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Exercise-modulated epigenetic markers and inflammatory response in COPD individuals: A pilot study

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ABSTRACT

The study investigated the effects of exercise on epigenetic signals and systemic cytokine levels in chronic obstructive pulmonary disease (COPD) individuals. Ten participants of a pulmonary rehabilitation program were submitted to 24 sessions of a supervisioned exercise protocol thrice-weekly (90 min/session). Blood samples were collected at baseline, after the 1st session, before and after the 24th session. A DNA hypomethylation status was observed after the 1st session when compared at baseline, while global histone H4 acetylation status was unaltered in any time-points evaluated. No significant changes were observed on cytokine levels after the 1st session. A significant enhancement on interleukin 6 (IL-6) and a decrease on transforming growth factor-beta (TGF-β) levels were found after the 24th session when compared to the pre 24th session. Moreover, 23 sessions of exercise were able to diminish significantly the basal levels of IL-6 and interleukin 8 (IL-8). These data suggest a potential role of epigenetic machinery in mediating the anti-inflammatory effects of exercise in COPD patients.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major and increasing cause of chronic morbidity and mortality contributing for 3 million deaths per year worldwide. In fact, the World Health Organization (WHO) has predicted a remarkable rise of COPD mortality for the next years being the third cause of death in 2030 (Acquaah-Mensah et al., 2012). Moreover, COPD is considered a global health problem causing enormous costs for care systems (GOLD, 2010).

Large part of COPD patients has a poor quality of life, which is related to the comorbidities allied to this disease. Acute exacerbations are also common in these patients, which affect the function of other organs and systems such as cardiovascular, blood, nervous and skeletal muscles. Thus, individuals with COPD show not only limitations in daily physical activities as well generally adopt a sedentary lifestyle (Miravitlles et al., 2012).

COPD is characterized by partially reversible and progressive airflow limitation, caused by noxious particles or gases such as cigarette smoke (CS) which is considered one of the major etiologic risk factor in the COPD pathogenesis (Miravitlles et al., 2012). Importantly, CS can induce both local and systemic chronic inflammatory-immune responses through the recruitment and activation of pro-inflammatory mediators in lung tissue and peripheral blood (Agustí, 2005). In this context, increased levels of circulating cytokines including interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor alfa (TNF-α) and interferon γ (IFN-γ) are observed in this population even when they are at a stable condition of the disease (Agustí, 2005). Although it is reported that interleukin 4 (IL-4) is able to inhibits the release of some pro-inflammatory markers such as TNF-α and IL-6, the link between IL-4 and these cytokines in COPD is poorly investigated.

Emerging findings have been pointed out that epigenetic mechanisms exert a pivotal role in COPD physiopathology (Krauss-Etschmann et al., 2015). Epigenetic has been defined as dynamic regulation of gene expression in response to external stimuli independent that is independent of changes in the underlying DNA sequence. DNA methylation and

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modifications of core histones are the most studied epigenetic mechanisms. DNA methylation, catalyzed by the DNA methyltransferases (DNMTs), is often associated with gene transcription silencing (Paulsen and Ferguson-Smith, 2001). Conversely, histone acetylation is associated with enhanced transcriptional activity, process controlled by histone acetyltransferases (HAT) and histone deacetylases (HDAC) enzymes, which respectively add and remove the acetyl groups (Kouzarides, 2007). In this context, it is known that histone acetylation imbalance contributes to the transcription of pro-inflammatory genes (Marwick et al., 2004)

Importantly, the decrease of HDAC activity was significantly correlated with smoking exposure and serum interleukin-8 (CXCL8) concentrations, suggesting that the modulation of histone acetylation status in peripheral blood mononuclear cells (PBMCs) can modify the response of systemic inflammation by smoking quantities (Chen et al., 2012). Furthermore, it has been described that disruptions of DNA methylation levels also are involved in the inflammatory status in COPD patients and might influence negatively the severity and progression of the disease (Murphy et al., 2015; Uddin et al., 2011).

