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EFEITOS DA REABILITAÇÃO PULMONAR SOBRE A FUNÇÃO ENDOTELIAL E SOBRE OS NÍVEIS PLASMÁTICOS DE BDNF EM PACIENTES COM DPOC

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre, como requisito para a obtenção do grau de Doutor em Ciências da Saúde.

Orientador: Prof. Dr. Pedro Dal Lago

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“*Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas graças a Deus, não sou o que era antes*”.

Marthin Luther King (1929 – 1968)

RESUMO

A doença pulmonar obstrutiva crônica (DPOC) apresenta caráter progressivo e sistêmico. Embora acometa principalmente os pulmões, a mortalidade parece estar mais relacionada à doença cardiovascular do que à pulmonar em si. A fisiopatologia da DPOC envolve múltiplas vias, com marcado desequilíbrio de diversos biomarcadores que apresentam alterações suscetíveis ao exercício. Evidências emergentes apontam o fator neurotrófico derivado do cérebro (BDNF) como um importante marcador na DPOC, estando relacionado a parâmetros de gravidade da doença. Os programas de reabilitação pulmonar (RP), os quais têm como elemento central o exercício físico, constituem uma importante ferramenta para a melhora do quadro clínico de pacientes com DPOC. Com base nesses fatos, a presente tese teve como objetivo conduzir dois estudos com os objetivos de avaliar (1) os efeitos da RP na função endotelial e rigidez arterial e (2) nos níveis plasmáticos de BDNF em pacientes com DPOC. No estudo 1, foi verificado que a função endotelial, a rigidez arterial e pressão arterial sistêmica não foram modificados com a reabilitação pulmonar. O estudo 2, demonstrou que uma única sessão de exercício em pacientes sedentários (efeito agudo) reduz os níveis de BDNF, porém esse efeito não se mantém com o treinamento e o treinamento não é capaz de reduzir os níveis basais de BDNF.

Ainda assim, ambos estudos demonstram que a RP melhorou a capacidade funcional, a dispneia, a qualidade de vida e reduziu o risco de morte nesses pacientes. Com bases nos atuais achados, pode-se concluir que ainda são necessários avanços no que diz respeito a intervenções capazes de modificar parâmetros cardiovasculares, bem como se faz importante o melhor entendimento da cinética do BDNF em relação ao exercício físico e seu entendimento com marcadores de severidade da DPOC.

Palavras-chave: Doença pulmonar obstrutiva crônica; Reabilitação pulmonar; Função endotelial; Rigidez arterial; Fator neurotrófico derivado do cérebro; Capacidade funcional; Risco de morte.

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is progressive and presents systemic character. Although it mainly affects the lungs, mortality seems to be more related to cardiovascular disease than to pulmonary disease itself. The pathophysiology of COPD involves multiple pathways, with marked imbalance of several biomarkers which present changes that are susceptible to exercise. Emerging evidence points to brain-derived neurotrophic factor (BDNF) as an important marker in COPD, being related to disease severity parameters. Pulmonary rehabilitation programs (RP), which have as their central element physical exercise, are important tools for improving the clinical condition of patients with COPD. Based on these facts, the present thesis aimed to investigate the effects of RP on (1) endothelial function and arterial stiffness and (2) on plasmatic levels of BDNF in patients with COPD. In study 1, it was found that endothelial function, arterial stiffness and systemic arterial pressure were not modified with pulmonary rehabilitation. Study 2 demonstrated that a single exercise session in sedentary patients (acute effect) reduces BDNF levels, but this effect is not maintained with training and training is not able to reduce basal levels of BDNF. Still, all studies show that a PR has improved functional capacity, dyspnea, quality of life and death risk. Based on the current findings, it is possible to conclude that advances are still needed regarding interventions capable of modifying the cardiovascular pathway, as well as a better understanding of BDNF kinetics regarding to physical exercise and its understanding with markers of COPD severity.

Key-words: Chronic obstructive pulmonary disease; Pulmonary rehabilitation; Endothelial function; Arterial stiffness; Brain-derived neurotrophic factor; Functional capacity; Risk of death.

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Lista de Abreviaturas

ADL	*Activity of Daily Living*, atividade de vida diária.
AIx	Augmentation index, medida de rigidez arterial.
AIx75	Augmentation index ajustado para frequência cardíaca de 75 batimentos por minuto.
ATS	*American Thoracic Society.*
AVD	Atividade de vida diária.
BDNF	*Brain-derived neurotrophic fator*; fator neurotrófico derivado do cérebro.
BMI	*Body-mass index,* índice de massa corporal.
BODE	*The body-mass index, airflow obstruction, dyspnea, and exercise index.*
COPD	*Chronic obstructive pulmonary disease.*
DPOC	Doença pulmonar obstrutiva crônica.
DBP	*Diastolic blood pressure*, pressão arterial diastólica.
ERS	*European Respiratory Society.*
FEV_1	*Forced expiratory volume in one second*, volume expiratório forçado em um segundo.
FMD	*Flow-mediated dilation*, dilatação fluxo-mediada.
FVC	*Forced vital capacity;* capacidade vital forçada
HR	*Heart rate*, frequência cardíaca.
ISCMPA	Irmandade Santa Casa de Misericórdia de Porto Alegre.
MAP	*Mean arterial pressure*, pressão arterial média.
min	Minutos.
mMRC	*Modified Medical Research Council scale.*
NO	*Nitric oxide*, óxido nítrico.
PAT	*Peripheral arterial tonometry*, tonometria arterial periférica.
PR	*Pulmonary rehabilitation*, reabilitação pulmonar.
PRP	Programa de reabilitação pulmonar
QV	Qualidade de vida.
SBP	*Systolic blood pressure*, pressão arterial sistólica.

SpO2	Saturação periférica de oxigênio.
TGlittre	*Glittre-Activity of Daily Living test.*
6MWT	*Six-minute walking test,* teste de caminhada de seis minutos.
%pred	*Percent of the predicted value;* percentual do valor previsto.

SUMÁRIO

1 CONTEXTUALIZAÇÃO

A doença pulmonar obstrutiva crônica (DPOC) constitui um importante problema de saúde pública, sendo uma das principais causas de mortalidade e morbidade do mundo. Essa doença, de caráter progressivo, é caracterizada essencialmente por obstrução crônica do fluxo aéreo, parcialmente reversível (VOGELMEIER et al., 2017). Embora a DPOC acometa principalmente os pulmões, cabe descrever que alterações causadas pelo processo inflamatório crônico não se restringem somente ao parênquima pulmonar; sendo observadas também alterações inflamatórias sistêmicas.

Os sintomas da DPOC, principalmente a dispneia, interferem em vários aspectos da vida do paciente, especialmente em relação às atividades de vida diária (AVD) e capacidade funcional destes pacientes (GARCIA-RIO et al., 2009), sendo que essa diminuição da capacidade funcional está relacionada a uma maior mortalidade (CELLI et al., 2004).

A doença cardiovascular é uma das principais causas de mortalidade na DPOC (MCGARVEY et al., 2007) e a taxa de morte súbita cardíaca é marcadamente maior nessa população (SIN; MAN, 2005). Além do estilo de vida sedentário, os efeitos sistêmicos do tabagismo e manifestações sistêmicas da doença, como a disfunção endotelial (CLARENBACH; SIEVI; KOHLER, 2017; MACLAY et al., 2009), podem aumentar o risco de mortalidade cardiovascular nesses pacientes (EICKHOFF et al., 2008; PATEL et al., 2013).

O endotélio desempenha um papel importante no controle do fluxo sanguíneo, coagulação, fibrinólise e inflamação. A disfunção endotelial pode ser avaliada por tonometria arterial periférica (PAT), uma técnica não invasiva que mede as alterações do volume pulsátil arterial do dedo durante a hiperemia reativa. PAT mede o índice de hiperemia reativa (RHI) e o índice de aumento (AIx), um marcador de rigidez arterial. Estudos identificaram que os pacientes com DPOC apresentam aumento da rigidez arterial e função vasomotora alterada (BARR et al., 2007; EICKHOFF et al., 2008). O aumento da rigidez arterial é um marcador independente de doença cardiovascular (LAURENT et al., 2001; ROMAN et al., 2000) e o RHI anormal está associado a maior risco

de eventos coronários e a uma ruptura mais suscetível de placas de ateroma (SCHOENENBERGER et al., 2012).

A fisiopatologia da DPOC envolve vias múltiplas, como a ativação de células inflamatórias circulantes e níveis aumentados de citocinas pró-inflamatórias (AGUSTÍ et al., 2003; RAHMAN et al., 1996) e o desequilíbrio de marcadores epigenéticos (SCHAMBERGER et al., 2014; ZONG; OUYANG; CHEN, 2015), que apresentaram alterações suscetíveis ao exercício (DA SILVA et al., 2017). Evidências experimentais e clínicas emergentes apontaram que o fator neurotrófico derivado do cérebro (BDNF) é um biomarcador importante na DPOC, com níveis periféricos mais elevados quando comparado ao grupo controle (PINTO-PLATA et al., 2007; STOLL et al., 2012).

BDNF é uma neurotrofina associada a vários processos, como neuroplasticidade, crescimento neuronal, diferenciação e reparo (MCALLISTER; KATZ; LO, 1999). Além disso, também está relacionado à fisiopatologia de várias doenças. O papel e a regulação do BDNF na DPOC ainda são incompletamente compreendidos. Foi proposto que esta neurotrofina parece desempenhar um papel fundamental nas condições inflamatórias agudas e crônicas das vias aéreas. Especificamente, pode aumentar o número e a função das células do músculo liso das vias aéreas (ARAVAMUDAN et al., 2012) está envolvido na hiperreatividade e tosse das vias aéreas (LOMMATZSCH et al., 2005, 2007). Além disso, Pinto-Plata e colegas (PINTO-PLATA et al., 2007) relataram que o BDNF se correlacionou com o volume expiratório forçado em um segundo (FEV_1), fator de transferência de monóxido de carbono, teste de caminhada de 6 minutos (6MWT), índice BODE e frequência de exacerbação.

Apesar de ser uma doença progressiva, a DPOC pode ser prevenida e tratada (SBPT, 2004). Os programas de reabilitação pulmonar (PRP), os quais têm como elemento central o exercício físico, constituem uma importante ferramenta para a melhora do quadro clínico de pacientes com DPOC. Especificamente, foi demonstrado que os pacientes submetidos a esta intervenção relatam diminuição da sensação de dispneia e fadiga, melhora da percepção da qualidade de vida, aumento da capacidade de exercício (LACASSE et al., 2006) e da força muscular (TROOSTERS; GOSSELINK;

DECRAMER, 2000). Sabe-se que PRPs mais longos, produzem efeitos mais duradouros. Após doze semanas de reabilitação pulmonar a capacidade funcional atinge um platô em pacientes com DPOC (SPRUIT et al., 2013).

Portanto, estudos sobre recursos que venham a incrementar os benefícios observados em participantes de PRP e, em particular, sobre aqueles que podem influenciar positivamente a mortalidade, a funcionalidade, bem como a qualidade de vida em pacientes com DPOC, são relevantes tanto para a prática clínica quanto para o conhecimento científico. Além de que, o conhecimento sobre as bases moleculares envolvidas com os efeitos benéficos do PRP em pacientes com DPOC pode ser útil no estabelecimento de novas estratégias de intervenção terapêutica e preventiva para esta população.

Sendo assim, na presente tese, foram desenvolvidos dois estudos que investigaram os efeitos da reabilitação pulmonar em pacientes com DPOC. O primeiro artigo se intitula "*Pulmonary Rehabilitation did not change endothelial function measured by Peripheral Arterial Tonometry in patients with COPD*", com foco principal na função endotelial avaliada pela PAT, e o segundo estudo se intitula "*Time-dependent effects of pulmonary rehabilitation on BDNF levels in patients with chronic obstructive pulmonary disease: a pilot clinical trial*", com foco principal nos níveis plasmáticos de BDNF. Os estudos desenvolvidos estão apresentados a seguir, em formato de artigo científico, de acordo com as normas das respetivas revistas para os quais foram submetidos.

