The effects of pulmonary rehabilitation program on oxidative stress biomarker and antioxidant enzymes activities in chronic obstructive pulmonary disease patients and in healthy subjects

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of pulmonary rehabilitation on oxidative stress biomarker (Thiobarbituric acid substances “TBARS”) and antioxidant enzymes activities (superoxide dismutase “SOD”, glutathione peroxidise “GPx” and catalase “CAT”) in patients with chronic obstructive pulmonary disease (COPD) compared to healthy subjects.

Material and Methods: The study included 26 patients with COPD and 12 healthy subjects. The patients were divided into 2 groups (16 trained and 10 untrained). Trained groups performed 45 min individualized exercise training enrolled to an 8 weeks pulmonary rehabilitation program 3 times a week. Prior to and after the programme, exercise testing and pulmonary function were evaluated. Antioxidant enzymes activities were measured by spectrophotometry and spectrofluorimetry was used for plasma levels of thiobarbituric acid substances.

Results: After the program, exercise capacity improved significantly in trained groups. At baseline, plasma TBARS were significantly increased in patients with COPD compared to the healthy subjects (p<0.05). Baseline values of SOD and CAT activities were significantly lower in patients with COPD than in healthy subjects. After rehabilitation, there were no significant differences in plasma TBARS between groups. However, SOD activity increased significantly in trained patients and in healthy subjects. GPx activity increased only in trained patients. There was no significant change for CAT activity for all groups.

Conclusions: This study suggests that pulmonary rehabilitation program with moderate physical training can activate antioxidant defence and regulate systemic oxidative stress release.

Keywords: Chronic obstructive pulmonary disease; healthy subjects; pulmonary rehabilitation; oxidative stress biomarker; antioxidant enzymes
INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major worldwide health problem that has an increasing prevalence and mortality (1). Oxidative stress, which can be defined as an increased exposure to oxidants and/or decreased antioxidant capacities, is widely recognized as a central feature of many diseases (2). Considerable evidence now links COPD with increased oxidative stress. Markers of oxidative stress are increasing even further during exacerbations of COPD and in patients with very severe form of this disease (3). At the same time, the antioxidant mechanisms attenuated in these patients, as indicated by reduced glutathione levels in the lungs (3), reduced glutathione peroxidase (GPx) activity in erythrocytes and lower antioxidant capacity in plasma (4,3). Regular exercise training associated with numerous physiological adaptations of the body, and consequently with numerous health benefits for exercisers. The decreasing of a wide range of reactive oxygen species associated diseases is one of the many health-related benefits of regular exercise. It is thought that this preventive effect of regular exercise, at least in part, is due to oxidative stress-induced adaptation (5). The oxidative challenge related adaptive process of exercise is systemic and includes increased antioxidant/damage repair enzyme activity, lower oxidative damage, and increased resistance to oxidative stress, due to the changes in redox homeostasis(5).

A major component of pulmonary rehabilitation to improve physical performance and health-related quality of life is exercise training (1). Regular exercise training in chronic heart failure (6,7) and coronary artery disease (8) has been shown to decrease lipid peroxidation and nitrotyrosine formation (6) as well as to promote an upregulation in antioxidant defence, evident by an increase in the activity of superoxide dismutase (SOD)(8), glutathione peroxidise (GPx) and catalase (CAT)(6).

In opposition to the above results, negative findings have been noted in a few exercise training studies using COPD patients, evident by decreases in reduced glutathione and increases in oxidized glutathione (9) and lipid peroxidation (10). For that reason, it is important to understand whether or not exercise training during a pulmonary rehabilitation program can modify oxidative stress biomarkers and antioxidant enzymes activities. Therefore, the aim of this study was to evaluate the effects of pulmonary rehabilitation on oxidative stress biomarker (Thiobarbituric acid substances “TBARS”) and antioxidant enzymes activities (SOD, GPx and CAT) in patients with COPD compared to healthy subjects.