It is well established that exercise is the basis of effective pulmonary rehabilitation programs (PRP) and includes aerobic and muscle training. It have been considered an important complement to conventional therapy in COPD, being a multidisciplinary non-pharmacological strategy that impacts positively the disease progression (Spruit et al., 2013). It is known that patients submitted to exercise training showed reduced symptoms, such as exertional dyspnea, optimize exercise tolerance and functional capacity improvement after intervention. These factors contribute to restore the highest level of independent function which impacts directly their quality of life (Spruit et al., 2013).

Despite these findings, the molecular mechanisms associated with these beneficial effects are not elucidated yet. Recent evidences have been demonstrating that diverse exercise protocols can modulate epigenetic signals in peripheral blood from different populations (Dorneles et al., 2016a,b; Zhang et al., 2015; Lavratti et al., 2017). However, to our knowledge, no studies reported the impact of exercise on epigenetic signals in COPD individuals.

Finally, emerging evidence has pointed out to a closer relationship between epigenetic mechanisms and inflammatory mediators in several respiratory diseases (Sakao and Tatsumi, 2011). In this sense, the phenotype of peripheral blood mononuclear cell (PBMC) is controlled through epigenetic signals that are involved in both activation and repression of specific genes involved in the immune response as well as in the production of inflammatory mediators (Shanmugam and Sethi, 2013). Regarding COPD, previous work demonstrated altered levels of histone deacetylase (HDAC) in immune cells from patients compared to matched controls (Tan et al., 2016; To et al., 2012). Interestingly, the histone hyperacetylation status in PBMC of COPD patients was also accompanied by higher expression of NF-κB and increased systemic levels of TNF-α and IL-8 (Ito et al., 2005). In addition, Chen et al. (2012) reported a negative correlation between plasmatic IL-8 and HDAC activity in PBMC. Nevertheless, to date, effects of exercise on epigenetic markers associated with inflammatory mediators in COPD individuals have been not investigated.

Since chronic exercise training has greater potential to reduce low grade inflammation in many chronic diseases, it is important to understanding the underlying mechanisms through exercise-induced anti-inflammatory effects. Specifically, emerging evidences suggest that epigenetic changes has been involved in the attenuation of inflammatory response of PBMCs following exercise in both healthy (Denham et al., 2016) and chronic patients such as schizophrenia (Lavratti et al., 2017). To date, the impact of exercise training in COPD patients has contradictory findings with both non-altered (Canavan et al., 2007; Ramos et al., 2014) or decreased (do Nascimento et al., 2015; Wang et al., 2014) inflammatory cytokine levels. To our knowledge, no study was performed focusing on epigenetic-inflammation highway in response to acute or chronic exercise in COPD individuals. Therefore, the present study aimed to examine the short and long term outcomes of exercise on epigenetic and inflammatory markers in peripheral blood of COPD patients.

2. Material and methods

2.1. Subjects

Thirteen COPD patients of both genders that were previously sedentary for at least one year were recruited for this study. All them were recruited from the PRP of the Pulmonology Service of the Irmandade Santa Casa de Misericórdia de Porto Alegre-RS, Brazil.

The following inclusion criteria were applied for the patients recruitment: GOLD stages 2–4 of severity of airflow limitation, history of smoking ≥ 20 pack-years, nonsmoker for at least six months, clinical stability in the four weeks prior to the study protocol and age ≥ 40 - years.

Any patient whose training was interrupted, current smoking, pulmonary disease other than COPD and comorbidities that would compromise their ability to perform any of the evaluations in the study were excluded.

The study was approved by the Human Research Ethics Committee of the Irmandade Santa Casa de Misericórdia de Porto Alegre (protocol number 40078114.9.0000.5335) and the Methodist University Center IPA (protocol number n°918.889/2014). All participants provided written informed consent prior to participation.

2.2. Exercise training protocol

Patients participated in a 24 exercise training sessions designed according to the American Thoracic Society (ATS) and the European Respiratory Society (ERS) (Spruit et al., 2013). The protocol involved endurance training and peripheral muscle strength three times per week (approximately 90 min each session). The endurance training was taken on a treadmill with 60% of the speed average of the six-minute walking test and the work load progression occurred according to the dyspnea reported on the modified Borg scale (Borg, 1982). The lower limbs training involved quadriceps and triceps sural. The upper limb training was carried in diagonal axes.

