y significativa con el SM en este grupo. En los individuos con EPOC, la posibilidad de tener un SM aumenta un 5% por cada incremento de los AOPP (μM/l) ($p = 0,04$). Se completó un análisis estadístico similar para los grupos de control, sin embargo, no se encontraron asociaciones significativas.

Discusión

El presente estudio añade al campo de estudio que: 1) la respuesta antioxidante está aumentada en el grupo de EPOC con y sin SM; 2) que solamente en el grupo EPOC-SM los antioxidantes están correlacionados con la función pulmonar; 3) que la oxidación proteica es mayor en el grupo EPOC-SM que en el EPOC sin SM, y 4) que el EO está asociado con la peor función pulmonar, la gravedad de la EPOC y la existencia de SM.

Nuestro estudio mostró que la prevalencia de SM era del 28% en sujetos con EPOC y del 25% en el grupo control. Estos resultados están en consonancia con los de otros estudios[21,22]. Una revisión sistemática puso de manifiesto que la prevalencia del SM oscila entre el 21 y el 58% en individuos con EPOC frente al 17-54% en los sujetos sin EPOC[5]. En el presente estudio se observó una prevalencia más alta de SM en individuos con EPOC grave y muy grave; así, el 36% de los casos de EPOC con SM se clasificaron como GOLD 1-2 y el 64% como GOLD 3-4. Díez-Manglano et al.[23] encontraron resultados semejantes; ellos observaron que el 60% de los sujetos clasificados como EPOC grave y muy grave fueron diagnosticados de SM. Además, en nuestro estudio se diagnosticaron más comorbilidades en los individuos con EPOC-SM y EPOC sin SM en comparación con los grupos control. Estos hallazgos concuerdan con los de otros estudios

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que han descrito la prevalencia de otras comorbilidades en pacientes con EPOC. Dichas comorbilidades se han asociado, en esta población, a una peor calidad de vida y pronóstico y a una mayor frecuencia de exacerbaciones[23–26].

En cuanto a los biomarcadores antioxidantes, se investigaron PON1, CAT y SH. La PON1 es una enzima antioxidante ligada al c-HDL en sangre periférica, habiéndose descrito que su actividad está disminuida en pacientes con EPOC[27]. Aunque no se detectó diferencia en la actividad de la PON1, sí observamos menores niveles de c-HDL en EPOC-SM frente a EPOC, lo que es parecido a lo descrito en otro estudio[28]. En este mismo sentido, las disminuciones de los niveles de c-HDL tienen un impacto clínico sobre la inflamación y el EO[29], ya que las HDL juegan un papel importante como un biomarcador antiinflamatorio y antioxidante[30].

La CAT es un antioxidante importante que desempeña un papel en la eliminación del H_2O_2 y su actividad previene los efectos dañinos del EO[31]. Como se observó en el presente estudio, también se encontraron niveles más bajos de CAT en un estudio con 202 pacientes con EPOC en comparación con 136 controles sanos[32]. Además, la CAT se relacionó negativamente con el cociente FEV_1/FVC en EPOC-SM ($r = -0,61$; $p < 0,05$) y dichos valores fueron incluso mayores a los descritos por Anes et al.[33] ($r = -0,39$). Una explicación posible es que los sujetos con SM tienen unos niveles de especies reactivas de oxígeno más altos[29], lo que puede dar lugar al daño epitelial en el pulmón y a una reducción de la función pulmonar, lo que puede traducirse en un aumento en la actividad de la CAT como respuesta compensatoria[31,32].

El radical SH es otro biomarcador antioxidante[34]. Hemos encontrado que el SH es un factor protector de la gravedad de la EPOC, reduciendo la prevalencia de los GOLD 3 y 4 en un 2% por cada unidad de SH (OR 0,98; IC 95% 0,96-1,001; $p = 0,036$). También se encontró relación del SH con el FEV_1% previsto ($r = 0,52$) y con la ratio FEV_1/FVC ($r = 0,77$) en los pacientes con EPOC-SM. En este contexto, Zinellu et al.[34] observaron que el grupo de proteína SH se correlacionaba positivamente con la función pulmonar en sujetos con EPOC; sin embargo, no compararon individuos con y sin SM. Más aún, no se ha descrito ninguna evidencia sobre la asociación del SH o de otro biomarcador antioxidante con la función pulmonar en EPOC-SM. Al estar relacionado este síndrome con la inflamación y el EO, podemos suponer que podría interferir en la capacidad antioxidante y en la función pulmonar de dichos pacientes.

Otro aspecto clave relacionado con la capacidad antioxidante fue que en pacientes con EPOC los niveles de SH eran más altos que en el grupo control. Este resultado se ha descrito en otros estudios previos[33,35] como una respuesta compensatoria para el exceso de oxidantes, que da lugar a niveles aumentados de glutatión en individuos con EPOC. A este respecto, el glutatión es uno de los componentes orgánicos que contiene los grupos tioles de proteínas, las cuales están compuesta por radicales SH[36]. Las proteínas tiol reducen el H_2O_2 y los LOOH[37]. Aunque los niveles de SH fueron mayores en los individuos con EPOC frente al grupo control, en nuestro estudio no se observaron diferencias significativas en cuanto a los niveles de LOOH.

Se observó que los niveles de proteínas de oxidación, medidos como AOPP, estaban aumentados en la EPOC con SM en comparación con la EPOC sin SM[15]. Ben Anes et al.[33] comunicaron unos resultados parecidos en esta enfermedad, sin embargo, su estudio no comparó a los pacientes con y sin SM. Más aún, los biomarcadores de EO se han investigado poco en EPOC con SM. Por tanto, no hay pruebas de investigación de los niveles AOPP en esta población con SM, en contraste con sujetos aparentemente sanos[38]. En nuestro estudio, los sujetos del grupo control con SM mostraron unos niveles más altos de estos biomarcadores en comparación con el grupo control sin SM. A este respecto, la evidencia mostró que los niveles de AOPP eran considerados como el determinante de SM más importante en ancianos[38]. Paralelamente, también encontramos esta asociación con AOPP en individuos con EPOC, lo que fue el factor más importante relacionado con la prevalencia del SM en EPOC, aumentando esta en el 5% por cada unidad de AOPP ($R^2 = 0,57$; OR 1,05; IC 95% 1,004-1,100; $p = 0,04$). Por tanto, esto puede apoyar el hecho de que los AOPP sean los principales factores asociados con el SM en la EPOC, y también se correlacionó con una peor función pulmonar en EPOC-SM (FEV_1%pred: $r = -0,68$ y FEV_1/FVC: $r = -0,56$) y con la gravedad de la EPOC (OR 1,03; IC 95% 1,002-1,05; $p = 0,037$).

Por lo tanto, teniendo en cuenta que el SM en la EPOC se ha asociado con una peor función pulmonar[26] y un peor pronóstico en individuos con EPOC[23–25], los nuevos hallazgos obtenidos son importantes en la literatura y en la práctica clínica, pues demuestran que la función pulmonar, la gravedad de la EPOC y la prevalencia del SM pueden estar relacionados con biomarcadores antioxidantes y oxidantes en pacientes con EPOC. De manera más breve, nuestro estudio investigó que la etiología de varias implicaciones clínicas puede estar asociada con biomarcadores antioxidantes y oxidantes.

