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Effect of physical training on cytokine expression in CD4+ T lymphocytes in subjects with stable COPD

Juliana S. Uzeloto, Alessandra C. de Toledo-Arruda, Bruna S.A. Silva, Aline M.M. Braz, Fabiano F. de Lima, Isis Grigoletto, Dionei Ramos, Marjorie A. Golim and Ercy M.C. Ramos

Abstract

Introduction: Although evidence suggests that physical exercise reduces systemic inflammation, at the plasma level, there are still contradictions in chronic obstructive pulmonary disease (COPD). In this sense, analysis of intracellular cytokines could clear off the effect of physical exercise on the inflammatory profile of these subjects.

Aim: The aim was to evaluate the effect of physical training on cytokine expression in CD4+ T lymphocytes from subjects with COPD.

Methods: This is a randomized controlled trial. Subjects with stable COPD were grouped into two groups, exercise and control. In total, 23 subjects with stable COPD were evaluated, of which 15 underwent aerobic strength training [physical exercise group (PEG)] and 8 underwent breathing exercises [respiratory physiotherapy group (RPG)]. Intracellular cytokines [interleukin (IL)-8, IL-13, IL-17, IL-6, IL-2, IL-10, and tumor necrosis factor alpha (TNF-α)] from CD4+ T lymphocytes were analyzed from peripheral blood through flow cytometry, before and after 8 weeks of intervention.

Results: The PEG and RPG groups had a mean age of 68 ± 5.96 and 72.25 ± 6.86 years and predicted forced expiratory volume in the first second (FEV_1) of 58.6 ± 15.99% and 39.75 ± 10.39%, respectively. It was possible to detect a significant reduction in IL-8 ($p = 0.0125$) and an increase in IL-13 ($p = 0.0014$) and an increase in TNF-α ($p < 0.001$) in both groups.

Conclusion: Eight weeks of physical training, both peripheral and respiratory, were able to reduce concentrations of IL-8 and to increase IL-13, and TNF-α in CD4+ T lymphocytes in subjects with stable COPD. The findings reinforce the benefits of interventions in subjects with COPD, revealing data not previously investigated.

Keywords: chronic obstructive pulmonary disease, flow cytometry, inflammation, physical exercise

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Correspondence to:
Juliana S. Uzeloto
Department of Physiotherapy, Postgraduate Program in Physiotherapy, Faculty of Science and Technology, São Paulo State University (UNESP), Rua Roberto Simonsen, 305, Presidente Prudente, São Paulo 19060-900, Brazil
juliana_uzeloto@hotmail.com

Alessandra C. de Toledo-Arruda
Department of Physiology and Pharmacology, Laboratory of Exercise Sciences, Fluminense Federal University, Rio de Janeiro, Brazil

Bruna S.A. Silva
Fabiano F. de Lima
Isis Grigoletto
Dionei Ramos
Ercy M.C. Ramos
Physiotherapy, Universidade Estadual Paulista Julio de Mesquita Filho, Presidente Prudente

Aline M.M. Braz
Universidade Estadual Paulista Julio de Mesquita Filho

Instituto de Biociencias Campus de Botucatu, Medical Biotechnology, Botucatu

Marjorie A. Golim
Botucatu Medical School, Postgraduate Program in Research & Development: Medical Biotechnology, Blood Center, Flow Cytometry Laboratory, São Paulo State University (UNESP), São Paulo, Brazil

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation, which is generally progressive and associated with an abnormal inflammatory response of the lungs to harmful particles and gases.[1] COPD is a disease that causes great impacts on health and economies, being the third leading cause of death worldwide,[2] which makes it the target of research for the control and treatment of the disease.