2.3. Blood collection

In order to analyze the short and long term effects of exercise on epigenetic and inflammatory markers, blood samples (approximately 12 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 peripheral blood mononuclear cells (PBMCs) were isolated from EDTA blood through the density gradient technique as described by Bicalho et al. (1981). For this purpose, whole blood samples were diluted in a proportion of 4:3 in phosphate-buffered saline (PBS, 136 mM, NaCl, 2.7 mMKCl, 7.8 mM Na2HPO4, 1.7 mM KH2PO4; pH 7.2-7.4) and centrifuged (1500 rpm, 21° C, 20 min) on Ficoll-Histopaque 1077 (Sigma, MO, USA). At this time, 1.5 ml of plasma was separated and frozen at −20 for circulating cytokines analysis. PBMCs were collected and washed 2 x in PBS (2000 rpm, 21° C, 8 min). Cells were counted by microscopy (100x) and cell viability always exceeded 95% of the total as assessed by their ability to exclude Trypan Blue (Sigma, USA). The pellet of cells and plasma were frozen at −80° until analysis. The determination of epigenetic and cytokine measurements were carried out by an observer blinded to the interventions of the study.

2.4. Determination of epigenetic parameters

Global DNA methylation levels in the plasma samples were quanti-

fied using MethylFlashTM Methylated DNA Quantification Kit (Epigentek, Catalog number P-1034, NY, USA) according to the manufacturer's information. The capture antibody in this kit binds to 5-methylcytosine, thus it was measured the total DNA methylation level as a percentage of total DNA present in each plasma sample. A standard curve with positive and negative controls was run with the samples. Then, 100 ng of DNA (1–8 ng/μl) was bound to the plate at 37 °C for 90 min. The methylated fraction of DNA was detected using capture and detection antibodies. The relative optical density (OD) units were quantified by reading the absorbance in a FLUOstar Optima microplate reader (Labtech). Methylated DNA amount was proportional to the OD measured. Absolute amount of methylated DNA was quantified from a standard curve, and the slope of the standard curve was used to calculate the percentage of methylated DNA (5-mC%) in the plasma samples.

The global histone H4 acetylation levels in PBMCs were determined using the Global Histone H4 Acetylation Assay Kit (Colorimetric Detection, catalog number P-4009, EpiQuik USA) according to the manufacturer's instructions. The samples were incubated with the capture antibody followed by incubation with detection antibody. After, were incubated with developing solution followed by the addition of the Stop Solution. The absorbance was measured on a spectrophotometer at a wavelength of 450 nm. The global histone H4 acetylation levels in PBMCs were expressed as ng/mg protein.

The protein concentration of each sample was measured by the Coomassie Blue method using bovine serum albumin as standard (Bradford, 1976).

2.5. Cytokine measurement

The levels of IL-4, IL-6, IL-8, INF-γ and TGF-β in the plasma samples were determined by Enzyme-Linked Immunosorbent Assay (ELISA) protocols according the manufacturer's recommendations. IL-4, IL-6, IL-8, INF-γ were analyzed using ELISA Development Kit of Peprotech Inc, New Jersey, USA, while TGF-b were quantified using ELISA commercial kit from eBioscience, San Diego, USA.

2.6. Exercise capacity

The six-minute walk test (6MWT) was carried out according to the recommendations of the ATS (ATS, 2002) to assess exercise capacity.

2.7. Dyspnea

Dyspnea was assessed with de Medical Research Council (MRC) 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 (Mahler and Wells, 1988).

2.8. Quality of life

The perception of quality of life was assessed by the St. George's Respiratory Questionnaire (SGRQ), which has been widely used for patients with chronic respiratory diseases. The SGRQ is a quality of life questionnaire related to disease-specific health, with three domains (symptoms, activity and impact of the disease) divided into 76 items. The scores of each answer are added together and the total is displayed as a percentage of maximum. Values above 10% reflect an impairment on quality of life in that domain or in the total score (de Sousa et al., 2000; Camelier et al., 2006).

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 exercise session.

