

## Do COPD and Healthy Subjects have Similar Acute Inflammatory Response Induced by Sub-maximum Effort Test?

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### Abstract

**Introduction:** COPD (chronic obstructive pulmonary disease) presents a low degree of systemic inflammation responsible for the disease's extra pulmonary consequences. Its inflammatory profile can be altered by acute exercise. However, acute effects of a sub maximum test, capable of reproducing activities of daily living (ADL) in sedentary COPD individuals, are not well-known

**Objective:** To evaluate if the six-minute walk test (6MWT) is capable of altering interleukin-6 (IL-6) blood levels, tumoral necrosis factor alfa (TNF- $\alpha$ ) and hypersensitive C reactive protein (hs-CRP) in relation to the basal level of COPD individuals.

**Methods:** 21 individuals with moderate and severe COPD and 8 healthy individuals, with no history of smoking, sedentary, matched by age were assessed regarding plasma levels of IL-6, TNF- $\alpha$  and hs-CRP before and right after a 6MWT. They also did spirometry and body composition analysis.

**Results:** 6MWT did not provoke significant alteration in IL-6 levels in COPD individuals (pre= $4.53 \pm 9.0$  pg/ml vs. post= $7.14 \pm 11.31$  pg/ml,  $p=0.11$ ), whereas in healthy individuals this increase was significant (pre= $1.56 \pm 6.45$  pg/ml vs. post= $4.37 \pm 8.0$  pg/ml,  $p<0.01$ ). COPD individuals present higher hs-CRP blood levels when compared to healthy subjects both in rest ( $8.15 \pm 9.68$  pg/ml vs.  $2.60 \pm 1.88$  pg/ml,  $p=0.02$ ), and after exercise ( $8.18 \pm 9.08$  pg/ml vs.  $2.62 \pm 1.85$  pg/ml,  $p=0.02$ ). TNF- $\alpha$  did not present difference between groups during rest (COPD= $2.13 \pm 1.03$  pg/ml vs. healthy= $2.00 \pm 0.59$  pg/ml,  $p>0.05$ ) as well as after 6MWT (COPD= $2.48 \pm 1.92$  pg/ml vs. healthy= $1.89 \pm 0.69$  pg/ml,  $p>0.05$ ), and their intragroup focus was not affected by the effort ( $p>0.05$ ).

**Conclusions:** In COPD patients, 6MWT does not induce acute inflammatory response of IL-6 at the same proportion of that of healthy subjects. Hs-CRP's and TNF $\alpha$ 's response to 6MWT was similar between groups. COPD patients presented higher concentrations of hs-CRP than healthy individuals.

**Keywords:** Chronic obstructive pulmonary disease; Exercise; Inflammation

### Introduction

Exercise intolerance from a chronic obstructive pulmonary disease (COPD) patient is intimately related to the survival of these individuals [1]. It is a multifactorial problem in which low degree systemic inflammation [2] and acute inflammatory response to physical effort may present an important role in the individual's incapacity to meet the high respiratory need [1]. Even outside the clinical decompensation period and in rest, there are high levels of inflammatory cells, cytokines and acute phase proteins in these individuals' peripheral blood, being the main Interleukin 6 (IL-6), a interleukin 8 (IL-8), tumoral necrosis factor alfa (TNF- $\alpha$ ) and C-reactive protein (CRP) [3,4].

Physical exercise, depending on its intensity, duration and muscular mass involved, is capable of provoking an increase in oxidative stress and inflammatory biomarkers levels in healthy individuals' peripheral blood [5,6] and in COPD carriers [7,8]. According to Van Helvoort et al. [9], after maximum exercise, in COPD patients, there is a significant increase of leukocytes in peripheral blood. Even in healthy athletes there is an elevation of inflammatory factors (IL-6, IL-8, CRP) after very intense or long physical exercise, such as marathons [6,10].

Acute inflammatory response to submaximum exercise, however, is less known in COPD individuals [11]. Some results indicate that these individuals present elevation in inflammatory mediator's blood levels during the performance of their activities of daily living (ADL) and that this frequent inflammatory response would be responsible for the worsening of extrapulmonary manifestations of the disease, such as, by instance, muscle deterioration [7].