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2 OBJETIVOS

2.1 OBJETIVOS GERAIS

Avaliar os efeitos da reabilitação pulmonar sobre a função endotelial e sobre os níveis plasmáticos de BDNF em pacientes com DPOC.

2.2 OBJETIVOS ESPECÍFICOS

Os objetivos específicos foram contemplados em cada um dos estudos desenvolvidos:

2.2.1 Estudo 1

O estudo 1 teve como objetivo investigar os efeitos de 24 e 48 sessões de reabilitação pulmonar sobre:

- Função endotelial;
- Rigidez arterial;
- Pressão arterial sistêmica;
- Frequência cardíaca;
- Capacidade Funcional;
- Marcador de mortalidade.

2.2.2 Estudo 2

O estudo 2 teve como objetivo investigar os efeitos de 24 sessões de reabilitação pulmonar sobre:

- Capacidade Funcional;
- Dispneia;

- Qualidade de vida;
- Marcador de mortalidade;
- Efeito agudo de uma única sessão ao início e ao final do programa de 24 sessões sobre os níveis plasmáticos de BDNF.

3 ESTUDO 1

Este estudo foi formatado conforme as normas do periódico "*Journal of Cardiopulmonary Rehabilitation and Prevention*" (ANEXO 7.2).

Pulmonary Rehabilitation did not change endothelial function measured by Peripheral Arterial Tonometry in patients with COPD

Short title: Pulmonary Rehabilitation and Peripheral Arterial Tonometry in COPD

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Figures: 01

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ABSTRACT

Purpose: To assess the impact of a pulmonary rehabilitation program (PRP) on endothelium function and arterial stiffness assessed by peripheral arterial tonometry (PAT) in patients with COPD.

Methods: Patients with COPD engaged a PRP thrice a week during 90 min/session for 24 sessions, then for 48 sessions twice a week. Reactive hyperemia index (RHI), augmentation index (AIx) and heart rate (HR) assed by PAT, systemic blood pressure, functional capacity (six-minute walking test and Glittre ADL-test) and risk of death (BODE index) were assessed at baseline and after 24 and 48 sessions of PRP.

Results: Twenty-one subjects were included and completed 24 PR sessions and 16 subjects completed 48 sessions It was observed a poorer AIx adjusted for HR in frequent COPD exacerbators ($p=0.02$). PRP improved functional capacity ($p<0.001$) and risk of death ($p=0.001$), but did not change HR, systemic arterial pressure, RHI and AIx regardless exacerbation frequency.

Conclusion: Exercise-based PRP improved functional capacity and risk of death, but did not change cardiovascular variables studied in patients with COPD.

Condensed abstract

This study assessed the effects of a pulmonary rehabilitation program (PRP) on endothelial function and arterial stiffness assed by peripheral arterial tonometry, and as well on functional capacity and risk of death in patients with COPD. PRP improved functional capacity and risk of death, but did not change cardiovascular variables.

Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of morbidity and mortality worldwide[1–3]. Cardiovascular disease is a major cause of mortality in COPD[4]. Systemic effects of smoking, sedentary lifestyle and systemic manifestations, such as chronic inflammation[5], and endothelial dysfunction[6] may enhance cardiovascular mortality risk in these patients[7,8].

The endothelium plays an important role in the control of blood flow, coagulation, fibrinolysis, and inflammation. Endothelial dysfunction can be assessed by peripheral arterial tonometry (PAT), a noninvasive technique that measures finger arterial pulsatile volume changes during reactive hyperemia. PAT measures the reactive hyperemia index (RHI) and the augmentation index (AIx), a marker for arterial stiffness. Studies have identified that patients with COPD present increased arterial stiffness[9] and altered vasomotor function[7,10]. Increased arterial stiffness is an independent marker of cardiovascular disease[11,12] and abnormal RHI is associated with greater risk of coronary events and more susceptible rupture of atheroma plaques[13].

Pulmonary rehabilitation (PR) based on exercise training is a key component of the treatment of COPD[14] and it is well stablished that it increases exercise capacity, reduces dyspnea, improves quality of life[15], and seems to be indirect associated to reduction of mortality[16]. At the same time, exercise has shown to be beneficial in patients with cardiovascular disease[17,18], including outcomes regarding endothelium function[19,20]. However, little is known about the effect of PR on endothelium function of patients with COPD.

In this context, the current study aimed to investigate whether PR has the potential to change endothelium function and arterial stiffness assessed by PAT in patients with COPD.

Methods

Subjects

Subjects with COPD were recruited from pulmonology outpatient service of Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA). Only engaged the study patients with Global Initiative for Obstructive Lung Disease (GOLD) stages 2, 3 and 4 of severity of airflow limitation[21], smoking history ≥ 20 pack-years, clinical stability in the 4 weeks prior to the study protocol and age ≥ 40 years. None of the subjects had been engaged in any exercise-training program at least one year before participating in the study. Patients with any pulmonary disease other than COPD, current smoking, and comorbidities (neurological, orthopaedical, cardiovascular) that would compromise the ability to perform any of the evaluations in the study were excluded. The study was approved by the Human Research Ethics Committee of the ISCMPA and Health Science Federal University of Porto Alegre (protocol numbers 1.413.342 and 836.248, respectively) and all participants signed an informed consent. This study was registered at *Registro Brasileiro de Ensaios Clínicos* / Brazilian Clinical Trials Registry (Identifier RBR-26pms3).

Protocol

Subjects were assessed for functional capacity, endothelial function (PAT), and risk of death at baseline, after 24 and 48 sessions of PR. Pulmonary function was assessed only at baseline. Subjects were also classified as exacerbator if they presented at least two ambulatorial exacerbations or at least one exacerbation leading to hospital admission[21]. The first 24 sessions of PR occurred three times a week, after that patients continued for more 24 sessions two times a week.

Pulmonary function test

Lung function was assessed in the Pulmonary Function Laboratory of the ISCMPA by specialized technicians, supervised and interpreted by pulmonologists with

a degree of accreditation in spirometry by the Brazilian Society for Pulmonology and Tisiology and in accordance with the American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines[22].

Functional Capacity

Functional capacity was assessed by the six minute-walking test (6MWT) and by the Glittre-Activity of Daily Living test (TGlittre).

The 6MWT was performed in accordance to the guidelines of the American Thoracic Society[23]. Heart rate (HR; Polar RS800, Oulu, Finland), pulse oxyhemoglobin saturation (SpO_2; Oxi-Go, Oximeter Plus, Roslyn Heights, NY, USA) and dyspnea (Borg CR10 scale)[24] were measured at the beginning, at the second and fourth minute, and at end of the test. Arterial blood pressure was measured at the beginning and at the end of the 6MWT. Predicted values for walking distance were calculated according to Britto et al.[25].

The Glittre-ADL test (TGlittre) is a multiple-task test validated for patients with COPD(26) that consists of completing five laps of a circuit of common ADL, such as: standing up form a chair, walking, carrying weight, climbing steps, moving objects on a shelf and sitting down. Subjects are instructed to complete the TGlittre as quickly as possible[26]. HR, SpO_2 and dyspnea were measured at the beginning of the test, at the end of each lap, and at the end of the test. Arterial blood pressure was measured at the beginning and at the end of the TGlittre. A reduction of 0.89 min in performing the test was used as an indication of performance improvement based on the responsiveness value found by Skumlien et al.[26].

BODE index

The body-mass index, airflow obstruction, dyspnea, and exercise (BODE) index, is a multidimensional scoring system which uses the four factors to predict risk

of death patients with COPD. The BODE index will result in a score of zero to ten dependent upon body-mass index (BMI, defined as weight/height^2), forced expiratory volume in one second (FEV_1), the degree of dyspnea measure by the mMRC[27], and the distance walked in the 6MWT. The score is divided into quartiles; the higher means higher risk of death[28].

Endothelial Function and Arterial Stiffness (Peripheral arterial tonometry - PAT)

Endothelial function was measured by the natural logarithm of RHI and arterial stiffness, expressed by the augmentation index (AIx) and by the augmentation index adjusted for 75 beats per minute (AIx75). Peripheral arterial tonometry signals were obtained noninvasively using a beat-to-beat plethysmographic recording of the digital arterial pulse-wave amplitude by pneumatic probes (EndoPAT 2000 device, Itamar Medical Ltd, Caesarea, Israel). The assessments were performed in a room with dimmed lights, noise and temperature control (22 – 24ºC), at supine position with a blood pressure cuff placed at the nondominant arm and both arms resting on arm support pads. After a 10-minute equilibration period, which is used as baseline, the cuff was inflated to 60mmHg over the systolic pressure or at least 200mmHg, but not higher than 300mmHg, for 5 minutes. After that, the cuff was deflated, and the recording continued for more 8 minutes. The dominant arm serves as control. The patients were instructed not to move their arms or fingers. Values of heart rate (HR), RHI, AIx and AIx75bpm were automatically calculated by the Itamar software. Before PAT analysis, systolic and diastolic arterial blood pressure (SBP and DBP, respectively) were measured with an arm sphygmomanometer with patient at sitting position. Mean arterial pressure (MAP) was calculated as (SBP + DBP^2)/3. All PAT assessments were performed after a 20 minutes resting-period, at the same period of the day under similar circumstances.

Normal reactive hyperemia is defined by a RHI > 0.51, values ≤ 0.51 are considered abnormal (Itamar product information). Arterial stiffness is considered normal by an AIx between -30% and -10%, increased arterial stiffness by an AIx between -10% and 10%, and abnormal by an AIx above 10%[29].

Pulmonary rehabilitation program

Subjects engaged in a 48-session outpatient multidisciplinary PR program. At the beginning of the protocol, all were submitted to medical evaluation and optimization of the medication and nutritional evaluation and guidance. The program also provided educational support on COPD, energy conservation techniques, and disease self-management.

The PR session involved endurance training and peripheral muscle strength (approximately 90 minutes each session). The endurance training was taken during 30 to 40 minutes on a treadmill with 60% of the speed average of the 6MWT and the work load progression occurred according to the dyspnea, which should be between 4 and 6 reported on the modified Borg scale[24]. The lower limbs training evolved quadriceps and triceps sural. The upper limbs training was carried in diagonal axes. The program was divided into two parts: months 1–2: 24 sessions three times a week; months 3–5: maintenance program of 48 sessions two times a week.

Statistical Analysis

Data distribution was checked with the Shapiro–Wilk test. Data were reported as mean ± SD or median (interquartile range). Comparisons between baseline, after the 24th and the 48th sessions considering frequent exacerbators and non-frequent exacerbators were analyzed using the Generalized Estimating Equations with Bonferroni post hoc analysis, considering Gamma model, except for the AIx and AIx75, which the Linear model was considered. Correlations were tested with the Pearson or

Spearman tests, according to data distribution. Statistical significance was set at $p<0.05$. Data were analyzed using SPSS 20.0 (SPSS, Chicago, IL, USA).

Results

Twenty-four patients were recruited for the study, 21 (7 men) were included and completed 24 PR sessions and 16 patients (6 men) completed 48 sessions (Figure 1). Only two subjects showed endothelial dysfunction (RHI ≤ 0.51) at baseline. However, 9 subjects presented increased arterial stiffness and 9 presented abnormal arterial stiffness. Subjects' baseline characteristics and medication used are shown on Table 1 and 2, respectively. There were no differences regarding baseline pulmonary function, functional capacity and endothelial function between frequent exacerbators and non-exacerbators ($p > 0.05$). Although, frequent exacerbators showed higher values for AIx75 (4.67 ± 16.5 vs 20.9 ± 12.9; $p = 0.02$). Even with a significant improvement in functional capacity and risk of death, PR did not change the endothelial function, arterial stiffness, heart rate and systemic arterial pressure (Table 3), irrespective of the frequency of exacerbations.

No correlation was found between baseline endothelial function and arterial stiffness with age, smoking history, BMI, 6MWT and TGlittre. Baseline SBP and MAP were correlated with FEV_1 ($r = -0.47$, $p = 0.031$; $r = -0.48$, $p = 0.029$; respectively) and with FEV_1%prev ($r = -0.54$, $p = 0.011$; $r = -0.46$, $p = 0.035$; respectively). DBP correlated with AIx ($r = 0.49$, $p = 0.023$) and with AIx75 ($r = 0.47$, $p = 0.034$) at baseline, but not after 24 nor 48 PR sessions.