Por último, el presente estudio tiene algunas limitaciones. Primera, la muestra de cada grupo fue pequeña, no igual y el muestreo fue de conveniencia; sin embargo, nuestro tamaño de muestra fue mayor que el de otros estudios[32,38]. Además, se encontraron fuertes correlaciones entre las variables estudiadas, y un cálculo de potencia retrospectivo reveló que la potencia del tamaño de la muestra del estudio era del 95% (alfa = 0,05) para detectar la diferencia de biomarcadores de estrés oxidativo entre los grupos (tamaño del efecto = 0,49). Segundo, fue un estudio observacional de corte transversal, por lo que la causalidad de las asociaciones debería ser interpretada con cuidado. A pesar de esto, las fortalezas del estudio fueron que proporcionaba nueva información al campo del EO en la EPOC con SM. Además, los grupos fueron controlados por los posibles efectos de las variables de confusión que pueden interferir en los biomarcadores del EO, como la ingesta de alcohol y el uso de suplementos antioxidantes. El diagnóstico del SM se hizo de acuerdo con los criterios internacionales[21].

Conclusión

En conclusión, en esta enfermedad el estrés oxidativo se asocia con la gravedad de la EPOC, una peor función pulmonar y la presencia de SM. Además, se observó una actividad antioxidante aumentada en los sujetos con EPOC frente a los del grupo control. En EPOC con SM, los biomarcadores de SO solo se correlacionaron con la función pulmonar. Finalmente, los sujetos de EPOC con SM mostraron unos niveles de proteínas de la oxidación más altos que los de EPOC sin SM.

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Financiación

Consejo Nacional de Desarrollo Científico y Tecnológico (CNPq) de Brasil. Identificación del financiamiento: 470853/2014; CNPq/MS/SCTIE/DECIT N.° 34/2014.

Conflicto de intereses

Los autores declaran que no tienen ningún conflicto de intereses.

Agradecimientos

Los autores agradecen al Consejo Nacional de Desarrollo Científico y Tecnológico (CNPq) de Brasil el apoyo financiero.

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Cómo citar este artículo: Sepúlveda Loyola WA, et al. ¿Está el estrés oxidativo asociado a la gravedad de la enfermedad, a la función pulmonar y al síndrome metabólico en la enfermedad pulmonar obstructiva crónica? Rev Clin Esp. 2019. https://doi.org/10.1016/j.rce.2019.04.007

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Cómo citar este artículo: Sepúlveda Loyola WA, et al. ¿Está el estrés oxidativo asociado a la gravedad de la enfermedad, a la función pulmonar y al síndrome metabólico en la enfermedad pulmonar obstructiva crónica? Rev Clin Esp. 2019. https://doi.org/10.1016/j.rce.2019.04.007

JAMDA xxx (2019) 1–6

JAMDA

journal homepage: www.jamda.com

Original Study

The Joint Occurrence of Osteoporosis and Sarcopenia (Osteosarcopenia): Definitions and Characteristics

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Keywords:
Aging
falls
fracture
osteoporosis
osteosarcopenia
physical performance
sarcopenia

A B S T R A C T

Objectives: We sought to examine the associations of osteosarcopenia with physical performance, balance, and falls and fractures in community-dwelling older adults. Additionally, we aimed to determine which clinical outcomes are associated with specific components of osteosarcopenia.
Design: Cross-sectional study.
Setting and Participants: 253 participants (77% women; aged 77.9 ± 0.42 years) who presented for a falls and fractures risk assessment in Melbourne, Australia.
Methods: Participants were mobile, community-dwelling older adults (≥65 years) free of cognitive impairment. Body composition (via dual-energy x-ray absorptiometry), physical performance [via Timed Up and Go (TUG) and Short Physical Performance Battery (SPPB)], and balance [via Four-Square Step test (FSS) and posturography] were examined. Falls in the past year and fractures in the past 5 years were self-reported. Osteosarcopenia was defined as (1) low bone mineral density (BMD) [T score <−1 standard deviation (SD)] combined with sarcopenia and (2) osteoporosis (BMD T score ≤−2.5 SD) combined with severe sarcopenia. For sarcopenia, we employed the criteria of the European Working Group on Sarcopenia in Older People (EWGSOP1), the revised criteria (EWGSOP2), and that of the Foundation for the National Institutes for Health (FNIH). Kruskal-Wallis and logistic regression tests were used for statistical analysis.
Results: Osteosarcopenia was associated with worse SPPB, TUG, FSS, limit of stability, and falls and fractures history. Additionally, osteosarcopenia (using the severe sarcopenia classification) conferred an increased rate of falls [odds ratios (ORs) from 2.83 to 3.63; $P < .05$ for all] and fractures (ORs from 3.86 to 4.38; $P < .05$ for all) when employing the EWGSOP2 and FNIH definitions, respectively.
Conclusions and Implications: Compared with the nonosteosarcopenic group, those with osteosarcopenia had greater impairment of physical performance and balance. The EWGSOP2 and FNIH criteria resulted in the strongest associations with physical performance and self-reported falls and fractures.

Osteosarcopenia is defined as the concomitant presence of osteoporosis or osteopenia combined with sarcopenia.[1,2] This new geriatric syndrome is associated with higher disability and rates of fracture and falls in older people[3,4] compared with either disease alone.[5]

Sarcopenia has recently been recognized as a disease in the International Disease Classification—10th Revision—Clinical Modification (ICD-10-CM) in 2016,[6] emphasizing the importance of this debilitating musculoskeletal condition that can increase the risk of disability and mortality in older people.[7] Severe sarcopenia in particular, defined as the concurrent presence of low muscle mass,

W.S.L. and S.P. contributed equally to this work.

This work was supported by a Visiting Fellow Grant from the Australian Institute for Musculoskeletal Science (AIMSS) to B.K. E.B.H. holds an Australian Medical Research Frontiers Fund (MRFF: MACH-RART scheme 2019) Fellowship. S.B.O. is supported by a National Health and Medical Council (NHMRC, of Australia) Career Development Fellowship (1107510). W.S.L. is supported by the Brazilian National Council for Scientific and Technological Development (CNPq). R.C. is supported by a PhD scholarship from the "Studienstiftung des deutschen Volkes."

The authors declare no conflicts of interest.

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https://doi.org/10.1016/j.jamda.2019.09.005
1525-8610/

physical performance, and strength,[8] may result in additional adverse outcomes including an increased risk of falls.[9] Furthermore, there is increasing evidence of crosstalk between muscle and bone.[2] In the case of sarcopenia, greater systemic inflammation and oxidative stress have been found, both of which are related to reduced bone mineral density (BMD).[10–12] With regard to osteopenia and osteoporosis, both are strongly associated with fracture risk as well as frailty,[13] resulting in declines in quality of life and increased mortality, particularly in those who experience hip fractures.[14]

Given the clinical outcomes associated with each of the 2 components of osteosarcopenia, the diagnosis of osteosarcopenia is imperative to enable clinical care.[15,16] Although the diagnostic criteria of osteopenia or osteoporosis are well established[13,17]; sarcopenia lacks an international consensus.[8,18–20] In addition, existing definitions of sarcopenia have shown minimal agreement.[21,22] This may impact the diagnosis and prevalence of osteosarcopenia, and the prioritization of interventions and maintenance of health outcomes.

At present, there is a paucity of data investigating the impact of osteosarcopenia (or severe sarcopenia alone) on physical performance in a population of high-risk community-dwelling older adults. As such, this study aims to (1) determine the associations between osteosarcopenia and clinical outcomes and (2) examine the association of severity of bone (osteopenia or osteoporosis) and muscle (sarcopenia vs severe sarcopenia) with these clinical outcomes in community-dwelling older adults. We hypothesized that osteosarcopenia is associated with worse physical function, balance, falls, and fractures, and that its impact increases when osteosarcopenia is diagnosed using osteoporosis combined with severe sarcopenia.

Methods

Study Population

This is a cross-sectional study of older adults (≥65 years old) who attended an assessment for falls and fracture risk at a clinic in Melbourne (Victoria, Australia). Inclusion criteria were as follows: ability to mobilize independently or with the use of gait aids, Mini-Mental State Examination score (>18/30), risk or history of falls and fractures (determined by general practitioner), and willingness to attend the clinic (the participation was voluntary). This study was approved by the Western Health local Human Ethics Research Committee (DB2017.13 and QA2018.80-46205).

