Universidade Estadual de Londrina

WALTER AQUILES SEPÚLVEDA LOYOLA

OXIDATIVE STRESS CORRELATES WITH CLINICAL MEASUREMENTS OF SARCOPENIA IN COPD

Londrina
2018

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Dissertação 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 Mestre em ciências da Reabilitação.

Orientadora: Prof[a]. Dr[a]. Vanessa Suziane Probst
Coorientadora: Prof[a]. Dr[a]. Carrie Chueri Ramos Galvan.

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BANCA EXAMINADORA

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Londrina, 28 de fevereiro de 2018.

Dedico este trabalho a Deus, a minha família, meus pais, meus irmãos, meus avós e minha bisavó que está no céu. Também a meus professores e amigos, que sempre acreditaram em mim.

RECONHECIMENTOS

“Gostaria de agradecer, primeiramente,
quem está sempre com a gente,
é Deus quem me acompanhou,
desde o momento em que nasci
dos meus primeiros passos,
ele estava caminhando do meu lado,
eu quando caí ele me alçou sua mão.

Sou o que sou e o que serei,
por a família que eu tive,
minha mãe, meu pai, avós e irmãos,
eles são o presente mais bonito que o céu
tem dado.

Graças às pessoas que Deus me
apresentou,
começando com Rosi, a mulher que me
apaixonou,
também dou graças a Vanessa,
uma maravilhosa mãe e mulher,
um anjo que está nesta terra para ajudar a
gente crescer.

E como as coisas são construídas com
alegria,
Vinicius, o amigo mais fiel veio à minha
vida,
meu colega e meu parceiro
que caminhou comigo neste processo.

E para criar conhecimento,
sempre se precisa de em uma equipe,
Larissa e Andrea eu aprecio o tempo,
o aprendizado que juntos criamos,
e os esforços a cada momento.

E quem diz que a Ciência, é séria ou é
chata?
ainda não conhece como a equipe do
Décio trabalha,
Kamila, Paula, Denise, Keiko e Mariano,
obrigado por sempre estender sua mão.

Agradeço também a LFIP e Fabio Pitta
Carrie mi coorientadora
também a Dirce uma ótima pessoa
Suhaila e familiares pessoas muito
preocupadas
e o Guto a mistura de conhecimento e
brincadeira
todos eles, pessoas muito boas
os momentos e aprendizagens,
nunca os esquecerei.

Por último, lembrar que
uma casa é mais do que apenas 4 paredes
são as pessoas que preencheram esse
espaço,
a vida me deu dois amigos incondicionais
família 04
Felipe e Fabinho dois amigos
dois novos irmãos
para você e para toda pessoa que falei
e todos os outros que não foram
mencionados
só posso dizer muito obrigado.”

Estilo Literário: Paya Chilena

PENSAMENTO

*“*O conhecimento, as experiências, e ainda as grandes pesquisas são somente palavras jogadas ao vento, quando não se tem Deus no coração, pois é Ele quem nos dá a sabedoria, para entender, que cada nova pesquisa é um ato de amor, para ajudar o próximo”.

Walter Aquiles

SEPÚLVEDA LOYOLA, Walter Aquiles. **Correlação entre estresse oxidativo com medidas clínicas da sarcopenia na DPOC.** 2017. 89 f. Dissertação (Mestrado em Ciências da Reabilitação) – Programa Associado UEL e UNOPAR – Universidade Estadual de Londrina, Londrina, 2018.

RESUMO

Introdução: Há evidências de que a Doença Pulmonar Obstrutiva Crônica (DPOC) é uma doença sistêmica que aumenta as espécies reativas de oxigênio e nitrogênio (ERO / ERN), causando estresse oxidativo (EO). ERO/ERN induzem danos na estrutura celular, como fibras musculares, que gera fraqueza muscular e sarcopenia, e prejudica a funcionalidade e a qualidade de vida. **Objetivo**: Analisar e comparar biomarcadores de estresse oxidativo entre indivíduos aparentemente saudáveis e indivíduos com DPOC, estabelecendo associações com medidas clínicas de sarcopenia. **Métodos:** Trinta e nove indivíduos com DPOC (69 ± 7 anos; 41% mulheres; VEF1: 49 ± 13% pré) e trinta e cinco indivíduos aparentemente saudáveis para o grupo controle (GC) (69 ± 7 anos; 43% mulheres; VEF1 : 98 ± 16% pred) foram incluídos. Todos os indivíduos passaram pelas avaliações de análise sanguínea, pressão inspiratória e expiratória máxima (PImáx e PEmáx, respectivamente), velocidade da marcha (VC), força de preensão manual (FPM), força do quadríceps (FQ), índice de massa muscular esquelética (IMME) e índice de massa livre de gordura (IMLG). Os critérios de sarcopenia foram estabelecidos de acordo com o grupo de trabalho europeu sobre sarcopenia em idosos. A investigação de biomarcadores de EO foi realizada por meio da análise de produtos oxidados de proteínas avançadas (AOPP), paraoxonase 1 (PON1), atividade superóxido dismutase (SOD), atividade da catalase dismutase (CAT), grupo sulfidrila (SH), metabólitos de óxido nítrico (NOX) e parâmetro antioxidante total (TRAP). **Resultados:** os indivíduos com DPOC apresentaram níveis mais elevados de atividade antioxidante, SH, PON-1 e SOD; P <0,02 para todos, em comparação ao GC. Na DPOC, a TRAP apresentou correlação positiva com IMLG (r = 0,5), IMME (r = 0,5), FQ (r = 0,64) e FPM (r = 0,51); P <0,05 para todos). AOPP apresentou correlação positiva com IMLG (r = 0,43), IMME (r = 0,52), FPM (r = 0,5), PImáx (r = 0,59) e PEmáx (r = 0,46); P <0,05 para todos). Variação em IMLG e VC foram explicados por TRAP e IMC (R2: 0,80 e R2: 0,51). A TRAP foi o único determinante da FQ (R2: 0,43; P = 0,004). IMME foi explicado por TRAP, AOPP e IMC (R2: 0,51; P = 0,0004). Os pontos de corte TRAP≤ 850 µM trolox e AOPP≤65 µM / l associaram-se ao risco de sarcopenia (OR: 8,3; IC95%: 1,372-49,6 e OR: 14; IC95%: 2,251-87,05, respectivamente; P <0,05 para ambos). **Conclusão:** Indivíduos com DPOC apresentaram níveis mais elevados de atividade antioxidante e maior prevalência de sarcopenia quando comparados a indivíduos aparentemente saudáveis. Medidas clínicas de sarcopenia foram correlacionadas com biomarcadores de EO, sendo TRAP e AOPP altamente associados a desfechos de sarcopenia nessa população.

Palavras-chave: Estresse oxidativo. Sarcopenia. DPOC.

SEPÚLVEDA LOYOLA, Walter Aquiles**. Oxidative stress correlates with clinical measurements of Sarcopenia in COPD.** 2018. 86 p. Dissertation (Master's Degree in Rehabilitation Sciences) – Programa Associado UEL e UNOPAR – Universidade Estadual de Londrina, Londrina, 2018.