Although COPD is clinically diagnosed through pulmonary factors, systemic alterations in this population have been described by several authors. The changes in body composition,[3] nutritional depletion,[4] oxidative stress,[5] and systemic inflammation[6] are highlighted. A pro-inflammatory profile in subjects with COPD is associated with a higher risk of exacerbation, morbidity, and mortality.[7]

In an attempt to reduce the deleterious effects caused by the disease, several treatments have

been developed for these subjects. Among the nonpharmacological options, physical exercise stands out (evidence A), for reducing symptoms such as fatigue and dyspnea, and improving exercise capacity and quality of life.[8] However, although physical exercise is identified as a potential tool to prevent and treat diseases, due to the anti-inflammatory effect,[9] evidence on the effects of physical exercise on the inflammatory profile of subjects with stable COPD is contradictory, pointing to a reduction,[10–12] increase,[13] or stability[14–18] of inflammation markers. Furthermore, these studies are based only on plasma analyses.[10–18]

Considering the complexity of the immune system and the systemic inflammatory response, the analysis of intracellular biomarkers can be used to investigate the production of cytokines in a specific cell population, allowing for more robust analyses.[19] Among the cells that integrate the inflammatory process, CD4+ T lymphocytes play an important role in the activation of other effector cells, by releasing activating cytokines.[20]

It should be noted that the evidence on the effect of physical exercise on systemic inflammation of subjects with COPD was carried out at the plasma level and with inconsistent results, even though physical exercise induces improvements in T lymphocyte function.[21] In this sense, the analysis of intracellular cytokines could provide an additional contribution to the effect of physical training on the inflammatory profile of these subjects, which until now is unknown, and which may assist in targeting treatments and prognosis.

Thus, the aim of this study was to evaluate the effect of combined physical training (aerobic and resistance) on the expression of cytokines in CD4+ T lymphocytes of subjects with stable COPD, and to investigate whether changes in inflammatory biomarkers when exercising are related to clinical and functional characteristics of these individuals.

Methods

Study participants and design

This is a subsample of a completed randomized controlled clinical study [Brazilian Clinical Trials Registry (ReBEC) ID: RBR-3zmh3r], in which 140 individuals with COPD were assessed for eligibility from medical and outpatient clinics. All volunteers were diagnosed according to the GOLD criteria.[1] The inclusion criteria were as follows: absence of exacerbation in the 3 months before collection, the absence of infectious and autoimmune diseases, home use of oxygen supplementation, and any condition that could prevent physical exercise. Subjects who refused to perform any of the evaluations, or who interrupted the proposed interventions, and samples which demonstrated errors during processing were excluded from this analysis (Figure 1). It is important to clarify that a large number of blood samples (63 samples from the initial moment) presented processing errors that, despite a lot of effort, were not able to be clarified.

The objectives and methods of the study were clarified to all volunteers, who, after agreeing, signed the informed consent form. The study was previously approved by the Research Ethics Committee (CAAE: 77909317.2.0000.5402), following the rules of the Declaration of Helsinki.

On the first visit to the laboratory, subjects were familiarized with all the procedures and techniques used in the study. Participants underwent pulmonary function assessment through spirometry, which was conducted to confirm the diagnosis of COPD,[1] using a portable spirometer MIR – Spirobank version 3.6. The interpretation of the results was carried out in accordance with the rules of the American Thoracic Society and European Respiratory Society,[22] and the values were interpreted as proposed by Neder *et al.* who investigated the Brazilian population.[23] The sensation of dyspnea was evaluated using the scale from the Medical Research Council (MRC),[24] and quality of life using the Chronic Respiratory Questionnaire (CRQ).[25] The Clinical COPD Questionnaire (CCQ), proposed by Van der Molen *et al.*,[26] was applied. This questionnaire refers to symptoms presented in the previous 7 days. Limitations during the performance of activities of daily living were assessed using the London Chest Activity of Daily Living (LCADL) scale.[27] The Berg Balance Scale (BBS), which evaluates the balance of subjects with chronic disorders, is valid and reliable, and was used to assess the postural control of subjects with COPD, as previously described.[28] To evaluate body composition, Octopolar InBody 720 electrical bioimpedance (Biospace, Seoul, Korea) was used. Skeletal muscle mass, body fat mass, and lean mass were

