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Research Article

Effect of Physical Exercise on the Level of DNA Damage in Chronic Obstructive Pulmonary Disease Patients

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This study assessed the chronic effects of physical exercise on the level of DNA damage and the susceptibility to exogenous mutagens in peripheral blood cells of chronic obstructive pulmonary disease (COPD) patients. The case-control study enrolled COPD patients separated into two groups (group of physical exercise (PE-COPD; $n = 15$); group of nonphysical exercise (COPD; $n = 36$)) and 51 controls. Peripheral blood was used to evaluate DNA damage by comet assay and lipid peroxidation by measurement of thiobarbituric acid reactive species (TBARS). The cytogenetic damage was evaluated by the buccal micronucleus cytome assay. The results showed that the TBARS values were significantly lower in PE-COPD than in COPD group. The residual DNA damage (induced by methyl methanesulphonate alkylating agent) in PE-COPD was similar to the controls group, in contrast to COPD group where it was significantly elevated. COPD group showed elevated frequency of nuclear buds (BUD) and condensed chromatin (CC) in relation to PE-COPD and control groups, which could indicate a deficiency in DNA repair and early apoptosis of the damaged cells. We concluded that the physical exercise for COPD patients leads to significant decrease of lipid peroxidation in blood plasma, decrease of susceptibility to exogenous mutagenic, and better efficiency in DNA repair.

1. Introduction

The chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality in countries with different levels of economic development, and it is estimated that in 2020 the COPD will become the third leading cause of death all over the world [1, 2]. COPD is currently defined as a preventable and treatable disease characterized by airflow limitation, resulting from an abnormal inflammatory reaction to inhaled particles from cigarette smoking and associated with comorbidities [2]. The COPD is multifactorial and its pathology often includes systemic inflammation and oxidative stress [3, 4].

The formation of reactive oxygen species (ROS) by cigarette smoke and inflammatory cells, generated in the pulmonary epithelium, has been associated with slowly progressive and irreversible decrease in forced expiratory volume in one second (FEV_1), loss of muscle mass, and muscle dysfunction [3, 4, 11] and probably modulates some of the systemic effects of COPD (i.e., skeletal muscles atrophy, osteoporosis, anemia, and cachexia [11, 12]). Some of the many different compounds in cigarette smoke can react

directly with cellular components to form ROS while other carcinogens must be activated to produce single- and double-strand breaks into DNA [13, 14].

Additionally, evidence about the effect of exercise-induced systemic oxidative stress in COPD patients is still lacking especially about possible differential effects of exercise at different intensities [15]. Endurance physical training is a principal constituent of the pulmonary rehabilitation programs yielding clear beneficial effects on skeletal muscles and on relevant clinical outcomes. Improved skeletal muscle bioenergetics after training has been associated with decreased inflammation in healthy subjects and in other chronic conditions, but data on COPD patients remain controversial [15, 16]. This is a relevant aspect since the intensity and duration of the general exercise training are key factors that may predict outcomes such as muscle oxidative capacity adaptations as well as the potential development of oxidative stress [16].

Moreover, no studies are currently available that have evaluated the effect of exercise on oxidative stress and DNA damage in COPD patients. The cellular processes of DNA damage induction and repair are fundamental for the maintenance of genome integrity, and the modulation of these processes can dramatically increase individual susceptibility to cancer [17, 18]. Comet assay has been used in various studies to investigate the DNA damage in connection with various diseases because it is a rapid, simple, and sensitive technique for measuring DNA breaks and repair in single cells [19–21]. The cytogenetic damage evaluated by buccal micronucleus cytome assay (BMCyt) is a sensitive biomarker that is widely accepted for chromosome damage evaluation [22]. The buccal epithelial cells are the first barrier for the inhalation or ingestion route that can metabolize proximate carcinogens to reactive products. About 90% of the human cancers originate from epithelial cells. Therefore, oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body.