ABSTRACT

Introduction: There is evidence that Chronic Obstructive Pulmonary Disease (COPD) is a systemic disease that increases reactive oxygen and nitrogen species (ROS/RNS), causing oxidative stress (OS). ROS/RNS induce damage in a cellular structure such as muscle fibers, producing muscle weakness and sarcopenia, impairing functionality and quality of life. **Objective:** To analyze and compare oxidative stress biomarkers between apparently healthy individuals and subjects with COPD, establishing associations with clinical measurements of sarcopenia. **Methods:** Thirty-nine subjects with COPD (69±7 years; 41% female; FEV_1: 49±13% pred) and thirty-five apparently healthy individuals for the control group (CG) (69±7years; 43% female; FEV_1: 98±16% pred) were included. All individuals were evaluated for blood analysis, maximal inspiratory and expiratory pressure (MIP and MEP, respectively), gait speed (GS), handgrip strength (HGS), quadriceps strength (QS), skeletal muscle mass index (SMMI) and fat-free mass index (FFMI). Sarcopenia criteria were established according to the European working group on sarcopenia in older people. Investigation of OS biomarkers was conducted *via* analysis of a*dvanced oxidation protein products* (AOPP), paraoxonase 1 (PON1), superoxide dismutase activity (SOD), catalase dismutase activity (CAT), sulfhydryl group (SH), nitric oxide metabolites (NOX) and total radical trapping antioxidant parameter (TRAP). **Results:** COPD subjects showed higher levels of antioxidant activity, SH, PON-1 and SOD; P<0.02 for all, in comparison to CG. In COPD, TRAP showed positive correlation with FFMI (r=0.5), SMMI (r=0.5), QS (r=0.64) and HGS (r=0.51); *P*<0.05 for all). 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); *P*<0.05 for all). Variation in FFMI and GS were explained by TRAP and BMI (R^2:0.80 and R^2:0.51). TRAP was the only determinant of QS (R^2:0.43; *P*=0.004). SMMI was explained by TRAP, AOPP and BMI (R^2:0.51; *P*=0.0004). The cut-off points TRAP≤ 850 µM trolox and AOPP≤65 µM/l were associated with risk of sarcopenia (OR: 8.3; 95% CI:1.372-49.6 and OR:14; 95% CI: 2.251-87.05, respectively; *P*<0.05 for both). **Conclusion:** COPD subjects showed higher levels of antioxidant activity and more sarcopenia prevalence when compared to apparently healthy individuals. Clinical measurements of sarcopenia were correlated with OS biomarkers, being TRAP and AOPP highly associated with sarcopenia outcomes in this population.

Keywords: Oxidative stress. Sarcopenia. COPD.

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LIST OF ABBREVIATIONS

ACCI	Age-adjusted Charlson comorbidity index
ADLs	Activities of daily living
ALM	Appendicular lean mass
AOPP	Advanced oxidation protein products
ASMI	Appendicular skeletal muscle mass index
AUC	Area under curve
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BODE	Body mass index, airflow Obstruction, dyspnea and exercise capacity
CAT	Catalase activity
CIs	Confidence intervals
CC	Calf circumference
COPD	Chronic obstructive pulmonary disease
DEXA	Dual-energy x-ray absorptiometry
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
GST	Glutathione-S-transferase
HDL-C	High-density lipoprotein cholesterol
HGF	Hand grip force
HR	Hazard ratio
IGF-1	Insulin-like growth factor 1
LDL-C	Low-density lipoprotein cholesterol
NF-kB	Nuclear factor-KB
NOX	Nitric oxide metabolites
OS	Oxidative stress
MDA	Malondialdehyde
MEP	Maximal expiratory pressure

MIP	Maximal inspiratory pressure
PON1	Paraoxonase 1
QS	Quadriceps strength
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ROC	Receiver-operating characteristic
SH	Sulfhydryl group
SM	Skeletal muscle mass
SMI	Skeletal muscle mass index
SOD	Superoxide dismutase activity
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TBARS	Thiobarbituric acid reactive substances
TNF-α	Tumor necrosis factor alpha
TRAP	Total radical trapping antioxidant parameter

SUMMARY

1 INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) 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[1]. 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[2–6]. Subjects with COPD have lower level 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 produce systemic inflammation[10] and oxidative stress[11], which are the mean factors associated with sarcopenia[9,12].

Sarcopenia has been defined by the European working group on sarcopenia in older people (EWSGOP) as a syndrome characterized by loss of skeletal muscle, muscle strength and physical performance, being the major cause of frailty in the elderly[13]. 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 higher and vary between 15%[15] to 55 %[16], depending on evaluation´s criteria[17].

Sarcopenia is associated with metabolic change, immobility, mitochondrial dysfunction[9,18], oxidative stress[12,19] and systemic inflammation[9] which contribute to age-related deficits in muscle[20], through increased damage to the cell[12].

COPD is a systemic disease and there is strong evidence showing that pathophysiological changes are related to an imbalance between oxidant and antioxidant, producing oxidative stress (OS)[11]. However, according to a recent systematic review[21], there are some contradicting results about some biomarkers compared with control groups such as advanced oxidation protein products (AOPP), sulfhydryl group (SH), superoxide dismutase activity (SOD) and catalase activity (CAT)[22–24]. These biomarkers have been reported to be increased, reduced or non-different between subjects with COPD and control group[21]. In addition, the antioxidant TRAP (total radical trapping antioxidant parameter) which have been

associated with increases in muscle strength in older people[25], has been little studied in COPD.

Although there is evidence showing that OS induces more inflammation[9,12], muscular atrophy[26] and sarcopenia in other population[26], no direct association has been shown between clinical measurements of sarcopenia (muscle mass, muscle strength and physical performance) and OS biomarkers in subjects with COPD.

2 OBJECTIVES

2.1 Primary Objective

The main objective was to analyze and compare oxidative stress biomarkers in subjects with COPD and apparently healthy individuals, establishing associations with muscle strength, muscle mass and physical performance.

2.1 Secondary Objective

A secondary objective was to compare the prevalence of sarcopenia, muscle weakness and muscle depletion between subjects with COPD and apparently healthy individuals.

3 RATIONALE

There is no evidence showing a direct association between clinical measurements of sarcopenia (muscle mass, muscle strength and physical performance) in subjects with COPD. Additionally, in subjects with COPD there are some conflicting results about the levels of some biomarkers compared to control group such as advanced oxidation protein products (AOPP), sulfhydryl group (SH), superoxide dismutase activity (SOD) and catalase activity (CAT), which have been reported to be increased, reduced or non-different between subjects with COPD and control group[21]. In addition, the antioxidant TRAP (total radical trapping antioxidant parameter) has been poorly studied in individuals with COPD. Therefore, it is important to explore about oxidative and antioxidative biomarkers in COPD, since they play a role in the development of new comorbidities and systemic inflammation, which could contribute to impair the prognosis of the disease. Moreover, since sarcopenia is the main cause of frailty in the elderly population, it is necessary to identify which are the biomarkers associated with muscle strength, muscle mass and physical performance, in order to better comprehend some mechanisms that produce sarcopenia in subjects with COPD.

4 HYPOTHESIS

It is expected that subjects with COPD present higher levels of oxidative biomarkers (NOX and AOPP) and lower levels of antioxidative biomarkers (TRAP, SH, PON1, CAT and SOD), producing oxidative stress due to the imbalance between oxidant and antioxidant species[11]. In addition, considering the evidence about prevalence of sarcopenia in individuals with COPD[15,16], it is likely that the prevalence of sarcopenia in this population would be higher than in apparently healthy subjects. Finally, other expected result would be that the oxidant biomarkers will be negatively correlated to clinical measurements of sarcopenia as well as the levels of antioxidant will be positively correlated to these outcomes in individuals with COPD.

5 LITERATURE REVIEW

5.1 COPD AND SARCOPENIA

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of death worldwide[27]. COPD is a highly prevalent disease affecting up to 10% of adults over 40 years old, leading to disability and impairing quality of life[7,27]. It has been associated with different non-respiratory disorders, such as cardiovascular and metabolic problems[28], physiological alterations[29], balance impairment[30], systemic inflammation[10], oxidative stress[21] and Sarcopenia[10,16].

Sarcopenia is a clinical syndrome, which has been defined by the European Working Group on Sarcopenia in Older People (EWGSOP)[13] as a syndrome characterized by loss of skeletal muscle mass, muscle strength and physical performance, being the major cause of frailty in the elderly[13,31]. The term sarcopenia is derived from the Greek words sarx (flesh) and penia (poverty)[32]. 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[33]. Therefore, sarcopenia is related to disability, severity and a poor prognosis in individuals with COPD[15,16].

