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# Oxygen therapy at low flow causes oxidative stress in chronic obstructive pulmonary disease: Prevention by N-acetyl cysteine

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**Abstract**

Exposure to high oxygen concentration produces toxicity by free radical release. We aimed to study: whether stable chronic obstructive pulmonary disease (COPD) patients present an unbalance in the blood redox status; the effect of oxygen administration on blood redox balance; the efficacy of N-acetyl-cysteine (NAC) treatment against the oxidative stress-induced by oxygen administration and whether it is dose-related. To this, 45 stable state III COPD patients were recruited and reduced glutathione (GSH) and oxidised glutathione (GSSG) in erythrocytes and thiol proteins (P-SH) and carbonyl proteins (PC) in both erythrocytes and plasma were evaluated. All COPD patients underwent 2 l/m oxygen for 18 h and NAC at 1200 or 1800 mg/day or placebo for 48 h starting with oxygen administration. Blood samples were collected at basal conditions, after 8 and 18 h of oxygen administration and 24 h after oxygen withdrawal. *Results*: COPD patients present an unstable redox equilibrium mainly due to plasma sulphydryl protein depletion. Oxygen administration oxidize erythrocyte GSH, decrease P-SH and increase PC levels in both plasma and erythrocytes. NAC administration counteract the oxidative stress and at the highest dose completely prevent protein oxidation. In conclusion, stable state III COPD patients present an unstable redox balance; long term low flow oxygen administration induces systemic oxidative stress, which is prevented by NAC treatment.

**Keywords:** *GSH, GSSG, N-acetylcysteine, COPD, blood redox status, oxygen administration*

**Abbreviations:** *COPD, chronic obstructive pulmonary disease; $FEV_1$, forced expiratory volume in one second; FVC, forced vital capacity; GSH, reduced glutathione; GSSG, oxidised glutathione; NAC, N-acetyl-cysteine; PC, carbonyl proteins; P-SH, thiol proteins; RBC, red blood cells*

## Introduction

Oxidative stress, due to increased production of reactive oxygen species (ROS) and/or reduced antioxidant defences, is involved in the pathogenesis of several inflammatory pulmonary diseases [21]. There is mounting evidence that a lung oxidant/antioxidant imbalance may play an important pathophysiologic role in chronic obstructive pulmonary disease (COPD), where increased oxidative stress has been demonstrated in exhaled breath [24] as well as in plasma [27]. Inactivation of antiproteinases, airspace epithelial injury, increased sequestration of neutrophils in the pulmonary microvasculature, and gene expression of proinflammatory mediators are considered key events related to oxidative stress in COPD [4].

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Glutathione (GSH) is an ubiquitous, essential tripeptide containing a sulphydryl group that enables it to protect cells against oxidants. GSH, which accounts for 90% of intracellular non-protein thiols, is a key intracellular reducing agent and is implicated in immune modulation and inflammatory conditions [19]. GSH is critical to lung cellular antioxidant defences particularly in the protection from oxidant injury [16]; moreover, GSH is of primary importance in protecting the erythrocyte membrane structure and function. There is evidence that the increased release of oxidants in COPD patients may induce erythrocyte alteration responsible for the deficient oxygenation of these patients [31,32].

The long-term administration of oxygen (>15 h per day) to patients with chronic respiratory failure is the only drug that has been demonstrated to increase survival and improve the quality of life (Evidence A, GOLD guidelines) [22,25]. However, the efficacy of home oxygen in COPD patients with moderate hypoxemia or with nocturnal desaturation has been questioned, as there is no obvious clinical benefit [8,9,12]. Long-term oxygen therapy is generally introduced in Stage III, severe COPD patients and in patients developing cor polmonale [33] and there are strict guidelines for its use [1]. Supplementary oxygen therapy may further increase oxidative stress, which result in enhancing inflammation and worsening the disease [2,18]; moreover, there is an increase in markers of oxidative stress in plasma and in airways after hyperbaric oxygen therapy in humans, indicating that hyperoxia may theoretically worsen pulmonary disease toxicity [2,5]. In addition, Barnes et al. have recently shown that 1-h supplementary oxygen in COPD patients increases 8-isoprostane and Interleukin-6 in breath condensate of mild COPD patients [7]. This suggests a potentially dangerous effect of oxygen supplementation, by increasing the inflammatory response and the progression of disease.