Physical Performance

Physical performance was evaluated by handgrip strength, gait speed, Timed Up and Go (TUG), 5 times sit-to-stand test, and Short Physical Performance Battery (SPPB). Handgrip strength was assessed in a seated position using a Jamar hydraulic dynamometer (Sammons Preston Inc, Saint Paul, MN) with the forearm and wrist resting on the chair arms. Participants performed 3 trials on each hand, alternating between them, with a rest of 30 seconds between tests. The highest value was recorded and used for analysis.[23]

Gait speed was measured using the GAITRite system (CIR Systems Inc, Havertown, PA) with the mat measuring 580 cm × 89 cm × 0.625 cm, and a sample rate of 120 Hz. Participants were instructed to walk at their usual gait speed, as previously reported,[24] with the fastest of 2 attempts used for analysis.

The TUG evaluated the time it takes to stand up from a chair, walk 3 m, turn, walk back to the chair, and sit down again at normal pace.[25]

Standardized operating procedures were followed for the SPPB,[26] which consisted of 3 timed components: standing balance, 4-m gait speed, and time to complete 5 chair-stands. Participant scores for each component were totaled between 0 and 12 and used for analysis. Poor performance was defined as TUG ≥ 20 seconds, SPPB ≤8 points, and 5 times sit-to-stand test score > 15 seconds.[18]

Dynamic and Static Balance

Dynamic balance was measured by Four-Square Step test (FSS) following validated guidelines.[27] Poor dynamic balance was defined as FSS score > 15 seconds.[24,27]

Static balance was assessed using the Balance Rehabilitation Unit (BRU; Medicaa, Uruguay), which provided results for limits of stability, center of pressure area, and sway velocity for 6 different conditions: (1) eyes opened on hard surface; (2) eyes closed on hard surface; (3) eyes closed on foam; (4) eyes opened with saccadic stimulation; (5) eyes opened with horizontal vestibular stimulation; and (6) eyes opened with vertical vestibular stimulation. Visual stimulus was displayed using 3D virtual-reality goggles with moving objects (conditions 4-6). Each condition lasted for 1 minute. To measure limits of stability, participants were instructed to shift their weight in anteroposterior and lateral directions using the ankle strategy for balance (without moving the feet or bending at the hip). Poor static balance was considered as limits of stability <170 cm^2.[24]

Self-reported falls (in the past year) and fractures (in the past 5 years), body mass index, and Charlson Age-Comorbidity Index were also assessed.[28]

Diagnosis of Osteosarcopenia

Cut-points used to diagnose osteosarcopenia are presented in Table 1. Several osteosarcopenia categories were created depending on the definition of sarcopenia employed. Osteosarcopenia was detected with the combination of low BMD and sarcopenia.[2] Low BMD was defined as a T score <−1 standard deviation according to World Health Organization criteria for osteopenia.[17] As a result, osteoporotic participants were included in this category. Sarcopenia was defined as low appendicular lean mass plus low muscle strength (LMS) or low physical performance (LPP).[8,18–20] The following definitions of sarcopenia were used: European Working Group on Sarcopenia in Older People (EWGSOP1), its revised criteria (EWGSOP2), and the Foundation for the National Institutes for Health (FNIH).[8,18,19]

For the second objective of this study, we selected a subgroup of osteoporotic participants using a T score of ≤−2.5 standard deviation and combined this with a severe sarcopenia group who fulfilled all 3 criteria for sarcopenia (low appendicular lean mass, LMS, and LPP).

Table 1
Different Cut-points Used to Identify Osteosarcopenia

Osteosarcopenia Definition	Low BMD	Low ALM		Low MS		Low PP
EWGSOP1	T score <−1	ASMI: <7.23 for men and <5.67 for women	AND	HGS: <30 kg for men and <20 kg for women	OR	GS ≤ 0.8 m/s
EWGSOP2		ASM: ≤20 kg for men and ≤15 kg for women		HGS: <27 kg for men and <16 kg for women		
FNIH		ASM/BMI: <0.789 for men and for <0.512 women		HGS: <26 kg for men and <16 kg for women		

ASM, appendicular skeletal mass; ASMI, appendicular skeletal mass index; BMI, body mass index; GS, gait speed; HGS, handgrip strength; MS, muscle strength; PP, physical performance.

Finally, we performed an analysis excluding measurements of lean mass (ie, only including those presenting with low BMD, LMS, and LPP) to examine the association of lean mass measures and clinical outcomes. Appendicular lean mass and areal BMD at 3 sites (lumbar spine, total hip, and femoral neck) were assessed using a Hologic Horizon dual-energy x-ray absorptiometry machine (Bedford, MA).

Statistical Analysis

Statistical analysis was performed using SPSS version 25 (SPSS, IBM Corp, Armonk, NY). Data are expressed as frequency and percentage, mean ± standard deviation, or median and interquartile range. The Kolmogorov-Smirnov test was used to analyze normality of data distribution. The McNemar test was used to compare the prevalence among the definitions of osteosarcopenia. Logistic regression was used to determine associations between the groups (1) BMD <-1 + sarcopenia, (2) BMD ≤ -2.5 + sarcopenia, (3) BMD <-1 + severe sarcopenia, (4) BMD ≤ -2.5 + sarcopenia, (5) BMD <-1 + LPP and LMS, and (6) BMD ≤ -2.5 + LPP and LMS with worse physical performance, dynamic and static balance, and falls and fracture history. The logistic regression was adjusted for age, gender, and Charlson Age-Comorbidity Index. Statistical significance was set as $P < .05$.

Results

A total of 253 individuals were included in this study (age: 77.9 ± 0.42 years; women 77%; body mass index 28.3 ± 6). For BMD, 14 (6%), 69 (27%), and 158 (62%) were classified as having normal BMD, osteopenia and osteoporosis, respectively (Table 2). Mean Charlson Age-Comorbidity Index was 4.6±1.8 points, with 45% presenting with a score ≥5 points. When assessing fall and fracture history, 130 (54%) participants presented with ≥2 falls in the past year and 37 (13%) reported ≥2 fractures.

Prevalence of Osteosarcopenia

Depending on the definition, the prevalence of osteosarcopenia varied between 11% and 21% (Figure 1). We observed significant differences among EWGSOP2 (21%) and FNIH (20%) compared with EWGSOP1 (11%) with divergences due to ALM ($P < .05$ for all). When using a T score of ≤ -2.5 combined with sarcopenia, the prevalence of osteosarcopenia was reduced and it ranged from 8% to 19%. We observed a difference among EWGSOP2 (19%) and FNIH (14%) compared to EWGSOP1 (8%) ($P < .05$ for all).

Prevalence of Osteosarcopenia in Those With Severe Sarcopenia

The prevalence of osteosarcopenia with a severe sarcopenia component for various definitions is reported in Figure 1. When using a BMD T score of < -1 and severe sarcopenia, prevalence varied from 9% to 11%. For a BMD T score of ≤ -2.5 combined with severe sarcopenia, the prevalence ranged from 5% to 9%. No significant differences between the prevalences were observed.

Associations Between Osteosarcopenia and Outcome Measures

Osteosarcopenia diagnosed using EWGSOP2 was associated with worse physical performance and falls, regardless of T score [odds ratio (OR) 0.49-3.07; $P < .05$ for all]. When based on the FNIH criteria, osteosarcopenia was associated with worse physical performance and dynamic balance only when it was combined with a T score of < -1 (OR 3.59-4.84; $P < .05$ for all). No significant associations were found with static balance. Individuals diagnosed with sarcopenia (EWGSOP1 and EWGSOP2) presented with a lower association with multiple falls when it was combined with a T score <-1 (Table 3).