Discussion

The current study showed that PR did not change the endothelial function assessed by PAT in patients with COPD, even with the improvement of functional

capacity. Meanwhile, the present study showed that exacerbator patients present higher arterial stiffness, that the severity of airflow limitation is related to higher SBP and MAP, and arterial stiffness is related to higher DBP.

Cardiovascular disease a major comorbidity in patients with COPD and it is related to endothelial dysfunction, which raises the mortality risk in these patients. Endothelial dysfunction is characterized as the arterial inability to sufficiently dilate in response to a proper endothelial stimulus. It is related to impaired nitric oxide (NO) bioavailability due to poor production by NO synthase and/or increase NO breakdown by reactive oxygen species. Its bioavailability is also related to the shear stress, an important activator of the NO synthase[30]. Endothelial dysfunction simultaneously affects different vascular territories, featuring a systemic condition[31].

The measurement of the flow-mediated dilation (FMD) of the brachial artery after occlusion by high resolution ultrasound is the gold standard[32] and the most common noninvasive technique of endothelial dysfunction assessment[33]. However, FMD is strongly operator-dependent and has a significant learning curve[34]. In addition, vasodilatation is only measured in one arm, which precludes the correction for autonomous activation of the nervous system. On the other hand, the PAT allows noninvasive measurement of endothelial function during reactive hyperemia, with minimal operator bias, using the other arm as control. Anyway, studies have shown correlation between FMD and PAT[35,36], probably because both techniques involve ischemia followed by reactive hyperemia. After the ischemia, the strong blood flow causes shear stress in the vessels releasing NO, leading to vasodilation and reactive hyperemia[33]. However, reactive hyperemia is a complex response to ischemia and is only partially dependent on NO[37].

Up to our knowledge, only one prior cross-sectional study assessed endothelial function (RHI) with PAT, but not arterial stiffness (AIx) in patients with COPD[38]. In which, the mean RHI was 0.43 ranging from -0.14 to 1.3. Although that sample had

lower RHI values than the current study, the airflow limitation was less severe. They also found that the 6MWT appears to be a predictor of endothelial dysfunction, while we did not find a correlation between RHI and 6MWT. This divergence in findings may be justified by the inclusion of current smokers and patients during exacerbation.

In a recent meta-analysis, Early et al.[39] showed that exercise training contributes to a significant increase in brachial artery FMD, especially interventions with greater duration and intensity. However, there were not any study with patients with COPD included in this review. Up to our knowledge, this is the first study using PAT to assess the effect of pulmonary rehabilitation on the RHI in patients with COPD. In addition, only few longitudinal studies used the PAT as a tool for endothelial function assessment.

It is important to point out that, although there were no changes in the RHI, the current sample did not present endothelial dysfunction. Cornelissen et al.[40] investigated the effect of exercise-based cardiac rehabilitation on endothelial function simultaneously by FMD and PAT in patients with coronary arterial disease. Surprisingly, exercise was associated with an improvement in endothelial function when assessed with FMD, but not with the PAT. That study did not find relation between baseline RHI measured by PAT and FMD. Which suggests that both methods might assess different physiological mechanisms.

Although the current sample showed normal endothelial function, they presented abnormal arterial stiffness at baseline. Arterial stiffness is considered a predictor not only of increased cardiovascular disease risk but also all-cause mortality[11]. It is characterized by diminished arterial compliance, which leads to faster reflection of the systolic wave from peripheral small arteries[41]. This increase in central pressure enhances the ventricular afterload and reduces coronary perfusion pressure, eventually leading to myocardial hypertrophy, ischemia and infarction[42]. An increase in arterial stiffness is a common feature of the aging process[43] and chronic conditions

such as systolic hypertension[44], cardiovascular disease[45], heart failure[46], diabetes[47], and COPD[48], wherein previous studies showed its correlation with airflow obstruction[7,49]. Despite we have not found a correlation between airflow limitation and arterial stiffness, we found a correlation of airflow limitation with SBP and MAP. As well, frequent exacerbators showed higher AIx75, and both AIx and AIx75 correlated with DBP. These findings corroborate the hypotheses raised by Dugac et al.[50], in which they hypothesize that persistent endothelial dysfunction in frequent exacerbators may lead to a progressive systemic vascular disease.

Even without a statistically significant change, arterial stiffness went from "abnormal" to "increased" after 24 PR sessions, but returning to "abnormal" after 48 sessions. As far as we know, few studies assessed the effect of exercise on arterial stiffness in patients with COPD and none used PAT for its assessment. The arterial stiffness is commonly assessed by the measurement of PWV in COPD patients[48].

The last systematic review about the exercise effects on PWV and AIx[51] showed that aerobic exercise reduces both measurements of arterial stiffness and the exercise effect tended to be greater in peripheral (brachial-ankle PWV) than in central (carotid-femoral PWV) indices. The same review showed that resistance and combined exercise training did not lead to beneficial effects on PWV or AIx. Furthermore, larger effects were observed in subjects with more arterial stiffness at baseline, and in trails with longer duration. Interestingly, the improvement in AIx was associated with exercise intensity rather than volume (frequency and duration of sessions). Nevertheless, in the current study, arterial stiffness became abnormal after reduction of the volume of the sessions. However, that review did not include any trail with patients with COPD or other chronic pulmonary disease.

Data regarding the effect of exercise-based interventions on arterial stiffness in patients with COPD are conflicting. Vivodtzev et al.[52] used the carotid-radial PWV assessment to evaluate the effect of 4 weeks of endurance training on the arterial

stiffness of 10 patients with COPD. However, the AIx was not calculated. The endurance training program reduced the carotid-radial PWV proportionally to the improvement in the 6MWT and in muscle endurance. Changes in fasting glucose and blood pressure were also related to the carotid-radial PWV improvement. Posteriorly, Gale et al.[53] showed improvement in aortic PWV after seven weeks of a state-of-the-art pulmonary rehabilitation program in 22 patients with COPD, but not on brachial PWV neither AIx. Like Vivodtzev et al.[52], a reduction on SBP was demonstrated, but not on HR. But, no change in brachial PWV neither in AIx was found after the pulmonary rehabilitation[53]. The authors relate these findings to the different properties of central and peripheral arteries. Corroborating, Vanfleteren et al.[54] did not find change in aortic PWV neither in AIx after a 40-session state-of-the-art pulmonary rehabilitation program in 129 COPD patients. While Gale et al.[53] demonstrated reduction on peripheral and central SBP, DBP, MAP and peripheral pulse pressure but not on central pulse pressure and HR; Vanfleteren et al.[54] only found reduction on central and peripheral pulse pressure and HR.

Although FMD seems to be reproducible in patients with COPD[55], an important limitation of endothelial function and arterial stiffness assessment reliability in COPD is the chronic use of inhaled bronchodilators. As mentioned before, all included subjects used inhaled bronchodilators and most of them used both β2-agonist and muscarinic antagonist (Table 2). It has been shown that β2-receptor stimulation lead to NO endothelial release[56] and β2-agonist inhaled at clinical standard doses reduces arterial stiffness[57]. In addition, evidence suggests that muscarinic M3 receptors on endothelial cells are responsible for acetylcholine (Ach)-dependent vasodilatation[58] and the forearm blood flow response to ACh is attenuated by the administration of a nonselective muscarinic antagonist[59]. Meanwhile, the reliability of PAT assessment in patients with COPD and its dose-response issues regarding bronchodilators still need further investigation.

In summary, exacerbators showed poorer arterial stiffness and PR was not able to change endothelial function neither arterial stiffness assessed by PAT in patients with COPD, regardless exacerbation frequency. However, information regarding PAT reliability and responsiveness in COPD and regarding interventions capable of reducing cardiovascular risk in these patients needs further investigation.

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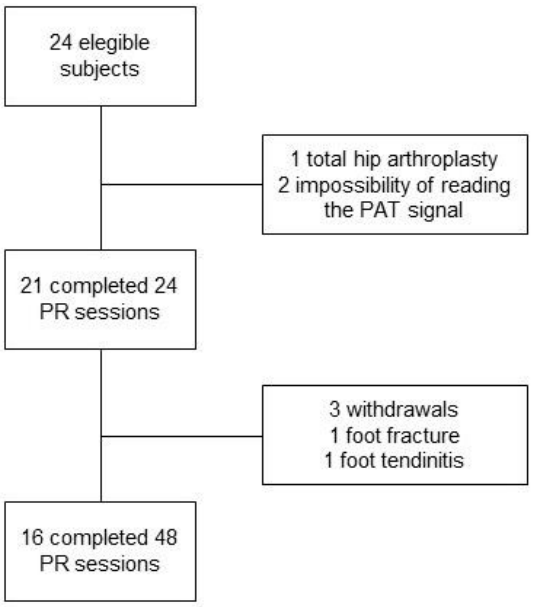

Figure 1. Flow diagram.

Table 1. Subjects characteristics at baseline

Characteristics	Subjects at baseline n=21
Age, years	64.6 ± 7.58
Long-term oxygen therapy, n	2
Frequent exacerbator, n	12
Smoking history, pack-years	43.0 (52.5)
FEV_1/FVC, %	0.48 ± 0.1
BMI, kg/m^2	26.0 ± 3.97
mMRC	4 (2)
FEV_1, l	0.82 (0.39)
FEV_1, %pred	34.4 ± 13.8
FVC, l	1.74 (0.9)
FVC, %pred	55.1 ± 11.6
GOLD 2, n	3
GOLD 3, n	8
GOLD 4, n	10

Data given as n (frequency), mean ± standard deviation or median (interquartile range). FEV_1: forced expiratory volume in one second, liter; FVC: forced vital capacity, liter; %pred: perceptual of the predicted value; GOLD: Global Initiative for Obstructive Lung Disease (GOLD) stages of airflow limitation; BMI: body mass index, weight/height2; mMRC: modified Medical Research Council scale.

Table 2. Medication

Medication	Subjects at baseline n=21
Long-acting β2-agonists	20
Short-acting β2-agonists	19
Long-acting muscarinic antagonists	18
Short-acting muscarinic antagonists	6
Xanthines	4
Angiotensin II Receptor Blockers	7
Angiotensin converting enzyme inhibitors	2
Calcium channel blockers	2
Statins	2
Inhaled corticosteroids	19
Systemic corticosteroids	1
Antidepressants	6

Table 3. Pulmonary Rehabilitation outcomes

	Baseline		After 24th session		After 48th sessions		p
	Mean ± SD	CI 95%	Mean ± SD	CI 95%	Mean ± SD	CI 95%	
RHI	0.79 ± 0.28	0.66 – 0.91	0.63 ± 0.31	0.48 – 0.77	0.67 ± 0.27	0.53 – 0.81	0.234
AIx#	13.0 (25.0)	4.27 – 18.3	6.0 (11.5)	-1.69 – 13.1	11.9 (19.8)	3.06 – 80.8	0.224
AIx75	14.0 ± 16.4	6.5 – 21.4	9.14 ± 17.1	1.37 – 19.9	13.8 ± 15.9	5.35 – 22.3	0.270
HR, bpm	79.2 ± 9.33	75.0 – 83.5	80.5 ± 10.7	75.6 – 85.4	77.8 ± 6.07	74.5 – 81.0	0.473
SBP, mmHg	128 ± 11.6	122 - 133	123 ± 13.4	117 - 129	130 ± 10.7§	124 – 135	0.036
DBP, mmHg#	80.0 (12.5)	75.2 – 83.4	80.0 (10.0)	76.5 – 83.5	80.0 (10.0)	71.9 – 80.7	0.241
MAP, mmHg	95.4 ± 8.85	91.4 – 99.4	94.3 ± 8.94	90.2 – 98.4	94.1 ± 6.24	90.7 – 97.4	0.639
6MWT, m	380 ± 107	323 – 437	434 ± 106‡	376 - 490	442 ± 115†	381 - 503	< 0.001
TGlittre, min#	4.98 (2.97)	4.25 – 6.38	4.11 (1.51)‡	3.50 – 4.84	3.93 (1.33)‡	3.28 – 4.56	< 0.001
BODE, score	6 (2.75)	4.55 – 6.45	4 (1.75)*	3.69 – 5.91	4 (3)	3.14 – 5.24	0.001
BODE, quartile	3 (1.75)	2.52 – 3.48	2 (1)**	1.95 – 2.81	2 (1)	1.87 – 2.89	0.001

Data given as mean ± standard deviation (SD) or #median (interquartile range); CI95%: confidence interval of 95% (lower – upper bound); RHI: reactive hyperemia index; AIx: augmentation index; AIx75: augmentation index adjusted for heart rate of 75 beats per minute; HR: heart rate, beats per minute; SBP: systolic blood pressure, millimeter of mercury (mmHg); DBP: diastolic blood pressure, mmHg; MAP: mean arterial pressure, mmHg; 6MWT: six-minute walking test, meters; TGlittre: Glittre ADL-test, minutes; BODE: The body-mass index, airflow obstruction, dyspnea, and exercise index.