We aimed to investigate if regular physical exercise can have some influence on the damage induction and DNA repair in COPD patients. The present study was, therefore, designed to assess the chronic effects of endurance physical exercise on the level of micronucleus formation in buccal mucosa and DNA damage in peripheral blood cells of COPD patients and controls, evaluated by comet assay. The oxidative stress was also evaluated by means of thiobarbituric acid reactive species (TBARS) in blood plasma.

2. Materials and Methods

Fifty-one COPD patients were included in this study, with average age of 65.33 ± 8.91 years, treated at the Santa Cruz Hospital by Research Group for Health Rehabilitation, south Brazil. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease Guidelines-GOLD [2], using clinical history, physical examination, and presence of airflow obstruction, defined as a ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) less than 70% of predicted value. The COPD patients were allocated into two groups: group of physical exercise (PE-COPD; $n = 15$, e.g., pulmonary rehabilitation program) and group of COPD patients (COPD; $n = 36$, e.g., only ambulatory care). At the beginning of the study, all participants of PE-COPD underwent pulmonary rehabilitation 2 times per week for 8 weeks (i.e., endurance physical training and education about the disease) [2]. The COPD patients were matched by gender and age with 51 control individuals (e.g., general population) without previous pulmonary disease and with no evidence of airflow obstruction ($FEV_1/FVC > 70\%$ and $FEV_1 > 80\%$ predicted) [2]. The study protocol was approved by the Ethics Committee of UNISC, number 2011/08. All individuals answered the personal health questionnaire and signed informed consent before the interview.

2.1. Blood Collection. Peripheral blood samples were collected at different time points from patients and control individuals, at rest, early in the morning, into two tubes with anticoagulant. One aliquot was used for the comet assay and the other aliquot to obtain blood plasma for the remaining analysis. The samples of all patients in the PE-COPD group were collected after at least 72 h without exercise to avoid the acute effect of exercise.

2.2. DNA Damage Evaluation by Comet Assay. The comet assay was performed under alkaline and neutral conditions [5, 6]. Aliquots of 10 μL freshly collected whole blood were mixed with 90 μL low melting point agarose (0.7% in phosphate buffer) and added to microscope slides precoated with 1.5% agarose. The slides were then incubated in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 20 mM NaOH, pH 10.2, 1% Triton X-100, and 10% DMSO). After 24 h at 4°C, the slides were removed from the lysis solution and placed in an electrophoresis unit filled with fresh electrophoresis buffer at 4°C. In the alkaline version of comet assay (10 M NaOH, 1 mM EDTA, and pH > 13), 20 minutes of denaturation and 15-minute electrophoresis time were used. For the neutral version of comet assay (3 M sodium acetate, 1 M Tris, and pH = 8.5), the denaturation time of 1 h and electrophoresis time of 1 h were used. In both versions of comet assay, after electrophoresis, the slides were neutralized (0.4 M Tris, pH 7.5) and washed in water. Slides were dried overnight at room temperature, then fixed and stained with silver nitrate [7]. For DNA damage evaluation, 100 cells per sample were analyzed by optical microscopy at 100x magnification. The cells were visually scored by measuring the DNA migration length and the amount of DNA in the tail into five classes, from undamaged −0 to maximally damaged −4, and a damage index (DI) value was calculated for each sample [7]. Damage index, thus, ranged from 0 (completely undamaged: 100 cells × 0) to 400 (with maximum damage: 100 cells × 4).

2.3. Detection of Oxidized Bases. We performed the modified comet assay using specific enzymes to expose oxidative damage. The enzyme formamidopyrimidine DNA glycosylase (FPG) recognizes the common oxidized purine 8-oxo-7,8-dihydroguanine and ring-opened purines [8], whereas

endonuclease III (ENDO III) converts oxidized pyrimidines to strand breaks [9]. After lysis, the slides were washed for 5 minutes each in enzyme buffer (40 mM HEPES-KOH, 1 M KCl, 5 mM EDTA, 2.5 mg/mL bovine serum albumin fraction V-BSA, and pH 8.0). The suspension was added to the slide, covered with coverslip, and incubated for 45 (ENDO III) and 30 minutes (FPG) at 37°C. Subsequent steps were the same as in the alkaline version of comet assay.