MATERIAL AND METHODS

Subjects

Twenty six patients with stage II–III disease according to the Global Initiative for Chronic Obstructive Lung Disease guidelines were consecutively recruited on admission to the department of physiology and lung testing and participated in an 8 weeks pulmonary rehabilitation program (1). All patients were ex-smokers, were not depleted, did not use oxygen supplementation, and had not experienced respiratory tract infection or exacerbation of their disease for at least 4 wk before the study. Exclusion criteria were no other chronic diseases, such as rheumatoid arthritis and chronic colitis. Also, patients with diabetes, cardiovascular diseases, renal diseases, liver diseases, or mental diseases were excluded from the study. Twelve healthy, age-matched, non-smoking participants were recruited as the healthy trained group. In addition, all participants were questioned on their dietary habits to ensure that none were taking antioxidants or vitamin supplements. All patients and healthy subjects gave a written consent to include in the study, which was approved by the medical ethics committee of “Farhat Hached” Hospital.

Experiment protocol

Before and after rehabilitation program, the participants underwent spirometry using a whole body plethysmograph. Exercise capacity was evaluated by an exercise testing in cycle ergometer (Ergoline, Bitz, Germany) using a gas analysis system (ZAN 600, Ergotest, ZAN Meßgrate GmbH, Germany). The participants
were required to participate to an 8 weeks pulmonary rehabilitation program 3 days per week. They received the same exercise training and education program. This consisted of two sessions of 30min/wk of seminars and discussions covering the following topics: relaxation, disease education, benefits advice, energy conversation, medication advice, chest clearance, and breathing control techniques. The training schedule was the same in the two groups, consisting of a 5 min warm-up followed by 10min of work and 5 min of active recovery, repeated over a 45 min session. Each subject was trained by an individualized program according to its target heart rate corresponding to the gas exchange threshold (40% \( \text{VO}_{2}\text{max} \) for COPD patients and 49% \( \text{VO}_{2}\text{max} \) for healthy subjects). Subjects were instructed to perform a stationary bicycle exercise using cycle ergometer (Ergomedic 828E, Monark, Sweden) and the warm-up, cool-down, and upper extremity exercises. During training, heart rate was continuously monitored by means of a cardiofrequency meter (Polar, S810). The cardiofrequency meter was set in such a way that subject could exercise within ± 5 beats/min of prescribed intensity. An alarm insured that the subject trained within the preselected range.

**Blood collection**

Fasting blood samples obtained by venepuncture were drawn into heparinized tubes. Red blood cells and plasma were separated by centrifugation at 3600 rpm for 10 min, coded and stored at −80 °C until analysis. All samples for all parameters studied were assayed in duplicate. Biochemical assays were conducted blind to clinical information. The biochemist did not know whether blood samples were from trained or untrained patients, or healthy subjects.

**Measurement of plasma TBARS**

Plasma TBARS were measured according to the method of Satoch (11) and Yagi (12) which is based on the thiobarbituric acid method. TBARS were expressed as \( \mu \text{mol/liter} \).

**Measurement of the antioxidant enzymes activities**

SOD activity was measured by the method of Marklund and Marklund (13). GPx activity was measured using the method of Gunzler et al (14), and to determine CAT activity Aebi's spectrophotometric method was used as described by Beers and Sizer (15). Results were expressed as U/g Hb.

**Statistical analysis**

The statistical analysis was performed with Statistica (Kernel Version 6. Stat Soft. France). Results are expressed as the means ± standard deviation. The data obtained for all antioxidants and oxidative stress markers were analyzed using a 2-ways analysis (ANOVA): 3 groups (trained COPD vs. Healthy subjects vs. COPD controls) X 2 times (baseline vs. after rehabilitation). Significant interactions and main effects were analyzed using Fisher LSD post hoc tests. When the 2-ways ANOVA showed no significant interaction (group X time) a Kruskal-Wallis ANOVA was performed and a Mann-Whitney test was used to compare every two groups. The effect of pulmonary rehabilitation was then tested using Wilcoxon signed-rank test (for paired samples). Finally, the correlations between plasma TBARS and RBC antioxidant enzyme (SOD, GPx and CAT) activities were calculated using Spearman Rank correlation coefficients. Differences were considered significant at \( p < 0.05 \).