Table 2
Clinical Characteristics of Study Participants

Variables	Sample Size	Mean ± SD or n (%)
Age, y	253	77.9 ± 0.42
Women, n (%)	253	196 (77)
ASM, kg/m^2	242	16.8 ± 4.2
ASMI	242	6.6 ± 1.2
Femoral T score	223	−2.11 ± 1.03
Lumbar T score	231	−1.19 ± 1.6
Arm T score	134	−2.23 ± 1.58
CACI total, score	234	4.6 ± 1.8
Number of falls in the last year	250	
0		10 (4)
1		106 (42)
>1		134 (54)
Number of fractures	246	
0		42 (17)
1		162 (66)
>1		42 (17)
BMI classifications	253	
<18.5		7 (3)
18.5-25		76 (30)
25-30		84 (33)
≥30		86 (34)
BMI		28.3 ± 6
Physical performance		
Handgrip strength, kg	251	22.39 ± 7.9
5-times sit-to-stand time, s	248	16.6 ± 6.8
Gait speed, m/s	247	0.73 ± 0.29
Timed Up and Go test score, s	240	17.9 ± 8.1
Short Physical Performance Battery score	247	7 ± 2.7
Dynamic balance		
Four Square Step test, s	120	19.1 ± 7.9
Static balance		
Limits of stability, cm^2	222	113.2 ± 65
Eyes open on hard surface		
CoP area, cm^2	222	6.9 ± 5.9
Oscillation, cm/s	222	1.7 ± 0.92
Eyes closed on hard surface		
CoP area, cm^2	199	7.6 ± 7
Oscillation, cm/s	199	2.3 ± 1.4
Eyes closed on foam		
CoP area, cm^2	110	25.4 ± 19.6
Oscillation, cm/s	110	4.6 ± 1.9
Saccadic dual task		
CoP area, cm^2	189	7.3 ± 8.2*
Oscillation, cm/s	189	2.6 ± 1.5
Horizontal vestibular interaction		
CoP area, cm^2	173	13.3 ±12.9
Oscillation, cm/s	173	3.7 ± 2.2
Vertical vestibular interaction		
CoP area, cm^2	166	11.3 ± 10.4
Oscillation, cm/s	166	3.6 ± 2.0

ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; BMI, body mass index; CACI, Charlson Age-Comorbidity Index; CoP, center of pressure; SD, standard deviation.

Larger Associations Between Osteosarcopenia With a Severe Sarcopenia and Outcome Measures

Regardless of definition, there was a significant association or trend toward association between osteosarcopenia with severe sarcopenia, SPPB, and TUG performance (OR 2.56-7.19; $P < .05$ for all). No significant associations were evident for 5 times sit-to-stand test time. When using EWGSOP2 criteria, there was an association with multiple falls irrespective of T score (OR 2.83-3.63; $P < .05$ for all). Osteosarcopenia with severe sarcopenia according to FNIH was significantly associated with multiple fractures regardless of T score (OR 3.86-4.38; $P < .05$ for all) (Table 3).

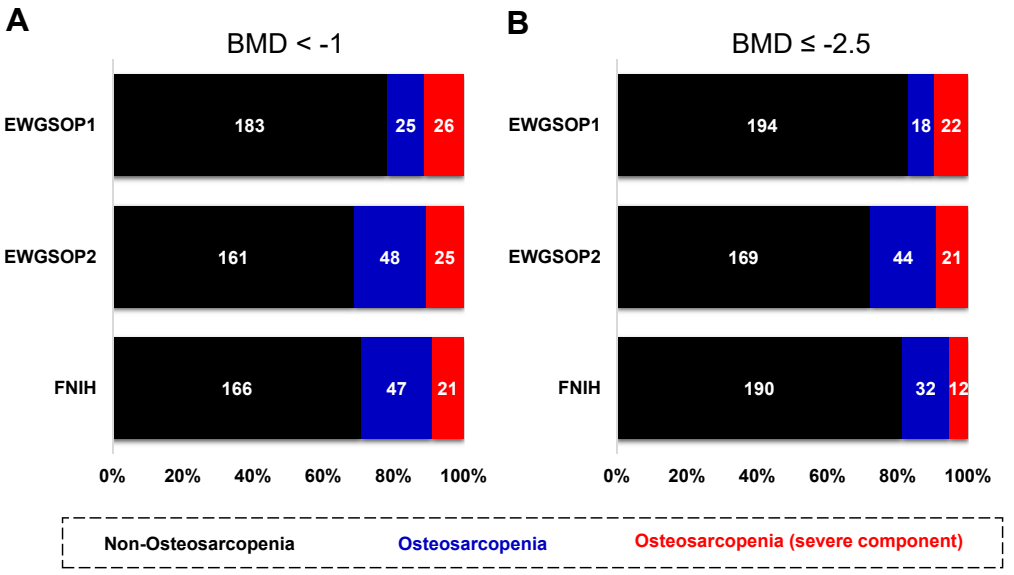

Fig. 1. Prevalence of osteosarcopenia and osteosarcopenia (with severe sarcopenia component) using different definitions and comparing with (A) BMD T score <1 SD and (B) BMD T score ≤2.5 SD.SD, standard deviation.

Exclusion of Lean Mass Further Increases Associations With Outcome Measures

Irrespective of the definition used, when we excluded low appendicular lean mass and combined LPP and LMS with low BMD, there was an association with worse physical performance (SPPB and TUG) and multiple falls (OR 2.18-7.89; $P < .05$ for all). When using the cut-points for LPP and LMS according to EWGSOP1 combined with low BMD, there was an additional association with balance measures (OR 2.20, 95% confidence interval: 1.3-6.0; $P < .05$) (Table 3).

Table 3
Associations Among Different Criteria to Detect Osteosarcopenia With Physical Performance, Dynamic and Static Balance