§Compared with after 24th session, p = 0.029.

‡Compared with baseline, p < 0.001.

†Compared with baseline, p = 0.024.

* Compared with baseline, p = 0.002.

** Compared with baseline, p = 0.003.

4 ESTUDO 2

Este estudo foi formatado conforme as normas do periódico "*Journal of Physical Activity & Health*" (ANEXO 7.3).

Original Research

Time-dependent effects of pulmonary rehabilitation on BDNF levels in patients with chronic obstructive pulmonary disease: a pilot clinical trial

Running head: Pulmonary rehabilitation and BDNF levels in COPD

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ABSTRACT

Background: COPD physiopathology involves multiple pathways and evidence have pointed out brain-derived neurotrophic factor (BDNF) as an important biomarker, being associated with parameters of disease severity. This study aimed to assess the impact of a pulmonary rehabilitation program (PRP) on exercise capacity, dyspnea, quality of life, and risk of death on COPD patients, as well to analyze the time course of PRP effects on BDNF levels.

Methods: Patients enrolled 24 sessions of PRP, including endurance training, upper and lower limbs training, educational and nutritional orientation. Exercise capacity, dyspnea, quality of life, and risk of death were assessed at baseline and after the PRP. Plasmatic BDNF levels were measured at baseline (immediately before the 1st session), after the 1st session, before and after the 24th session.

Results: Sixteen patients were included. PRP improved exercise capacity and quality of life, reduced dyspnea and risk of death. It was observed a reduction on BDNF levels after the 1st session and an increase between the end of the 1st session and the beginning of the 24th session.

Conclusion: PRP improved exercise capacity, dyspnea, quality of life and risk of death. Exercise acutely reduced BDNF levels, effect that was nullified by the long-term intervention.

INTRODUTION

Chronic obstructive pulmonary disease (COPD), characterized by airflow limitation with persistent respiratory symptoms and systemic manifestations is considered the fourth leading cause of death worldwide. Patients with COPD show pulmonary function impairment, dyspnea, and peripheral muscle dysfunction, contributing to exercise intolerance and reduced activities of daily living[1]. Moreover, a high prevalence of psychiatric symptoms such as depression is observed among COPD patients, which is pointed out as relevant comorbidities in these individuals[2].

The physiopathology of COPD involves multiple pathways such as the activation of circulating inflammatory cells and increased levels of proinflammatory cytokines[3,4] and the imbalance of epigenetic markers[5,6], which showed changes susceptible to exercise[7]. Emerging experimental and clinical evidence have pointed out that brain-derived neurotrophic factor (BDNF) is an important biomarker in COPD, with higher peripheral levels when compared to control group[8,9].

BDNF is a neurotrophin associated with several processes such as neuroplasticity, neuronal growth, differentiation and repair[10]. Furthermore, seems to exert a major role in cognition, emotion, mood as well is related to the pathophysiology of several diseases including neuropsychiatric conditions[11,12]. Although it is found throughout the brain, BDNF can cross the blood-brain barrier in a bi-directional manner and peripheral levels seems to present a strong correlation with the cerebrospinal fluid levels[13]. Therefore, peripheral BDNF levels have been used as a biomarker in several clinical studies[14,15].

Despite these findings regarding brain function, the role and regulation of BDNF in COPD is still incompletely understood. It has been proposed that this neurotrophin seems to play a pivotal role in acute and chronic inflammatory conditions of the airways. Specifically, it can enhance the number and function of airway smooth

muscle cells[16] and is involved in the airway hyperresponsiveness and cough[17,18]. In addition, Pinto-Plata and colleagues[8] reported that BDNF correlated with forced expiratory volume in one second (FEV_1), carbon monoxide transfer factor, 6-minute walk test (6MWT) performance, BODE index and exacerbation frequency.

Pulmonary rehabilitation programs (PRP) is an important strategy in promoting beneficial effects to COPD patients. It consists of a multidisciplinary intervention that includes mainly physical exercise training and educational sessions to recover independence and functional capacity[19]. Several studies have reported that COPD patients submitted to PRP show exercise capacity enhancement, an improvement on quality of life, reduction in the frequency and duration of hospitalizations and in dyspnea[19].

In this context, there is a growing body of evidence reporting that exercise seems to induce time-dependent responses in BDNF in different populations[20–28]. However, no studies have investigated the training effects on BDNF in patients with COPD. Therefore, this study aimed to analyze the time course of the PRP effects on BDNF modulation, specifically, at baseline, after the 1st session, before and after the 24th session and to investigate the impact of a PRP on exercise capacity, quality of life, dyspnea and on the BODE index in COPD patients.

METHODS

Patients with COPD confirmed spirometry (GOLD stages 2, 3 and 4 of the severity of airflow limitation[1]) were recruited from the Pulmonology Service of the Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), RS/Brazil. Subjects should be clinical stable in the four weeks before the study protocol, nonsmoker for at least six months and present smoking history ≥ 20 pack-years, and age ≥ 40 years. Current smoking, patients who participated in any exercise program in

the last 12 months, who had other pulmonary disease than COPD or other diseases such as musculoskeletal, neurologic or cardiovascular that would compromise their ability to perform any of the evaluations in the study were considered exclusion criteria for the study. Subjects were also classified as frequent exacerbator if they presented at least two exacerbations or at least one exacerbation leading to hospital admission. Exercise capacity, dyspnea and quality of life were assessed at the same day, from two to four days before the beginning of the intervention and from two to four days after the last PRP session.

This study was approved by the Human Research Ethics Committee of the ISCMPA (protocol number 40078114.9.0000.5335) and the Methodist University Center IPA (protocol number nº918.889/2014) and all subjects provided written informed consent. The current study has been registered at The Brazilian Clinical Trials Registry/Registro Brasileiro de Ensaios Clínicos (ReBEC; registration number RBR-26pms3).

Pulmonary function test

Data on lung function are collected from medical records no later than six months prior to protocol. All tests were performed before and after bronchodilator inhalation at the Pulmonary Function Laboratory of ISCMPA according to the recommendations of the American Thoracic Society (ATS)/European Respiratory Society (ERS)[29]. The equation proposed for the Brazilian population was used to calculate the predicted values[30].

BDNF measurement

To analyze the short and long-term effects of PRP on BDNF levels, blood samples (approximately 5 ml) were taken in the antecubital vein of individuals at different time-points: baseline, immediately after the 1st session, before and

immediately after the 24th session. The blood samples were centrifuged, and the plasma was stored at -20ºC until the analysis moment.

Plasma BDNF levels were determined by the ELISA method, from Sigma-Aldrich commercial kit (catalog number RAB0026) according to manufacturer's instructions. Briefly, the sample and BDNF specific standards were added to ELISA microplate and incubated for 2.5 hours at room temperature. Subsequently, the solutions were discarded, and the same plate was washed four times with wash buffer (PBS, Tween 20 0.01%). After washing, the secondary antibody bound to biotin was added, and incubated for 1 hour at room temperature with gentle agitation. The plate was again washed with wash buffer, and streptavidin solution was added, and the plate was incubated at room temperature for 45 minutes with gentle agitation. The solution was discarded, and the plate passed through the washing process. Tetramethylbenzidine was added, and then it was incubated for 30 minutes at room temperature, light deprivation, with gentle agitation. The stop solution was added, and the plate was read in a spectrophotometer at a wavelength of 450nm. The plasma BDNF levels were expressed as ng/ml.

Exercise capacity

Exercise capacity was assessed by the 6-minute walking test (6MWT) conducted according to the recommendations of the ATS[31]. Two tests were performed with an interval of 30 minutes and the best performance was used for the analysis.

Dyspnea

Dyspnea was assessed with the modified Medical Research Council (mMRC) scale that rates the degree of dyspnea on activities of daily living. Its score goes from 0 to 4, and the maximum score indicates greater dyspnea[32].

Quality of life

Quality of life was assessed by the St. George's Respiratory Questionnaire (SGRQ), a disease-specific questionnaire, validated in Brazil[33]. The SGRQ consists of three domains (symptoms, activity, and impact of the disease) divided into 76 items with scores from 0 to 100. The highest score is indicative of poor quality of life in any one of the domains in total calculation. Values above 10% reflect an impairment of quality of life in that domain or the total score.

BODE index

The body mass index (B), degree of obstruction (O), perception of dyspnea (D) and exercise capacity (E) index (BODE index) is a multidimensional grading system that predicts the risk of death from any cause and from respiratory causes among patients with COPD. The BODE index was calculated as previously described by Celli and Cote[34], and its score goes from 0 to 10 divided into quartiles. The higher quartile means higher risk of death.

Pulmonary Rehabilitation Program (PRP)

Patients engaged in a supervised 24-exercise-training program designed according to the ATS and the ERS[19]. The protocol involved endurance training and peripheral muscle strength three times per week (approximately 90 minutes each session). The endurance training was performed on a treadmill with 30 minutes of load initially determined at 60% of the speed average of the 6MWT. The work load progression occurred according to the dyspnea report (4 to 6 on the modified Borg scale[35]). The lower limbs training involved quadriceps and triceps sural with free weight and/or in the extensor chair (2 sets of 10 to 15 repetitions). The upper limb training was carried in diagonal axes with free weights or elastic bands, each diagonal was

performed in two sets, lasting two minutes each. In addition, patients received nutritional guidance and educational orientation regarding disease self-management.

Statistical Analysis

Normal distribution was checked with the Shapiro–Wilk test. Data were reported as mean (SD) or median (25–75% interquartile range). The Friedman test with Bonferroni post hoc analysis were used to compare BDNF levels at the beginning and the end of the first and the 24th sessions. Changes on data regarding exercise capacity, quality of life and depression were assessed with the Wilcoxon test. Comparisons between frequent exacerbators and non-frequent exacerbators were tested with Mann-Whitney's U test. Correlations were tested with the Spearman test. Statistical significance was set at $p<0.05$. Data were analyzed using SPSS 20.0 (SPSS, Chicago, IL, USA) and the graph was made with GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Twenty patients engaged the study and sixteen completed the intervention (two deaths and two withdraws). Subjects characteristics are shown in Table 1.

Baseline BDNF levels (before the first PRP session) did not correlated with baseline exercise capacity, quality of life, dyspnea, the BODE index neither with pulmonary function ($p > 0.05$).

Exercise capacity, dyspnea, the BODE index (score and quartile), SGRQ total score of quality of life and its activities domain improved significantly after the PRP intervention when compared to baseline (Table 2). The body mass index did not change after the PRP [25.1 (4.2) *vs.* 25.1 (4.5) kg/m^2; $p = 1.0$].

Post hoc analysis revealed a reduction on BDNF levels after the 1st session compared to baseline ($p < 0.001$) and increase between the end of the 1st session and the beginning of the 24th session ($p = 0.006$). No significant difference was found between the baseline and the end of the 24th session (Figure 1). The change on BDNF levels in the first session was inversely correlated with its levels at de beginning and at the end of the session (Figure 2).

Baseline characteristics and PRP's effects did not differ from frequent exacerbators from non-frequent exacerbators.