**RESULTS**

**Subjects**

Characteristics of the study groups before and after rehabilitation are presented in Table 1. There were no significant differences in anthropometric data between the three groups. Pulmonary function and exercise capacity were impaired in the patients with COPD when compared to the healthy subjects (\( P < 0.001 \)). After rehabilitation program, there were no significant differences in pulmonary parameters within groups. Trained COPD and healthy subjects responded to the rehabilitation program
by a significant increase in peak $\dot{V}O_2$ ($P=0.012$) and peak workload ($P=0.039$). No significant change was found for heart rate and aerobic threshold ($P>0.05$).

**Plasma TBARS**

Figure 1 showed the effect of the rehabilitation program on plasma TBARS in healthy subjects and in patients with COPD. At baseline, plasma TBARS were significantly different between groups (1.51±0.46 µmol/L vs. 2.37±0.52 µmol/L vs. 2.2±0.57 µmol/L, respectively for healthy subjects, trained and untrained COPD, $P=0.01$). Healthy subjects had significantly lower plasma TBARS than trained COPD ($P<0.001$) and untrained COPD ($P<0.01$). After rehabilitation, plasma TBARS values increased slightly but not significantly in healthy subjects and trained COPD (1.51±0.46 µmol/L vs. 1.68±0.49 µmol/L, $P>0.05$ and 2.37±0.52 µmol/L vs 2.52±0.83 µmol/L, $P>0.05$ respectively).

**Antioxidant enzyme activities**

Antioxidant enzyme activities (SOD, GPx and CAT) before and after rehabilitation program are presented respectively in Figures 2, 3 and 4. Baseline levels showed that SOD and CAT activities were higher in the healthy subjects than trained and untrained COPD patients (32.67±12.15U/mgHb vs. 17.49±16.24 U/mgHb vs. 20.43±6.13 U/mgHb, $P=0.0033$ and, 245.93±47.47U/gHb vs. 182.74±26.53U/gHb vs. 190.60±64.97U/gHb, $P=0.0002$ respectively for SOD and CAT activities). GPx activity did not differ significantly between groups (50±20.55U/gHb vs.37±20.38U/gHb vs. 43.63±28.04U/gHb, $P=0.129$ respectively for healthy subjects trained and untrained COPD patients). After rehabilitation, GPx and SOD activities showed significant differences between groups (54.98±10.15µmol/L vs. 46.36±18.33µmol/L vs. 40.97±16.06µmol/L, $P=0.0001$ and, 32.67±12.15µmol/L vs. 24.69±5.1µmol/L vs. 23.7±4.2µmol/L, $P=0.0002$, respectively for healthy subjects, trained and untrained COPD patients).