5.2 SARCOPENIA CRITERIA IN COPD

The main sarcopenia criteria is loss in muscle mass. The gold-standard measurement of this outcome is the dual-energy x-ray absorptiometry (DEXA)[13]. However, muscle mass can also be assessed with magnetic resonance imaging (MRI) and bioelectrical impedance analysis (BIA) to estimate skeletal muscle

mass index (SMI) and fat free mass index (FFMI). For this reason, there is a divergent prevalence of sarcopenia in older people, since it depends of the methodology, the skeletal muscle mass cut-off point, and the characteristics of the studied population[9]. For example, Janssen et al.[34], classified the presence of sarcopenia using skeletal muscle mass index (SMI), considering SMI among one to two standard deviations of young adult values to identify subjects with class I sarcopenia, and subjects with values lower than two standard deviations to identify class II sarcopenia. It is important to note that in this study SMI was predicted using a bioelectric impedance equation[34]. On the other hand, Newman et al.[35] used DEXA, and a different statistical method to classify sarcopenia, obtaining the following cut-off point for SMI: 7.23 Kg/height^2 (men) and 5.67 Kg/height^2(women). However, the cut-off point values found by Newman et al.[35] were similar with the study of Janssen et al.[34], which have been associate to disability and frailty[36]. In subjects with COPD, Byun et al.[10] quantified muscle mass with BIA and they defined low muscle mass as having an SMI at least two standard deviations below normal sex-specific mean in young people. Other studies[37,38] among COPD and sarcopenia used DEXA according to criteria defined by Baumgartner et al.[37], using appendicular skeletal muscle mass index (ASMI) calculated as the sum of skeletal muscle mass in the arms and legs divided by the square of height (Kg/m^2). Rutten et al.[39], compared the FFMI by BIA and DEXA in 1087 subjects with COPD, and predicted by multivariate analysis a new formula to calculate SMI by BIA in this population. Muscle wasting has been defined according to the following cut-off points in individuals with COPD: Schols et al..[40] (FFMI<16 Kg/m^2 for men, 15 Kg/m^2 for women), Vestbo et al..[41] (FFMI<17.1 Kg/m^2 for men, 14.6 Kg/m^2 for women), and Coin et al..[42] (FFMI<17.8 Kg/m^2 for men, 14.6 Kg/m^2 for women), being the cut-off point of Vestbo et al.. considered as the most optimal to identify muscle wasting in COPD[39]. Calf circumference (CC) is another measurement related to muscle mass. Borda et al..[43] assessed muscle mass with CC in individuals with COPD and asthma, defining lower muscle mass as values < 31 cm in women and men[43]. However, CC has a low sensitivity in comparison with the other methods[44].

The main effect of a reduction in muscle mass is declining in the muscle strength and physical performance, which are other criteria to diagnose sarcopenia[9,13]. Muscle strength has been assessed with hand grip force (HGF) using

a hydraulic or digital dynamometer, according to the EWGSOP[13]. Low muscle strength has been defined by EWGSOP as HGF ≤30 Kg for men and ≤20 Kg for women[13,45]. In subjects with COPD, Jones et al.[15] and and Byun et al.[10], have used these values to identify lower muscle strength in this population. Sarcopenia stage is characterized by low muscle mass, plus low muscle strength or low physical performance, for this reason the EWGSOP suggests to use the three criteria to diagnose sarcopenia[13]. However, some authors only considered lower muscle mass criteria to diagnose sarcopenia in individuals with COPD[16,38,46,47] (table1).

Physical performance has been measured using gait speed (GS) in four meters, which is a functional test where the subject has to walk with common gait speed in four meters, the average speed of two walks is used for analysis [45]. Low physical performance has been defined by EWGSOP as GS < 0.8 m/sec for both genders[13,45]. In addition, in COPD population, there are two studies which classified sarcopenia using the three criteria (muscle mass, muscle strength and physical performance[15,43]. However, the study of Jones et al..[15] is the only one that considers the measurements and methods suggested by the EWGSOP to find individuals with sarcopenia (table1), since Borda et al.[43] assessed muscle mass with calf circumference.

Table 1: Criteria used in subjects with COPD to identity sarcopenia in different studies.

Criteria	Measurement Methods	Cut-off point	Reference
Lower muscle mass	DXA	For men ALM (in Kg) = −28.15 + 27.49 × height (in m) + 0.1106 × fat mass (in Kg) For women ALM (in Kg) = −19.78 + 20.00 × height (in m) + 0.1554 × fat mass (in Kg) • Baumgartner criteria for individuals with a BMI < 22 Kg/m2. • Newman criteria for individuals with a BMI ≥ 22 Kg/m2.	Costa et al. ([16])
Lower muscle mass	BIA	For men SMI of ≤8.50 Kg/m2 For Women SMI of ≤5.75 Kg/m2 *According to (EWSGOP)[13].	Jones et al. ([15])
Lower muscle strength	HGF	For men HGF <30 Kg For women <20 Kg for women *According to (EWSGOP)[13].	
Lower physical performance	GS	In 4 meter GS <0.8 m/s *According to (EWSGOP)[13].	

Lower muscle mass	BIA	SMMI at least two standard deviations (SDs) below normal sex-specific means in young persons. (KNHANES).	Byun ([10])
Lower muscle strength	HGF	For men HGF <30 Kg For women <20 Kg for women *According to (EWSGOP)[13].	
Lower muscle mass	DEXA	ASMI For men SMI<7.23 Kg/m^2 For women SMI <5.67 Kg/m^2 *According to (EWSGOP)[13].	Van de Bool et al.([46])
Lower muscle mass	DEXA	ASMI at least two standard deviations (SDs) (sarcopenia) and between 2SDs and 1SD (presarcopenia) below the mean value of a young male reference group aged 20–39 years (KNHANES).	Hwang et al. ([38])
Lower muscle mass	DEXA	ASMI For men SMI<7.23 Kg/m^2 For women SMI <5.67 Kg/m^2 *According to (EWSGOP)[13].	Cebron Lipovec et al. ([47])
Lower muscle	BIO	SMI Lower than the 10th percentile from the general population of the UK Bio- bank[48].	Joppa et al. ([49])

mass			
Lower Muscle mass	CC	Calf circumference < 31 cm.	Borda et al. ([43])
Lower Muscle strength	HGF	Lower the last quintile in Colombian population.	
Lower physical performance	GS	Lower the last quartile in Colombian population.	

Legend: SDs: standard deviations; BIO: bioelectrical impedance analysis; DEXA: dual-energy x-ray absorptiometry; ALM: appendicular lean mass; KNHANES: Korean National Health and Nutrition Examination Survey; EWSGOP: European Working Group on Sarcopenia in Older People; GS: gait speed; HGF: hand grip force; CC: calf circumference; ASMI: appendicular skeletal muscle index; SMI: skeletal muscle mass index.

5.3 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[26]. 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[12,32]. These factors are also present in subjects with COPD and can potentially lead to sarcopenia in this disease (Figure 1)[10,11,50].