Administration of sulphydryl containing compounds may limit the $O_2$-induced oxidative stress alterations by increasing GSH levels. N-acetyl-cysteine (NAC), a cysteine-donor compound, acts as a cellular precursor of GSH and becomes deacetylated in the gut to cysteine after oral administration [23]. NAC may also favour the reduction of cystine to cysteine, which is an important mechanism for intracellular GSH elevation in lungs *in vivo*. It reduces disulfide bonds but also has the potential to interact directly with oxidants.

The aims of the present study were to investigate whether an oxidant/antioxidant imbalance occurs in stable severe COPD and whether oxygen therapy at low flow (2 l/m) induces systemic oxidative stress in these patients. In addition, the purpose of this study was to confirm the hypothesis that administration of NAC could exert positive effect in counteracting the systemic oxidative stress in patients undergoing oxygen therapy. We therefore measured plasma and erythrocyte redox status in stable state III COPD patients during oxygen administration with and without treatment with different doses of NAC.

## Materials and methods

### *Study population*

TWe studied 45 male patients, affected by state III, severe COPD, recruited from the Department of Respiratory Disease of University of Foggia (Italy). The diagnosis of COPD was based on GOLD Guidelines [25]. Patients were clinically stable with no worsening of symptoms within the previous 4 weeks. All patients presented a forced expiratory volume in one second ($FEV_1$) < 50% of predicted value and $FEV_1$/forced vital capacity (FVC) ratio <70% with a $PaO_2$ < 60 mmHg within the previous 2 months and were never treated with long term oxygen therapy. All COPD patients were ex-smokers (having stopped from at least 6 months) and were tested at enrolment for the breath CO measurement (MicroCO Sensor Medics, Italy). All patients were in stable condition at the time of the study and free from acute exacerbations of symptoms and from upper respiratory tract infections in the 3 months preceding the study. Patients were treated with inhaled β-adrenergic agonists and/or anticholinergics and/or theofilline. Fifteen healthy non-smoker male subjects were considered as a control group. After the subjects had given written informed consent, they underwent a clinical examination, blood gas, redox and lung function analysis, 4 h before starting oxygen administration (basal). The $FEV_1$, FVC, $FEV_1$/FVC ratio and blood gas analysis were measured using a Morgan Flexillo System spirometer and Instrumentation Laboratory, CGM 1312 Blood Gas Manager, respectively. Pulmonary function tests and blood gas analysis of all subjects are summarized in Table I. The forty-five COPD patients were then randomly

Table I. Lung function of COPD patients and health controls at the enrolment.

| | *n* | Sex | Duration of disease (Yr) | Age (Yr) | $FEV_1$% predicted | $FEV_1$/FVC (%) | PaO*2* | pCO*2* | Hematocrit, vol% |
|---|---|---|---|---|---|---|---|---|---|
| COPD | 45 | Male | 9.3 ± 1.4 | 62.8 ± 2.5 | 41.6 ± 9.3 | 59.9 ± 6.8 | 54.1 ± 2.5 | 47 ± 6 | 0.45 ± 0.32 |
| Healthy patients | 15 | Male | – | 61.2 ± 3.8 | 99 ± 1.8 | 101 ± 1.7 | 84.3 ± 9.2 | 39.6 ± 2.3 | 0.42 ± 0.29 |

Data are expressed as means ± SDM.
COPD: Chronic obstructive pulmonary disease; $FEV_1$: Forced expiratory volume in one second; $FEV_1$/FVC (%): Forced expiratory volume in one second/Forced vital capacity.

allocated (using a computer-generated list) to three groups ($n = 15$) receiving NAC orally 1200 or 1800 mg/day or placebo in two daily administration for 48 h starting with $O_2$ administration. All the tablets were identical in appearance. The forty-five COPD patients breathed 2 l/min ($FiO_2 = 24\%$) oxygen for 18 h continuously through nasal prongs. Blood samples were collected before $O_2$ administration ($T_0$), during oxygen breathing ($T_8$, $T_{18}$) and 24 h after oxygen withdrawal ($T_{42}$). At the end of study blood gas analysis, $FEV_1$ and FVC were also measured. A subject initially selected as control way out from the study because the intolerance to oxygen, after 2 h of oxygen breathing and was substituted. The study was approved by the Local Ethical Committee and was conformed to the guidelines of the Declaration of Helsinki.