Classification of Osteosarcopenia	Worse Physical Performance			Worse Dynamic and Static Balance		Multiple Falls	Multiple Fractures
	SPPB Score ≤ 8, OR (95% CI)	TUG Time ≥ 20 s, OR (95% CI)	5STS Time > 15 s, OR (95% CI)	FSS Score > 15 s, OR (95% CI)	LOS < 120 cm, OR (95% CI)	≥2 Falls, OR (95% CI)	≥2 Fractures, OR (95% CI)
BMD <−1 + sarcopenia according to							
EWGSOP1	1.29 (0.5-3.6)	0.84 (0.3-2.3)	0.85 (0.3-2.7)	1.32 (0.3-5.3)	2.64 (0.7-9.6)	0.28 (0.1-0.7)*	0.48 (0.1-2.2)
EWGSOP2	3.06 (1.2-7.6)*	3.07 (1.5-6.2)*	1.95 (0.7-5.7)	2.81 (0.6-12.6)	1.96 (0.8-4.8)	0.49 (0.2-0.9)*	0.74 (0.3-1.9)
FNIH	3.59 (1.4-9.3)*	1.29 (0.7-2.6)	3.70 (1.1-13.3)*	4.84 (1.1-21.6)*	1.87 (0.7-4.5)	0.69 (0.3-1.4)	0.45 (0.2-1.4)
BMD ≤−2.5 + sarcopenia according to							
EWGSOP1	1.63 (0.5-5.4)	0.75 (0.2-2.5)	0.75 (0.2-2.7)	0.93 (0.2-4.3)	2.62 (0.6-12.2)	0.71 (0.1-0.6)*	0.66 (0.14-3.1)
EWGSOP2	3.45 (1.3-9.0)*	3.06 (1.5-6.3)*	2.26 (0.7-7.2)	2.30 (0.5-10.9)	2.11 (0.8-5.4)	0.49 (0.2-1)**	0.85 (0.32-2.2)
FNIH	2.41 (0.8-6.8)**	1.35 (0.6-3.3)	3.47 (0.8-15.9)	2.27 (0.4-12.6)	1.51 (0.6-4.1)	0.88 (0.4-1.9)	0.52 (0.1-1.8)
BMD <−1 + severe sarcopenia according to							
EWGSOP1	7.19 (1.6-33.2)*	2.32 (0.9-5.6)**	1.7 (0.3-8.6)	—	2.37 (0.7-7.6)	2.48 (0.9-6.4)	1.61 (0.5-4.8)
EWGSOP2	5.71 (1.3-25.8)*	2.36 (0.9-5.7)**	0.85 (0.2-3.5)	—	4.36 (0.9-19.8)**	2.83 (1.1-7.6)*	0.82 (0.2-2.9)
FNIH	3.66 (0.9-13.5)**	3.83 (1.4-10.2)*	0.88 (0.21-3.68)	—	2.73 (0.7-10.2)	1.93 (0.7-5.2)	3.86 (1.3-11.3)*
BMD ≤−2.5 + severe sarcopenia according to							
EWGSOP1	6.22 (1.3-29.2)*	2.56 (1.0-6.5)*	1.72 (0.3-8.6)	—	1.97 (0.6-6.4)	2.61 (0.9-7.2)**	1.39 (0.4-4.5)
EWGSOP2	3.99 (0.9-18.3)**	3.38 (1.2-9.2)*	0.85 (0.2-3.5)	—	3.21 (0.7-14.9)	3.63 (1.1-11.6)*	0.69 (0.1-3.2)
FNIH	2.39 (0.5-11.9)	3.39 (0.9-12.1)**	0.91 (0.2-4.9)	—	2.08 (0.4-10.5)	2.75 (0.7-10.9)	4.38 (1.1-17.4)*
BMD <−1 + LPP and LMS according to							
EWGSOP1	7.89 (3.3- 18.7)***	5.14 (2.7-9.7)***	2.10 (0.8-5.7)	—	2.20 (1.3-6.0)*	2.60 (1.4-4.8)*	1.92 (0.9-4.1)**
EWGSOP2	7.85 (2.3-27.1)***	3.60 (1.7-7.5)***	1.25 (0.4-4.1)	—	2.35 (0.9-6.1)**	2.89 (1.3-6.3)*	1.75 (0.7-4.1)
FNIH	6.97 (2.0-24.1)*	3.46 (1.6-7.3)***	1.10 (0.3-3.7)	—	2.54 (9.1-7.1)**	2.92 (1.3-6.5)*	1.91 (0.8-4.6)
BMD ≤−2.5 + LPP and LMS according to							
EWGSOP1	5.63 (2.2-14.2)***	3.45 (1.8-6.7)***	2.12 (0.7-6.1)	—	2.17 (0.9-4.9)**	2.18 (1.4-4.2)*	1.82 (0.8-3.9)
EWGSOP2	6.02 (1.3-26.9)*	3.27 (1.4-7.9)*	1.25 (0.3-4.9)	—	1.90 (0.6-6.0)	3.23 (1.2-8.6)*	1.95 (0.7-5.4)
FNIH	5.53 (1.2-24.8)*	2.87 (1.2-6.9)*	1.06 (0.3-4.2)	—	1.69 (0.5-5.4)	2.92 (1.1-7.8)*	2.06 (0.7-5.8)

CI, confidence interval; LOS, limits of stability; 5STS, 5 times sit-to-stand test.
Adjusted for age, gender, and Charlson Age-Comorbidity Index.
For FSS, ORs could not be calculated for several definitions because of the small sample size.
Statistical significance *$P < .05$; **$P < .09$; ***$P \leq .001$.

Discussion

We aimed to determine the clinical implications of combined osteopenia or osteoporosis and sarcopenia (osteosarcopenia) in older adults. In agreement with current literature, we found that individuals diagnosed with osteosarcopenia not only presented with greater impairments in strength, lower limb performance, and balance, but were also associated with higher rates of falls and fractures. With respect to these outcomes, our findings suggest that the definition of the "osteo" component of osteosarcopenia (BMD < -1 or ≤ -2.5) may not significantly affect clinical outcomes. However, the sarcopenia definition may affect results on clinical outcomes in individuals with osteosarcopenia. Therefore, presence of a severe sarcopenia component was associated with adverse events such as falls and fractures.

Minimal Differences Between "Osteo" Definitions

The diagnosis of osteopenia or osteoporosis has been well established over the past few decades,[13,17] with a T score of < -1 indicative of osteopenia and ≤ -2.5 osteoporosis. The consistent and routine use of these cut-points in clinical practice enables effective diagnosis and implementation of interventions. Despite the links between osteoporosis, fracture, and poor clinical outcomes,[29] we did not find differences in fracture rates in osteopenic compared to osteoporotic classifications. In corroboration, and notwithstanding the limited sample size, studies have reported discrepancies in reported fractures and BMD, with osteopenic older adults experiencing fracture rates similar to, and in some cases greater than, those diagnosed with osteoporosis.[30] Given these findings, it appears the use of T scores ≤ -1.0 to diagnose osteosarcopenic participants may be sufficient to capture those at greater risk of fractures. Interestingly, we report greater associations with multiple fractures when using the FNIH criteria to diagnose osteosarcopenia irrespective of the "osteo" component combined with severe sarcopenia. However, no associations were observed when employing EWGSOP 1 or 2, which may be due to the limited number of participants (17%) reporting multiple fractures. Finally, similarities were evident in the physical performance of osteosarcopenic older adults regardless of BMD.

Osteosarcopenia With a Severe Sarcopenia Component

Several definitions of sarcopenia[8,18–20,31] have resulted in differing prevalence estimates, in line with previous studies.[21] These differences in prevalence may be attributed to variation in cut-points used for muscle strength and ALM, including the methods of correcting ALM for height-squared and body mass index. Despite this, we noted an overall increase in OR for all outcome measures assessed, which was more notable and somewhat expected for physical performance measures. Static and dynamic balance also displayed further declines in those presenting with osteosarcopenia with a severe sarcopenia component, although we failed to assess the OR for dynamic balance due to the large proportion (53% of participants) who were unable to complete the FSS test. Although the impact of sarcopenia and severe sarcopenia on static balance has not been well studied, there is well-established evidence demonstrating that resistance training can improve both postural control[32] and muscle mass in older adults.[33] In regard to falls, compared with other groups, older persons diagnosed as osteosarcopenic with severe sarcopenia displayed a higher likelihood of falls (OR 1.93-3.63), but only study participants diagnosed using the EWGSOP2 definition provided significant results. These results are in agreement with other studies that have showed up to 3-fold increase in falls risk in sarcopenic older adults.[34] Although the limitations in assessing risk for multiple falls have been previously discussed, this study found participants diagnosed as osteosarcopenic according to the FNIH criteria presented with significantly greater fracture rates. In this sense, considering the evidence of the increased fracture risk in overweight and obese individuals,[35,36] this finding may in part be explained by the greater distribution of overweight and obese older adults using the FNIH criteria (76%, compared to 18% and 38% using EWGSOP1 and EWGSOP2, respectively), which has been reported previously.[37] Our findings were also in agreement with another study under the term "dysmobility syndrome" (combination of osteosarcopenia and overweight or obesity), where a greater percentage of falls and fracture history has been reported.[38]