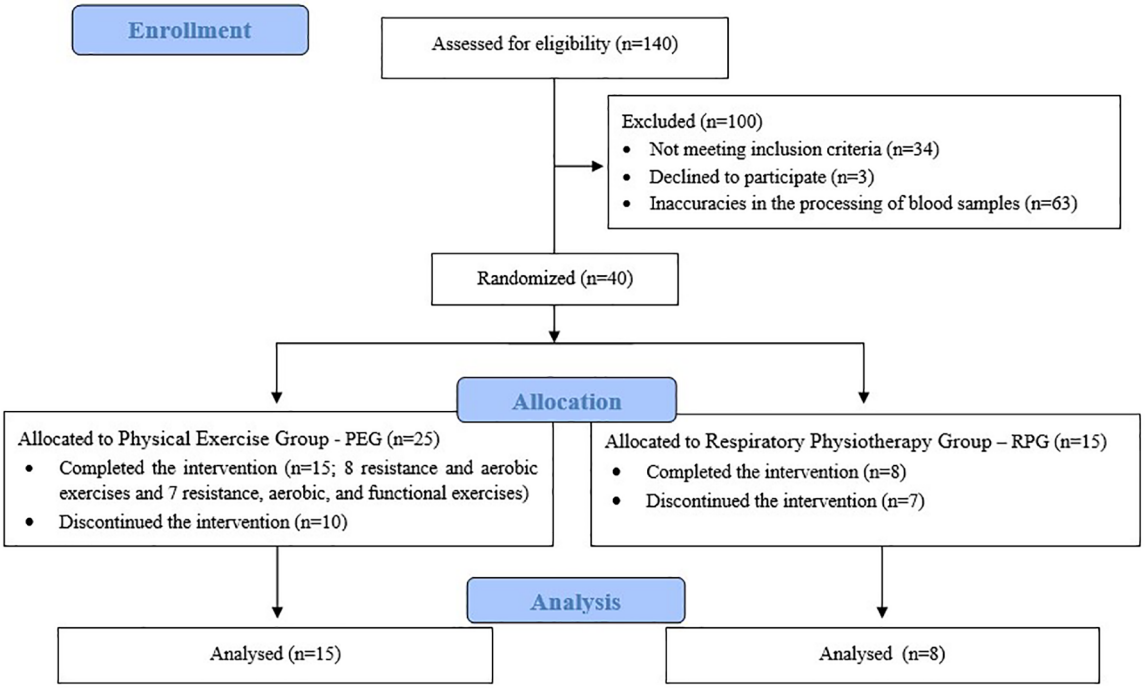

Figure 1. Flowchart of sample selection.

evaluated. The data were electronically imported into Excel, using Lookin'Body 3.0 software (Biospace). To analyze habitual daily physical activity, an Actigraph triaxial motion sensor, model GT3X (Actigraph LLC, Pensacola, FL) was used. The individuals were instructed to wear the equipment for 7 days and remove it only when in contact with water (personal hygiene or water activities) and for night sleep. For data analysis, specific *software* was used, ActiLife 5 – Data Analysis Software by Actigraph. The measurement of muscle strength was performed in the dominant hemibody by means of a digital dynamometer, brand Force Gauge, model FG-100 kg (Tobarra, Spain), and the results were expressed in Newtons (N). The volunteers were instructed to perform elbow flexion and knee flexion and extension movements, resisted by a steel cable attached to the dynamometer. Each movement was repeated 3 times with an interval of 1 min between attempts and the highest measured value was recorded.[29] For the assessment of functional exercise capacity, participants performed the 6-minute walk test (6MWT), carried out in accordance with the guidelines established by the American Thoracic Society,[30] and the Glittre Activities of Daily Living test (Glittre ADL), following the previous standards of Karloh *et al.*[31] It is important to highlight that a higher value in the 6MWT represents greater functional capacity, while a lower value in the Glittre ADL represents greater functional capacity.