### *Assays*

Reduced and oxidized glutathione (GSH and GSSG) were measured in erythrocytes by the method of Fariss and Reed [10]. The content of sulphydryl proteins (P-SH) in plasma and erythrocytes was assessed spectrophotometrically with a modification of the Ellman procedure [13].

Plasma for oxidized protein (PC) assessment was prepared according to the method of Levine et al. [15]. Erythrocytes were separated by washing with saline solution followed by centrifugation at 3000*g* for 5 min. To avoid contamination by other blood cells, the supernatant and the first layer of sedimented cells were removed by aspiration. Packed erythrocytes were broken with 1:10 distilled water for GSH and GSSG determination and with 1:10 solution containing 300 µl 2 N HCL and acetone for carbonyl proteins and P-SH measurement [13]. Protein concentration was assessed by the Peterson method [26] or BIO-RAD kit assay. All measurements were performed in triplicate.

### *Statistical analysis*

Data were expressed as mean ± standard deviation of the mean (SDM). The *t*-test for independent samples was applied after Fisher test evaluation to compare healthy controls to COPD patients. The difference between the means of the treatment groups and at different time were analysed by ANOVA and Repeated-ANOVA analysis, respectively, after Kolgomorov-Smirnov test for the Gaussian distribution evaluation. The Tukey Multiple Comparisons Test for all pairs of columns was also applied as post test. In all instances, $p < 0.05$ was taken as the lowest level of significance. The SPSS software package (SPSS, Inc., Chicago, IL) was used to perform all the statistical analyses.

## Results

### *Oxidative status in COPD and healthy controls*

The baseline characteristics of the blood redox pattern of patients and healthy controls are shown in Table II. A significant decrease in plasma protein thiols (P-SH) was observed in COPD group ($p = 0.0024$, $t = 3.147$, $df = 58$). GSH, GSSG and carbonyl proteins did not show any major difference in COPD patients as compared to healthy controls at the enrolment.

### *Blood oxidative status in COPD patients during oxygen therapy*

Blood gases change during $O_2$ administration in COPD patients is reported in Table III. GSH and GSSG were measured in erythrocytes and P-SH and carbonyl proteins (PC) in both erythrocytes and plasma, from COPD patients breathing $O_2$ treated with placebo. Erythrocyte GSH significantly decreased 8 h after $O_2$ administration and continued to fall during the following 10 h of oxygen breathing. Twenty four hours after oxygen suspension the erythrocyte GSH levels started to recover without reaching, however, the basal level ($p < 0.0001$, $F = 94.632$, $df = 59$) (Figure 1). The same effect was observed in both erythrocyte and plasma P-SH level: the decrease was significant 8 h after oxygen administration and worsened at 18 h, and slowly started to return to the basal level 24 h after oxygen withdrawal (Figures 3 and 4). An opposite effect was observed in erythrocyte GSSG, which significantly

Table II. Erythrocytes and plasma redox status of health controls and COPD at enrolment.

| | Health controls | COPD | *p* |
|---|---|---|---|
| *n* | 15 | 45 | |
| RBC GSH (µM) | 741 ± 101 | 769.5 ± 89 | ns |
| RBC GSSG (µM) | 23.57 ± 8.59 | 25.3 ± 6.470 | ns |
| RBC P-SH (nmol/mg prot) | 0.168 ± 0.032 | 0.149 ± 0.036 | ns |
| RBC carbonyls (nmol/mg prot) | 1.75 ± 0.28 | 1.8 ± 0.15 | ns |
| Plasma P-SH (nmol/mg prot) | 2.51 ± 0.74 | 1.74 ± 0.74 | 0.0024 |
| Plasma PC (nmol/mg prot) | 1.46 ± 0.3 | 1.56 ± 0.41 | ns |

Data are expressed as means ± SDM.
COPD: Chronic obstructive pulmonary disease; RBD: Red blood cells; P-SH: sulphydril proteins; PC: carbonyl proteins.