2.4. Susceptibility to DNA Damage. For the assessment of susceptibility to exogenous DNA damage, whole blood cells were treated with the alkylating agent methyl methanesulphonate—MMS (8×10^{-5} M) for 1 h or 3 h at 37°C prior to slides preparation. The percentage of residual DNA damage after 3-hour MMS treatment was calculated using the value of 1-hour MMS treatment for each subject as 100%. MMS is direct DNA alkylation agent widely used as positive control in genotoxicity testing.

2.5. Measurement of Lipid Peroxidation. Lipid peroxidation was monitored by the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, which has been widely adopted as a sensitive method for measuring lipid peroxidation. First, 1000 μL of 5% trichloroacetic acid were added to 250 μL of supernatants and centrifuged at 7000 ×g for 10 min. Then, 1000 μL of sulfuric acid (3M) were mixed with 1000 μL of thiobarbituric acid solution. The reaction mixture was incubated in a boiling water bath for 15 minutes and cooled at room temperature. Then, 3500 μL of *n*-butanol were added and centrifuged at 7000 ×g for 5 minutes. The absorbance was read at 532 nm [10]. Results were expressed as nmol/g of protein. Total protein levels were evaluated using the Biuret Total Proteins kit from Labtest (Labtest Diagnostica S.A., Brazil).

2.6. Buccal Micronucleus Cytome Assay (BMCyt). Buccal cell samples were collected and processed in accordance with Thomas et al. [23]. For each subject were prepared two tube containers for left cheek (LC) and right cheek (RC) cells, each containing 1000 μL of methanol. The cells were collected rotating a cytobrush 20 times in a circular and spiral motion against the inner surface of the cheek wall. The head of cytobrush was placed into the respective buffer, thereby producing a cloudy suspension of buccal cells. The cell suspensions were stored at 4°C, until processing. Afterwards, the cells were centrifuged for 3 minutes at 315 ×g at room temperature. The supernatant was aspirate leaving approximately 300 μL of cell suspension and replaced with 500 μL of buccal cell fixation buffer. The cells were briefly vortexed and centrifuged again for 3 min at 315 ×g at room temperature. The supernatant was aspirated and the cells resuspended in another 500 μL of buccal cell fixation buffer. For further cellular disaggregation and slide preparations, 5 μL of DMSO was added to each 100 μL of cell suspension. The fixed cells were hydrolyzed in HCl and stained according to the *Feulgen* method [23]. The scoring criteria for the distinct cell types and nuclear anomalies in the BMCyt assay were intended for classifying buccal cells into categories that distinguish between "normal" cells (basal cell) and cells that are considered "abnormal" on the basis of cytological and nuclear features, which are indicative of DNA damage (micronucleated: MN; nuclear bud: BUD), cytokinesis failure (binucleated: BI), or cell death (condensed chromatin: CC; karyorrhectic: KR; pyknotic: PY; karyolitic: KL). The 2,000 cells per sample were scored to determine the frequency of these cell types [23].

2.7. Statistical Analysis. The statistical analyses were performed using the statistical package SPSS 18.0 and $P < 0.05$ was considered statistically significant. Categorical variables were compared using χ^2 test. The comparison among multiple groups was performed by one-way analysis of variance (ANOVA) with post hoc Tukey's multiple comparison test and Kruskal-Wallis test with post hoc Dunn's multiple comparison test. The correlations between the parameters studied were evaluated by Spearman's test.

3. Results

The general characteristics of the COPD, PE-COPD, and control groups are shown in Table 1. The COPD patients and matched controls were similar in terms of age, gender, race, and comorbidities but differed in relation to body mass index (BMI), smoking status, cigarettes smoked per year, and smoking duration. The smoking-related variables were higher in the COPD patients.

The pulmonary function and comet assay results are shown in Table 2. The basal DI in the alkaline comet assay (detects DNA single- and double-strand breaks and alkali-labile sites) and in the neutral comet assay (detects DNA double-strand breaks) was significantly elevated in PE-COPD in relation to the COPD patients. Also, modified comet assay (with FPG and ENDO III enzymes) showed significantly higher damage index in PE-COPD than in COPD and control groups.