### Table 1. Characteristics of patients with COPD and healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n=12)</th>
<th>Trained COPD (n=16)</th>
<th>Untrained COPD (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age; Yr</td>
<td>58 ± 7</td>
<td>59 ± 6</td>
<td>61±1</td>
</tr>
<tr>
<td>BMI; kg/m$^2$</td>
<td>26.3 ± 1.2</td>
<td>24.6± 5.1</td>
<td>23.7± 4.2</td>
</tr>
<tr>
<td>FEV$_1$,% Predicted</td>
<td>88.66 ± 9.0</td>
<td>43.67 ± 8.45</td>
<td>45.15±9.9</td>
</tr>
<tr>
<td>FVC,% Predicted</td>
<td>82.75 ± 13.29</td>
<td>51.34 ± 10.8</td>
<td>49.45±12.2</td>
</tr>
<tr>
<td>FVC /FEV$_1$ Ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>82.8± 9</td>
<td>55.1 ± 4</td>
<td>56.9±2</td>
</tr>
<tr>
<td>Post</td>
<td>84.2± 10</td>
<td>58.6 ± 7</td>
<td>57.3±5</td>
</tr>
<tr>
<td>TLC%</td>
<td>113 ± 9</td>
<td>133 ± 18</td>
<td>129 ± 14</td>
</tr>
<tr>
<td>$\dot{V}O_2$ Peak (ml/min/Kg)</td>
<td></td>
<td></td>
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<tr>
<td>Before</td>
<td>29.03 ± 6.15</td>
<td>25.14±4.02$^\tau$</td>
<td>24.35±1.89$^\prime$</td>
</tr>
<tr>
<td>After</td>
<td>32.6±4.32</td>
<td>27.21±3.12</td>
<td>-</td>
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<tr>
<td>Peak workload (W)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Before</td>
<td>187.4±14.2$^\prime$</td>
<td>94.6±18.1$^\prime$</td>
<td>90.4±22.3$^\prime$</td>
</tr>
<tr>
<td>After</td>
<td>202.13±22.01</td>
<td>113.2±12.5</td>
<td>-</td>
</tr>
<tr>
<td>HR $AT$ (bat/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>132 ± 10.23</td>
<td>108.5±12.1$^\prime$</td>
<td>113±8.4$^\prime$</td>
</tr>
<tr>
<td>After</td>
<td>140±14.51$^\tau$</td>
<td>111±18.79</td>
<td>-</td>
</tr>
<tr>
<td>Peak HR (bat/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>156±18</td>
<td>136±5</td>
<td>129±6$^c$</td>
</tr>
<tr>
<td>After</td>
<td>152±24</td>
<td>131±7</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means ± SD, BMI: body mass index; FEV$_1$: forced expiratory volume in 1 second; FVC: forced vital capacity; HR $AT$: heart rate (Anaerobic threshold); $\dot{V}O_2$peak: oxygen uptake; TLC: total lung capacity; Before/After rehabilitation program. Significantly different when compared before and after rehabilitation program of the same group (p<0.05), $^\prime$ Significantly different when compared before and after rehabilitation program between group (p<0.05)
However, CAT activity did not differ between groups (235.93±19.74U/gHb vs. 203.58±32.72U/gHb vs. 184.65±16.97U/gHb, P=0.067, respectively for healthy subjects, trained and untrained COPD patients).

The values of GPx activity increased significantly in trained COPD (37±20.38U/gHb vs. 46.36±18.33 U/gHb, P=0.034). A significant increase was also showed for SOD activity in both healthy subjects and trained COPD (32.67±12.15 U/mgHb vs. 42.98±16.06U/mgHb, P=0.04 and 17.49±16.24 U/mgHb vs. 37.08±7.53 U/mgHb, P=0.025, respectively).

CAT activity increased slightly but not significantly in trained COPD.

Correlations between TBARS and antioxidant enzyme activities

No correlations were found between plasma TBARS and antioxidant enzyme activities (SOD, GPx and CAT) in the study groups before and after rehabilitation program. Moreover, after rehabilitation program, GPx activity correlated positively with CAT activity in trained COPD patients (r=0.28, p=0)

![Figure 1: Plasma TBARS concentrations of healthy subjects, trained and untrained COPD patients before and after rehabilitation program.](image)

![Figure 2: Superoxide dismutase (SOD) activity of healthy subjects, trained and untrained COPD patients before and after rehabilitation program.](image)

![Figure 3: Glutathione peroxidise (GPx) activity of healthy subjects, trained and untrained COPD patients before and after rehabilitation program.](image)

![Figure 4: Catalase (CAT) activity of healthy subjects, trained and untrained COPD patients before and after rehabilitation program.](image)
DISCUSSION

The aim of this study was to evaluate the effects of pulmonary rehabilitation on oxidative stress biomarker and antioxidant enzymes activities in patients with COPD compared to healthy subjects.