Table III. Blood gases change during time in COPD patients breathing 24% $FiO_2$ oxygen.

| | $T_0$ | $T_8$ | $T_{18}$ | $T_{42}$ | $p$ |
|---|---|---|---|---|---|
| $PaO_2$ | 54.1 ± 2.5 | 66.8 ± 7.4 | 67.9 ± 6.6 | 54.2 ± 2.6 | $<0.001$* |
| $PaCO_2$ | 47 ± 6 | 50.3 ± 7.4 | 49.5 ± 7.4 | 46.1 ± 5.8 | $<0.01^{\circ}$ |
| Sat Hb, % | 87 ± 4 | 93 ± 6 | 96 ± 5 | 97 ± 4 | $<0.001$^ |

Data are expressed as means ± SDM.
p refers to Repeated measures-ANOVA: *$p < 0.001$ ($T_0$ vs $T_8$ and $T_{18}$ and $T_8$ vs $T_{42}$); °$p < 0.05$ ($T_8$ vs $T_{42}$); ^$p < 0.001$ ($T_0$ vs $T_8$, $T_{18}$ and $T_{42}$).

increased 8 and 18 h after oxygen administration and slowly returned to normal values 24 after oxygen suspension ($p < 0.0001$, $F = 21.326$, df = 59) (Figure 2). Erythrocyte protein carbonyls significantly increased after 8 h of oxygen administration with a further rise (two times) in the following 10 h. They partially decreased after oxygen suspension and at 18 h their concentrations were significantly higher than the basal level ($p < 0.0001$; $F = 80.648$ df = 59) (Figure 5). Similarly, plasma carbonyl proteins were increased at 8 and at 18 h of oxygen administration but did not decrease after oxygen withdrawal (Figure 6) ($p < 0.0001$; $F = 52.404$, df = 59) (Figure 6).

*Effect of NAC administration*

In order to explore the effect of NAC treatment on the systemic oxidative status in COPD patients undergoing $O_2$ therapy, GSH and GSSG were measured in erythrocytes and P-SH and PC in both erythrocytes and plasma, from COPD patients treated with NAC. NAC was administered orally at the dose of 1200 or 1800 mg/day starting simultaneously with $O_2$ and continuing for 48 h.

As shown in Figure 1, the erythrocyte GSH decrease was significantly lower in 1200 and 1800 NAC mg/day groups as compared with placebo and the difference was more evident 18 h after oxygen breathing ($p < 0.0001$, $F = 128.18$, df = 44). In addition, in the 1800 NAC mg/day group, GSH level did not decrease significantly at 18 h as compared to 8 h and returned to the basal level 24 h after oxygen suspension. It should be pointed-out, however, that NAC 1800 mg was shown to be more effective than NAC 1200 mg in preventing the fall ($p = 0.001$ at $T_{18}$) and in restoring the pool of GSH ($p = 0.01$ at $T_{42}$). The same effect occurred for erythrocyte and plasma P-SH levels: in the NAC groups both erythrocyte and plasma P-SH concentrations were significantly higher than placebo 8 and 18 h after oxygen administration. In addition, NAC rapidly restored the erythrocyte P-SH content 24 h after oxygen withdrawal but only 1800 mg/day completely prevented the thiol protein fall in red blood cells (RBC) (Figures 3 and 4).