Although the six-minute walk test (6MWT) is widely used, standardized and known to reflect ADL of COPD individuals [12],

little is known about the systemic inflammatory reactions induced by it in this population. Therefore, the objective of the present study was to compare blood levels of IL-6, TNF- $\alpha$  and hs-CRP in rest and after the 6MWT in COPD patients and healthy sedentary individuals.

## Methodology

Study type case-control developed with 21 COPD patients and 8 healthy individuals with no smoking record, recruited from public hospitals and private practices and among the patients' circle of friends with pulmonary disease.

**Inclusion criteria:** clinical stability for at least one month prior to the beginning of protocol, record of smoking above 20 packs/year, with interruption for at least 6 months, moderate or severe disease according to the classification from the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1]. For the control group: no record of active smoking and normal spirometry exam. Exclusion criteria were active smoking, use of systemic glucocorticoids or simvastatin and presence of associated diseases such as myocardiopathies, heart failure, coronary artery disease, bronchial asthma, musculoskeletal disorders, tuberculosis, rheumatoid arthritis or any inflammatory disease, use of orthopedic prosthetics and inclusion in pulmonary rehabilitation program or any supervised regular physical activity practice

Every individual was informed on the proceedings of the study and signed a free informed consent. The study was approved by the Research Ethics Committee of the Clinicas Hospital of the Federal University of Goiás under protocol 105/2010.

Volunteers accomplished a pre and post bronchodilator spirometry exam (spirometer EasyOne<sup>®</sup>, NDD, Switzerland), according to the guidelines of the American Thoracic Society [13], using the anticipated values established by Pereira et al. [14], two 6MWT, collection of peripheral blood after fifteen minutes of rest and right after the second 6MWT, body composition evaluation (skin folds) and they answered the MRC scale. 6MWT was performed following the criteria established by the American Thoracic Society [12], differing only due to the fact that the two recommended tests were done on different days (so that there would be no influence of the first test on inflammatory biomarkers), the first for learning and the second for data collection.

Interruptions occurred in 6MWT due to the subjective sensation of effort in the sample studied. There was no oxygen supplementation during the test. Before initiating 6MWT, and after its completion, it was measured the following parameters: systemic blood pressure, heart rate (HR), respiratory rate (RR), peripheral oxygen saturation (SpO<sub>2</sub>) and subjective perception of effort, assessed by modified Borg scale. At every two minutes HR, SpO<sub>2</sub> and score on Borg scale were verified.

Plasmatic levels of IL-6 and TNF- $\alpha$  were obtained through ELISA with pre-established procedures by Kit BD OptEIA, in agreement with manufacturer's instructions. Detection limits were 4.7 pg/ml and 3.1 pg/ml, respectively. Whereas hs-CRP was quantified from plasma samples, through the turbidimetry method with Cobas Mira Plus<sup>®</sup> in automated system, being the minimum detection limit 0.2 mg/l.

To perform the skinfold measurement a Lange adipometer as well as the technique describe by Harrison et al were used [15]. Body fat percentage was estimated through body density calculation according to Durnin and Womersley's equation [16].

For the statistical analysis, comparison between both groups was evaluated with Student's t test Mann Whitney U test (two-tailed), according to the nature of variables. It was utilized the  $\chi^2$  test to compare comorbidity and sex prevalence between groups. Differences were considered statistically significant when  $p < 0.05$ .

Sample calculation was done through t test to determine if the mean increase in biomarkers serum levels in COPD patients submitted to 6MWT differs significantly from the mean increase in serum level of a control group [17]. To be able to detect a 10% difference or higher between the means of both groups, regarding the utilization of a proportion of 1 control to 2 individuals with COPD commonly employed in the literature [8], the sample calculation showed the need to include 17 individuals in the COPD group and 8 individuals in the control group. It was decided to include 20% more individuals in the COPD group due to the risk of losses throughout the study.

## Results

The sample was constituted of 29 individuals, 21 with COPD and 8 controls. Regarding severity, COPD group was composed by 11 (52.38%) severe patients and 10 (47.62%) moderate ones, according to GOLD classification, 2010.