DISCUSSION

We demonstrated for the first time that an exercise-based PRP could modulate peripheral BDNF levels. Specifically, our results showed a significant reduction of this neurotrophin after the first training session when compared to the baseline. However, this acute effect was nullified in response to long-term PRP intervention. As expected, the current study also demonstrated the beneficial impact of PRP on quality of life, dyspnea, and exercise capacity improvements, as well in reducing the risk of death measured by the BODE index.

Literature is divergent regarding the acute and chronic effects of exercise on BDNF levels. Several studies showed acute increases on BDNF after a single endurance exercise bout in healthy subjects[20,22–24,28], while others showed no change[21,36,37]. Conflicting evidence also exists regarding the chronic impact, with at least two studies showing an increase after endurance training[20,37], while others reported that neither endurance training[21,38] nor resistance training[38,39] alters this neurotrophin.

Similar to our experimental design, other works also investigated the time course of BDNF levels in response to exercise. Yarrow and colleagues[40] showed, in

healthy young men, that endurance exercise acutely increases serum BDNF levels at baseline and after 5-weeks, demonstrating both acute and chronic changes. In addition, Castellano et al[21] reported no changes on BDNF levels 30 minutes after an endurance exercise session in patients with multiple sclerosis, while BDNF levels decreased after two hours resting, remaining decreased until 3 hours after the exercise session. Interestingly, the authors showed that BDNF remains unaltered after 8 weeks after the intervention, suggesting an adaptation following chronic exposure. Thus, these data led us to hypothesize that different from healthy individuals, in patients with chronic diseases such as COPD and multiple sclerosis, BDNF modulation seems to occur in a time-dependent manner, changing only acutely in response to exercise and reaching a homeostasis as an adaptation to exercise training.

According to Yarrow and colleagues[40] the acute reduction on BDNF levels may also could suggest (1) a greater tissue uptake and/or biding with tropomyosin-related kinase receptors in central and peripheral tissues, (2) greater clearance of BDNF from the circulation, and/or (3) reduced secretion of BDNF during recovery. Meanwhile, further studies are necessary to investigate the early-phase effects of training on BDNF levels in COPD patients.

It has been proposed that BDNF is stored mainly in platelets and is released from the platelets under inflammatory environments[41]. However, we recently showed that a single PRP session did not change peripheral inflammatory markers[7]. Interestingly, there is growing evidence demonstrating a suppression of BDNF production by corticosteroids[18,42]. Therefore, an important feature that may explain the sharp reduction of the peripheral BDNF levels after the exercise found in the current study could be the increase in cortisol levels after the physical stress, since cortisol seems to inhibit BDNF[43–45].

While platelets are considered the main source of peripheral circulating BDNF[46–48], monocytes and activated T and C lymphocytes are considered secondary

sources[49]. In this context, a single training session in previous sedentary COPD patients may change their immunological profile, which may be related to the acute exercise effect in sedentary patients with COPD.

As previously described, BDNF plays a significant role in acute and chronic inflammatory conditions of the airways. Previous studies[8,9] showed that patients with COPD present elevated BDNF levels when compared to matched controls. In fact, BDNF is considered to induce neuronal hyperreactivity leading to hyperresponsiveness and cough[17,18] and to increase quantity and function of airway smooth cells[16], considering a marker of neuromuscular dysfunction of the airways. Besides, BDNF is associated with lung function, exercise capacity and mortality risk[8] and might be related to the SGRQ Impact score[50] in patients with COPD. Altogether, these findings raise the hypothesis that reductions in BDNF levels after a single exercise session might have a protective effect in COPD individuals. Notwithstanding, PRP induced changes on exercise capacity and risk of death without changing the BDNF levels.

Another important finding of the current study is that the lower the BDNF level, the higher is the change induced by the first PRP session. Although we did not find a correlation between any of the clinical features, this finding reinforces the hypothesis of previous studies that BDNF may be a marker of disease severity and impact[8,9,50] and even for treatment responsiveness. The physiological relevance of this findings in COPD individuals must be investigate in future studies.

We acknowledge that the current study has some limitations such as the lack of a control group and the small sample size. Pulmonary rehabilitation has enough evidence to support our findings regarding exercise capacity, quality of life and risk of death in patients with COPD. Even though it was a pilot study with a small sample size, we found a statistical power of 92.5% (CI 95%) in the acute effect of PRP on BDNF levels. We know that the sample size is not sufficient to demonstrate the chronic effect

of PRP on BDNF levels and its correlations between clinical parameters. Nevertheless, it is important to reinforce that this is a real-time prospective study.

CONCLUSIONS

In summary, exercise acutely reduced BDNF levels, but this effect that was nullified by the long-term intervention. In addition, our study supports the evidence that PRP improves exercise capacity, quality of life and the BODE index in COPD patients. However, these beneficial effects are not linked to BDNF regulation, since this neurotrophin is able to respond uniquely in an acute way (single session). We might propose an exercise adaptation-related change in the BDNF dose–response, although further studies are needed to a better understanding of the BDNF modulation pathways in individuals with COPD.

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Table 1. Participants characteristics (n = 16)

Characteristics	Mean ± SD
Age, years	68.5 (6.7)
Male/female, n	10/6
Frequent exacerbator, n	8
Smoking history, pack-years	60.1 (31.4)
Tabaco abstinence, years	12.4 (6.4)
FEV_1, l	1.06 (0.44)
FEV_1, %pred	39.0 (14.0)
FVC, l	2.17 (0.71)
FVC, %pred	60.1 (12.0)
FEV_1/FVC, %	0.48 (0.08)
BMI, kg/m^2	25.1 (4.9)

Data given as n or mean (standard deviation). FEV_1: forced expiratory volume in one second, liter; FVC: forced vital capacity, liter; %pred: percent of the predicted value; BMI: body mass index, $weight/height^2$.

Table 2. Exercise-based PRP effects on clinical outcomes.

Characteristics	Baseline	After PRP	p
Exercise Capacity (6MWT, m)	416.0 (76.5)	480.4 (84.8)	0.003
Dyspnea (mMRC)	4.0 (2.3 – 4)	2 (1.3 – 3)	0.011
Bode index, score	4.81 (1.91)	4.06 (1.53)	0.012
Bode index, quartile	3.0 (2.0 – 3.0)	2 (2.0 – 3.0)	0.008
SGRQ total score	53.9 (40.8 – 64.6)	49.1 (35.7 – 51.4)	0.007
SGRQ Symptoms	52.0 (22.7)	40.2 (21.9)	0.069
SGRQ Activities	77.6 (13.9)	64.5 (16.2)	0.005
SGRQ Impact	38.8 (17.1)	31.6 (12.0)	0.070

Data shown as mean (standard deviation) or median (25 – 75% interquartile range). Baseline: immediately before the first session; After PRP: immediately before the 24th session; Exercise capacity: distance walked during the six-minute walking test (6MWT) in meters; Dyspnea: score of the perception on the modified Medical Research Council (mMRC); BODE index: score and quartile of the body mass index (B), degree of obstruction (O), perception of dyspnea (D) and exercise capacity (E) index; SGRQ: scores of the Saint George Respiratory Questionnaire.

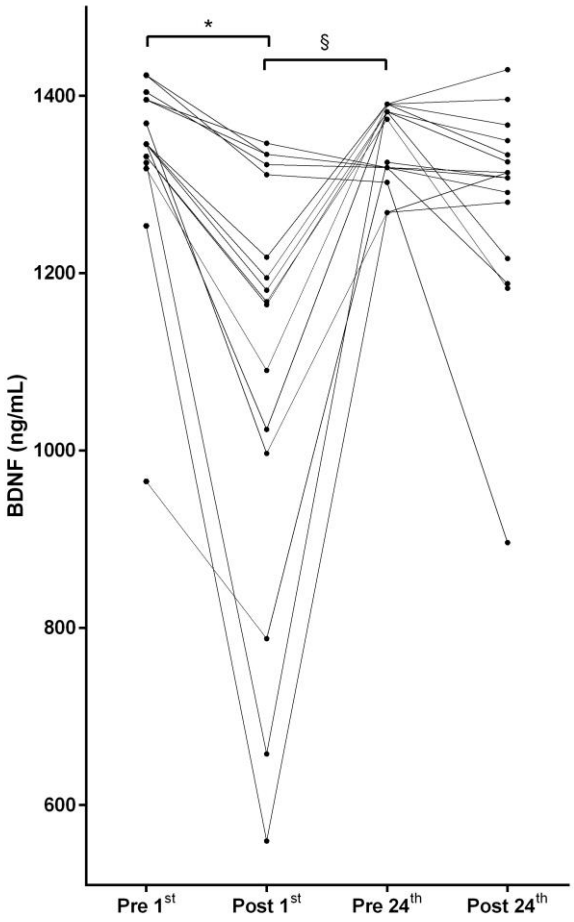

Figure 1 – Exercise-based PRP effect on plasmatic BDNF levels.

BDNF: Brain-derived neurotrophic factor. Plasmatic BDNF levels at baseline [immediately before the first session – Pre 1st; 1345.5 (1326.2 – 1395.5) ng/mL]; immediately after the first session [Post 1st; 1174.3 (1003.3 – 1319.7) ng/mL]; immediately before the 24th session [Pre 24th; 1349.2 (1318.9 – 1388.4) ng/mL] and immediately after the 24th session [Post 24th; 1310.4 (1232.2 – 1345.5) ng/mL].

* Reduction on BDNF levels when compared to the beginning of the first session (acute effect); $p < 0.001$.

§ Increase on BDNF levels when compared to the end of the first PRP session; $p = 0.006$ (Friedman test followed by Bonferroni *post hoc*).

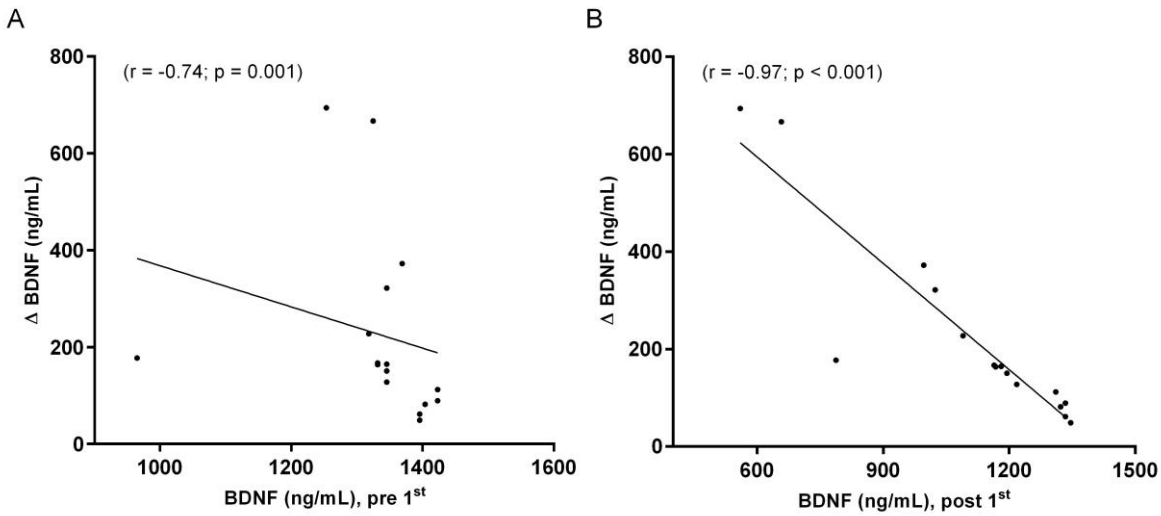

Figure 2 – Correlations between plasmatic BDNF levels and the acute effect of PRP.

BDNF: Brain-derived neurotrophic factor. A: Correlation between the change in plasmatic BDNF levels at the first session and the BDNF levels at baseline (immediately before the first session). B: Correlation between the change in plasmatic BDNF levels at the first session and the BDNF levels immediately after the first session.

5 CONCLUSÕES

Os estudos provenientes desta tese foram elaborados com o objetivo de contribuir na construção do conhecimento sobre os efeitos da reabilitação pulmonar em pacientes com DPOC.