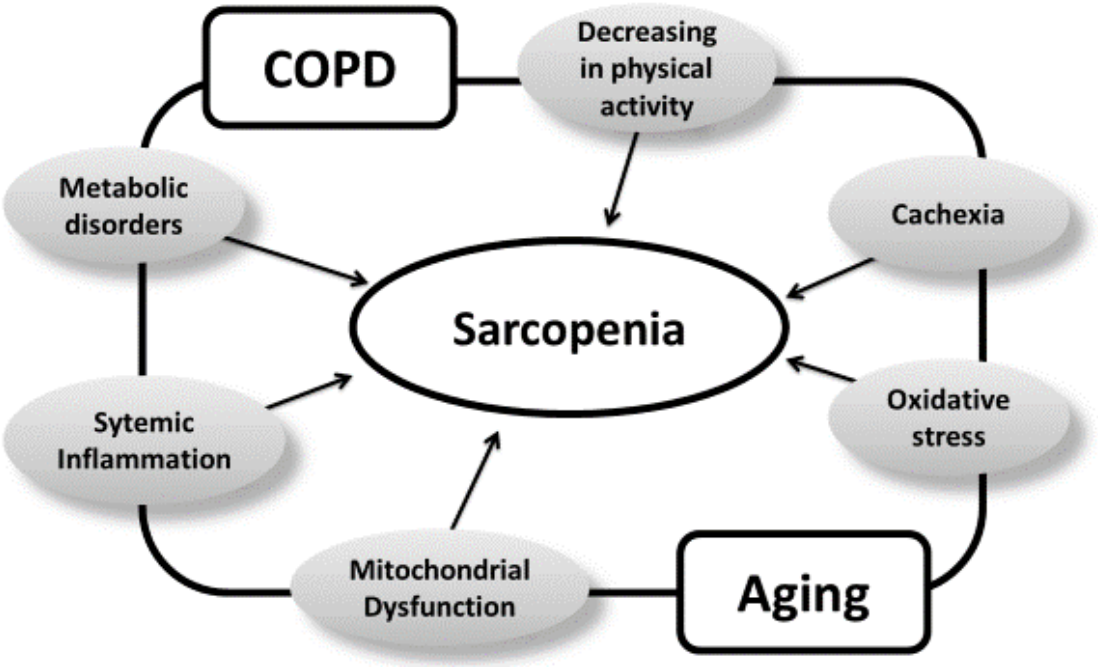

Figure 1. Features associated with sarcopenia among COPD and aging. COPD and Aging have been related to metabolic disorders, systemic inflammation, mitochondrial dysfunction, lower levels of physical activity, cachexia and oxidative stress. All these factors induce Sarcopenia.

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, and indirectly genetic mutations or polymorphism associated and sarcopenia (figure 2)[9,11,12,51]. Oxidative stress, chronic inflammation and mitochondrial dysfunction play important roles in muscle atrophy, because these factors affect the balance among protein synthesis and breakdown[12,18]. Oxidative stress is an imbalance between oxidant and antioxidant species[52]. 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[12]. TNF- α is one of the markers that induce cellular apoptosis in the muscle, which is associated with muscle catabolism, declining both muscle mass and strength[53]. TNF-α causes muscle weakness through two mechanisms: accelerated protein loss and contractile dysfunction[53]. The systemic inflammation and oxidative stress decreases 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[54,55]. The role of IGF-1 is to signal thought Akt pathway inducing protein synthesis by stimulation of mammalian target of rapamycin (mTOR)[26]. 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 (figure 3). IGF-1 can reduce with aging and chronic diseases, reducing the activity of Akt and mTOR[12]. These cascades signaled by oxidative stress and inflammatory biomarkers are produced during the aging process and it is exaggerated in the presence of chronic diseases, which is associated with sarcopenia[11,50].

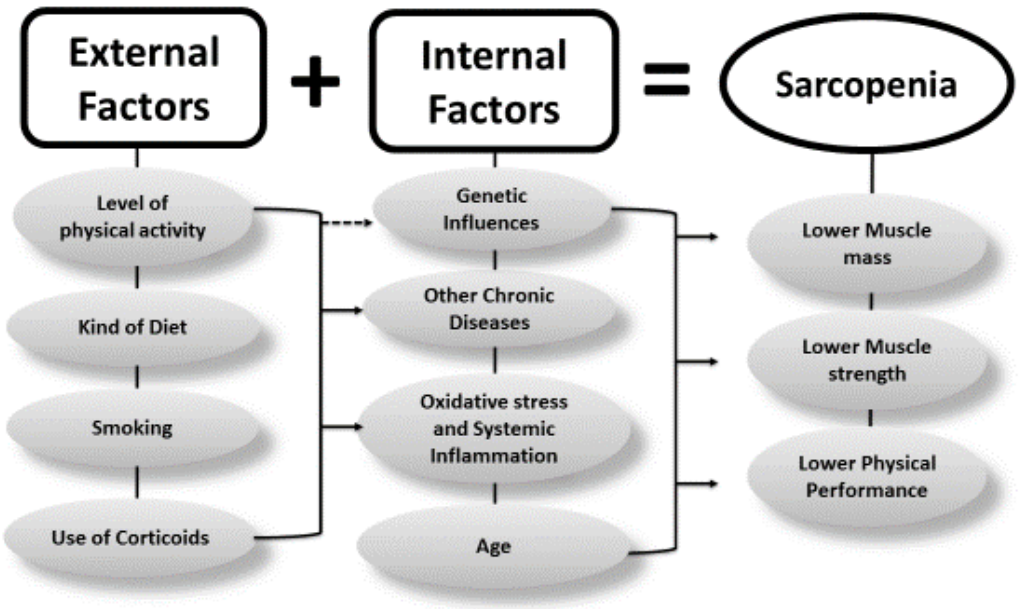

Figure 2. Factors associated with sarcopenia in COPD. Sarcopenia is induced by external and internal factors, which have direct association (continue line) or indirect association (dashed line) with each other and with sarcopenia.

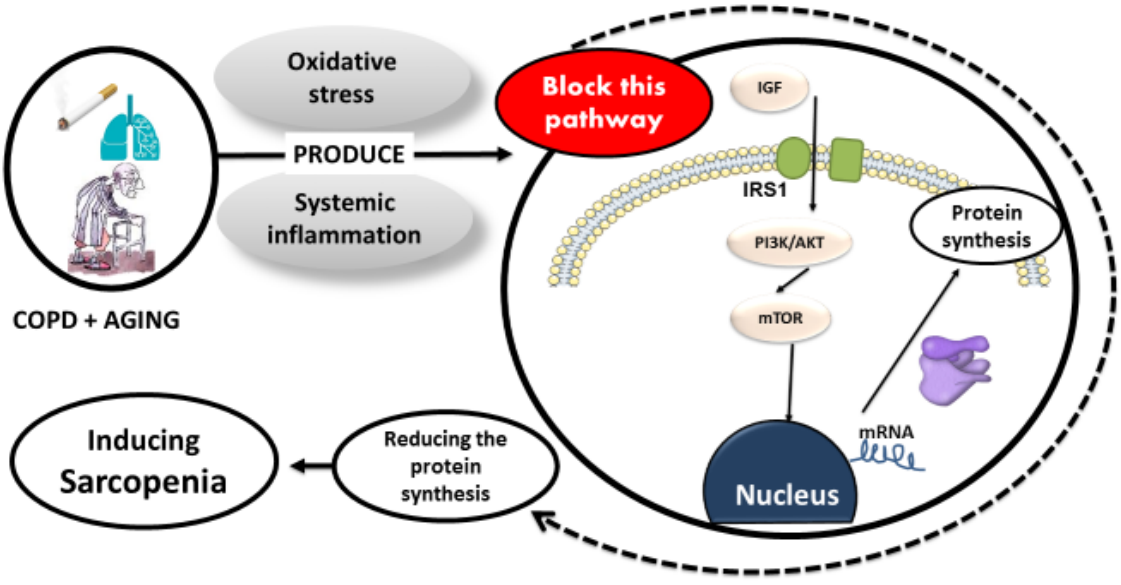

Figure 3. COPD and Aging induce Sarcopenia. COPD and Aging are associated with oxidative stress and systemic inflammation, they block the IGF/PI3K/AKT/mTOR pathway, reducing the protein synthesis, therefore, inducing Sarcopenia.

5.4 ANTIOXIDANT AND OXIDANT BIOMARKERS IN COPD

Cesari et al.[56] 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.[57] 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)[23,24,58,59]. 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 control group[21,60].

Subjects with COPD have an oxidant-antioxidant imbalance[23,58,59]. The levels of oxidative stress biomarkers are increased in individuals with COPD. [21,23,24,58–63]. Lipid peroxidation and protein oxidation are consequences of oxidative stress and cause of oxidative damage[21,59]. Thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) are the most commonly biomarkers of lipid peroxidation studied in subjects with COPD[59]. Niraj Dhakal et al.[59], Raut et al.[62] and Wozniak et al.[60] reported higher levels of MDA and TARBS in subjects with COPD compared to control group. In contrast with that, Syrine et al.[63] and Jammes et al.[64] found no differences in MDA and TBARS levels, respectively, among individuals with COPD and their counterparts. In COPD, the degree of oxidant-mediated protein damage has been investigated through the presence of advanced oxidation protein products (AOPP)[17]. Stanojkovic et al.[65] 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.