Concerning mortality risk estimated by BODE index the mean was  $3.95 \pm 2.66$  points, being 10 (47.62%) classified in the first quartile, 03 (14.29%) in the second, 06 (28.57%) in the third and 02 (9.52%) in the fourth quartile. Groups presented similarity regarding sex distribution, ( $\chi^2=2.77$ ,  $p=0.10$ ), being the percentage of men in the COPD group 80.9% and in the control group 50%.

Both groups characteristics are shown in Table 1. Groups were similar regarding age, weight, height, BMI and body fat percentage diverging in relation to pulmonary function variables and amount of cigarettes smoked ( $p < 0.05$ ).

Comorbidities were investigated and there was no difference between COPD and control group ( $p > 0.05$ ), being the most often: systemic blood pressure, gastroesophageal reflux, dyslipidemia, sleep apnea, hypothyroidism, arrhythmia and gastritis.

When comparing performance during 6MWT between COPD and control groups, it was verified that COPD group obtained a lower mean in distance covered ( $386.31 \pm 127.50$  m vs.  $505.90 \pm 99.13$  m,  $p=0.01$ ), more frequent report of dyspnea, evaluated by Borg scale, both before ( $0.86 \pm 1.03$  vs. 0.0,  $p=0.01$ ) and after the test ( $3.76 \pm 2.25$  vs.  $1.60 \pm 1.71$ ,  $p < 0.01$ ), higher rest HR ( $86.00 \pm 13.42$  bpm vs.  $71.00 \pm 9.4$  bpm,  $p < 0.01$ ), but no difference in the final test ( $108.71 \pm 16.54$  bpm vs.  $103.10 \pm 14.91$  bpm,  $p=0.37$ ), lower SpO<sub>2</sub> before ( $93.59 \pm 2.28\%$  vs.  $96.60 \pm 1.50\%$ ,  $p=0.01$ ) and at the end of the test ( $89.28 \pm 4.73\%$  vs.  $96.10 \pm 1.10\%$ ,  $p < 0.001$ ) and more elevated rest RR ( $18.72 \pm 3.71$  breaths/minute vs.  $15.80 \pm 2.74$  breaths/minute,  $p=0.03$ ) and at the end of the walk ( $24.36 \pm 4.10$  breaths/minute vs.  $20.80 \pm 3.91$  breaths/minute,  $p=0.02$ ).

The accomplishment of 6MWT did not cause significant alteration of IL6, Hs-CRP and TNF- $\alpha$  ( $p > 0.05$ ) in COPD group. Only the control group presented a significant increase of IL-6. Hs-CRP levels in COPD group was higher than that of the control group both before ( $p=0.02$ ) and after 6MWT ( $p=0.02$ ). There was no difference between groups regarding other biomarkers investigated in rest (IL-6,  $p=0.36$ ; TNF- $\alpha$ ,  $p=0.66$ ) or after the walk (IL-6,  $p=0.47$ ; TNF- $\alpha$ ,  $p=0.23$ ) (Table 2).

	COPD	Control	p
Age (years)	69.7 ± 9.01 (43/79)	68.4 ± 5.9 (60/76)	0.66
Years/Packs	55.5 ± 28.7 (20/120)	0 ± 0 (0/0)	<0.001*
Weight (Kg)	70.04 ± 18.2 (36/115)	70.1 ± 10.4 (50/84)	0.99
Height (m)	1.6 ± 0.1 (1.5/1.8)	1.6 ± 0.1 (1.4/1.7)	0.58
BMI (kg/m <sup>2</sup> )	26.1 ± 7.1 (15.4/42.2)	26.7 ± 4.8(21.1/35.1)	0.82
% Body fat	26.1 ± 10.1 (12.5/47.5)	36.4 ± 10.3 (24.7/46.7)	0.15
FEV1 (liters)	1.3 ± 0.5 (0.5/2.3)	2.7 ± 0.6 (1.7/3.7)	<0.001*
FEV1 (% of predicted)	45.3 ± 13.8 (17.3/7)	102.3 ± 15.8 (86.1/130.7)	<0.001*
FVC (liters)	2.6 ± 0.8 (1.4/4.5)	3.1 ± 0.8 (2.0/4.2)	0
FVC (% of predicted)	77.9 ± 18.4 (46/112)	96.8 ± 9.8 (84.5/108.1)	0.14
FEV1/ FVC (%)	54.5 ± 14.6 (20.9/69.7)	96.8 ± 9.8 (97.1/113.4)	<0.001*
MRC	3.1 ± 1.4 (1/5)	-	-