O estudo 1 teve a finalidade de investigar os efeitos de 24 e 48 sessões de reabilitação pulmonar sobre a função endotelial, rigidez arterial e frequência cardíaca avaliada por meio da PAT, além dos efeitos sobre pressão arterial sistêmica, capacidade funcional e risco de morte. Esse foi o primeiro estudo a investigar os efeitos da reabilitação pulmonar na função endotelial avaliada por PAT em pacientes com DPOC. Nesse estudo, foi verificado que os pacientes, em sua maioria, não apresentavam disfunção endotelial, porém apresentavam rigidez arterial aumentada ou anormal. A rigidez arterial foi significativamente pior em exacerbadores frequentes. Corroborando a literatura, foi visto que a reabilitação pulmonar melhorou a capacidade funcional e o risco de morte. Entretanto, os parâmetros cardiovasculares não foram modificados com a reabilitação pulmonar. Ainda assim, informações sobre a confiabilidade e a responsividade da PAT na DPOC, principalmente em relação ao efeito dose-resposta de broncodilatadores, precisam de uma investigação mais aprofundada.

O estudo 2, estudo piloto, avaliou o efeito agudo e crônico de 24 sessões de reabilitação pulmonar nos níveis plasmáticos de BDNF, bem como o efeito crônico na capacidade funcional, dispneia, risco de morte e qualidade de vida. Como demonstrado por outros estudos, a reabilitação pulmonar melhorou a capacidade funcional, a dispneia, a qualidade de vida e reduziu o risco de morte nesses pacientes. Pela primeira vez na literatura, foi demonstrado que uma única sessão de exercício em pacientes sedentários (efeito agudo) reduz os níveis de BDNF, porém esse efeito não se mantém com o treinamento e o treinamento não é capaz de reduzir os níveis basais de BDNF. Esse estudo também demonstrou não haver diferenças nos níveis basais de BDNF nem na resposta ao exercício entre exacerbadores frequentes e não-exacerbadores. Entretanto, é importante ressaltar

que esse estudo se trata de um estudo piloto e estudos futuros são necessários para melhor compreensão dos efeitos do exercício nessa neurotrofina.

De forma genérica, com base nos estudos apresentados, pode-se concluir que ainda são necessários avanços no que diz respeito a intervenções capazes de modificar parâmetros cardiovasculares em pacientes com DPOC, principalmente no que diz respeito à função endotelial e ao risco de eventos cardiovasculares. Também se faz importante o melhor entendimento da cinética do BDNF em relação ao exercício físico e seu entendimento com marcadores de severidade da DPOC.

6 APÊNDICE

6.1 TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Reabilitação na Doença Pulmonar Obstrutiva Crônica

23 de setembro de 2015

3ª versão

A doença pulmonar obstrutiva crônica (DPOC) é uma doença respiratória progressiva caracterizada por manifestações sistêmicas. Estas geram limitações funcionais e comportamentais, com consequente diminuição na capacidade do indivíduo de realizar atividades de vida diária (AVD), afetando sua de qualidade de vida (QV). Neste contexto, a reabilitação pulmonar já está bem estabelecida como um tratamento complementar eficaz para minimizar os sintomas e melhorar o estado funcional do indivíduo, resultando em melhora da realização de AVD e QV. Sabe-se que 12 semanas, tempo mínimo preconizado para a duração de um programa de reabilitação pulmonar, não é suficiente para que os pacientes se tornem mais ativos em seu cotidiano, apesar de melhorar a capacidade funcional e QV.

Sendo assim o sr(a) está sendo convidado(a) a participar de uma pesquisa que tem por objetivo avaliar o efeito de um programa de reabilitação pulmonar na capacidade funcional, na realização de atividade física de vida diária, na qualidade de vida, depressão, memória e marcadores sanguíneos neurofisiológicos e inflamatórios em pacientes com DPOC. O título do estudo é "**REABILITAÇÃO NA DOENÇA PULMONAR OBSTRUTIVA CRÔNICA**" e será conduzido no Laboratório de Fisioterapia da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) e no Pavilhão Pereira Filho da Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA).

A sua participação na pesquisa iniciará após a leitura, o esclarecimento de possíveis dúvidas e do seu consentimento livre e esclarecido por escrito. A assinatura do Termo de Consentimento Livre e Esclarecido será feita em duas vias, permanecendo uma delas com o participante.

Você será informado sobre os procedimentos e resultados da participação na pesquisa e receberá esclarecimento sobre as dúvidas que possam surgir dela.

Fases do Estudo:

ENTREVISTA: coletaremos seus dados de identificação e algumas informações referentes ao seu histórico da doença.

AVALIAÇÃO:

Dia 1: Aplicação de escalas e questionários referentes a falta de ar, impacto da doença, qualidade de vida, memória e depressão, será coletado sangue, medida de peso e altura e avaliação da função pulmonar (espirometria – "exame do sopro"). Para realizar a avaliação da função pulmonar é necessário inalar um medicamento broncodilatador (salbutamol) e esse medicamento pode causar aumento da frequência cardíaca, que será monitorada durante a avaliação. A coleta de sangue pode causar a formação de um hematoma e o desconforto relacionado à coleta de sangue. O senhor(a) deverá fazer jejum de 4 horas para realizar a coleta de sangue. O primeiro dia de avaliação terá duração aproximada de uma hora e trinta minutos.

Dia 2: serão avaliadas força, resistência e oxigenação de quadríceps (músculo da coxa) e oxigenação de membro superiro (antebraço) e a função dos seus vasos sanguíneos (função endotelial). A força e a resistência serão avaliadas num equipamento chamado dinamômetro isocinético e a oxigenação será avaliada de forma não invasiva com aparelho que utiliza luz infravermelha, conhecido como "espectroscopia infravermelho próximo - NIRS". A função endotelial será avaliada através da tonometria de pulso. Será colocado um sensor na ponta de cada dedo indicador, enquanto é feita uma pressão em de seus braços com um manguito de medir pressão (esfigmomanômetro). O segundo dia de avaliação terá duração aproximada de duas horas.

Dia 3: teste de caminhada e de atividade de vida diária, sendo esse último um teste que envolve caminhar, carregar uma mochila, subir e descer degraus, sentar e levantar. O terceiro dia de avaliação terá duração aproximada de duas horas.

Dia 4: teste cardiopulmonar em esteira, com velocidade de acordo com seu desempenho no teste de caminhada. O quarto dia de avaliação terá duração aproximada de uma hora.

Dias 5 e 6: monitoração das atividades de vida diária por meio de um aparelho acoplado em um cinto, chamado acelerômetro triaxial. A monitoração é feita em casa, pois o(a) Sr(a) levará o aparelho consigo. A avaliação será realizada durante dois dias

consecutivos, por 12h diárias. A devolução do aparelho será combinada entre a pesquisadora e o participante, podendo a pesquisadora buscar o aparelho na residência do participante.

REABILITAÇÃO PULMONAR: consiste em um programa de exercício físico que envolve exercício em esteira ou bicicleta e fortalecimento de braços e pernas. A reabilitação terá duração de seis meses (24 semanas) e será realizada três vezes por semana, com duração de uma hora e meia. Quinzenalmente o(a) senhor(a) e sua família serão convidados a assistirem a uma aula sobre sua doença e cuidados relacionados a ela.

REAVALIAÇÃO: Após 12, 18 e 24 semanas o senhor passará novamente pela avaliação. Depois do encerramento do programa de reabilitação pulmonar o senhor será convidado a realizar novas avaliações aos 3, 6, 9 e 12 meses após a alta.

No primeiro dia de reabilitação pulmonar, na 24ª sessão e na 48ª sessão, uma coleta de sangue será realizada no inícioe no final da sessão.

1. Benefícios e Riscos:

Os possíveis benefícios deste estudo são a diminuição da sensação de falta de ar e cansaço nos membros inferiores (pernas) e superiores (braços), assim como a melhora da realização das atividades de vida diária e qualidade de vida. Já os eventuais riscos do presente estudo são relacionados à prática de atividade física, como: cansaço, cãibra e dores nos músculos, no transcorrer e após a realização dos exercícios. Esses se tornarão menos frequentes e intensos, à medida que os pacientes forem aumentando o número de sessões. Para minimizar os riscos, os pacientes terão a pressão arterial, frequência cardíaca, sensação de falta de ar e de fadiga muscular monitoradas ao longo das sessões de treinamento. Referente à coleta de sangue, a mesma será realizada por um profissional devidamente qualificado e habilitado, bem como o local previamente preparado, higienizado e refrigerado para a realização do procedimento; respeitando os critérios biossegurança.

2. Danos reacionados à pesquisa:

Durante a pesquisa os participantes receberão acompanhamento da pesquisadora ou de alguém da sua equipe na realização das avaliações, reabilitação pulmonar e assistência no caso de alguma

lesão decorrente da participação na pesquisa. Está garantida a plena assistência ao paciente pelos pesquisadores responsáveis, caso haja necessidade.

3. Custos:

A participação em todos os procedimentos da pesquisa não implicará no pagamento de qualquer taxa. Todos os exames realizados na pesquisa serão cobertos pelos pesquisadores. Caso seja necessário, as despesas com transportes e alimentação nos dias de participação serão ressarcidas.

4. Participação voluntária:

A participação na pesquisa será voluntária. Concordando ou recusando em participar, não serão obtidas vantagens ou desvantagens no atendimento e tratamento no serviço de saúde no qual os pacientes são atendidos. Ninguém será obrigado a responder a todas as perguntas e realizar todas as avaliações e exercícios, podendo interromper ou cancelá-los a qualquer momento sem nenhum constrangimento. Sua participação no estudo em nada afetará o seu atendimento no hospital ISCMPA.

5. Privacidade e Confidencialidade:

As informações coletadas na pesquisa permanecerão no anonimato e em nenhum momento seu nome será revalado. Apenas a pesquisadora ou alguém autorizado por ela terá acesso aos dados de identificação. Sua participação no estudo em nada afetará o seu atendimento no hospital ISCMPA.

Em caso de dúvidas, entrar em contato:

Pesquisadora assistente: MSc. Cintia Laura Pereira de Araujo. Telefone: (51) 8128-4078.

Investigador principal: Dr. Pedro Dal Lago. Telefone: (51) 9961-7331.

Comitê de ética em Pesquisa da Irmandade Santa Casa de Misericórdia de Porto Alegre – sob coordenação do Dr. Cláudio Telöken.

Telefone: (51) 3214-8571; e-mail:cep@santacasa.tche.br

Rua Prof. Annes Dias, 295, Porto Alegre/RS.

Horário de Atendimento ao Público: 2ª a 6ª das 9h às 12h e das 13h30 às 17h.

Ao assinar abaixo, você confirma que leu as afirmações contidas nesse termo de consentimento, que foram explicados os procedimentos do estudo, que teve a oportunidade de fazer perguntas, que está satisfeito com as explicações fornecidas e que decidiu participar voluntariamente deste estudo. Uma via será entregue a você e outra será arquivada pelo investigador principal.

_____ _____

Nome do Sujeito da Pesquisa (letra de forma) Data

_____

Assinatura do Sujeito da Pesquisa

Dr. Pedro Dal Lago _____

Nome Pesquisador Data

_____

Assinatura e Carimbo do Pesquisador

7 ANEXO

7.1 ANEXO I: PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA EM PESQUISA

DADOS DA EMENDA

Título da Pesquisa: Reabilitação na Doença Pulmonar Obstrutiva Crônica - aspectos funcionais, cognitivos e epigenéticos **Pesquisador:** Pedro Dal Lago **Área Temática:**

Versão: 3

CAAE: 40078114.9.0000.5335

Instituição Proponente: ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.413.342

Apresentação do Projeto:

Trata-se de um estudo prospectivo, longitudinal, quase-experimental de abordagem quantitativa.