Finally, there are few studies that reported associations between antioxidant markers and sarcopenia or physical activity in individuals with COPD[63]. The majority of the studies correlated oxidative stress biomarkers with body mass index, lung function, nutritional status, exacerbations or GOLD stages[22,59,63,65,66]. The

research of Byun and collaborators[10] is the only one which reported association among some inflammatory biomarkers with sarcopenia in subjects with COPD. However, there are no studies reporting associations between sarcopenia and oxidative stress in this population. Therefore, since sarcopenia is the main cause of frailty in older people[13] and it is related with mortality and poor prognosis in individuals with COPD[16,43,47], new studies are necessary to identify oxidant or antioxidant biomarkers related with muscle strength, muscle mass and physical performance, in order to comprehend some mechanisms that produce sarcopenia in this disease.

6 ARTICLE

Oxidative stress correlates with clinical measurements of Sarcopenia in COPD

Artigo original formatado de acordo com as normas do periódico *Respir Med*
Fator de Impacto: 3,526; Qualis: A2.

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Abstract:

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a systemic disease that increases reactive oxygen species, causing oxidative stress (OS). OS may induce damage

in muscle fibers, producing sarcopenia, impairing functionality and quality of life. **Objective:** To analyze OS biomarkers between apparently healthy individuals (control group) and subjects with COPD, establishing associations with sarcopenia. **Design:** Cross-sectional study. **Participants:** 39 COPD subjects (69±7 years; 41% female; FEV_1: 49±13% pred) and 35 for control group (CG) (69±7years; 43% female; FEV_1: 98±16% pred). **Measurements:** The following OS biomarkers after blood analysis: advanced oxidation protein products (AOPP), paraoxonase 1 (PON1), superoxide dismutase activity (SOD), catalase dismutase activity (CAT), sulfhydryl group (SH), nitric oxide metabolites (NOX) and total radical trapping antioxidant parameter (TRAP). Maximal inspiratory and expiratory pressure (MIP and MEP, respectively), gait speed (GS), handgrip strength (HGS), quadriceps strength (QS), skeletal muscle mass index (SMMI) and fat-free mass index (FFMI). European criteria were used to detect Sarcopenia. **Results:** COPD subjects showed higher levels of antioxidant activity, SH, PON-1 and SOD; P<0.02 for all. In COPD, TRAP showed positive correlation with FFMI (r=0.5), SMMI (r=0.5), QS (r=0.64) and HGS (r=0.51) and 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); *P*<0.05 for all. FFMI and GS were explained by TRAP and BMI (R^2:0.80 and R^2:0.51; *P*<0.004). TRAP was the only determinant of QS (R^2:0.43; *P*=0.004). SMMI was explained by TRAP, AOPP and BMI (R^2:0.51; *P*=0.0004). The cut-off points TRAP≤ 850 µM trolox and AOPP≤65 µM/l were associated with risk of sarcopenia (OR: 8.3; 95% CI:1.372-49.6 and OR:14; 95% CI: 2.251-87.05, respectively; *P*<0.05 for both). **Conclusion:** COPD subjects showed higher antioxidant activity and more sarcopenia prevalence compared to CG. Clinical measurements of sarcopenia were correlated to OS biomarkers, being TRAP and AOPP highly associated with sarcopenia outcomes in this population.

Keywords: Oxidative stress, Sarcopenia, COPD.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a condition characterized by extrapulmonary changes, which affect functionality and quality of life[1,2]. Mainly, because COPD subjects have a lower level of physical activity, which can decrease muscle mass and generate muscle weakness[3–5]. Muscle weakness increases in the presence of chronic diseases and is directly related to sarcopenia[6].

Sarcopenia is defined as a reduction in muscle strength, physical performance and muscle mass[6]. This is the major cause of frailty [5,6] and has a prevalence of approximately 5–13% in the elderly[7]. The prevalence of sarcopenia in COPD is higher and varies between 15%[8] to 40%[4], depending on classification's criteria[6]. This geriatric syndrome is associated with metabolic change, immobility, mitochondrial dysfunction[5,9], oxidative stress (OS) [10,11] and systemic inflammation[3], which contribute to age-related alterations in the muscle[12], *via* increased damage to the cells[10].

There is strong evidence in COPD showing pathophysiological changes related to the oxidant / antioxidant disbalance to increase OS[13–15]. Depending on the selection of the OS biomarker, however, literature is still inconclusive. 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 [13,16–18].

It is likely that the OS biomarkers altered in sarcopenia are associated with clinical measurements used to diagnose sarcopenia (muscle strength, muscle mass, and physical performance) as biomarkers induce more inflammation and muscle atrophy[19]. The association (or lack thereof) between these outcomes, however, remains to be demonstrated. The aim of this study was to analyze and compare oxidative stress biomarkers between apparently healthy individuals and subjects with COPD, establishing associations with clinical measurements of sarcopenia.

MATERIAL AND METHODS

Patients with diagnosis of COPD[20] (55 ≥ years old) were recruited from the University Hospital of Londrina State University in Londrina, Brazil. Subjects with exacerbation during last two weeks, diagnosis of bronchial asthma, the presence of neurological or psychiatric diseases, arthritis, heart failure, alcohol dependence and use of antioxidant supplements were excluded. The control group included apparently healthy individuals recruited from the community who were matched for gender, age and ethnicity to the subjects with COPD. The study was approved by the university ethics review board and all participants provided written informed consent (1.830.048).

Measurements

Pulmonary function was assessed with whole-body plethysmography Elite Series®, Plethysmograph-MedGraphics). Measurements were performed according to the American Thoracic Society/European Respiratory Society guidelines[20], with FEV_1, FVC, and FEV_1,/FVC ratio and reference values were those described for the Brazilian population[21]. The quantity 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.[22].

Diagnosis of sarcopenia was done according to the European working group of sarcopenia in older people (EWGSOP) using the presence of low muscle mass and low muscle function (handgrip strength and physical performance)[6]. Muscle mass was quantified with 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 by the formula of Kyle et at[23]. Lower fat-free mass index was considered as FFMI <20.35kg/m^2 for men and 14.65 kg/m^2 for women[24]. SMM was estimated using the equation developed by Janssen et al.[25]. Low muscle mass was classified as SMMI ≤10.75 Kg/m^2 for men and ≤6.75 kg/m^2 for

women[6]. 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 each) was used as a maximal force value. Low muscle strength for HGS was defined as ≤30 Kg in men and ≤20 Kg in women[6]. Physical performance was evaluated with gait speed (GS) in 4 meters. The average speed of two walks was used for analysis. Low physical performance was defined as GS ≤0.8 m/sec[6].

Other measurements of muscle function associated with sarcopenia were also investigated. 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[26]. In short, participants performed a minimum of four and a maximum of five maximal voluntary isometric contractions of the quadriceps for six seconds. Less than 5% variability was established between the highest values. The highest value was considered for analysis[26]. 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 procedure[27]. 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.

Oxidative stress biomarkers were analyzed from blood samples. Blood was drawn from subjects after an overnight fast (10h). Samples were centrifuged at 3000 rpm for 10 minutes to separate blood components (serum and hematocrit). Total radical-trapping antioxidant parameter (TRAP)[28], nitric oxide metabolites (NOx)[29],superoxide dismutase activity (SOD) in erythrocytes[30], catalase activity (CAT) in erythrocytes[31], sulfhydryl groups (SH)[32], advanced oxidation protein products (AOPP)[33] and paraoxonase 1 activity (PON1)[34] were used as oxidative stress biomarkers and glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides were used as metabolic risk factors assayed following previous studies[28–34].