BMI: Body mass index; % Body fat: percentage of body fat; FEV<sub>1</sub>: Forced expiratory volume in 1s; FEV<sub>1</sub> % of predicted: Forced expiratory volume in 1s percentage preview; FVC: forced vital capacity; FVC % of predicted: forced vital capacity percentage preview; BMI: Body Mass Index; MRC: Medical Research Council. \*p Statistically significant, t test

**Table 1:** Comparison between groups regarding demographic characteristics, anthropometric and lung function.

	COPD (n=21)			Control (n=08)		
	Before 6MWT	After 6MWT	p	Before 6MWT	After 6MWT	p
hs-CRPa	8.15 ± 9.68	8.18 ± 9.80	0.18	2.60 ± 1.88*	2.62 ± 1.85*	0.71
IL-6	4.53 ± 9.0	7.14 ± 11.31	0.11	1.56 ± 6.45	4.37 ± 8.0	0.01**
TNFα	2.13 ± 1.03	2.48 ± 1.92	0.37	2.0 ± 0.59	1.89 ± 0.69	0.73

Mean ± SD. \*p <0.05, Mann-Whitney test: Comparison between control group and COPD (Before 6MWT vs. Before 6MWT, After 6MWT vs. After 6MWT); \*\*p: Wilcoxon test: comparison within the same group before and after 6MWT.

**Table 2:** Comparison of blood concentrations of inflammatory biomarkers before and after the test of the six-minute walk within and between groups.

## Discussion

The present study aimed at evaluating if 6MWT alters blood levels of IL-6, TNF-α and Hs-CRP between COPD and health subjects. Some interesting findings were observed. 6MWT cause a significant anti-inflammatory response (increase in IL-6 concentration) in the control group and not in COPD group. Moreover, COPD individuals presented higher levels of hs-CRP both in rest and after 6MWT.

The higher rest HR in COPD group indicates worse health prognosis [18]. At the end of 6MWT, however, HR was not different between groups, showing that the test presented a similar overload, once groups were matched by age. Nevertheless, if taken into consideration the respiratory parameters, such as RR, SpO<sub>2</sub> and the higher effort subjective perception, the respiratory system limitation of COPD individuals becomes clear. According to Pitta et al. [19], COPD carriers usually get into a vicious cycle of inactivity, performing less daily movements than healthy individuals, which generates more physical incapacity and more intensity of respiratory symptoms,

resulting in worse functional capacity, shorter distance covered during 6MWT, as demonstrated in the present study.

In addition to worse performance of COPD carriers during 6MWT, the same did not promote significant increase in IL-6 levels in COPD group. The production of this interleukin *in-vivo* is stimulated chiefly by monocytes, macrophages, fibroblasts and vascular endothelial cells, as well as adipose tissue, responsible for the release of 10 to 35% of all circulating IL-6 [20]. However, cells such as osteoblasts, T lymphocytes, B lymphocytes, neutrophils, eosinophils, mastocytes and skeletal and smooth muscular cells are also capable of producing it [21]. IL-6 is the cytokine produced in the biggest scale during exercise because the muscle contraction itself stimulates its release [22].