O estudo baseia-se nas seguintes hipóteses 1. Ocorrem alterações na capacidade e desempenho funcional, na qualidade de vida, na memória e na depressão, em marcadores epigenéticos, no BDNF ou em mediadores inflamatórios sistêmicos dos pacientes no período entre três e seis meses de reabilitação pulmonar pacientes em DPOC moderada a muito grave. Hipótese 2: As alterações na capacidade e desempenho funcional, na qualidade de vida, na memória e na depressão, em marcadores epigenéticos, no BDNF ou em mediadores inflamatórios sistêmicos decorrentes da reabilitação pulmonar perduram após a alta do programa.

As avaliações do projeto propostas serão: Avaliar performance e capacidade funcional antes e após 12, 18 e 24 semanas de reabilitação pulmonar em pacientes com DPOC moderada a muito grave. Avaliar a duração do efeito da reabilitação

pulmonar na performance e capacidade funcional aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Avaliar o risco de morte (Índice BODE) antes e após 12, 18 e 24 semanas de reabilitação pulmonar em pacientes com DPOC moderada a muito grave.

Avaliar a duração do efeito da reabilitação pulmonar no risco de morte (Índice BODE) aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Avaliar a performance da memória, a percepção de qualidade de vida e o índice de depressão de pacientes com DOPC antes e após 12, 18 e 24 semanas do PRP.

Avaliar a duração do efeito da reabilitação pulmonar na performance da memória, a percepção de qualidade de vida e o índice de depressão aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Determinar os níveis de acetilação global de histonas, a atividade das enzimas HAT e HDAC, os níveis de metilação global de DNA, a atividade das enzimas DNMT1 e DNMT3b (marcadores epigenéticos), os níveis de BDNF, os níveis de miostatina e os níveis de marcadores inflamatórios em pacientes com DOPC antes e após a primeira e a última sessão de reabilitação pulmonar.

Avaliar a duração do efeito da reabilitação nos marcadores epigenéticos, nos níveis de BDNF, nos níveis de miostatina e em marcadores inflamatórios (TNF- , IL-6. IL-1 e IL-10) aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Investigar associação entre o efeito da reabilitação pulmonar na performance e capacidade funcional, nos marcadores inflamatórios, epigenéticos e achados comportamentais (memória e depressão) e no risco de morte em pacientes com DPOC moderada a muito grave.

A população da pesquisa será composta por pacientes com DPOC moderada a muito grave, de ambos os sexos. Serão adotados como critérios de inclusão: estabilidade clínica no mês prévio ao início do protocolo e idade > ou igual a 40 anos. Os critérios de exclusão serão: tabagismo atual, presença de doenças associadas que impossibilitem a realização de alguma das avaliaçoes do estudo ou

que limitem a progressão do treinamento e não assinatura do TCLE (Termo de Consentimento Livre e Esclarecido).

Objetivo da Pesquisa:

Objetivos Gerais:

Avaliar alterações entre 12 e 24 semanas de reabilitação pulmonar na capacidade funcional e na realização de AFVD e a duração de tais efeitos após a alta do PRP em pacientes com DPOC moderada a muito grave.

Avaliar o efeito da reabilitação pulmonar sobre os marcadores epigenéticos e sobre os níveis de BDNF de pacientes com DPOC moderada a muito grave.

Objetivos Específicos:

Avaliar performance e capacidade funcional antes e após 12, 18 e 24 semanas de reabilitação pulmonar em pacientes com DPOC moderada a muito grave.

Avaliar a duração do efeito da reabilitação pulmonar na performance e capacidade funcional aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Avaliar o risco de morte (Índice BODE) antes e após 12, 18 e 24 semanas de reabilitação pulmonar em pacientes com DPOC moderada a muito grave.

Avaliar a duração do efeito da reabilitação pulmonar no risco de morte (Índice BODE) aos três, seis, nove e doze meses após o encerramento na participação no PRP. Avaliar a performance da memória, a percepção de qualidade de vida e o índice de depressão de pacientes com DOPC antes e após 12, 18 e 24 semanas do PRP. Avaliar a duração do efeito da reabilitação pulmonar na performance da memória, a

percepção de qualidade de vida e o índice de depressão aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Determinar os níveis de acetilação global de histonas, a atividade das enzimas HAT e HDAC, os níveis de metilação global de DNA, a atividade das enzimas DNMT1 e DNMT3b (marcadores epigenéticos), os níveis de BDNF, os níveis de miostatina e os níveis de marcadores inflamatórios em pacientes com DOPC antes e após a primeira e a última sessão de reabilitação pulmonar.

Avaliar a duração do efeito da reabilitação nos marcadores epigenéticos, nos níveis de BDNF, nos níveis de miostatina e em marcadores inflamatórios (TNF- , IL-6. IL-1 e IL-10) aos três, seis, nove e doze meses após o encerramento na participação no PRP.

Investigar associação entre o efeito da reabilitação pulmonar na performance e capacidade funcional, nos marcadores inflamatórios, epigenéticos e achados comportamentais (memória e depressão) e no risco de morte em pacientes com DPOC moderada a muito grave.

Avaliação dos Riscos e Benefícios:

Conforme descrito no projeto estudos sobre recursos que venham a incrementar os benefícios observados em participantes de PRP e, em particular, sobre aqueles que podem influenciar positivamente a performance e a capacidade funcional, qualidade de vida e seus aspectos neurofisiológicos em pacientes com DPOC, são relevantes tanto para a prática clínica quanto para o conhecimento científico, auxiliando, assim, no estabelecimento de estratégias de intervenção que beneficiem os indivíduos acometidos pela doença.

Comentários e Considerações sobre a Pesquisa:

Estudo com pacientes com DPOC que tem como objetivo principal o efeito de um programa de reabilitação pulmonar na capacidade funcional, na realização de atividade física de vida diária, depressão, memória e marcadores sanguíneos, neurofisiológicos e inflamatórios em pacientes com doença pulmonar obstrutiva crônica.

Considerações sobre os Termos de apresentação obrigatória:

Foi encaminhado ao CEP (Comitê de Ética em Pesquisa) desta Instituição emenda solicitando alterações de alguns itens referentes ao projeto.

Foram realizadas alterações no projeto e apresentado/discutido em reunião com os demais membros do CEP.

Recomendações:

Não há recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado.

Considerações Finais a critério do CEP:

Após avaliação das alterações efetuadas no estudo acima descrito, o presente Comitê não encontrou óbices quanto à implementação das mesmas.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_615792_E1.pdf	10/11/2015 14:56:41		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_novembro2015.pdf	10/11/2015 14:34:58	Viviane Rostirola Elsner	Aceito
Outros	Emenda_novembro2015.pdf	10/11/2015 14:34:15	Viviane Rostirola Elsner	Aceito
Cronograma	cronograma_novembro2015.pdf	10/11/2015 14:33:11	Viviane Rostirola Elsner	Aceito
Projeto Detalhado / Brochura Investigador	projeto_detalhado_novembro2015.pdf	10/11/2015 14:32:14	Viviane Rostirola Elsner	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE - cep ISCMPA versão 2.pdf	18/03/2015 19:50:56		Aceito
Outros	ORÇAMENTO.pdf	18/03/2015 19:32:35		Aceito
Projeto Detalhado	projeto_detalhado 18.03.15.pdf	18/03/2015		Aceito
/ Brochura Investigador	projeto_detalhado 18.03.15.pdf	19:31:37		Aceito
Folha de Rosto	Folha de rosto cep iscmpa.jpg	13/12/2014 21:21:10		Aceito
Outros	Formulário de inscrição de pesquisa .pdf	13/12/2014 21:11:34		Aceito
Outros	Declaração de autorização da chefia responsável - Dr. Felicetti.jpg	13/12/2014 21:10:45		Aceito

Outros	Autorização lab fisioterapia.jpg	13/12/2014 21:09:18		Aceito
Outros	Declaração de Concordância IPA.pdf	09/12/2014 09:31:00		Aceito
Outros	Termo de compromisso para a utilização de prontuários.jpg	08/12/2014 11:32:55		Aceito
Outros	Declaraçõa de risco e benefícios.jpg	08/12/2014 11:31:16		Aceito
Outros	Declaração de uso e publicação de dados.jpg	08/12/2014 11:30:49		Aceito
Outros	Declaração de uso de materiais.jpg	08/12/2014 11:30:30		Aceito
Outros	Declaração de isenção de ônus à instituição.jpg	08/12/2014 11:30:08		Aceito
Outros	Declaração de confidencialidade do sujeito do estudo.jpg	08/12/2014 11:29:47		Aceito
Outros	Declaração UFCSPA.pdf	08/12/2014 10:58:33		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PORTO ALEGRE, 18 de fevereiro de 2016

Assinado por:

Carlos Henrique Munhoz Olea

(Coordenador)

7.2 ANEXO II: NORMAS PARA SUBMISSÃO NO PERIÓDICO "*JOURNAL OF CARDIOPULMONARY REHABILITATION AND PREVENTION*"

Ethical/Legal Considerations

A submitted manuscript must be an original contribution not previously published (except as an abstract or a preliminary report), must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language, without the consent of Wolters Kluwer Health. Each person listed as an author is expected to have participated in the study to a significant extent. Although the editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with the Journal, its editors, or the publisher.

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Preparation of Manuscript

Manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. All content in The Journal of Cardiopulmonary Rehabilitation and Prevention is printed only in English.

Manuscript Submission

Authors are invited to submit original investigations, scientific reviews, brief reports, and case reports in all areas relating to the prevention and management of cardiopulmonary disease. These areas include but are not limited to cardiac and/or pulmonary rehabilitation, primary and secondary prevention, epidemiology, and exercise testing and training.

All manuscripts must be submitted on-line through the Journal Web site at http://jcrp.edmgr.com/. First-time users: Please click the Register button from the menu above and enter the requested information. On successful registration, you will be sent an E - mail indicating your user name and password. Print a copy of this information for future reference. Note: If you have received an E - mail from us with an assigned user ID and password, or if you are a repeat user, do not register again. Just log in. Once you have an assigned ID and password, you do not have to re-

register, even if your status changes (that is, author, reviewer, or editor). Authors: Please click the log-in button from the menu at the top of the page and log in to the system as an Author. Submit your manuscript according to the author instructions. You will be able to track the progress of your manuscript through the system. If you experience any problems, please contact Kate Maude, Editorial Coordinator at jcrp@smithbucklin.com. Requests for help and other questions will be addressed in the order received.

As of January 1, 2013, JCRP no longer requires that manuscripts be submitted in a blinded format. Author names, institutions, funding information, etc, are permissible within manuscript text.

If possible, all tables and figures should be included at the end of the text. The word count for the text-only portion for original investigations should be limited to 3000 words. A shortened form of the title should be included at the top of each manuscript page after the title page. A structured abstract and condensed abstract should be included for all manuscripts. Manuscripts are received with the understanding that they have not been previously published and are not currently under consideration for publication in any other journal. Manuscripts will be acknowledged upon receipt; those accepted for publication are subject to copy editing. The name, address, home and work telephone numbers, fax number, and e-mail address of the author responsible for correspondence regarding the manuscript should be included in an accompanying cover letter.

Acknowledgments must be given when material from other publications is included. Copies of the authors' and publishers' permission letters should be included with the manuscript. Provide names of author(s), title of article, title of journal or book, volume number, page(s), month, and year.

Figures:

A) Creating Digital Artwork

Learn about the publication requirements for Digital Artwork: http://links.lww.com/ES/A42

Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).

Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

B) Digital Artwork Guideline Checklist

Here are the basics to have in place before submitting your digital artwork:

Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.

Crop out any white or black space surrounding the image.

Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.

Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.

Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.

Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

Remember:

Cite figures consecutively in your manuscript.

Number figures in the figure legend in the order in which they are discussed.

Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.

Color Figures

The journal accepts for publication color figures that will enhance an article. Authors who submit color figures will receive an estimate of the cost for color reproduction. If they decide not to pay for color reproduction, they can request that the figures be converted to black and white at no charge.

Tables: Create tables using the table creating and editing feature of the word processing software (ie, Microsoft Word). Do not use Excel or comparable spreadsheet programs. Group all tables at the end of the manuscript, or supply them together in a separate file. Cite tables consecutively in the text, and number them in

that order. Key each on a separate sheet, and include the table title, appropriate column heads, and explanatory legends (including definitions of any abbreviations used). Do not embed tables within the body of the manuscript. They should be self-explanatory and should supplement, rather than duplicate, the material in the text.