The subjects in the clinical trial were randomized into three groups: (1) resistance, aerobic, and functional exercises, (2) resistance and aerobic exercises, and (3) breathing exercises, according to the published protocol.[32] Groups I and II performed three weekly sessions consisting of dynamic general stretching, 30 min of aerobic training on a treadmill (intensity of 80% of the 6MWT, with 5% progression, maintaining the sensation of dyspnea on the Borg scale between four and six), and resistance training of upper and lower limbs [initial load of 60% of one-repetition maximum (1RM) with a 5% progression every four sessions, reaching 80% of 1RM at the end of the protocol]. The only difference between the physical exercise protocols was that group I performed functional training in a circuit format (instead of a treadmill) in the third session. Group III was submitted only to usual care involving inhalation therapy, pulmonary deflation techniques, diaphragmatic awareness, and inspiratory muscle exercises, twice a week.

For this study, the physical exercise groups (I and II) were pooled, considering the similarity in the load and intensity of the protocols and with the aim of investigating the effect of physical exercise on the inflammatory profile of the evaluated sample, named the physical exercise group (PEG), and compared with subjects who performed only breathing exercises, named the respiratory physiotherapy group (RPG).

Lymphocyte and cytokine markers

Peripheral blood samples were collected for analysis of inflammation markers, before and after 8 weeks of intervention. The procedures performed for labeling CD3+ (anti-CD3 FITC) and CD4+ (anti-CD4 PerCP-CY5.5) as well as for labeling the specific monoclonal antibodies for the cytokines of interest [anti-interleukin (IL)-8 PE, IL-13 APC, IL-17 PE, IL-6 APC, IL-2 APC, IL-10 PE, tumor necrosis factor (TNF) APC] were previously described.[33] All reagents used in the analyses were from Becton Dickinson, San Diego, CA.

Analysis of cells and cytokine markers

The four-color FACSCalibur® cytometer (Becton Dickinson) was used for sample acquisition and analysis, standardizing a total of 3×10^5 events collected per tube. The strategy for identifying lymphocytes as well as for evaluating the expression of intracytoplasmic cytokines has been previously described.[33] The analyses were performed in CellQuest PRO® and FlowJo® software, with the results expressed in percentage values.

Statistical analysis

For data analysis, the statistical program SPSS 22.0 was used. To assess the normality of the data, the Shapiro–Wilk test was applied. For comparisons between groups, at a single moment, the Student's t test or Mann–Whitney test was performed, according to the normality of the data. Correlation analyses were performed using Pearson tests, for parametric data, and Spearman correlation, for nonparametric data. To interpret the correlation coefficient, the following values were adopted: very strong ($r \geqslant 0.9$); strong ($0.9 > r \geqslant 0.7$); moderate ($0.7 > r \geqslant 0.5$); or weak ($r < 0.5$).[34] The comparison of cytokine expression between groups (PEG and RPG) and time (preintervention and postintervention) was investigated using two-way analysis of variance (ANOVA).

Results

A total of 23 volunteers with COPD were evaluated in the study. The baseline characteristics of all participants are shown in Table 1.

In the two-way analysis, presented in detail in Table 2, a reduction in the proportion of CD4+ T lymphocytes that expressed IL-8 and an increase in IL-13 and tumor necrosis factor alpha (TNF-α) were observed after the interventions, with no differences between groups.

In the correlations performed among the individuals of the PEG, between the behavior of the cytokines before and after training (delta) and clinical and functional variables, it was observed that the worst symptoms, measured by the CCQ ($p = 0.004$; $r = 0.689$) and LCADL ($p = 0.034$; $r = 0.550$), are related to greater reductions in CD4+ IL-8 + and older age ($p = 0.001$; $r = -0.769$). In addition, greater strength of the elbow flexor muscles ($p = 0.041$; $r = -0.532$) is related to lower reductions in CD4+ IL-8+. Furthermore, better functional capacity, measured by the 6MWT ($p = 0.027$; $r = -0.570$) and Glittre ADL ($p = 0.007$; $r = 0.660$), and better balance, assessed by the BBS ($p = 0.037$; $r = -0.542$), were related to smaller variations in IL-17+ in CD4+ T lymphocytes (Figure 2). The rest of the clinical and functional variables did not demonstrate significant correlations with the variation in the cytokine profile in CD4+ T lymphocytes after physical exercise (Supplemental Table S1 – 10.6084/m9.figshare.14035400).