NAC administration significantly decreased the oxidation of GSH to GSSG at both 1200 and 1800 mg/day doses (Figure 2). The difference between NAC and placebo administration, already statistically significant at 8 h, was further evident after 18 h of oxygen administration. The effect of NAC on GSH oxidation was dose-dependent: GSSG was lower in the 1800 mg than in the 1200 mg group and this difference was evident in the first 8 h of oxygen breathing. NAC treatment significantly protected plasma and erythrocyte proteins from oxidative damage. In fact, as shown in Figures 5 and 6, the level of plasma carbonyl proteins in 1800 mg NAC group was almost unaffected by oxygen and only a moderate change was observed at $T_8$ in 1200 mg NAC group. At the erythrocyte level, where the oxygen damaging effect was observed 18 h after oxygen was started, administration of both 1200 and 1800 mg of NAC, limited the rise in protein carbonyl formation

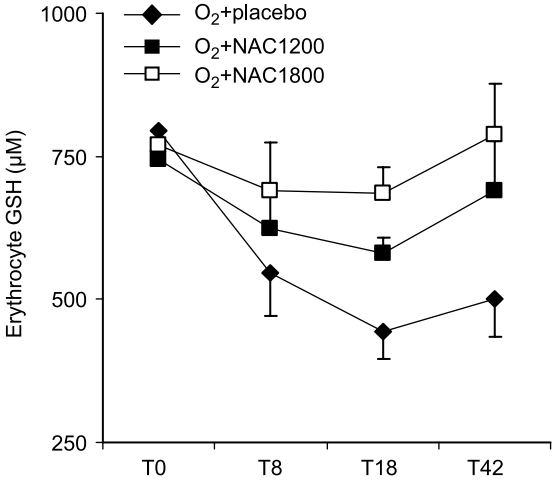


Figure 1. Effect of low flow oxygen administration on reduced glutathione (GSH) level in erythrocytes from controls ($O_2$ + placebo) and treated patients ($O_2$ + NAC 1200 or $O_2$ + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to repeated-ANOVA for the difference intra-group (A) and to ANOVA for the difference between groups (B). In both cases, the Tukey-Kramer multi comparison test was applied as a post-test. (A) Intra-group (Repeated-ANOVA) *$O_2$ + placebo*: $F = 64.145$; df = 59; $p < 0.0001$; $T_0$ vs $T_8$, $T_{18}$ and $T_{42}$, $p = 0.001$; $T_8$ vs $T_{18}$, $p = 0.01$; *$O_2$ + NAC 1200* mg/day: $F = 16.4$; df = 59; $p < 0.005$; $T_0$ vs $T_8$ and $T_{18}$, $p < 0.001$; *$O_2$ + NAC 1800* mg/day: $F = 6867$; df = 59; $p < 0{,}001$; $T_0$ vs $T_8$ and $T_{18}$, $p = 0.05$; $T_{42}$ vs $T_8$ and $T_{18}$, $p = 0.05$; (B) Between groups (ANOVA): $T_0$: ns; $T_8$: $F = 14.267$, $p < 0.001$; df = 44. $O_2$ + placebo vs NAC 1200: $p = 0.05$ and vs NAC 1800: $p = 0.001$; $T_{18}$: $F = 128.18$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.001$; $T_{42}$: $F = 48.465$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.01$.