2.9. Statistical analysis

Data were tested for normality with the Shapiro-Wilk test. Parametric variables (cytokine levels, exercise capacity and quality of life) were presented as mean ± standard deviation (SD). The global levels of histone H4 acetylation, DNA methylation and dyspnea were considered non-parametric and were presented as median (interquartile range). One-way ANOVA for repeated measurements were performed to compare the cytokine levels at baseline and immediately after the 1st and the 24th exercise session. If a significant effect was observed, a Bonferroni post-hoc test was applied for multiple comparisons. For those non-parametric variables, Kruskall Wallis test followed by Dunn's post-hoc was used. Correlations between inflammatory variables and epigenetic markers checked with Spearmańs test. Changes in exercise capacity and quality of life were assessed by the Student *t*-test for paired samples and changes in dyspnea were assessed by the Wilcoxon test. The level of statistical significance was set at $p < 0.05$. Data were analyzed using SPSS 20.0 (SPSS, Chicago, IL, USA).

3. Results

Of the 13 participants recruited into the study, 10 successfully completed the 24 exercise sessions. Three patients withdrew for undisclosed reasons. The sample characterization is described in Table 1. Patients improved exercise capacity and quality of life perception regarding the activity domain score and the total score (Table 2).

The exercise did not change global histone H4 acetylation levels in any time-points evaluated ($p < 0.05$; Fig. 1). However, as highlighted in Fig. 2, a remarkable reduction on global DNA methylation levels was observed immediately after the 1st session compared to the basal period [0.04 (0.0401–0.0421) to 0.03 (0.0324–0.0399), $p = 0.007$]. This effect was not found after the 24th session [0.0398 (0.0372–0.0410) to 0.0375 (0.0359–0.0400), $p > 0.05$].

Interestingly, correlations between the inflammatory markers and epigenetic parameters were observed (Fig. 3). At 24th session, the basal values of global histone H4 acetylation levels were negatively correlated with basal IL-4 levels ($r = -0.65$; $p = 0.04$; Fig. 3A) and positively correlated with IL-8 levels ($r = 0.85$; $p < 0.01$; Fig. 3B). However, no correlations were found between inflammatory or epigenetic markers and functional status data ($p > 0.05$).

Finally, Table 3 shows the short and long term cytokine responses to exercise. No significant changes were observed on cytokine levels immediately after the 1st session ($p > 0.05$). After 23 sessions of exercise, COPD patients exhibited lower levels of IL-6 ($p = 0.003$) and

Table 1
Participants characteristics (n = 13).

Characteristics	N	Mean	SD
Age, years		68.5	6.49
Male (female), N	7(6)		
Smoking history, pack-years		70.7	40.2
Tabaco abstinence, years		11.6	6.87
FEV_1, l		0.90	0.33
FEV_1, %pred		35.1	11.9
FVC, l		1.90	0.59
FVC, %pred		55.5	12.9
FEV_1/FVC, %		0.50	0.09
GOLD 2, N	2		
GOLD 3, N	5		
GOLD 4, N	6		
BMI, kg/m^2		26.9	3.88

FEV1, I: Forced Expiratory Volume in One Second in liters; FEV1, %: Forced Expiratory Volume in percentage; FVC, I: Forced Vital Capacity in liters; FEV1/FVC, %: the ratio of FEV1 to FVC in percentage.
*Data given as mean ± SD unless otherwise indicated.

Table 2
Effect of exercise on the functional status.

Characteristics	Baseline	After PRP	p
Exercise Capacity (6MWT, m)	416,8 ± 69,9	493,3 ± 99,7	0,004
Dyspnea (MRC)	4 (2–4)	2 (1–4)	0,071
Quality of Life total score (SGRQ)	53,7 ± 16,5	43,4 ± 11,7	0,041
Symptoms	50,0 ± 22,6	43,1 ± 22,5	0,441
Activities	80,1 ± 15,4	63,6 ± 17,0	0,002
Impact	39,8 ± 18,7	32,1 ± 10,1	0,185

Exercise capacity: distance walked during the six-minute walk test (6MWT) in meters; Dyspnea: score of the perception on the Medical Research Council (MRC); Quality of life: score on the Santi George Respiratory Questionnaire (SGRQ). Data shown as mean ± standard deviation or median (interquartile range). Changes in exercise capacity and quality of life were assessed by the Student *t*-test and changes in dyspnea were assessed by the Wilcoxon test.