Discussion

This is the first known study to evaluate the effects of physical exercise on the behavior of intracellular T lymphocyte cytokines in subjects with stable COPD. Few studies have investigated the immunomodulatory effect of physical exercise in a general manner,[21] and investigations with subjects with COPD are unknown.

According to the findings of this study, a combined aerobic and resistance exercise program as well as breathing exercises was able to reduce the proportion of T lymphocytes that expressed IL-8+ and increase IL-13+ expression in

Table 1. Sample characterization.

	PEG ($N = 15$)	RPG ($N = 8$)	*p* value
Male/Female, *n* (%)	7 (46.7)/8 (53.3)	5 (62.5)/3 (37.5)	0.667
Age (years)	65 (65–72)	73.00 (67.25–77.25)	0.098
BMI (kg/m^2)	27.00 ± 5.20	25.12 ± 4.94	0.412
FEV_1 (% of predicted)	58.60 ± 15.99	39.75 ± 10.39	0.007
FVC (% of predicted)	80.33 ± 16.70	70.88 ± 12.60	0.177
FEV_1/FVC (%)	56.89 ± 8.01	44.29 ± 12.39	0.007
MRC	3.07 ± 1.44	3.38 ± 1.30	0.619
CRQ	20.34 ± 4.17	18.79 ± 4.85	0.431
CCQ	11.20 ± 7.38	19.75 ± 14.86	0.077
LCADL	17.00 (15.00–22.00)	16.50 (14.50–24.25)	0.974
BBS	56.00 (54.00–56.00)	55.00 (52.25–55.00)	0.158
Fat mass (kg)	26.94 ± 9.64	23.43 ± 8.50	0.396
Skeletal muscle mass (kg)	23.85 ± 4.94	23.09 ± 5.47	0.737
Lean mass (kg)	41.56 ± 7.90	40.46 ± 8.70	0.762
Steps per day	4547.43 ± 2121.56	2908.93 ± 1661.42	0.073
Muscle strength of elbow flexors (N)	92.00 ± 35.18	102.43 ± 40.60	0.528
Muscle strength of knee flexors (N)	121.93 ± 34.12	126.35 ± 51.78	0.807
Muscle strength of knee extensors (N)	174.92 ± 57.31	186.03 ± 57.58	0.663
6MWT distance (m)	496.00 ± 62.77	421.50 ± 164.32	0.129
Glittre ADL (min)	4.67 (4.21–5.23)	5.30 (4.61–13.40)	0.087

6MWT, 6-minute walk test; BBS, Berg Balance Scale; BMI, body mass index; CCQ, Clinical COPD Questionnaire; CRQ, Chronic Respiratory Questionnaire; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; Glittre ADL, Glittre Activities of Daily Living test; LCADL, London Chest Activity of Daily Living; MRC, Medical Research Council; N, Newton; PEG, physical exercise group; RPG, respiratory physiotherapy group.
Data expressed as mean ± standard deviation or median (range: 25–75%), according to normality.

individuals with stable COPD. Furthermore, increased expression of TNF-α+ is observed over time in this population, regardless of the interventions performed. In addition, the findings indicate that worse symptoms, younger age, and lower muscle strength of elbow flexors were related to greater changes in IL-8 after physical exercise. Finally, major changes in IL-17+ after the exercise program are correlated with less physical functionality.

Physical exercise is indicated as a treatment for several diseases, as it has numerous benefits to the healthy and sick organism.[35] In COPD, specifically, even without altering lung function, physical exercise is able to improve functional capacity and muscle strength, and reduce symptoms of dyspnea. The most likely explanation for these gains is the possible anti-inflammatory effect of physical exercise.[36] However, studies carried out in an attempt to clarify the anti-inflammatory

Table 2. Comparison of the cytokine profile in CD4+ T lymphocytes, before and after the intervention, between groups.