The DNA damage index after 3 h MMS treatment decreased in relation to the 1 h MMS treatment value in the control group (Table 2). In contrast, the damage index at 3 h MMS treatment remained constant in PE-COPD and increased in COPD group in relation to the 1 h value. To investigate these differences, the changes in DI after 3 h treatment in relation to the 1 h DI value were calculated for each person. This parameter, denominated residual DNA damage, showed that the DNA damage detected after 3 h treatment in PE-COPD does not increase in relation to the 1 h value (considered as 100%) as observed in COPD patients (Figure 1). The residual DNA damage values were lower in control and PE-COPD groups than in COPD group.

As observed in Figure 2, the PE-COPD group showed a significant decrease of TBARS compared to COPD patients. Mean frequencies of basal buccal cells, cells with micronuclei (MN), nuclear buds (BUD), and condensed chromatin (CC) in patients and controls are shown in Figure 3. No difference was found for the frequency of MN, BUD, and CC in PE-COPD group in relation to the control group, whereas increased BUD and CC formation were observed in

Table 1: General and clinical characteristic in the COPD patients and control group.

Characteristic	COPD ($n = 36$)	PE-COPD ($n = 15$)	Control ($n = 51$)	$^{*}P$ value
Sex				
Males, n (%)	21 (58)	9 (60)	28 (55)	NS
Females, n (%)	15 (42)	6 (40)	23 (45)	NS
White ethnicity, n (%)	32 (89)	13 (87)	50 (98)	NS
Age (years)[a]	65.36 ± 9.48	65.27 ± 7.65	63.61 ± 9.39	NS
BMI (kg/m^2)[a]	26.88 ± 5.61	23.01 ± 5.14*	26.81 ± 3.88	0.025
Smoking habit				
Cigarettes-year[b]	10698 (1095–25550)$^{\#}$	7924 (1095–21900)	5814 (1095–14600)	NS
Smoking status				
Never/former/current	4/21/11$^{\#}$	1/13/1	22/25/4	NS
Smoking duration				
>30 years, n (%)	27 (84)$^{\#}$	9 (64)	9 (31)	NS
Medications				
COPD drugs, n (%)	33 (92)	15 (100)	—	NS
Corticosteroid, n (%)	16 (66)	20 (74)	—	NS
Antihypertensive, n (%)	22 (61)	5 (32)	27 (53)	NS
Oxygen therapy, n (%)	7 (20)	5 (34)	—	NS
Comorbidities	26 (71)	19 (70)	38 (74)	NS
SAH, n (%)	10	8	11	NS
Heart disease, n (%)	5	5	2	—
Diabetes, n (%)	4	4	1	—
COPD status				
Mild, n (%)	7 (20)	1 (6)	—	NS
Moderate, n (%)	10 (28)	6 (40)	—	NS
Severe, n (%)	12 (33)	4 (27)	—	NS
Very severe, n (%)	7 (19)	4 (27)	—	NS

[a]Data are presented as mean ± SD; [b]median (minimum–maximum); BMI: body mass index; SAH: systemic arterial hypertension; $^{*}P < 0.05$, COPD compared with the PE-COPD; $^{\#}P < 0.05$, COPD or PE-COPD compared with the control group.

Table 2: Pulmonary function and comet assay results in the COPD, PE-COPD patients, and control group.