IL-8 ($p = 0.05$) compared to pre 1st session. In addition, the acute response to 24th session was able to induce a significant enhancement of systemic IL-6 ($p = 0.017$) and a significant decrease on TGF-β levels ($p = 0.043$) after exercise. No significant changes were found in IL-4 nor INF-γ following 1 or 24 exercise sessions ($p > 0.05$).

4. Discussion

In the current study, we demonstrated the impact of exercise on epigenetic parameters in peripheral blood of moderate-to-very severe COPD patients. It's noteworthy that, the exercise together with epigenetic changes seems to modulate systemic inflammatory markers.

Clinical data available in the literature regarding exercise outcomes on DNA methylation status investigate only the effects of a single bout or chronic protocols. Therefore, another novel finding that emerged from this study is the time course of the exercise on this epigenetic marker, since we evaluated both short (1 session) and long-terms (24 sessions) effects.

It was demonstrated that plasma DNA methylation levels were acutely demethylated after the 1st exercise session in COPD individuals. In agreement, Barrès et al. (2012) demonstrated that a single session of training at high intensity treadmill (80% of maximal aerobic capacity) globally decreased DNA methylation levels in muscle tissue of healthy adults previously sedentary. On the other hand, other study conducted with well-trained young men reported no changes in DNA methylation levels immediately after a single aerobic exercise protocol (treadmill at 60% of VO_2 max for 120 min, 5 km fasting) (Robson-Ansley et al., 2014). Taken together, these findings suggest that the DNA methylation status in response to single bouts of exercise, independent of the healthy condition, might depends on the training status. Specifically, sedentary individuals seem to be more sensitive to this modulation compared to well-trained ones.

Although we observed a DNA hypomethylation after the 1st exercise session, no delayed response was found since this epigenetic marker remained unchanged after 24 exercise sessions. In contrast, Denham et al. (2015) showed that 3 months twice a week of a concurrent exercise training protocol increased DNA methylation levels in sperm of young healthy men. Collectively, different from the short-term response, these findings led us to hypothesize that the chronic exercise protocols responses on DNA methylation status might be influenced by health conditions, being less evident in illness situations such as COPD. Future studies should investigate the exact mechanisms and pathways involved with these different responses in sedentary and trained individuals to elucidate this question.

It should be noted that epigenetic mechanisms are not isolated events (Gupta et al., 2010). Therefore, beyond the measurement of DNA methylation levels, we also aimed to investigate the effect of exercise on global histone H4 acetylation levels. We found that this parameter was unchanged in any time-point evaluated. In contrast, Zimmer et al. (2014) showed that a single session of 30 min at a bicycle ergometer on a moderate intensity induced a hyperacetylation histone H4 status in PBMC of non-hodgkin lymphoma individuals. In addition, recent data from our laboratory reported that a single session of a high intensity interval exercise protocol during 31 min significantly increased the global HDAC activity in PBMC of obese individuals (Dorneles et al., 2016a, 2016b). Importantly, the PRP is characterized by 90 min of concurrent training composed by endurance and resistance exercises. Collectively, these data raise the possibility that exercise effects on histone acetylation status seems to be more sensitive to aerobic and shorter durations sessions.

Some insights have been obtained into the key role of higher IL-6 and IL-8 levels in the systemic inflammation of COPD patients (Hurst et al., 2006). Hence, it is known that chronic inflammation can contribute to muscle wasting, cachexia and poor functional performance in this population (Hurst et al., 2006). In this context, Spruit et al. (2003) demonstrated an inverse relationship between muscle force and increasing IL-8 during COPD exacerbations.

In the general healthy population, circulating levels of IL-6 increases in response of a single bout of exercise and these elevations seem to promote an anti-inflammatory environment (Petersen et al., 2008). On