Further Differences With Removal of Appendicular Lean Mass Measures

The use of dual-energy x-ray absorptiometry to determine appendicular lean mass involves the direct measurement of bone and fat mass, followed by calculations to quantify lean mass.[39] This method is influenced by vasculature, connective tissue, and partially fat-infiltrated muscle,[39] which may account for the lack of effect of muscle mass on clinical outcomes reported in various studies.[40,41] Given this evidence, we decided to exclude ALM measures from sarcopenia definitions and combine low BMD, LMS, and LPP to assess whether associations with physical performance, balance, and outcomes change. In doing so, we found even stronger associations with performance, balance, and falls when compared to severe sarcopenia. In particular, the OR for falls showed a significant association for all measures. These findings highlight the lack of an accurate "gold standard" for assessing muscle mass and the need for further research in this area, with several new methods recently proposed, including the use of D3-creatine and intramuscular and marrow fat.[42,43]

Strengths and Limitations

This study provides relevant information for clinical practice by reporting the impact of different definitions of osteosarcopenia on clinical outcomes and their associations with physical performance, balance, falls, and fracture history. Compared with previous studies,[1,5,44] we combined different criteria to diagnose osteosarcopenia, including a severity component. Finally, the assessments used to measure physical performance[23,25,26] and static and dynamic balance[18,24] are validated in older adults, and we also used a gold standard to measure osteopenia and osteoporosis,[17,39] which is also commonly accepted to define sarcopenia.[39] However, there were several limitations in this study. First, given the cross-sectional study design, we are unable to comment on causality. Second, as our study sample consisted primarily of women, this may skew results given that women have shown increased falls risk, however, results remained significant after adjustment for gender. Finally, the majority of participants presented with a fall or fracture history, with 95% reporting a fall in the past year and 81% experiencing a fracture. Given a lack of participants experiencing no falls or fractures, associations reported in this study may have been increased, but still are representative of the target population.

Conclusions and Implications

In conclusion, osteosarcopenia was strongly associated with worse physical performance and falls and fractures history in community-dwelling older adults. Additionally, osteosarcopenia with a severe sarcopenia component was associated with increased falls when based on EWGSOP2 and fractures when using the FNIH definition. In this study, the assessment of lean mass and the definition used of osteopenia and osteoporosis did not significantly affect results, with severe sarcopenia being the driving factor. Future longitudinal studies should be developed to monitor an individual's progression from a healthy state to the individual or combined components of

osteosarcopenia to establish the best definition of this syndrome and enhance assessment of the clinical impacts of osteosarcopenia on functionality and falls and fracture risk.

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Geriatrics Gerontology INTERNATIONAL Medline Indexed

Geriatr Gerontol Int 2017

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Effects of an exercise model based on functional circuits in an older population with different levels of social participation

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Aim: To investigate whether being part of a community organization interfered with older adults' overall functionality. The magnitude of responses to a community exercise program based on functional circuits in socially active and socially non-active older adults was also investigated.

Methods: A total of 200 older adults aged ≥60 years from Requinoa, Chile, participated in the study. Participants were separated into two groups according to the level of social participation: socially active (SA) and socially non-active (SNA). During an evaluation, data regarding the presence of comorbidities (Charlson Comorbidity Index), disability level (Modified Health Assessment Questionnaire), dynamic balance (Timed Up and Go test), muscle force (handgrip dynamometry) and sociodemographic conditions were collected. Participants followed an exercise program consisting of a functional exercise circuit including balance, resistance and aerobic exercises, twice a week, for 12 weeks.

Results: Both the Charlson Comorbidity Index and disability were higher in SNA compared with SA participants on inclusion. Both groups improved disability (Δ–0.25 patients [−0.5 – −0.0625 patients] for SNA and (Δ–0.125 patients [−0.5–0 patients] for SA) and balance (Δ–2 s [−3–0 s] for SNA and (Δ–1 s [−3–0 s] for SA) after training ($P < 0.05$ for all). Changes from baseline were statistically higher in the SNA group. Handgrip force improved only in participants in the SNA group (Δ1.7 kg [0.6–2.8 kg], $P = 0.0001$). No differences, however, were observed between the magnitude of improvements of handgrip between groups.

Conclusions: Although socially non-active older adults had more comorbidity and disability than their socially active counterparts, they showed a higher response to a community exercise program. **Geriatr Gerontol Int 2017; ••: ••–••.**

Keywords: aging, exercise, health status, physical function, social participation.

Introduction

Aging leads to a reduction in aerobic capacity, strength, balance and flexibility.[1] These alterations have a direct impact on functionality, potentially causing sarcopenia and frailty, especially when comorbidities and sedentarism are present.[2] In addition, factors such as socioeconomic status, education, social participation, race, and ethnicity might contribute to variation in biological and health outcomes.[3,4] It has been estimated that by 2030, 25% of the world population will be composed of adults aged >70 years.[5] This predicted shift of life expectancy will force governments to change policies of public health. Further than the resources to pharmacological treatments, the new policies will need to include the development of prevention strategies and health promotion, such as community programs.[6]

In order for older adults to achieve healthy aging, two widely known protective factors must be incorporated in the daily routine: social relationship (SR) and physical activity (PA).[7] Evidence shows that SR affect a range of health outcomes, including mental and physical health, health habits, and mortality risk.[7–9] A recent meta-analysis found that individuals with inadequate SR had a 50% greater risk of mortality than their counterparts.[10]

Family support, marital status and social participation in community organizations (CO) have been identified to potentially influence physical activity.[7] Commonly, older adults participate in religious groups, sports, senior centers and different community organizations.[11] The participation in physical activity programs is translated into health

Accepted for publication 17 July 2017.

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benefits by increasing aerobic capacity, strength, balance and preventing the risk of falls.[3,12–14] Benefits are also observed in reducing comorbidities,[3] promoting bone mineralization, improving memory and learning.[15] A bottleneck is the rarely offered individualized exercise prescription in community exercise programs. The combination of limited availability of health professionals and increased demand (a typical scenario in Latin America, with limited economical resources) hinders a more tailored exercise program to participants.[16] Furthermore, community programs deal with a very heterogeneous population with different comorbidities, social status and even participation in CO. Therefore, it is reasonable to hypothesize that differences in functionality among older adults with different levels of social participation exist.

The aim of the present study was to investigate whether being part of a CO interfered with participants' functionality. Additionally, the magnitude of responses to a community exercise program based on functional circuits in socially active (SA) and socially non-active (SNA) older adults was investigated.

Methods

A clinical study was carried out with older adults from the commune of Requinoa, Chile. Individuals would be included if were aged >60 years and were classified as independent according to the functional scale for older people designed by Chilean Ministry of Health,[17] and were registered in the primary care system of their city commune. Individuals were ineligible for the study if were unable to walk, had a history of recent hip fracture or stroke or had any of the absolute contraindications to exercising from the American College of Sports Medicine guidelines for exercise testing and prescription.[18] The study was approved by the ethics committee of the Primary Care Center of the commune, and all participants provided written informed consent to participation in the program. Participants in this study were volunteers and were separated into two groups according to the level of social participation: SA and SNA. SA was defined as a person who attends the CO at least once a week. SNA was defined as a person who does not attend the CO any time a week. The CO considered were: religious groups, sporting activities, cultural activities, neighborhood associations and senior centers.

Measurements

Handgrip force was assessed with individuals in a standing position using a hydraulic hand dynamometer (Jamar Dynamometer; Jamar Plus + Digital 563213; Lafayette Instrument Company, Lafayette, IN, USA). The shoulder was positioned at adducted, and neutrally rotated position with the elbow at 90° and the forearm and wrist at a neutral position.[19] Three repetitions were carried out on the right hand with 30-s rests between tests. The highest value (in kilograms) from the three attempts was used as the maximal force.