Supplemental Digital Content (SDC): Authors may submit SDC via Editorial Manager to WKH journals that enhance their article's text to be considered for online posting. SDC may include standard media such as text documents, graphs, audio, video, etc. On the Attach Files page of the submission process, please select Supplemental Audio, Video, or Data for your uploaded file as the Submission Item. If an article with SDC is accepted, our production staff will create a URL with the SDC file. The URL will be placed in the call-out within the article. SDC files are not copy-edited by WKH staff, they will be presented digitally as submitted. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

SDC Call-outs

Supplemental Digital Content must be cited consecutively in the text of the submitted manuscript. Citations should include the type of material submitted (Audio, Figure, Table, etc.), be clearly labeled as "Supplemental Digital Content," include the sequential list number, and provide a description of the supplemental content. All descriptive text should be included in the call-out as it will not appear elsewhere in the article.

Example:

We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive.

List of Supplemental Digital Content

A listing of Supplemental Digital Content must be submitted at the end of the manuscript file. Include the SDC number and file type of the Supplemental Digital Content. This text will be removed by our production staff and not be published.

Example:

Supplemental Digital Content 1. wmv

SDC File Requirements

All acceptable file types are permissible up to 10 MBs. For audio or video files greater than 10 MBs, authors should first query the journal office for approval. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

BRIEF REPORTS AND CASE REPORTS

Brief reports and case reports will be considered for publication in JCRP. These reports should be in the areas of cardiac and/or pulmonary rehabilitation, primary and secondary prevention, epidemiology, and exercise testing and training. Reports should be limited to 2000 words for the text-only portion, 15 references, and no more than a total of two tables or figures. The title page must be labeled "Case Report" or "Brief Report." A structured abstract and condensed abstract should be included for Brief Reports. Brief Reports should include the following subheadings: Introduction, Methods, Results, and Discussion. Case Reports should be divided into three sections: Details of the Clinical Case, Discussion, and Summary. Provide a summary sentence at the end. Negative decisions will not be accompanied by a full review. Accepted manuscripts may require revisions.

SCIENTIFIC REVIEW

Scientific Reviews may be submitted on topics relating to the prevention and/or management of cardiopulmonary disease. Word count is generally 3000-4000 words and references should be limited to 75 or less. A structured abstract and condensed abstract should accompany review articles. Title page of review article must be labeled "Review Article". Review articles should follow this outline: 1) title page; 2) title page without author names or institution; 3) structured and condensed abstracts; 4) introduction and statement of purpose; 5) review of relevant literature as

appropriate to article; 6) discussion; 7) application to practice; 8) summary; and 9) references.

ABBREVIATIONS

Abbreviations should be limited to five commonly used terms or phrases per manuscript. Abbreviations should be spelled out at the first mention in the abstract and then again in the body of the text. The abbreviation should follow in parentheses. A term or phrase should be used more than five times to merit abbreviation.

TITLE PAGE

Information on the title page should include the full name, academic degree, hospital or university affiliation of each author and a word count for text only (excluding references). If an author's present affiliation is different from that under which the work was done, both should be given. The name, address, phone, fax, and e-mail of the corresponding author should be provided.

The title page must also include disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

FORMAT AND ABSTRACTS

Original investigations should follow this outline: 1) title page; 2) structured abstract and condensed abstract; 3) introduction and statement of purpose; 4) patients (or subjects) and methods; 5) results; 6) discussion; 7) references; 8) tables; and 9) figure legends.

All submissions should be accompanied by two abstracts: a structured abstract of 250 words or less and a condensed abstract of no more than 50 words for use in the Table of Contents. The structured abstract should consist of four paragraphs, labeled Purpose, Methods, Results, and Conclusions. They should

briefly describe, respectively, the rationale for the study, how the study was conducted, the salient results, and what the authors conclude from the findings.

REFERENCES

References should be listed in the order in which they appear in the article and should be typed double-spaced. Authors are responsible for the completeness and accuracy of all references. Journal references should include authors' surnames followed by initials (without punctuation), title of article, name of journal as abbreviated in Index Medicus (if not included in Index Medicus, the journal name should be spelled out), year of publication, volume number, and inclusive page numbers. If there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first three authors and et al is adequate. Personal communications and unpublished data should be included within the text of the manuscript or as footnotes, not as references. References should be formatted as shown in the American Medical Association Manual of Style 10th edition.

LETTERS TO THE EDITOR

Letters will be published as space permits and at the discretion of the editors.

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Manuscripts must conform to "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (N Engl J Med. 1997;336:309-315).

Manuscripts may not contain previously published material or be under consideration for publication elsewhere.

If an author has work that is in preparation, has been previously submitted or published, or is in press and potentially overlaps the submitted manuscript, the work must be submitted as an attachment with the current submission.

All sources of support must be cited on the title page. Sources of support and potential conflicts of interest must be stated in the submission letter.

A statement of submission must accompany the manuscript. It should state the following: "All authors have read and approved submission of the manuscript and the manuscript has not been published and is not being considered for publication elsewhere in whole or part in any language except as an abstract."

Word count for the text-only portion of the manuscript should be stated in the title page.

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A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, WKH will identify to the National Library of Medicine (NLM) articles that require deposit and will transmit the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Wellcome Trust, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The revised Copyright Transfer Agreement provides the mechanism.

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FAQ for open access: http://www.wkopenhealth.com/openaccessfaq.php

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Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional, and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading “Conflicts of Interest and Source of Funding.” For example:

Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker’s bureau for Organization X—the CME organizers for Company A. For the remaining authors none were declared.

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Disponível em: < http://edmgr.ovid.com/jcrp/accounts/ifauth.htm> Acesso: 27/12/2017.

7.3 ANEXO III: NORMAS PARA SUBMISSÃO NO PERIÓDICO "*JOURNAL OF PHYSICAL ACTIVITY & HEALTH*"

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b. Drafting the article or revising it critically for important intellectual content; and
c. Final approval of the version to be published.

Conditions a, b, and c must all be met. Individuals who do not meet the above criteria may be listed in the acknowledgments section of the manuscript.

*Uniform requirements for manuscripts submitted to biomedical journals. New England Journal of Medicine, 1991, 324, 424–428.

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All types of manuscripts submitted to JPAH are judged on the following primary criteria: adherence to accepted scientific principles and methods, the significant or novel contribution to research or practice in the field of physical activity, clarity and conciseness of writing, and interest to the readership. There are no page charges to contributors.

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Standardized Publication Reporting Guides

JPAH highly recommends that authors refer to relevant published reporting guidelines for different types of research studies. Examples of reporting guidelines include:

Consolidated Standards of Reporting Trials (CONSORT)
Meta-analysis of Observational Studies in Epidemiology (MOOSE)
Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)
STrengthening the Reporting of OBservational studies in Epidemiology (STROBE)

Improving the Quality of Web Surveys: The Checklist for Reporting Results of Internet E-Surveys (CHERRIES)

Manuscripts must be submitted in Microsoft Word® (*.doc) or rich text (*.rtf) format only. Do not submit a .pdf file. Graphics should be submitted in .tif or .jpg formats only. Before submitting, authors should complete the Manuscript Submission Checklist (see below). Authors may be asked to provide Human Kinetics with photo-ready graphics and/or a hard copy of the text. Authors are responsible for confirming the accuracy of the final copy, particularly the accuracy of references, and to retain a duplicate copy to guard against loss. Final review of the pre-published text is the responsibility of the authors. Authors of manuscripts accepted for publication must transfer copyright to Human Kinetics, as applicable.

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Manuscript Types

Original Research. A manuscript describing the methods and results of a research study (quantitative or qualitative), including the background and purpose of the study, a detailed description of the research design and methods, clear and comprehensive presentation of results, and discussion of the salient findings.

Public Health Practice. A manuscript describing the development or evaluation of a public health intervention to increase or promote physical activity in a community setting, or a study that describes translation of research to practice.

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The order of submission must be (1) Title page, (2) Abstract, (3) Text, (4) Acknowledgments, (5) Funding source, (6) References, (7) Tables, (8) Figures/Graphics.

Title page. The manuscript must include a title page that provides the full title, a brief running head, manuscript type (see definitions above), three to five key words not used in the title of the manuscript, abstract word count, manuscript word count (inclusive of all pages except the abstract and title page), and date of manuscript submission. Do not include author names on the title page.

Abstract. All manuscripts must have a structured abstract of no more than 200 words. Required headings are (1) Background, (2) Methods, (3) Results, and (4) Conclusions.

Text. The entire manuscript must be double-spaced, including the abstract, references, and tables. Line numbers must appear on each page in the left margin. A brief running head is to be included on the upper right corner of each page; page numbers must appear on the bottom right corner of each page.

For studies involving human subjects, the Methods section must include statements regarding institutional approval of the protocol and obtaining informed consent. For studies using animals, the Methods section must include a statement regarding institutional approval and compliance with governmental policies and regulations regarding animal welfare.

Acknowledgments. Provide the names, affiliations, and the nature of the contribution for all persons not included as an author who played a critical role in the study.

Funding source/trial registration. Details of all funding sources for the work should be provided (including agency name, grant numbers, etc.). Provide the registry name and registration number for all clinical trials (see JPAH Ethics Policies below).

Example: “This work was supported by a grant (grant #) from the National Cancer Institute, National Institutes of Health. This study is registered at www.clinicaltrials.gov (No. xxxxx).”

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Journal articles: Surname of first author, initials, then surname and initials of each coauthor; title of article (capitalize only the first word and proper nouns), name of the journal (italicized and abbreviated according to style of Index Medicus), year, volume, and inclusive page numbers.

Melby CL, Osterberg K, Resch A, Davy B, Johnson S, Davy K. Effect of carbohydrate ingestion during exercise on post-exercise substrate oxidation and energy intake. Int J Sport Nutr Exerc Metab. 2002;12:294–309.

Book references: Author(s) as above, title of book (italicized and all major words capitalized), city and state/province of publication, publisher, and year.

Pearl AJ. The Female Athlete. Champaign, Ill: Human Kinetics; 1993.

Chapter in an edited book: Same as book references, but add the name of the chapter author(s) and title of chapter (capitalize first word and proper nouns) before the book information and inclusive page numbers.

Perrin DH. The evaluation process in rehabilitation. In: Prentice WE, ed. Rehabilitation Techniques in Sports Medicine. 2nd ed. St Louis, Mo: Mosby Year Book; 1994:253–276.

Tables. Each table must be accompanied by an explanatory title so that it is intelligible without specific reference to the text. Column headings and all units of measure must be labeled clearly within each table; abbreviations and acronyms must be fully explained in the table or footnotes without reference to the text.

Figures/Graphics. Graphics should be prepared with clean, crisp lines, and be camera-ready. For shading, stripe patterns or solids (black and white) are better choices than colors. Graphics created on standard computer programs will be accepted. Graphics should be submitted in .tif or .jpg formats only. Each figure and photo must be properly identified. A hard copy may be requested. If photos are used, they should be black and white, clear, and show good contrast.

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Before submitting a first or revised manuscript, the following criteria must be met:

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Line numbers appear in left margin
Page numbers appear in bottom right corner
Brief running head appears in upper right corner

Title page does not include author names or affiliations
Abstract is formatted and contains fewer than 200 words
Page count under limit for the manuscript type (15, 25, or 30 pages)
Fewer than 10 tables/figures
References are formatted per AMA guidelines
Submitting Author Revisions
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The following types of manuscripts will be given the lowest priority and are the most likely to be rejected without review:

Small, cross-sectional, descriptive studies without any innovative features (e.g., the association between physical activity and body mass index)
Pilot studies
Studies having no control or reference group
Studies in which physical activity is merely a covariable of interest

Methodological studies with no health-related outcome (e.g., associations among three types of accelerometers)
The types of studies given the highest priority are the following:

Etiologic or experimental studies testing a specific hypothesis or highlighting a specific mechanism relating physical activity or inactivity to health and function
Prospective or longitudinal studies
Evaluation studies of effective public health practice
Studies that are truly innovative and reflect progressive thinking
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