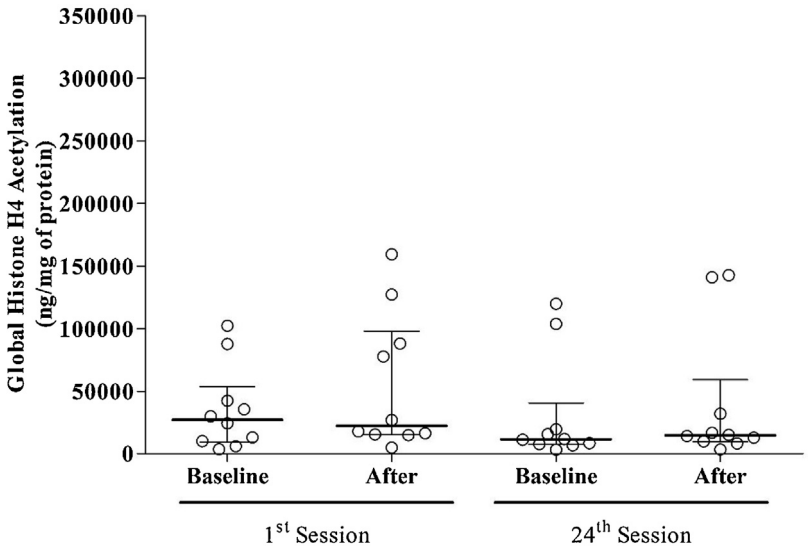

Fig. 1. Global histone H4 acetylation levels in PBMCs from COPD individuals in response to exercise. Kruskall Wallis test ($p > 0.05$).

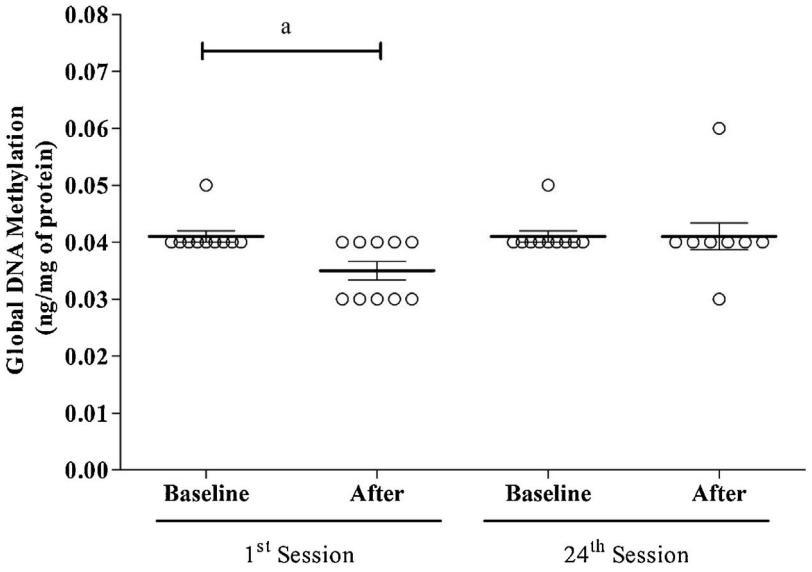

Fig. 2. Global plasma DNA methylation levels in COPD individuals in response to exercise. Kruskall Wallis test followed by Dunn's Post-hoc ($p = 0.007$). *Statistically different from the baseline period (1st session).

the other hand, previous studies showed a potential for exacerbated circulating cytokine concentrations post-exercise as reported in chronic conditions such COPD, chronic kidney disease, and cystic fibrosis (Ionescu et al., 2006). However, we demonstrated that a single exercise session was not able to induce a substantial peripheral inflammatory response in the patients. Our data are in accordance to those obtained by Olfert et al. (2014) who reported no significant increase on TNF-α, IL-6 or CRP levels in alpha-1-antitrypsin-deficient (AATP) or non-AATP COPD patients submitted to one hour of 50% knee extensor exercise. However, Dorneles et al. (2016a,b) demonstrated that a single bout of six-minute walk test elevated IL-4 and IL-6 levels in very severe COPD patients, but no in moderate or severe COPD. Instead, the systemic levels of cytokines were unchanged after the first session of rehabilitation protocol, that was composed by prolonged aerobic exercise plus resistance exercise in the same session. Collectively, these findings suggest that the variables related to the protocol of exercise, i.e. intensity, volume and duration, are key determinants to modulate inflammatory mediators.