Statistical Analysis

Statistical analysis was performed using SPSS (IBM Co., USA). The Kolmogorov–Smirnov test was used to analyze normality of data distribution. Since data showed normal distribution, they were expressed as mean ± SD. Comparisons were done using Chi square test and unpaired Student t-tests with 95% confidence intervals (CIs). Pearson's correlation coefficient was used to assess relationships of strength, muscle mass and gait speed with OS biomarkers. Linear regression analysis was performed to find significant determinants between OS biomarkers and clinical measurements of sarcopenia. Logistic regression analysis was used to identify factors most associated with sarcopenia in COPD. Receiver-operating characteristic (ROC) curves, the area under the curve (AUC), sensitivity and specificity were used to identify best cut-offs discriminating sarcopenia of the following variables: QS, MIP and MEP, antioxidant and oxidant biomarkers. Statistical significance was set at $P<0.05$.

RESULTS

Seventy-four subjects (thirty-nine patients with COPD and thirty-five apparently healthy subjects) were included. Socio-demographic characteristics, age-adjusted, Charlson comorbidity index 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 CG ($P=0.02$ and 0.01, respectively). QS and MIP were worse in COPD than CG ($P<0.001$ and <0.05, respectively).

Antioxidant and oxidant biomarkers are reported in Figures 1 and 2. The following biomarkers were 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 difference between groups was observed for TRAP, AOPP and NOX.

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.

Selection of presented cut-offs used in the logistic regression was 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)[4,24]. The selection of cut-offs for the outcomes without reference values was done via the ROC analysis. 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. Factors significantly associated with the development of sarcopenia in individuals with COPD were: age, gender (female), BMI, FFMI, MIP, MEP, QS, TRAP, AOPP and HDL ($p<0.05$ 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. Prevalence of sarcopenia in the present sample of subjects with COPD (23%) was similar with previous studies reporting prevalence rates between 15%[8] to 40%[4] and significantly higher than the observed in the control group (3%). Also, patients with COPD presented higher scores in the age-adjusted Charlson comorbidity index (more comorbidities with larger weights). Of note, the abovementioned differences between COPD and the control group were expected and previously described[35].

COPD has been related to systemic inflammation and oxidative stress[3,13,14]. In the present study, the sulfhydryl group (SH) was significantly increased in COPD subjects. In line with our findings, Nadeem et al.[16] 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[36]. PON1 is an enzyme with antioxidative and antiatherogenic properties, which is associated with high-density lipoprotein (HDL)[37]. Its activity was found reduced in different diseases associated with oxidative stress such as COPD[13,14]. 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[37]. 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 III-IV, a group of subjects with known higher levels of HDL[38].

SOD is an important antioxidant enzyme that inhibits superoxide anion (O_2^-) and protects aerobic cells from oxidative stress[17]. We observed higher levels of superoxide dismutase in patients with COPD. Similar findings have been reported in other studies[16,17]. SOD is the only enzymatic system-decomposing O_2^- to H_2O_2 and it plays a significant role especially in the lung and muscular cells[39]. 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). Therefore, it can be hypothesized that higher SOD activity is associated with lower inspiratory muscle strength in subjects with COPD. Since a reduction in MIP induces respiratory overload, it might be related to an increase in SOD activity, as this enzyme is sensitive to the stress in the respiratory system[39]. Another important antioxidant enzyme is catalase. Literature, however, is still controversial about its levels in subjects with COPD[13,17,18]. Wozniak et al. evaluated 73 patients with COPD and observed no differences in CAT activity compared to the control group[17]. In contrast, Vibhuti et al. dosed the antioxidant capacity in 202 subjects with COPD and found a significant reduction in CAT activity in comparison to healthy controls[18]. 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[18]. Nitric oxide (NO) is an important element of vascular homeostasis[40]. OS reduces the synthesis of NO impairing the endothelial and pulmonary function[36,40]. Anes et al.[36] found a significant reduction in products of NO (NOx) levels in subjects with COPD compared to control group and observed an association between NOx and airflow obstruction. In our study, although 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 to estimate protein damage[33]. This is the first study to investigate correlations between AOPP and sarcopenia measurements in COPD. AOPP was positively correlated with muscle mass and strength (FFMI: 0.43; SMMI: 0.51; HGS: 0.5, MIP: 0.6 and MEP: 0.46). A likely explanation for the correlations is that increased OS in

COPD increases the proteins oxidation levels. Higher muscle mass and strength implies in more protein substrate to be oxidized[5,41] and therefore higher AOPP levels (a biomarker associated with oxidation of albumin, fibrinogen and lipoproteins[42]). This could also help understanding why lower values of AOPP (≤65 μM/l) were associated with sarcopenia in subjects with COPD.

In a multivariate analysis TRAP, AOPP and BMI were the variables explaining the increase in SMMI (R^2: 0.51). In our 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[42]. In the present study, this biomarkers combined with other outcomes explained 80% of FFMI, 51% of QS and 43% of GS. Therefore, 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[6]. 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 studies are encouraged to confirm these results.

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. This was a cross-sectional study and therefore causality of associations should be interpreted with caution. 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].

CONCLUSIONS

Subjects with COPD showed higher levels of antioxidant activity and more sarcopenia prevalence when compared to apparently healthy individuals. Clinical measurements of sarcopenia were correlated with OS biomarkers, being lower antioxidative capacity and protein oxidation associated with more prevalence of sarcopenia in COPD.

ACKNOWLEDGMENTS

The authors would like to thank all professionals from the clinical hospital at Londrina State University, Brazil and all patients and volunteers from the community for their collaboration.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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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*
GOLD I-II, n (%)	18 (46%)	-
GOLD III-IV, n (%)	21 (54%)	-
Charlson Comorbidity index		
Heart Failure	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-C (mg/dl)	115 ± 38	110 ± 35

HDL-C (mg/dl)	65 ± 34	54 ± 12
Triglycerides (mg/dl)	130 ± 88	132 ± 96

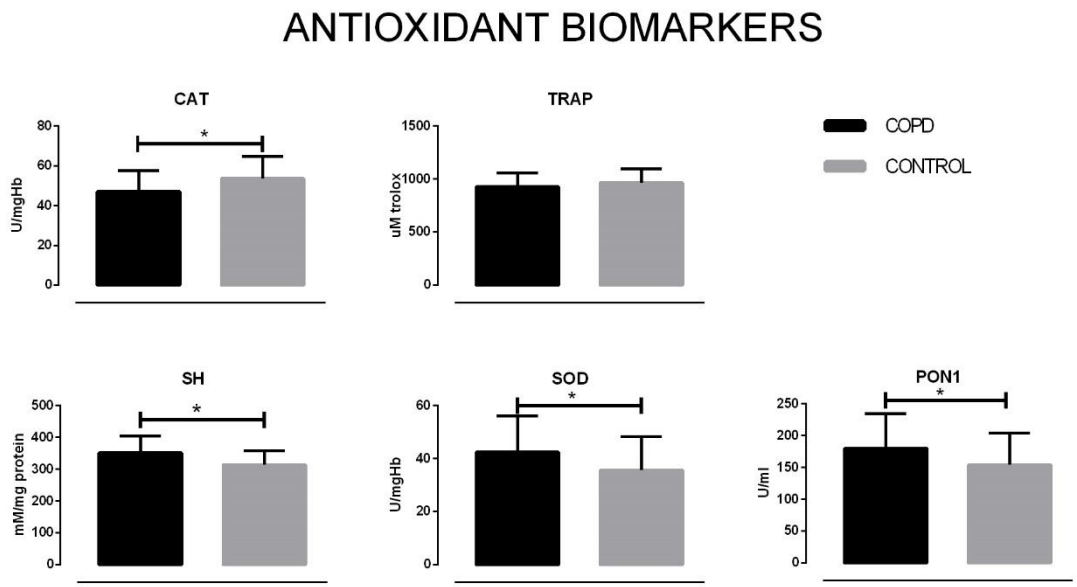
Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; *P≤0.05; **P≤0.001.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