In the present study, our results show a significant increase in plasma TBARS levels as markers of oxidative stress in COPD patients compared to the healthy subjects. This result is in agreement with those of Mercken et al. (16), who revealed a significant increase in resting plasma malondialdehyde (MDA) levels in patients with COPD compared with age-matched healthy subjects. Others studies showed elevated levels of other markers of lipid peroxidation such as urinary and plasma concentrations of 8-8 isoprostane, urinary MDA (16), exhaled ethane in patients with COPD (3). As a result of oxidant stress, lipid peroxides are formed due the peroxidation of unsaturated fatty acids present on cell membranes. Our observation of increased levels of products of lipid peroxidation in plasma, together with a fall in antioxidant capacity, strongly supports the contention that there is increased oxidative stress in patients with stable COPD.

After rehabilitation, levels of plasma TBARS did not change significantly in patients with COPD and in healthy subjects. Thus, plasma TBARS were not impaired by training (17). This result is in disagreement with those of Pinho et al (10) who showed a significant increase in resting plasma TBARS after the exercise training program. In deed, it should be noted that exercise training is not always associated with a change in baseline levels of lipid peroxidation indices, as noted in plasma (18), erythrocytes (19,20), and skeletal muscle (21,22). The effects of exercise training on oxidative stress are not clear. The magnitude of oxidative damage may be related to the power of the pro-oxidant attack (intensity and duration of physical exercise) and capacity of the individual exerciser’s antioxidant system (23). On the other hand, lipid peroxidation by measurement of plasma TBARS, which is a nonspecific oxidative stress index that should be used with caution. We used a fluorometric method, which was sufficiently sensitive and reproducible to provide a valid estimation of oxidative stress as previously discussed (24).

Regarding the enzymatic antioxidants, the results of this study show that concentrations of SOD and CAT were significantly lower in patients with COPD compared than in healthy subjects. However, GPx activity did not differ between groups. Different results were found for the antioxidative defences in COPD patients. Nadeem et al. showed that red cell antioxidant enzyme activities were altered, with GPx having lower, SOD having greater and CAT having similar activity in patients as compared to non-smoking healthy controls (4). Duthie et al. found lower GPx activities in erythrocytes of smokers compared to non-smokers (25). In contrast, Sadowska et al. showed an increase in GPx and SOD activities during exacerbation when compared with stable COPD patients (26). In addition, other reports have found that erythrocyte SOD activity was more elevated in COPD patients and in healthy smokers than in healthy non-smokers (27, 28, 3). In contrast, one study showed a reduction in erythrocyte SOD activity in COPD patients, probably because of an increase in consumption of antioxidants (29). A decreased activity of SOD and GPx was also found in alveolar macrophages of elderly smokers when compared with nonsmokers (30). However, in Chinese patients with COPD, we found no differences in erythrocyte SOD activity but elevated erythrocyte catalase activity when compared to healthy Chinese smokers matched for age and pack-years smoked (31). The discrepancy in findings could be due to several factors, such as differences in the techniques used to measure the antioxidant enzyme levels, differences in the materials tested (erythrocytes vs. plasma vs. serum), sampling of patients at different stages of disease progression, different course of illness, different ethnic origin, lifestyle or dietary pattern.