Dynamic balance and agility was assessed with the Timed Up and Go test (TUG). Participants stood up from a seated position, walked 3 m, turned around and returned to the seated position on a chair. After a practice trial, the shortest time (in seconds) of two trials was recorded and used as the TUG score.[20]

The index of disability was measured by the Modified Health Assessment Questionnaire (MHAQ). The MHAQ assesses the degree of difficulty, satisfaction with function and changes over the past 6 months in eight different items (dressing, arising, eating, walking, hygiene, reaching, gripping, and getting in and out of car).[21] Participants choose between four options in each of the items (without any difficulty, with some difficulty, with much difficulty, unable to do). The questionnaire was administered during a face-to-face interview, always by the same professional.

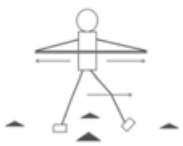
The Charlson Comorbidity Index (CCI) was used to quantify the overall burden of comorbidities. The index includes 19 medical conditions with corresponding weights. Comorbidities were weighted and scored using an algorithm proposed by Charlson *et al.*[22] Afterwards, the total score was adjusted by age (age-adjusted CCI [ACCI]).[23]

Interventions

The exercise program consisted of 35–45-min sessions on non-consecutive days, twice a week, for 12 weeks (24 sessions). Each session started with a warm-up (e.g. jogging, walking, flexibility exercise etc.) or group dynamics, and followed a standardized functional exercise circuit (FEC) consisted of aerobic, resistance, balance and coordination exercises applied as functional tasks in 15 stations (1 minute of duration each). All the activities were performed in training groups with a limit of 15 participants (including individuals belonging to SNA and SA). Subjects had to complete the functional exercise circuit twice. Details of all included activities of the program are provided in Table 1.

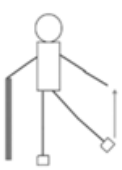
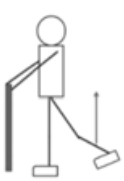
For the exercise prescription, a participant was evaluated for 1 min in each station, where she/he was requested to carry out either the maximum number of repetitions (MNR) or the maximum level of difficulty according to the characteristic of the task. The percentage of requirement for each task was calculated according to the initial evaluation. The sequence was: first week 50% of MNR, third week 60% of MNR, fifth week 70% of MNR, seventh week 90% of MNR, ninth week 110% of MNR and 11th week 150% of MNR. Exercise stations including balance exercises were not evaluated with repetitions, but instead by level of difficulty, and progression of these tasks were carried out by reducing stability. Exercise sessions were always supervised by the same professionals.

Table 1 Functional exercise circuit

Exercise	Aim	Description of task	Progression from initial test (week)
	Agility Coordination Aerobic Capacity	Walking over 5 obstacles, 50-cm apart from each other, forwards and backwards. Instructed to repeat this according to the percentage of MNR. The maximum number of laps at the initial evaluation was used as MNR.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Coordination	Standing: keeping one foot on the center, moving the other foot in the direction of the cones (forward, back, left and right). Simultaneously to the lower limb exercises, arms must perform abduction and adduction when the lower limb returns to the center. Instructed to repeat with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Coordination	Walking over 5 marked lines on the floor, 50-cm apart from each other, in a lateral gait, left and right. Repeat this according to the percentage of MNR. MNR was calculated from the maximum number of laps in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Strength	Standing: grip the resistance wheel, keeping your back straight at all time. Repeat it according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Strength	Sitting on a chair: biceps pulley from 90° to 120° of elbow flexion. Repeat it according to the percentage of MNR. MNR was calculated with the moderate resistance chosen by the patient at baseline.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Aerobic capacity	Standing: stationary gait with the elevation of each lower limb. Repeat with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetitions between both lower limbs in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR

(*Continues*)

Table 1 (Continued)

Exercise	Aim	Description of task	Progression from initial test (week)
	Strength	Sitting on a recumbent chair: abdominal crunches ranging from 45° to 90° position. Repeat it according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Coordination Accuracy	Standing: take the ball with the poles and hold it, then leave it in the plastic pot and repeat it. The level of difficulty and progression were done adding other tasks.	1st–3rd keep the ball for 10 s in the initial position. 4th –9th keep the ball and walk for 15 s. 10th–12th keep the ball and walk with obstacles as long as possible.
	Strength Power	Standing: rest your hands on the back of the chair for stability if necessary and perform a squat. Repeat it according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Strength	Standing: rest your hands on the back of a chair for stability. Raise one leg to the side as far as is comfortable, keeping your back and hips straight. Avoid tilting to the other leg. Repeat it with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Strength	Standing: rest your hands on the back of a chair for stability. Standing upright, raise your leg backwards, keeping it straight. Keep the back straight as you take your leg back. Repeat it with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1st–2nd 50% of MNR 3rd–4th 60% of MNR 5th–6th 70% of MNR 7th–8th 90% of MNR 9th–10th 110% of MNR 11th–12th 150% of MNR
	Balance	Standing: put one foot over the balance board, and keep your back straight and the other foot on the floor. Hold the balance for 30 s. In the beginning you can rest your hands on the back of the chair for more stability. The level of difficulty and	1st–3rd keep the position for 30 s. 4th–9th keep the position for 30 s, with an object in both hands.

(*Continues*)

Table 1 (Continued)

Exercise	Aim	Description of task	Progression from initial test (week)
		progression were done adding other tasks.	10^{th}–12^{th} keep the position for 30 s, and move your other leg forward and backward.
	Aerobic Capacity	Sitting on a chair: run a static bike at a speed of at least 60 r.p.m., at a light load. The progression was done adding a progressive load during the training.	1^{st}–2^{nd} 1 3^{rd}–4^{th} 2 5^{th}–6^{th} 3 7^{th}–8^{th} 4 9^{th}–10^{th} 5 1^{th}–12^{th} 6
	Balance	Standing: put one foot over the balance-disc, and keep your back straight and lift the other foot; keeping the balance for 15 s. Change your leg. In the beginning you can rest your hands on the back of the chair for more stability. The level of difficulty and progression were done adding other tasks.	1^{st}–3^{rd} keep the position for 15 seconds. 4^{th}–9^{th} keep the position for 15 s, with an object in a hand. 10^{th}–12^{th} keep the position for 15 s, and move your other leg forward and backward.
	Strength Power Coordination	Pass behind your back the elastic band and stretch forward with your arms, doing lunges, one leg at a time. Keeping your back straight. Repeat it with each leg according to the percentage of MNR. MNR was calculated from the maximum number of repetitions in the initial evaluation.	1^{st}–2^{nd} 50% of MNR 3^{rd}–4^{th} 60% of MNR 5^{th}–6^{th} 70% of MNR 7^{th}–8^{th} 90% of MNR 9^{th}–10^{th} 110% of MNR 11^{th}–12^{th} 150% of MNR

MNR, maximum number of repetitions.

Statistical analysis

The software used for the statistical analysis was Statistical Package for the Social Sciences (SPSS) version 19.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA). Data normality was evaluated using the Kolmogorov–Smirnov test, and expressed as the mean and standard deviation or median and interquartile range according to the data distribution. Paired comparisons (baseline *vs* post program) were carried out by the paired *t*-test or Wilcoxon test according to the distribution of data. The comparison between SA and SNA was carried out by the unpaired *t*-test or Mann–Whitney test, also following data distribution. Categorical data were compared using the χ^2-test. The level of significance was established as P 0.05.

Results

A total of 200 individuals were initially included in the exercise program, separated into 15 training groups. A total of 164 patients (73 ± 6 years, body mass index 28 ± 4 kg/m^2) completed all the 24 sessions of the program (66% of SNA and 82% of SA). There were no significant differences of the dropout rate between SNA and SA (P = 0.091). Sociodemographic characteristic of the participants are reported in Table 2. There were no significant differences in bodyweight or body composition between the groups. There were more women (P = 0.031) and participants with higher education (P = 0.019) in the SA group. The ACCI was higher in the SNA group compared with the SA group (P = 0.04). Amongst SNA participants, 51% had an ACCI score of 2–3, and 49% had an ACCI score of 4–5. Amongst SA participants, 64% had an ACCI score 2–3, and 36% had an ACCI score of 4–5 (Table 2). At the initial evaluation, the index of disability was higher in the SNA group compared with the SA group ($P < 0.05$; Table 3).