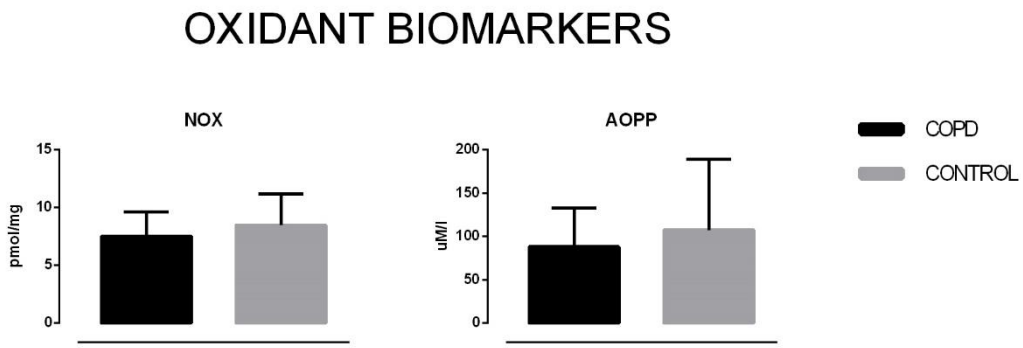
Data are expressed as mean ± standard deviation. COPD: Chronic pulmonary obstructive disease; QS: quadriceps strength; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; FFM: fat-free mass; FFMI: fat-free mass index; SM: skeletal muscle mass; SMMI: skeletal muscle mass index; HGS: handgrip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

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.

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			

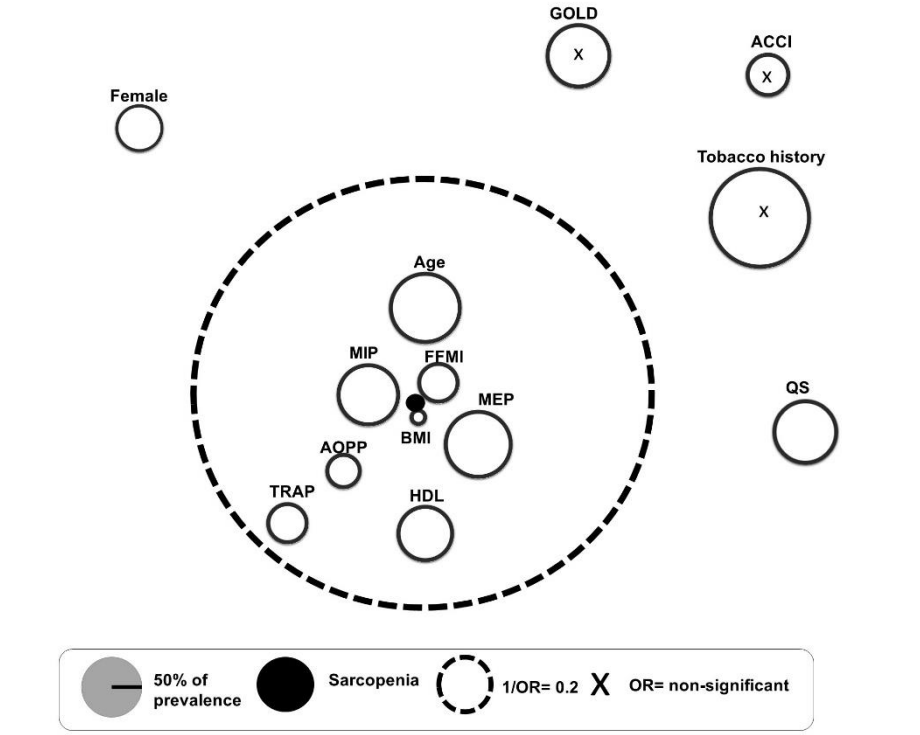
COPD: Chronic pulmonary obstructive disease; FFMI: fat-free mass index; SMMI: skeletal muscle mass index; QS: quadriceps strength; GS: gait speed; HGS: handgrip strength; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; TRAP: total radical trapping antioxidant parameter; AOPP: advanced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

Table 4: ROC curve analysis to detect individuals with sarcopenia

	TRAP	AOPP	HDL	QS	MIP	MEP
Cut off	≤ 885	≤ 65	≥57.5	≤ 26.15	≤ 77.5	≤115
	µM trolox	µM/l	mg/dl	Kg	cmH_2O	cmH_2O
Sensitivity	78%	68%	89%	100%	90%	70%
Specificity	74%	75%	66%	63%	63.3%	69%
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.

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).

FINAL CONCLUSION

This study showed that individuals with COPD presented increase in the levels of antioxidant biomarkers in blood and higher prevalence of sarcopenia in comparison with apparently healthy subjects. In addition, the majority of the measurements of sarcopenia were associated with oxidative stress biomarkers, being AOPP and TRAP highly correlated to muscle strength, muscle mass and gait speed, which are the criteria to identify sarcopenia in older people according to the EWGSOP.

Finally, considering the findings of the study, it is possible to identify three main points. Firstly, the recommendation of the EWGSOP should be used in order to diagnose sarcopenia. Since many studies considered only muscle mass as a criteria to define sarcopenia, this could lead to an overestimation of its prevalence. Secondly, oxidative stress biomarkers are one of the factors associated with sarcopenia in COPD, and increase in antioxidant activity could be related to compensatory response to oxidative stress. Finally, more studies are necessary to comprehend the relation between TRAP with measurements of sarcopenia, since this antioxidant biomarker has been little studied in individuals with COPD and deserves to be further explored.

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APPENDING

APPENDING A

Spirometry evaluation sheet

Espirometria

Nome: _____ Data: ____/____/____

Idade: _____ Peso: _____ Altura: _____

Avaliador: _____

Treinamento: Endurance e força ( ) RCTP ( )

Avaliação: Pré-tto ( ) 3 meses ( ) 6 meses ( ) 6 meses pós-tto ( )

Espirometria

Pré-Bd CVF (L / %): _____ Pós-Bd CVF (L / %): _____

VEF_1 (L / %): _____ VEF_1 (L / %): _____

VEF_1/CVF: _____ VEF_1/CVF: _____

Qualidade: _____ Qualidade: _____

Laudo: _____ GOLD: _____

OBS.1: O laudo é dado segundo as Diretrizes para Teste de Função Pulmonar, da Sociedade Brasileira de Pneumologia e Tisiologia (2002), enquanto o GOLD é segundo as diretrizes do *Global Initiative for Chronic Obstructive Lung Disease*.

OBS.2: Os valores de referência deverão ser segundo - Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007; 33(4):397-406.

Observações:

_____

APPENDING B

Strength evaluation sheet

Avaliação da Força Muscular

Data: _____/_____/_____ Avaliador:_____

Dados Pessoais

Nome: _____

Destro ( ) Canhoto ( )

Entrada da barra de quadríceps: Superior ( ) Inferior ( )

Peso: _____ Altura: _____ IMC: _____

1RM	Kg
Quadríceps Femoral	
Bíceps Braquial	
Tríceps Braquial	

APENDING C

Calculation of volume of blood to do the specific test

Divisão das amostras para fazer os testes		
PRECEDENTE	EVALUATION	VOLUME (µL)
SORO	TRAP AOOP PON-1	700
SORO	FOX NO SH	600
SORO	LOOH	300
SORO	SOBRA	SOBRA
SORO	MDA	400
SORO	INTERLEUCINAS	400
PLASMA	SOBRA	SOBRA
PLASMA	SOBRA	SOBRA
HEMÁCIA	SOD CATALASE	HEMÁCIA
HEMÁCIA	B.C	B.C
HEMÁCIA	GSH	HEMÁCIA
HEMÁCIA	SOBRA	SOBRA

ANNEX

ANNEX A

Informed consent

Termo de Consentimento Livre e Esclarecido

“Estratégias para a manutenção do equilíbrio corporal em DPOC: associação da fraqueza muscular respiratória e hiperinsuflação pulmonar com déficit de equilíbrio, um estudo controlado”