Characteristics	COPD ($n = 36$)	PE-COPD ($n = 15$)	Control ($n = 51$)	$^{*}P$ value
FEV_1 (% predicted)	41.78 ± 18.60$^{\#}$	45.60 ± 20.42$^{\#}$	86.14 ± 11.72	NS
FVC (% predicted)	60.28 ± 19.34$^{\#}$	66.80 ± 15.04$^{\#}$	90.75 ± 13.67	NS
FEV_1/FVC (% predicted)	69.11 ± 20.23$^{\#}$	65.07 ± 18.17$^{\#}$	105.24 ± 70.28	NS
Damage index—alkaline comet assay	32.28 ± 24.96	47.33 ± 24.03$^{\#}$	26.65 ± 27.96	NS
Damage index—1 h of MMS treatment	131.67 ± 64.54	81.80 ± 23.19*	103.12 ± 60.38	0.019
Damage index—3 h of MMS treatment	190.47 ± 105.17$^{\#}$	83.13 ± 43.58*	60.61 ± 61.45	0.000
Damage index—neutral comet assay	38.47 ± 32.59	68.27 ± 19.70*	37.49 ± 38.05	0.001
Damage index—FPG comet assay	37.78 ± 26.05	71.60 ± 23.61$^{*\#}$	39.78 ± 23.60	0.001
Damage index—ENDO III comet assay	38.94 ± 26.70	70.67 ± 16.72$^{*\#}$	40.15 ± 34.04	0.002

Data are presented as mean ± SD; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $^{*}P < 0.05$, COPD compared with the PE-COPD; $^{\#}P < 0.05$, COPD or PE-COPD compared with the control group.

COPD patients. Both COPD and PE-COPD groups showed increment of basal cells in relation to the control group, which is more pronounced in the physical exercise group.

The results of the comet assay and TBARS in the COPD groups stratified by disease status (Table 3) do not show different pattern of the respective group before stratification. No significant difference was found in BMCyt analysis for COPD patients stratified by disease status, probably due to the small sample size.

Table 4 shows the correlations between the parameters studied in patients and controls.

Disease indicators FEV_1 and FEV_1/FVC correlate negatively with the frequency of karyorrhectic cells (apoptosis cells) in PE-COPD and COPD patients and with the frequency of BUD in COPD patients suggesting that patients with more severe COPD present an increment in cytogenetic damage and early cell death by apoptosis. The basal DI in the neutral comet assay (detecting double-strand breaks) in

Table 3: DNA damage and TBARS in the COPD and PE-COPD by status diseases.

Disease status	Group	Damage index-comet assay					TBARS[a]
		Neutral	Alkaline	FPG	ENDO III	Residual damage	
Mild and moderated	COPD (n = 17)	24.80 ± 24.69	24.20 ± 19.14	11.53 ± 12.68	13.33 ± 13.82	160.53 ± 90.23	11.41 ± 4.10
	PE-COPD (n = 07)	72.14 ± 25.47	58.43 ± 27.59	26.57 ± 15.89	34.14 ± 25.02	94.71 ± 26.76	6.35 ± 4.24
	P value	**0.024**	**0.028**	**0.022**	**NS**	**NS**	**0.007**
Severe	COPD (n = 12)	31.50 ± 29.07	27.25 ± 22.67	6.36 ± 7.65	17.50 ± 21.36	160.25 ± 70.36	13.43 ± 3.64
	PE-COPD (n = 04)	59.00 ± 17.20	39.25 ± 18.02	28.50 ± 19.74	37.25 ± 7.58	120.75 ± 86.06	6.05 ± 4.01
	P value	**NS**	**NS**	**0.004**	**0.049**	**NS**	**0.031**
Very severe	COPD (n = 07)	55.50 ± 24.88	40.17 ± 31.30	28.50 ± 27.07	13.00 ± 16.22	196.00 ± 68.02	10.85 ± 4.98
	PE-COPD (n = 04)	74.50 ± 4.50	36.00 ± 17.32	45.25 ± 31.71	39.50 ± 27.76	104.75 ± 38.42	8.47 ± 5.69
	P value	**NS**	**NS**	**0.027**	**NS**	**NS**	**NS**

Data are presented as mean ± SD; [a]nmol/mg of protein; n: sample numbers; NS: not significant.

Table 4: Correlations between the parameters analyzed in patients and controls.