For some time IL-6 was associated only with its pro-inflammatory action, with an important role in bacterial infections, being essential in increasing TNF-α blood levels, also important in fever induction and exacerbation in COPD [6,23]. However, in the last decade it has been classified as anti-inflammatory, if its increase is promoted by muscle

contraction [22]. Moreover, initially, it was also associated the plasmatic increase of IL-6 to maximum, prolonged exercises with muscular lesion followed by repair mechanisms with the invasion of macrophages. Today, it is known that muscle deterioration may lead to IL-6 concentration increase through this mechanism, however late and in lower magnitude. It is believed that the rapid increase of IL-6 levels in the blood occurs due to its release by muscle contraction during exercise [22], and results in beneficial actions to the health [24], such as: action on glucose homeostase and fat metabolism during physical exercise, TNF- $\alpha$  inhibition [25] and IL-10 stimulation [24]. Possibly, it has also influence over liver glucose metabolism to maintain it's in the blood while there is an increase of its need during exercise [26]. Another important role of IL-6 is the fact that it is a powerful lipolytic factor during exercise, promoting the increase of free fatty acids for the production of energy in the skeletal muscular cell during physical effort and increasing lipid oxidation by the human organism [25]. Moreover, it was verified that the increase in IL-6 gene expression, caused by exercise is an inhibiting factor for TNF- $\alpha$  production,

attenuating TNF- $\alpha$  plasma increase in physical effort. This may be considered a positive biological mechanism, once TNF- $\alpha$  is involved in the development of cell resistance to insulin action in atherosclerotic processes [24]. Finally, IL-6 released by exercise promotes increase of IL-10 in blood circulation, responsible for the synthesis suppression of several pro-inflammatory cytokines released by different cells, mainly by neutrophils and mononuclear cells [27].

A recent study, in which it was verified IL-6 levels in different degrees of COPD before and after 6MWT, it was observed that very severe individuals presented higher IL-6 concentrations after the test and that the inflammatory response to the test was discrete regardless the severity [28]. In the present study, according to Figure 1, although the COPD group presents increase in absolute values of this interleukin's concentration after exercise was not significant. Some factors may have contributed to that: first, almost half the sample was constituted of individuals with moderate degree; secondly, this group covered the shortest distance during the test.