Table 3 shows the changes of assessed outcomes after the exercise program. There was a statistically significant reduction in disability measured with the MHAQ, as well as an improvement of dynamic balance and agility measured by the TUG ($P < 0.05$ for all outcomes in both

Table 2 Sociodemographic characteristics of socially non-active and socially active participants

Variable	Group	
	SNA (n = 41)	SA (n = 123)
Age (years)	74 ± 6	71 ± 5
Female, n (%)	26 (63%)	101(82%)*
BMI (kg/m^2)	28 ± 4	27 ± 5
Marital status		
Married	19 (46%)	68 (55%)
Widowed	3 (8%)	11 (9%)
Single	19 (46%)	42 (34%)
Educational level		
Less than primary education	27 (66%)	80 (65%)
Primary education	13 (32%)	24 (20%)
Secondary education	1 (2%)	4(3%)
Higher education	0 (0%)	15(12%)*
ACCI		
Mean scores	3.5 ± 1	3 ± 1*
0–1, n (% of total)	0 (0%)	0 (0%)
2–3, n (% of total)	21 (51%)	79 (64%)
4–5, n (% of total)	20 (49%)	44 (36%)
≥6, n (% of total)	0 (0%)	0 (0%)

Data are expressed as mean ± standard deviation or absolute number and frequency. *Statistically significant ($P \leq 0.05$). ACCI, age-adjusted Charlson Comorbidity; BMI, Body mass index; SA, socially active group; SNA, socially non-active group.

groups). In comparison with the SA, SNA showed a statistically higher improvement in both MHAQ (Δ–0.25 patients [−0.5 to −0.0625patients] for SNA and (Δ–0.125 patients [−0.5–0 patients] for SA, P = 0.0002) and TUG (Δ–2 s [−3–0 s] for SNA and Δ–1 s [−3–0 s] for SA, P = 0.0093). Handgrip force improved in SNA (Δ1.7 kg [0.6–2.8 kg], P = 0.0001), whereas no significant improvement was observed in SA (Δ1.7 kg [−4.0–6.1 kg], $P > 0.05$). No differences, however, were observed between the magnitude of improvements of handgrip between groups.

Discussion

The present study found that SNA individuals presented higher levels of disability and comorbidities in comparison with SA individuals. Despite this, participants in the SNA group were able to improve muscle force after a specific training program, which was not the case in participants in the SA group. Similar improvements in disability and dynamic balance were observed in both the SA and SNA groups. However, more pronounced improvements were observed in the SNA group.

Social ties has been significantly associated with cardiovascular disease, high blood pleasure, cancer, and changes in immune and endocrine function.[7,24] Brummett *et al.*, found that socially isolated adults with coronary artery disease had a relative risk of 2.43 (95% CI 1.39–3.19, P = 0.001) for cardiac mortality.[8] Furthermore, Kiecolt-Glaser *et al.* reported that low quality or quantity of social relationships is associated with an increase of inflammatory biomarkers and impaired immune function, through negative emotion.[4] Biomarkers of inflammation are a triggering factor for developing different chronic disease, such as diabetes mellitus, hypertension, metabolic syndrome, pulmonary and cardiovascular disease.[25] In the present study, SNA participants had more comorbidities and disability. Although it is not possible to verify the causality of activity status on the onset of allied diseases/comorbidities, it can be hypothesized that the higher prevalence of comorbidities in SNA can be somehow linked with social isolation, as there is evidence that social interaction benefits immune, endocrine and cardiovascular functions.[7,26]

Another important finding is that the functional exercise circuit induced improvements in both groups for gait speed and dynamic balance evaluated by the TUG, and decreased the disability evaluated by MHAQ. This is consistent with previous studies on the positive effects of exercise on older adults, such as improvement in force, gait speed, dynamic balance and prevention of disabilities and comorbidities.[3,13–15,27] However, it is worth pointing out that exercise generated greater benefits in SNA participants than in the SA participants, and that handgrip force post-training only improved in SNA participants. A possible explanation could be that the SNA participants had a higher disability than the SA participants at the initial evaluation, leaving more room for improvement in SNA participants than in SA participants. Importantly, the training program allowed SNA

Table 3 Baseline (week 0) characteristics of socially non-active and socially active participants

Measures	SNA (n = 41)		SA (n = 123)	
	Pre	Post	Pre	Post
Handgrip force (kg)	23 (19–33)	24 (20–36)*	23 (19–27)	24 (20–28)
Disability	0.375 (0.125–0.582)	0 (0–0.125)*	0 (0–0.125)**	0 (0.125–33)*
MHAQ				
Timed Up and Go (s)	10 (8–11)	8 (7–9)*	9 (8–10)	8 (7–9)*

Data are expressed as median [interquartile range]. *Statistically significant inter group; **Statistically significant intragroup ($P \leq 0.05$). MHAQ, Modified Health Assessment Questionnaire; SA, socially active group; SNA, socially non-active group.

participants to carry out physical activities and interact with other people twice a week, as people from both groups were included in the exercise sessions (SNA and SA). It can be hypothesized that the participation of SNA individuals together with SA individuals could have induced the adherence to two protective factors for healthy aging: physical activity and social relationship.[28]

Participation in group activities and social support has a positive impact on people's health.[10,28] In fact, the World Health Organization has recognized that the prevention of social isolation and loneliness is considered necessary to maintain functionality, and to prevent disability and frailty in older people.[29] It can be assumed that the older adults from the SNA group were people without social participation who probably experienced loneliness. Hence, another positive effect of the program was to integrate non-active people into groups that allowed them to interact with other individuals, making them feel part of the community.

Commonly, participants of exercise programs in the community are socially active, and have more access to information about different programs and benefits. In the present study, even though it was not statistically significant, there was a trend of higher dropout rates in the SNA group ($P = 0.091$). This could be related to several issues, such as a lower level of information or access to such programs, lower educational level and marital status (fewer married participants).[30] Nevertheless, older adults who decided to participate improved their functionality even more than those from the SA group.

A great deal of scientific evidence shows that social participation affects health and mortality.[9,10,24,26] However, there is little evidence about the effects of social participation on functional capacity in older people. Although it is not possible to imply causality, the present study is among the very few that provide evidence of the association of social participation and functional capacity in older adults, and different responses to exercise programs in this population.

A potential limitation of the present study was the lack of investigation of training effects on a broader scope of outcomes. Holt-Lunstad *et al*. suggested that other factors could influence social status (e.g. family support, economical status and emotional function).[10] Another limitation was a lower number of men attending CO. Commonly, members of CO in Chile are women, because men generally continue working after retirement or do not show interest in belonging to any organization, for causes not yet completely elucidated and that should be studied in future research. Therefore, future studies should address new health strategies that could be developed in order to increase the participation of men and also SNA older adults.

In conclusion, the present findings show that despite the fact that SNA older adults are more disabled and with more comorbidity than their socially active counterparts, they had a higher response in terms of balance and disability to a community exercise program based on functional circuits. In fact, SNA participants showed more prominent improvements in terms of muscle force.

Acknowledgements

This work was supported by Primary Health Care from the Commune of Requinoa, and by the Program "*Adultos mayores mas autovalentes*" from the Ministry of Health, Chile.

The authors acknowledge the Directive from Community Organization for Older People, Municipality of Requinoa, Health Service O'Higgins and University of Aconcagua, Chile, for its collaboration, and physiotherapist Gloria Luna from Del Rosario University, Colombia, for having inspired this kind of exercise.

Disclosure statement

The authors declare no conflict of interest.

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