Parameters	COPD		PE-COPD	
	Spearman's rho	P value	Spearman's rho	P value
FEV_1/FVC—BUD	−0.385	**0.027**	−0.385	0.210
FEV_1/FVC—KR	−0.399	**0.021**	−0.611	**0.016**
FEV_1—KR	−0.277	0.119	0.527	**0.043**
Basal DI in neutral comet assay—BUD	0.360	**0.039**	0.524	**0.045**
DI following 3 h MMS treatment—KL	−0.551	**0.001**	0.172	0.539
% Residual damage—KR	0.528	**0.002**	0.183	0.514

FEV_1/FVC: ratio of forced expiratory volume in one second to forced vital capacity; BUD: nuclear buds; KR: karyorrhectic cells; KL: karyolytic cells; DI: damage index; MMS: methyl methanesulphonate; % residual damage, calculated for DI after 3 h MMS treatment for each subject taking the value of DI after 1 h MMS treatment as 100%.

COPD and PE-COPD patients correlates positively with BUD induction, suggesting that the kind of DNA damage occurred could progress to permanent DNA damage. Also, in COPD group the percentage of residual damage correlates positively with the frequency of KR cells reinforcing the induction of apoptosis in the pathogenesis of COPD.

4. Discussion

The pathogenesis of COPD leads to an increase in the oxidative burst in lungs, and evidence suggest that this increase is implicated in pathogenic processes such as damage to pulmonary cells, mucus hypersecretion, antiprotease inactivation, and exacerbation of lung inflammation [2]. Recently, it has been suggested that the oxidative stress may extend beyond the lung to generate the systemic manifestation of COPD [16]. Regular physical exercise, which has been proven to increase mean life span, could also serve as a stimulating stressor [24]. However, mounting epidemiological data have proven that exercise decreases the incidence of oxidative stress associated diseases [24]. Previous study showed that muscle oxidative stress levels would be increased in severe COPD only in the initial phase of adaptation to training (first 2 or 3 weeks), probably in the context of a transient antioxidant insufficiency. Indeed, abnormal adaptations to keeping redox balance have been reported in other series of COPD patients [16]. This argument is also consistent with existing literature data showing that long-term exercise training programs facilitate physiological changes, decreasing the impact on systemic oxidative stress [16].

Our results showed increased DNA damage in PE-COPD patients (Table 2), which disagree with the data reported by Mercken et al. [15]. These authors observed a decrease in ROS-induced DNA damage after submaximal exercise test following pulmonary rehabilitation. The phenomenon was attributed to an improved training status of the patients with COPD that increased exercise capacity and physical activity level after pulmonary rehabilitation [15]. Another study reported that the extent of DNA damage was dependent on the training status of the healthy participants, as trained participants had less DNA damage after exercise compared with untrained participants [25].

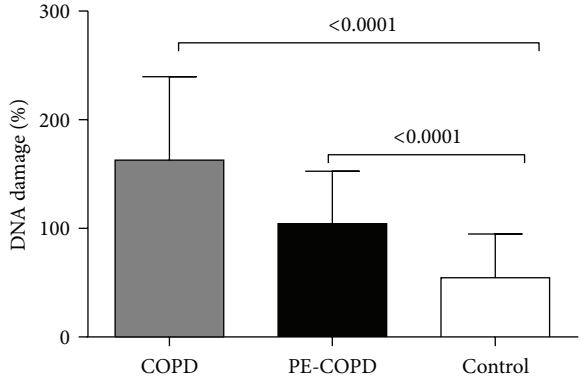

Figure 1: Residual DNA damage in blood cells of patients and healthy controls following 3 h MMS treatment. The percentage of residual DNA damage after 3 h MMS treatment was calculated considering the value of 1 h MMS treatment for each subject as 100%. Data are presented as mean ± SD, COPD patients—gray bars, PE-COPD patients—black bars, and controls—white bars. Statistical analysis was performed by Kruskal-Wallis test with post hoc Dunn's multiple comparison test.