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo (a) para participar da pesquisa **“Estratégias para a manutenção do equilíbrio corporal em DPOC: associação da fraqueza muscular respiratória e hiperinsuflação pulmonar com déficit de equilíbrio, um estudo controlado”**, a ser realizada na Universidade Estadual de Londrina, Londrina-PR**.** O objetivo da pesquisa é investigar as estratégias utilizadas para a manutenção do equilíbrio corporal em pacientes com doença pulmonar obstrutiva crônica e comparar com as estratégias presentes em indivíduos saudáveis**.** Sua participação é muito importante e ela se daria da seguinte forma: todos os participantes serão submetidos à avaliação do equilíbrio corporal estático (plataforma de força) associado à eletromiografia dos músculos do tronco e membros inferiores; função pulmonar (pletismografia); força muscular respiratória (pressões respiratórias máximas) e periférica (dinamometria); composição corporal (bioimpedância elétrica); nível de atividade física na vida diária (monitores de atividade física); capacidade funcional de exercício (teste de caminhada de 6 minutos); qualidade de vida, sensação de dispneia e sintomas de ansiedade e depressão (questionários).

Esclarecemos que sua participação é totalmente voluntária, podendo o (a) senhor (a): recusar-se a participar, ou mesmo desistir a qualquer momento, sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Esclarecemos, também, que suas informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

Esclarecemos ainda, que o(a) senhor(a) não pagará e nem será remunerado(a) por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação.

Como benefícios advindos deste estudo, esperamos contribuir para o conhecimento científico da área. Além disso, caso qualquer alteração de saúde seja identificada, os participantes serão encaminhados para o serviço de saúde apropriado para o acompanhamento. Quanto aos riscos, nenhum dos procedimentos apresentam risco direto para a integridade física ou moral dos participantes.

Caso o(a) senhor(a) tenha dúvidas ou necessite de maiores esclarecimentos poderá nos contatar (pesquisadora responsável: Vanessa Suziane Probst; endereço: Avenida Robert Koch, 60 - Laboratório de Pesquisa em Fisioterapia Pulmonar; telefone: (43) 33712477 ou (43) 91613022; e-mail: vanessaprobst@uol.com.br), ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, situado junto ao LABESC – Laboratório Escola, no Campus Universitário, telefone 3371-5455, e-mail: cep268@uel.br.

Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas devidamente preenchida, assinada e entregue ao (à) senhor(a).

Londrina, ___ de _____de 201_.

Pesquisador Responsável

RG:_____

_____, tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____

Data:_____

Obs.: Caso o participante da pesquisa seja menor de idade, o texto deve estar voltado para os pais e deve ser incluído ainda, campo para assinatura do menor e do responsável.

ANNEX B

Acceptance of the ethics committee.

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Estratégias para manutenção do equilíbrio corporal em DPOC: associação de fraqueza muscular inspiratória e hiperinsuflação pulmonar com déficit de equilíbrio, um estudo controlado.

Pesquisador: Vanessa Suziane Probst

Área Temática:

Versão: 3

CAAE: 49998315.5.0000.5231

Instituição Proponente: Departamento de Fisioterapia

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 1.830.048

Apresentação do Projeto:

Trata-se de emenda ao projeto intitulado "Estratégias para manutenção do equilíbrio corporal em DPOC: associação de fraqueza muscular inspiratória e hiperinsuflação pulmonar com déficit de equilíbrio, um estudo controlado."

No qual a pesquisadora solicita a inclusão de novas análises.

No projeto da pesquisa inicial, o objetivo é explicar as estratégias para a manutenção do equilíbrio corporal em pacientes com DPOC e investigar se a fraqueza muscular inspiratória e a hiperinsuflação pulmonar tem associação com o déficit de equilíbrio apresentado por essa população. No entanto, com o intuito de ampliar ainda mais o conhecimento na área, a equipe envolvida no projeto decidiu por incluir novas metodologias que irão fornecer dados para elucidação das razões pelas quais os pacientes com DPOC tem pior equilíbrio e estão mais propensos às quedas, quando comparados a idosos saudáveis.

Desta forma justifica-se a inclusão do estudo da sarcopenia, a qual é definida como uma diminuição da força, rendimento e controle muscular e tem sido apontada como uma das principais causas da fragilidade em idosos. A sarcopenia está associada ao estresse oxidativo, disfunção mitocondrial, alteração metabólica, inflamação sistêmica, imobilidade e envelhecimento, e recentemente também foi correlacionada com polimorfismos

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UF: PR **Município:** LONDRINA
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genéticos.
Assim sendo a pesquisadora solicita a adição de avaliações relacionadas com níveis de marcadores de estresse oxidativo, de inflamação e polimorfismos genéticos que permitam estabelecer correlações entre as avaliações de eletromiografia, controle postural e rendimento físico.
Para tanto será feita coleta de sangue, por profissional capacitado vinculado ao HU-UEL para a avaliação do estresse oxidativo. A amostra de sangue também será utilizada para a avaliação da inflamação por meio da osagem das interleucinas IL-6 e fator de necrose tumoral (TNF) pelo método de ELISA.
Para a avaliação dos polimorfismos genéticos, será coletada uma amostra de saliva para análise das células epiteliais da mucosa bucal e posterior extração de DNA. A análise do polimorfismo do gene IL-6, IL-10, TNF-, Vitamina D e F-box protein 32 será realizada por
meio da reação em cadeia da polimerase (PCR) em tempo real.

Para essas análises extras foram incluídos no projeto os professores:
- Décio Sabbatini Barbosa: o qual será responsável pela supervisão das análises de estresse oxidativo e marcadores inflamatórios.
- Regina Celia Poli Frederico: a qual supervisionará as análises de polimorfismos.

Objetivo da Pesquisa:
O objetivo da emenda é elucidação das razões pelas quais os pacientes com DPOC tem pior equilíbrio e estão mais propensos às quedas, quando comparados a idosos saudáveis.
Para isso será investigada a sarcopenia, em especial sua relação com o estrese oxidativo, disfunção mitocondrial, alteração metabólica, inflamação sistêmica, imobilidade e envelhecimento, e também com polimorfismos genéticos.

Avaliação dos Riscos e Benefícios:
A pesquisadora afirma no TCLE que nenhum dos procedimentos adicionais apresenta risco direto para a integridade física ou moral dos participantes. Talvez, em alguns casos, um pequeno hematoma poderá ser gerado pelo processo da coleta de sangue. Contudo a pesquisadora se responsabiliza pela assistencia integral e imediata dos participantes caso eles sintam algum desconforto.
Como benefícios a pesquisadora afirma que com este estudo será possível contribuir para o
conhecimento científico da área.
A pesquisadora afirma que caso qualquer alteração de saúde seja identificada, os participantes serão encaminhados para o serviço de saúde apropriado para o acompanhamento.

Comentários e Considerações sobre a Pesquisa:

emenda bem justificada com base na literatura.

Considerações sobre os Termos de apresentação obrigatória:

A pesquisadora apresentou um novo TCLE para as novas metodologias propostas, considerando os possíveis riscos das novas análises e assumindo a responsabilidade pelos mesmos.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_750910E1.pdf	14/11/2016 11:01:59		Aceito
Declaração de Pesquisadores	declaracao_emenda.pdf	14/11/2016 10:59:22	Vanessa Suziane Probst	Aceito
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Outros	Concessao.pdf	04/09/2015 17:11:49	Vanessa Suziane Probst	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	04/09/2015 17:06:38	Vanessa Suziane Probst	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

LONDRINA, 22 de Novembro de 2016

Assinado por:
Rosana Lopes
(Coordenador)

Endereço: LABESC - Sala 14
Bairro: Campus Universitário **CEP:** 86.057-970
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ANNEX C

Author information of Respiratory Medicine.

RESPIRATORY MEDICINE

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

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