Many of the long term exercise benefits are linked to its anti-inflammatory effects, which include the reduction on pro inflammatory mediators such as IL-6 and IL-8 (Gleeson et al., 2011). In our study, 23 exercise sessions were able to reduce significantly systemic concentrations of these markers in order to 23.91% and 31.94%, respectively. Our data corroborate previous studies demonstrating decreases in pro inflammatory cytokines after many rehabilitation program forms (Ramos et al., 2014) as well reinforces the potential of chronic exercise training to attenuate the low-grade inflammation in chronic disease, such as COPD, as demonstrated through low IL- 6 and IL-8 levels after 23 exercise sessions.

Spruit et al. (2005) have demonstrated the influence of IL-8 levels modulation in COPD rehabilitation. The authors described an inverse relationship between baseline circulating levels of IL-8 with the changes in health-related quality of life and the total score of the COPD Questionnaire after 12 weeks of exercise training program. In addition, IL-8 seems to be linked to muscle weakness development in both hospitalized and stable COPD subjects during acute exacerbations (Spruit et al., 2003). Collectively, these data suggest that IL-8 may be a key focus during pulmonary rehabilitation due their role on worsening of the disease. It is important to note that the results of Spruit et al. (2005) only showed a relationship between inflammatory mediators and quality of life in female patients. Thus, the decrease of IL-8 after 23 sessions in our study seems to be beneficial for improvements in the clinical status of COPD patients.

Another important finding that emerged this study is that immediately after the 24th session, a significant increase in IL-6 levels in parallel with decreasing TGF-β levels was observed. It should be emphasized that IL-6 in response to exercise may have important anti-inflammatory effects, being described as a myokine secreted by muscle tissue (Pedersen and Febbraio, 2008). Indeed, the myokine secreted after acute exercise is dependent of (amount of muscle mass) muscle's mass amount and the intensity of the exercise (Febbraio and Pedersen, 2005). Once chronic exercise was associated to improvements on functional capacity and reduced myopathy on COPD, we can believe that the elevation of myokines, such as IL-6, during and after exercise is dependent of reduction on tissue-inflammation and enhancement of muscle mass. In addition, healthy subjects showed increased expression of IL-6 concomitant with anti-inflammatory cytokines, such as IL-10 and IL-1ra, after aerobic exercise, creating a systemic anti-inflammatory environment that can contribute to enhancement of myokine secretion (Pedersen and Febbraio, 2008). Corroborating, studies showed a lack of elevations of pro inflammatory cytokines, like TNF-α, in response to acute exercise after a training program (Canavan et al., 2007). The uncontrolled increase of systemic TGF-β on COPD patients is related to higher fibrosis and clinical severity (Chiang et al., 2014). According to the data, our study is the first to demonstrate reduction on systemic TGF-β levels after acute exercise in trained COPD patients. Previously, Li et al. (2014) showed that COPD rats, induced by cigarette smoke and bacterial infections exposures, reduced their TGF-β levels after 32 weeks of traditional Chinese medicine. Thus, decreasing TGF-β after 24 exercise sessions may suggest a better inflammatory control on COPD.

The effects of exercise training on exercise capacity, quality of life and dyspnea have been extensively studied (McCarthy et al., 2015; Spruit et al., 2013). Our findings regarding exercise capacity and the total score of quality of life and the activity domain of the SGRQ agree with the literature (McCarthy et al., 2015). However, no significant difference on dyspnea and on the symptons and impact domains of the SGRQ were found, probably due to the small study sample.

5. Conclusions

Summarizing, this pilot study provides the first evidence reporting that the exercise is able to modulate DNA methylation status and cytokine levels in peripheral blood of COPD individuals accompanied