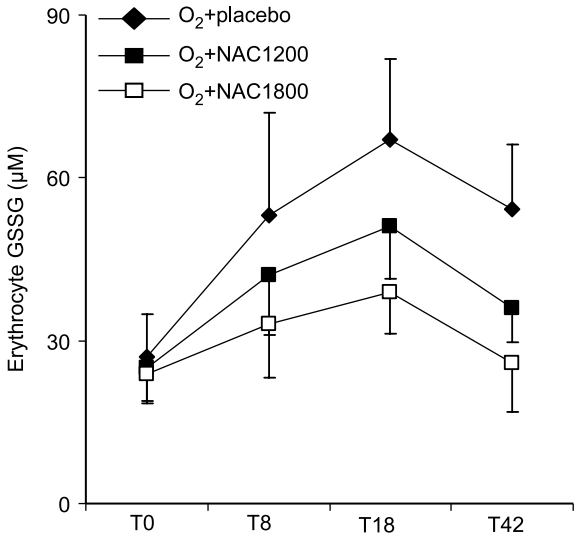


Figure 2. Effect of low flow oxygen administration on oxidized glutathione (GSSG) level in erythrocytes from controls ($O_2$ + placebo) and treated patients ($O_2$ + NAC 1200 or $O_2$ + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to repeated-ANOVA for the difference intra-group (A) and to ANOVA for the difference between groups (B). In both cases, the Tukey-Kramer multi comparison test was applied as post-test. (A) Intra-group (Repeated-ANOVA) *$O_2$ + placebo*: $F = 21.326$; df = 59; $p < 0.0001$; $T_0$ vs $T_8$, $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{18}$: $p = 0.05$; *$O_2$ + NAC 1200* mg/day: $F = 24.533$, df = 59; $p < 0.005$; $T_0$ vs $T_8$ and $T_{18}$: $p < 0.001$; $T_0$ vs $T_{42}$: $p = 0.01$; $T_{18}$ vs $T_{42}$: $p = 0.001$; *$O_2$ + NAC 1800* mg/day: $F = 10.918$; $p < 0.0001$; df = 59; $T_0$ vs $T_8$: $p = 0.05$; $T_0$ vs $T_{18}$: $p = 0.001$; $T_{18}$ vs $T_{42}$: $p = 0.001$; (B) Between group (ANOVA): $T_0$: ns; $T_8$: $F = 7.806$; $p < 0.001$; df = 44. $O_2$ + placebo vs NAC 1800: $p = 0.001$; $T_{18}$: $F = 23.492$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.05$; $T_{42}$: $F = 35.271$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.05$.

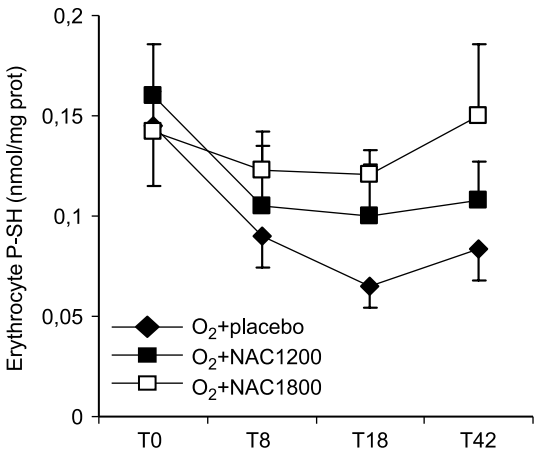


Figure 3. Effect of low flow oxygen administration on protein thiols levels in erythrocytes from controls ($O_2$ + placebo) and treated patients ($O_2$ + NAC 1200 or $O_2$ + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to repeated-ANOVA for the difference intragroup (A) and to ANOVA for the difference between groups (B). In both cases the Tukey-Kramer multi comparison test was applied as post-test. (A) Intra-group (repeated-ANOVA) *$O_2$ + placebo*: $F = 46.337$; df = 59; $p < 0.0001$; $T_0$ vs $T_8$, $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{18}$: $p = 0.01$; *$O_2$ + NAC 1200* mg/day: $F = 18.39$ df = 59; $p = 0.005$; $T_0$ vs $T_8$ and $T_{18}$: $p < 0.001$; *$O_2$ + NAC 1800* mg/day: $F = 5.543$; $p < 0.0001$; df = 59; $T_8$ vs $T_{42}$: $p = 0.01$; $T_{18}$ vs $T_{42}$: $p = 0.01$; (B) Between group (ANOVA): $T_0$: ns; $T_8$: $F = 8.098$; $p = 0.0011$; df = 44. $O_2$ + placebo vs NAC 1800: $p = 0.001$; $T_{18}$: $F = 38.273$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.01$; $T_{42}$: $F = 26.535$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200: $p = 0.05$ and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC $p = 0.001$.

(Figure 5). Of note, although the level of carbonyl proteins was significantly different in the placebo compared to NAC groups, in erythrocytes it was still higher than baseline 24 h after oxygen withdrawal.

No differences in $FEV_1$ and in FVC were observed in the three groups of COPD patients after 18 h breathing oxygen.