Regarding the effect of rehabilitation program on enzymatic antioxidants, our results show a significant increase in the SOD activity for both trained groups, GPx activity increased only in trained patients and CAT activity did not change.
after the program. The studies on the effect of exercise training enrolled to a rehabilitation program on antioxidant status are also rare and contradictory. These findings are in agreement with several previous studies in rodents (32), healthy (33) and coronary artery diseased humans (6,8) demonstrating an improvement in antioxidative status after short-term exercise or long-term training. Based on this finding, we can assume that regular exercise can serve as a stimulus to allow for the upregulation in endogenous antioxidant defence, in much the same was as does some forms of exercise (34, 35). In deed, Naghizadeh et al. are in agreement with our results showing that SOD activity increased more than CAT activity after exercise (34). This supports that SOD, as the first line defence enzyme in red cells, is the enzyme whose activity most oftenly and easily changes due to regular exercise. Other studies reported unchanged SOD and GPx activities after intermittent sprint cycle training in healthy subjects (36), and in trained rats (20). Opposing, Leeuwenburgh et al. (37) found that a 10-week exercise program increased GPx and SOD activities in the deep portion of vastus lateralis muscle. It is well known that in exercise training, individual systemic oxidative stress is lower (38), while muscle antioxidant enzymes activities are higher when compared to the untrained subjects (5). The exercise training induced increase in the antioxidant protection in skeletal muscles is mainly dedicated to the up-regulation of the antioxidant enzymes content/activity such as mitochondrial SOD, GPx and g-glutamylcysteine synthetase via reactive oxygen species-dependent signalling pathways (5). In addition, improvement of succeeding antioxidant defence lines requests harder work and further improving of aerobic capacity. The exercise intensity level is crucial to induce adaptation processes and may explain the conflicting results of previous studies. Recently, it has been demonstrated that the moderate intensity exercise, in which small increase in reactive oxygen species production is observed (39), exerts beneficial effects on the antioxidant protection (23). As proposed by some authors (40, 23), the contradictory signalling function of reactive oxygen species might be explained on the basis of the hormesis theory (40) according to which a low dose of substance is stimulatory and a high dose of this substance is inhibitory. Hence, low level of reactive oxygen species production like during moderate intensity exercise (23) influence signalling pathways and can induce adaptive responses that protect against a subsequent stronger stress (40). Therefore, we postulate that the exercise training induced increase in the red cells SOD and GPx content, as demonstrated in our study was due to up-regulation of reactive oxygen species - dependent signalling pathways in response to the applied moderate activity.

Our study showed a positive correlation between GPx and CAT activities in trained COPD patients. This confirms that antioxidant enzymes act in a cooperative or synergistic way to ensure global cell protection (3). Indeed, SOD detoxifies superoxide radicals and converts them to hydrogen peroxide which is further converted to water by CAT and GPx.

The effects of rehabilitation program seem interestingly by increasing SOD activity which plays a protective role in preventing cells from peroxynitrite formation (41). In addition, the increase of GPx activity only in COPD patients appear to be related to the muscle wasting commonly observed in these individuals (42), which suggested that muscle wasted COPD may be more susceptible to oxidative stress markers attack and requires the intervention of other antioxidative enzymes. On the other hand, high GPx activity can protect against cells damage induced by free radicals and provides an effective protective mechanism against cytosolic injury because it eliminates hydrogen peroxide and lipid peroxides (3).

Several limitations should be considered when interpreting these results. First, local measurements of antioxidative status in muscle tissue are preferred over blood analysis, as oxidative stress is primarily induced by the mitochondria. However, indirect antioxidative status assessment from blood samples is commonly accepted and widely applied, while it also has important practical advantages (8). In addition, both SOD and GPx measurements are dependent on specific substrates (selenium and manganese, respectively), which are influenced by dietary intake. Participants must registered detailed food intake prior to baseline

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measurements, and were asked to keep this similar before and after rehabilitation program measurements. In the same way, the classical determination of TBARS is limited because is a nonspecific oxidative stress index that should be used with caution because under oxidative stress conditions, malondialdehyde, hydroperoxides and certain carbohydrates and amino acids may yield products that could react with thiobarbituric acid (43). Finally, the relatively small sample size unlikely confounded our results.

This study confirmed the imbalance between the antioxidant enzyme activities and the oxidative stress in patients with COPD which might play a role in the weakness related to these patients. Pulmonary rehabilitation with moderate physical training could be beneficial for patients with COPD by inducing changes in antioxidant activity able to regulate oxidative stress damage. Further analysis could explain responses to the exercise training in COPD patients and in healthy individuals.

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Conflict of interest

We have no conflict of interest.

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