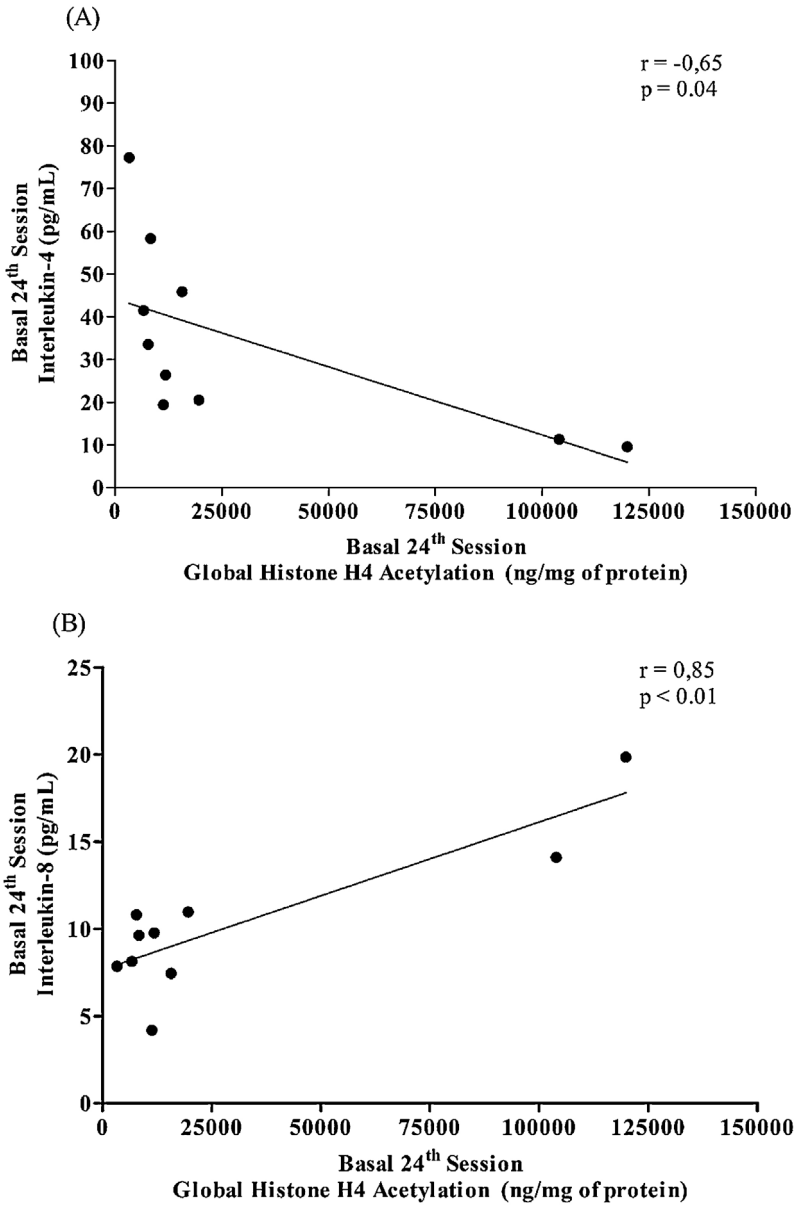

Fig. 3. (A) Correlation between basal 24th session values of global histone H4 acetylation and IL-4. Speraman test ($r = -0.65$; $p = 0.04$).(B) Correlation between basal 24th session values of global histone H4 acetylation and IL-8. Spearman test ($r = 0.85$; $p < 0.01$)

by exercise capacity improvement, suggesting a potential role of epigenetic machinery in the anti-inflammatory effects mediated by exercise in this population.

The primary limitation of this study is the small sample size. However, we believe that these innovative and preliminarily findings might encourage future investigations to verify the influence of exercise on epigenetic and inflammatory response in COPD individuals with more robust number of patients in order to elucidate the exact pathways behind this phenomenon.

Table 3
The effect of exercise on circulating cytokine at different time-points.

	1st Session		24th Session	
	Pre	Post	Pre	Post
Interleukin-4 (pg/mL)	33.17 ± 15.30	33.68 ± 16.63	34.40 ± 18.76	35.30 ± 17.60
Interleukin-6 (pg/mL)	43.82 ± 8.58	47.05 ± 12.08	33.34 ± 8.06[a,b]	43.54 ± 8.41
Interleukin-8 (pg/mL)	15.09 ± 6.20	12.58 ± 5.03	10.27 ± 3.69[a]	12.18 ± 4.39
Interferon-γ (pg/mL)	25.60 ± 5.13	27.45 ± 7.70	20.76 ± 6.78	24.61 ± 7.58
Transforming Growth Factor-β1 (pg/mL)	358.62 ± 196.57	291.63 ± 207.42	404.00 ± 166.89[b]	232.70 ± 116.65

Data given as mean ± SD.
[a] Indicates significant difference compared to the pre 1st session period ($p < 0.05$).
[b] Indicates significant difference compared to post 24th session time ($p < 0.05$).

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