## Discussion

The present study demonstrates that the exposure of stable COPD patients to low-flow oxygen therapy leads to an impairment in circulating and erythrocytes antioxidant defense. In addition, this study confirms that an unstable oxidant/antioxidant balance occurs in COPD patients. Maintenance of high GSH level and an elevated GSH/GSSG ratio are essential for overall health and may provide a good index for oxidative stress. A lower content of GSH has been reported in the pulmonary tissue of patients affected by COPD [30]. Several reports have shown that neutrophils sequestered in the pulmonary microcirculation may be a source of oxidative stress, which may have a role in inducing systemic oxidative stress in COPD patients, particularly during exacerbation [17,28]. Taken together, these findings suggest that the same pathogenic mechanism leading to the alteration of antioxidant lung system in COPD patients may be also responsible for the oxidative derangement observed in the blood.

Two recent studies observed that plasma antioxidant capacity was significantly decreased in smokers and in COPD patients, conceivably due to a profound depletion of plasma sulphydryl proteins [28,29]. According to previous reports, in the present study, we show that stable state III COPD patients present a significant decrease in plasma thiol protein content as compared to normal healthy controls.

In addition, our study demonstrates that stable state III COPD patients present an unstable equilibrium which is lost during exposure to a pro-oxidant agent, such as oxygen administration. Very recently, we have shown that 1-h supplementary oxygen increases oxidative stress and inflammation in the airways of healthy subjects and COPD patients [7]. Several previous studies have shown that hyperoxia may

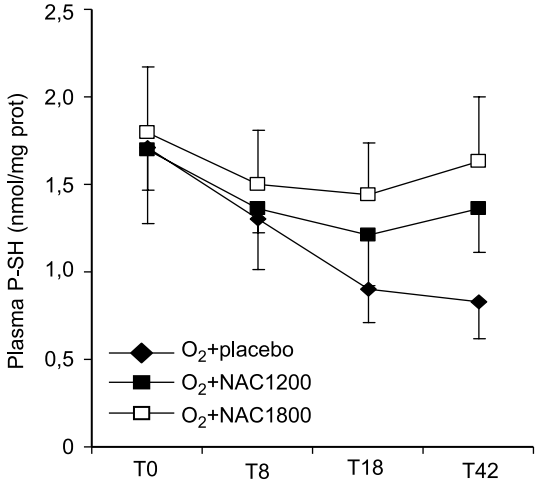


Figure 4. Effect of low flow oxygen administration on plasma protein thiols (P-SH) levels from controls ($O_2$ + placebo) and treated patients ($O_2$ + NAC 1200 or $O_2$ + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to Repeated-ANOVA for the difference intra-group (A) and to ANOVA for the difference between groups (B) In both cases the Tukey-Kramer multi comparison test was applied as post-test. (A) Intra-group (Repeated-ANOVA); $O_2 + placebo$: $F = 29{,}9$; df = 59; $p < 0.001$; $T_0$ vs $T_8$, $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{18}$: $p = 0.01$ and vs $T_{42}$: $p = 0.001$; $O_2 + NAC\ 1200$ mg/day: $F = 11{,}866$ df = 59; $p < 0.001$; $T_0$ vs $T_8$ and $T_{42}$: $p < 0.01$; $T_0$ vs $T_{18}$: $p = 0.001$; $O_2$ + NAC 1800 mg/day: ns; (B) Between group (ANOVA): $T_0$: ns; $T_8$: ns; $T_{18}$: $F = 16.678$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200: $p = 0.01$ and vs NAC 1800: $p = 0.001$; $T_{42}$: $F = 30.389$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200: $p = 0.05$ and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC $p = 0.05$.