		Baseline	Final		*p* value (two-way ANOVA)
IL-8 (%)	PEG	15.12 ± 18.27	4.86 ± 3.81	Group	0.8278
				Time	0.0125*
	RPG	17.07 ± 21.03	4.89 ± 4.58	Group × Time	0.8166
IL-13 (%)	PEG	11.42 ± 2.70	16.55 ± 3.51	Group	0.0963
				Time	0.0014*
	RPG	11.12 ± 2.65	13.03 ± 4.49	Group × Time	0.1061
IL-17 (%)	PEG	0.16 ± 0.14	0.21 ± 0.20	Group	0.0741
				Time	0.6485
	RPG	0.28 ± 0.19	0.29 ± 0.17	Group × Time	0.7477
IL-6 (%)	PEG	12.33 ± 2.87	14.70 ± 3.25	Group	0.4923
				Time	0.1010
	RPG	12.09 ± 5.32	12.92 ± 5.15	Group × Time	0.4179
IL-2 (%)	PEG	22.84 ± 6.98	26.23 ± 7.98	Group	0.7030
				Time	0.4973
	RPG	24.95 ± 10.35	25.67 ± 8.45	Group × Time	0.6578
IL-10 (%)	PEG	0.19 ± 0.17	0.29 ± 0.29	Group	0.3241
				Time	0.2836
	RPG	0.33 ± 0.26	0.44 ± 0.76	Group × Time	0.9594
TNF-α (%)	PEG	9.28 ± 2.40	13.94 ± 3.06	Group	0.8444
				Time	0.0001*
	RPG	9.37 ± 1.78	13.50 ± 4.22	Group × Time	0.7782

ANOVA, analysis of variance; IL, interleukin; PEG, physical exercise group; RPG, respiratory physiotherapy group; TNF-α, tumor necrosis factor alpha; %, percentual.
*$p < 0.05$.

effect of exercise on COPD present contradictory results.[37]

Regarding the main signaling cytokines, authors did not observe any alterations in the behavior of IL-6,[10,11,13–17] TNF-α,[10,14–17] and IL-10[18] of subjects with COPD after an exercise program.

An important factor to be considered for alterations in the inflammatory profile after a physical training program is the intensity of the exercise performed. Nimmo *et al.*,[38] in a literature review, point out that aerobic training performed at moderate to high intensity (above 70% of maximum aerobic capacity), and associated with strength exercises, promotes the best anti-inflammatory effects.

In this study, a reduction in the proportion of T lymphocytes that expressed IL-8+ was observed