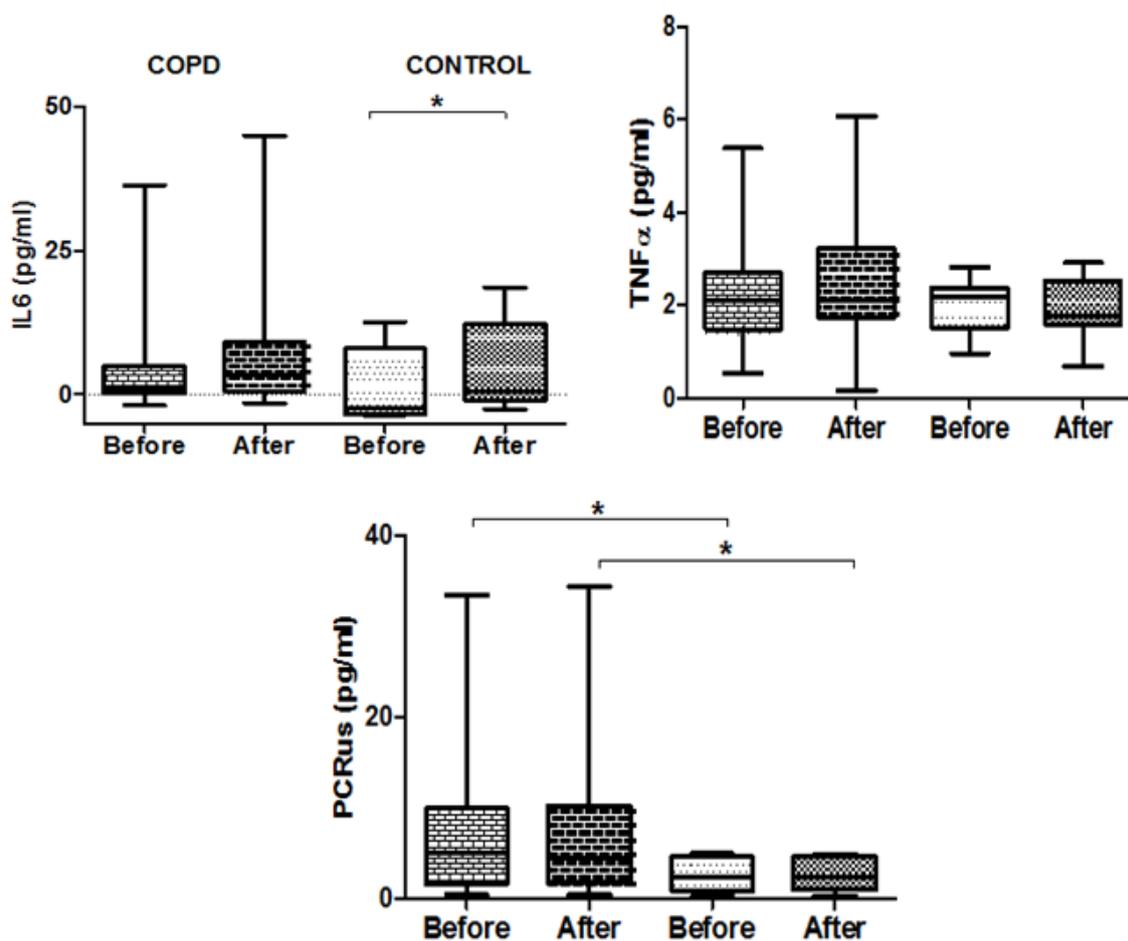


Figure 1: Presents the mean inflammatory biomarkers before and after 6MWT in the COPD group and Control, \*p<0.05.

COPD group presented the highest levels of Hs-CRP. This result is in accordance with other authors [2,29] and partially justifies the low degree status of systemic inflammation of these patients. However, in no group was there an acute increase of this biomarker as a

consequence of exercise. This fact was also verified by Van Helvoort al. [7], after the performance of maximum exercise in COPD patients with and without muscle depletion. For healthy elderly acute increase

of CRP was reported, however, one day after the execution of long-duration exercise [30].

TNF- $\alpha$  blood levels did not differ between groups and was not influenced by 6MWT in an acute way. Results agree with those of Dorneles et al. [28] who could not verify a difference between groups with different degrees of COPD after 6MWT, and with the data presented by Papaioannou et al. [31] that did not reveal differences regarding the blood level of this biomarker in stable COPD individuals and healthy controls. However, other studies, with more consistency methodology, perceived this difference [2,32]. As opposed to the present study, it was verified in COPD individuals and increase in TNF- $\alpha$  plasma levels after eleven minutes of moderate intensity ergometric exercise [8]. The present study, in turn, although the mean was numerically higher after 6MWT in COPD group, verified no significant difference.

The present study presents some limitations. Even though every individual presents rest SpO<sub>2</sub> higher than 90%, some COPD individuals reduced SpO<sub>2</sub> by more than 4%, which define desaturation during physical effort. That could lead to a confounding factor, once tissue hypoxia could also induce to the increase of IL-6 levels. However, the intention was for individuals to reproduce their ADL and, in their daily chores, they do not use oxygen therapy. Another limiting factor is that COPD group covered different degrees (moderate and severe) which may have influenced in the inflammatory profile response to exercise.

Despite the limitations, the present study brings novel contributions as it helps to demystify the fact that submaximum exercise in ADL may generate muscle deterioration in COPD patients and contribute to the worsening of the disease. COPD individuals need to improve the level of physical activity to obtain better quality of life, even if it is done in their own home environment, executing ADL. According to Pita et al. [19], COPD patients stay three times longer in bed or lying and almost half of the time walking when compared to healthy individuals. This behavior needs to be confronted and doing more ADL may be the solution, since every day it seems safer to execute sub maximum exercise from the inflammatory point of view.

We suggest that more studies, of wider scope, be performed investigating the inflammatory profile after varied intensities exercise, as well as different degrees and functional capacities, even executing their own ADL, so that it can be clarified how the exercise-inflammatory response relation occurs in COPD, since to obtain a better quality of life and longer survival rates this group needs to have the best possible physical capacity.

Therefore, in COPD individuals, even though 6MWT causes more cardiorespiratory overload and tiredness, it does not induce the same acute anti-inflammatory response of IL-6 at the same proportion of healthy individuals and for the inflammatory mediator's hs-CRP and TNF $\alpha$  the response to 6MWT is similar to that of healthy individuals.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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