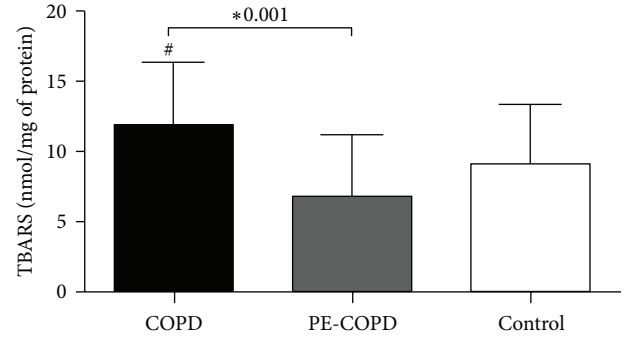

Figure 2: Concentration of TBARS in blood plasma of patients and healthy controls. Data are presented as mean ± SD, COPD patients—gray bars, PE-COPD patients—black bars, and controls—white bars. $^{*}P < 0.001$; COPD compared with the PE-COPD; $^{\#}P < 0.05$, COPD and PE-COPD compared with the control group. Statistical analysis was performed by Kruskal-Wallis test with post hoc Dunn's multiple comparison test.

Also, an exercise-induced DNA damage in athletes after exhaustive treadmill running or a marathon race has been reported, whereas no enhanced DNA damage was found in healthy control subjects after moderate exercise, not exceeding the anaerobic threshold [15]. Regarding to this aspect, it has been suggested that DNA damage after physical activity can only be detected in exercise protocols exceeding the proband's anaerobic threshold [26], and the intensity of physical activity may determine unwanted metabolic changes. Light-to-moderate physical activity has been described as an inducer of transient redox unbalance [27]. In our study, PE-COPD practiced submaximal aerobic exercise in ergometer for lower limbs, 02 times per week, that is, light-to-moderate physical activity.

In the present study, the MMS-induced DNA damage was transient in control group, probably due to induction of DNA repair process. Interestingly, PE-COPD group showed decreased susceptibility to 1 h MMS treatment compared to COPD and control groups. Moreover, the DNA damage in PE-COPD patients remains constant after 3 h MMS treatment, suggesting that the observed low susceptibility may result from previously activated repair process. The residual damage caused by MMS treatment in the control group was also lower than in COPD patients, which could indicate ongoing repair process (Figure 1). For COPD group, the residual DNA damage further increased indicating the induction of persistent DNA damage that could reflect DNA repair inhibition. This DNA damage may be transient and repaired later [27] or the damaged cells could be eliminated by apoptosis. The high standard deviations for the DNA damage values in comet assay found in our study could be attributed to the great genetic heterogeneity, which is a critical aspect in human biomonitoring studies [28].

The values of TBARS were significantly lower in PE-COPD than in COPD group indicating an adaptive cellular response to exercise-induced oxidative stress (Figure 2). These data are in agreement with those of Radak et al. [24] showing that regular exercise-induced adaptation attenuates the age-associated increase in the levels of 8-OHdG in muscle cells and increases the activity of DNA repair as well as a resistance against oxidative stress. Our results also showed a decrease in residual damage and TBARS induction in PE-COPD in all disease stages in relation to COPD (Table 3), suggesting an adaptive response induced by the physical exercise. In this context, the increased strand break formation, detected in all modalities of comet assay in PE-COPD in relation to COPD, could reflect DNA strand breaks formed as intermediates of increased repair activity in the PE-COPD.

On the other hand, it has been suggested that DNA damage detected in COPD patients is possibly not directly induced by reactive oxygen species but is mediated by secondary factors [26]. This effect can be attributed to the differences in pulmonary ventilation between the maximal and submaximal exercise tests [15]. As the ventilatory demands increase more during the maximal than submaximal exercise tests, COPD patients tend to take more shallow breaths and to breathe with higher frequency, resulting in dynamic lung hyperinflation. This results in increased physiologic dead space ventilation with an attendant drop in alveolar oxygen partial pressure, causing hypoxia and DNA damage [15].