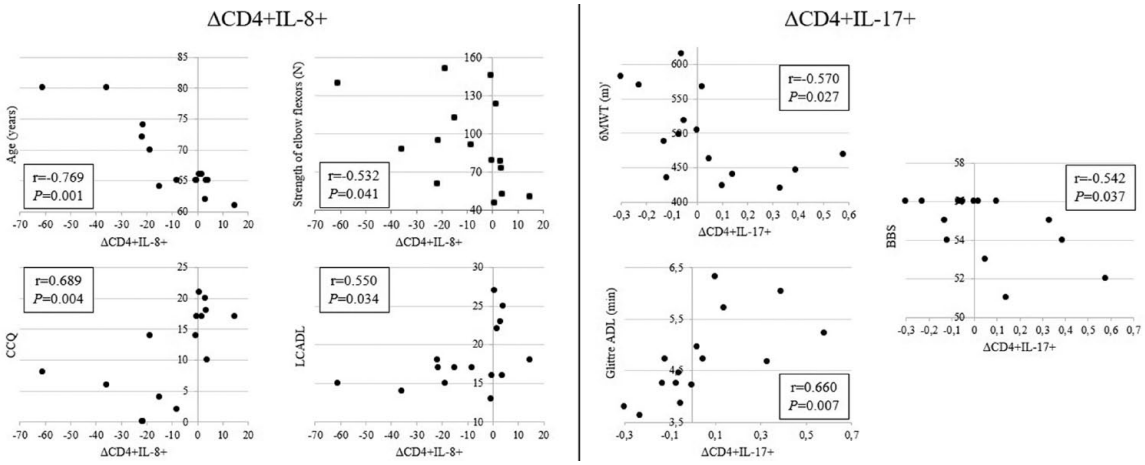

Figure 2. Graphical representation of correlation between change of interleukin expression in CD4+ T lymphocytes and clinical and functional characteristics.
6MWT: 6-minute walk test; BBS, Berg Balance Scale; CCQ, Clinical COPD Questionnaire; Glittre ADL, Glittre Activities of Daily Living test; LCADL, London Chest Activity of Daily Living; Δ, difference between final and baseline.

after 8 weeks of combined exercise (aerobic at 80% of the 6MWT and resisted with 60% of 1RM, both with progressive protocols based on the perception of dyspnea) and also after 8 weeks of breathing exercises, including inhalation therapy, pulmonary deflation techniques, diaphragmatic awareness, and inspiratory muscle exercise. Some authors have observed similar behavior of IL-8 in relation to physical exercise in subjects with COPD. Among the studies that observed plasma reductions in IL-8, Whang *et al.*[10] and do Nascimento *et al.*[11] applied 8-week interventions, with walks performed at 80% of VO_2 peak and combined exercises (aerobic at 85% of Incremental Shuttle Walk Test and strength with loads based on perceived effort) in the home, respectively. In the study of da Silva *et al.*,[12] the reduction was observed after 12 weeks of combined exercises (aerobic at 60% of the 6MWT and strength exercises with 15RM).

IL-8 is a cytokine with chemoattractant properties to immune cells and is associated with increased mucus production and airway remodeling processes.[39] In this sense, the reduction in IL-8 after an exercise program, both peripheral and respiratory, may represent improvement in the clinical status of subjects with COPD.

In addition, greater alterations in the proportion of lymphocytes that expressed IL-8 after exercise were correlated with worse symptoms, younger age, and lower muscle strength of elbow flexors. Spruit *et al.*[40] point out that IL-8 could be an important focus in the evaluation of subjects with COPD during pulmonary rehabilitation, due to its role in worsening the disease. Thus, the decrease in IL-8 after the physical training program in this study represents a benefit and is further enhanced in subjects with COPD of younger age, with worse symptoms, and lower muscle strength of the upper limbs.

On the other hand, greater expression of CD4+ IL-13 T lymphocytes was observed after the interventions in this study. In COPD, the role of IL-13 is still uncertain.[41] Knudsen *et al.*,[42] in a high-impact experimental study, clarified the role of IL-13 in physical training. The authors point out an important role of IL-13 in biogenesis and mitochondrial respiration, as well as in the increase in the number of muscle oxidative fibers, resistance capacity, and glucose tolerance in resistance training. In this sense, the observed increase in IL-13 in both groups can be justified by the need for this cytokine in signals for metabolic adjustments of muscle preparation to sustain physical activity, as pointed out by Knudsen *et al.*[42]

To our knowledge, there is no previous evidence in the literature regarding the influence of breathing exercises in reducing inflammation. However, it is known that interventions with only breathing exercises, from 4 weeks onwards, improve exercise capacity in people with COPD, possibly by increasing exercise tolerance.[43]

High concentrations of TNF-α in subjects with COPD, even if stable, are almost a consensus.[39,44,45] This cytokine presents pleiotropic action, that is, numerous functions; however, the pro-inflammatory role stands out. In COPD, TNF-α amplifies inflammatory responses, affecting the release of other cytokines.[39] Interestingly, in this study, volunteers from both groups evaluated showed an increase in TNF-α after the interventions, corroborating with the findings of Ryrsø *et al.*[13] who also observed an increase in TNF-α after a physical training program in subjects with COPD. In contrast, previous studies[10,14–17] pointed out that physical exercise was not able to reduce the concentration of this cytokine in subjects with COPD.

Whereas high concentrations of TNF-α are associated with greater disease severity,[44] it is assumed that TNF-α is a potential inflammatory marker, indicative of disease progression. Therefore, despite the relevant anti-inflammatory effect of exercise,[9] the behavior of TNF-α after physical exercise in subjects with COPD needs further investigation.

IL-17 is presented as a pro-inflammatory cytokine, which in COPD appears to play an important role in the pathophysiology of emphysema.[46] Studies demonstrate an increase in IL-17 in subjects with COPD, and also show an association of this cytokine with the severity of the disease.[47,48] On the other hand, IL-17 is expressed with the objective of recruiting neutrophils to fight infections, thus presenting an important function in the body's protection mechanism against pathogens.[49,50] However, the effect of exercise on IL-17 in COPD is unknown. In this study, despite the observed increase in IL17+, no significant changes were detected after the interventions. On the other hand, it was possible to observe a relationship between the functionality of the subjects with the alterations in IL-17+ after physical exercise, where subjects with less functionality presented greater alterations in this cytokine described as pro-inflammatory.

One factor that cannot be ruled out is the difference in lung function observed between the groups, where the RPG presented greater airway obstruction. However, although lung function is a crucial factor in characterizing subjects with COPD, approaches should not be limited to this factor, as the disease affects systems other than the pulmonary system.[51]

As previously mentioned, the originality of the study in relation to the investigation of the immunomodulatory effect of physical exercise,21 and especially with subjects with COPD, indicates that this analysis can assist in the investigation of the real anti-inflammatory effects of exercise, both peripheral and respiratory, in this population and thereby open the way for new research that aims to elucidate the mechanisms by which physical exercise can act on the inflammatory profile of these subjects.

Although we present unprecedented results, limitations can be pointed out and should be considered when interpreting this study. The absence of a control group without COPD, similar in age and anthropometric characteristics, would be interesting for comparisons of inflammatory biomarkers and analysis of disease impairment. In addition, there was loss around 40% of participants during the study and analysis was only based on those who completed the interventions. The small sample number, resulting mainly from the large loss of data due to inaccuracies in the processing of blood samples, can be raised as a major limitation of the study. Therefore, studies with a larger sample size, also encompassing a population without changes in lung function, are necessary to consolidate the results found.

It was concluded that interventions, both peripheral physical exercise and respiratory exercise (usual care), were beneficial in reducing pro-inflammatory expression, by reducing IL-8, by increasing IL-13 expression, despite the increase in TNF-α in CD4+ T lymphocytes in subjects with stable COPD. Furthermore, symptoms, age, and muscle strength of elbow flexors are related to alterations in IL-8 after physical exercise and functionality with alterations in IL-17+ after the exercise program.

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Author contributions

Juliana S. Uzeloto: Conceptualization; Data curation; Formal analysis; Funding acquisition;

Investigation; Methodology; Project administration; Visualization; Writing – original draft; Writing – review & editing.

Alessandra C. de Toledo-Arruda: Conceptualization; Methodology; Supervision; Validation; Writing – review & editing.

Bruna S.A. Silva: Conceptualization; Data curation; Investigation; Methodology; Writing – review & editing.

Aline M.M. Braz: Data curation; Investigation; Methodology; Software; Visualization; Writing – review & editing.

Fabiano F. de Lima: Data curation; Investigation; Project administration; Writing – review & editing.

Isis Grigoletto: Data curation; Investigation; Project administration; Writing – review & editing.

Dionei Ramos: Resources; Supervision; Writing – review & editing.

Marjorie A. Golim: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Writing – review & editing.

Ercy M.C. Ramos: Conceptualization; Funding acquisition; Resources; Supervision; Validation; Visualization; Writing – review & editing.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Juliana S. Uzeloto https://orcid.org/0000-0003-0158-3693

Supplemental material

Supplemental material for this article is available online.

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