For the first time, our study showed that the supervised physical exercise during pulmonary rehabilitation for COPD patients decreased the susceptibility to exogenous mutagens and systemic exercise-induced oxidative stress. The observed improvement could be attributed to exercise-induced adaptive responses involving a more efficient oxidative metabolism, an increased capacity of endogenous antioxidative systems, interference with the oxidative damage repair/eliminating systems, and modulation of redox-sensitive gene expression and protein assembly [15, 24]. The ROS formation during exercise evokes specific adaptation, such as increased antioxidant capacity/oxidative damage-repairing enzyme activity, increased resistance to oxidative stress, and lower levels of oxidative damage. This specific

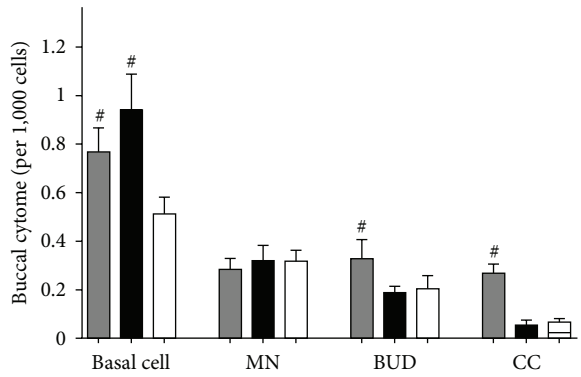

Figure 3: Frequency of buccal cell types scored in the BMCyt assay of patients and healthy controls. Data are presented as mean ± SD, COPD patients—gray bars, PE-COPD patients—black, and controls—white bars. MN: micronuclei; BUD: nuclear buds; CC: condensed chromatin cells. $^{\#}P < 0.05$, COPD and PE-COPD compared with the control group. Statistical analysis was performed by Kruskal-Wallis test with post hoc Dunn's multiple comparison test.

adaptation seems to be systemic [24]. The effects of exercise could modulate the regulation of the DNA repair process. The adaptive mechanism is initiated by transcription factors, resulting in increased level of antioxidant enzymes, more effective repair, and proteasome degradation of damaged proteins. The molecular adaptation then leads to an improved physiological function and enhanced resistance to oxidative stress [24].

The alteration of repair efficiency has already been reported in breast and lung cancers [29–31]. In some cases, low basal DNA damage in comet assay could indicate toxic condition to the cells (i.e., chronic oxidative stress) leading to a decrease in genotoxicity [29]. Such decrease in basal DNA damage in lymphocytes (lower than in controls) was associated with repair inhibition and possible elimination of highly damaged cells by apoptosis [29].

The hypothesis of apoptosis induction is supported by the results obtained in this study in BMCyt assay (Figure 3) that showed higher frequency of BUD and CC in buccal cells of COPD patients, which could indicate a deficiency in DNA repair and early apoptosis of damaged cells. The presence of BUD cells suggests the elimination of amplified DNA or DNA repair complexes from the nucleus, whereas CC cells are indicative of early stage of apoptosis [23]. Altered repair capacity in COPD patients is also evidenced by the enhanced susceptibility to MMS-induced DNA damage detected in our study by comet assay.

The correlations observed between the comet assay and BMCyt assay data (Table 4) confirmed that the deterioration of pulmonary function correlates with increased frequency of karyolytic cells (indicating death cell) and formation of cells with BUD (indicating less efficient DNA repair). Moreover, the BUD frequency in COPD patients correlates positively with the DI in neutral comet assay, indicating the involvement of DNA double-strand breaks formation in this process. In conditions of repair inhibition, the cells with DNA damaged could be eliminated by apoptosis. An increased apoptosis in COPD patients has been detected to persist even after smoking cessation [32], and disturbed balance between apoptosis and structural regeneration in lung cells contributes to the pathogenesis of COPD [33, 34].

In conclusion, our results showed that PE-COPD patients presented significantly elevated basal DNA damage in peripheral blood cells, as detected by the comet assay, in relation to the COPD and control groups. However, PE-COPD patients were less susceptible to exogenous DNA damage induction by MMS treatment and did not present an elevated frequency of BUD and CC cells as noted in COPD patients. Furthermore, it is possible to conclude that physical exercise for COPD leads to a significant decrease of lipid peroxidation in blood plasma, decreased formation of BUD and CC buccal cells, and more effective DNA repair.

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