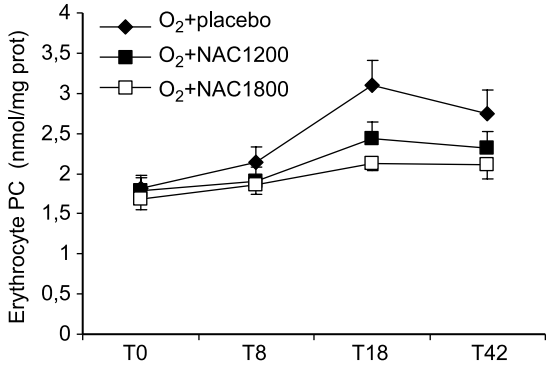


Figure 5. Effect of low flow oxygen administration on oxidized proteins in erythrocytes from controls ($O_2$ + placebo) and treated patients ($O_2$ + NAC 1200 or $O_2$ + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to Repeated-ANOVA for the difference intragroup (A) and to ANOVA for the difference between groups (B) In both cases the Tukey-Kramer multi comparison test was applied as post-test. (A) Intra-group (Repeated-ANOVA); $O_2 + placebo$: $F = 80.648$; df = 59; $p < 0.0001$; $T_0$ vs $T_8$: $p = 0.01$ and vs $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{18}$ nad vs $T_{42}$: $p = 0.001$; $T_{18}$ vs $T_{42}$: $p = 0.01$; $O_2 + NAC\ 1200$ mg/day: $F = 39.492$ df = 59; $p = 0.005$; $T_0$ vs $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{42}$: $p = 0.001$; $O_2 + NAC\ 1800$ mg/day: $F = 36.172$; $p < 0.0001$; df = 59; $T_0$ vs $T_8$: $p = 0.01$; $T_0$ vs $T_{18}$ and $T_{42}$: $p = 0.001$; **B)** Between group (ANOVA): $T_0$: ns; $T_8$: $F = 12.629$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and NAC 1800: $p = 0.001$; $T_{18}$: $F = 74.838$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.01$; $T_{42}$: $F = 27.304$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and NAC 1800: $p = 0.001$.

increase lung oxidative stress [2,11,34]. However, to our knowledge, this is the first report showing the effect of low flow $O_2$ administration on the circulating redox status in stable COPD patients. Previously, Viña et al. [35] reported that light exercise causes an oxidation of GSH in COPD patients, which can be partially prevented by low-flow oxygen therapy. In contrast, our study show that long term (18 h) and low flow $O_2$ administration is associated with GSH oxidation. The apparent disparity of results is explainable on the difference oxygen time exposure of the study. In fact, in the paper of Viña, COPD patients performed 10 min of light exercise breathing oxygen and blood GSH was evaluated immediately after the end of the effort. In contrast, we evaluated the effect of long term administration of low flow $O_2$ therapy. Our study show that $O_2$ administration is associated with GSH oxidation in erythrocytes and P-SH consumption both in plasma and erythrocytes 8 h after administration. Since RBC cannot increase the expression of γ-glutamylcisteine synthetase, the ratelimiting step in GSH synthesis, to synthesize *ex novo* GSH, the maintenance of normal P-SH values becomes of crucial importance. In addition, oxygen oxidizes plasmatic as well as erythrocyte proteins, increasing the systemic oxidative damage. NAC is the most widely investigated drug with antioxidant properties that has been used in both experimental and clinical settings which are relevant to COPD [3,20]. In this study we have shown that NAC can effectively counteract the oxidative alterations induced by $O_2$ treatment even at blood level. In fact, NAC reacts directly with electrophiles, thus providing antioxidant and cytoprotective effect against free radical mediated injury. It is efficiently absorbed after oral administration and most of the effects are consequent to the increase in tissue and circulating levels of thiols. Oral NAC administration supports the synthesis of GSH in subjects in which the demand for GSH is increased [6]. Our study seems to confirm this view. In addition, the choice of the appropriate dose deserves consideration: only the highest dosage used in our study was able to completely prevent the erythrocytes protein oxidation induced by $O_2$ administration. The protection exerted by NAC against protein oxidation supports the hypothesis that NAC prevents ultrastructural changes of erythrocytes occurring in COPD patients [32]. A further increase of dosage, however, could theoretically expose the patient to an increased risk of cystine toxicity. The NAC, indeed, is deacetylated after intestinal absorbtion and the delivered cysteine, if in excess, is rapidly oxidized to cystine, which follows alternative routes [14].

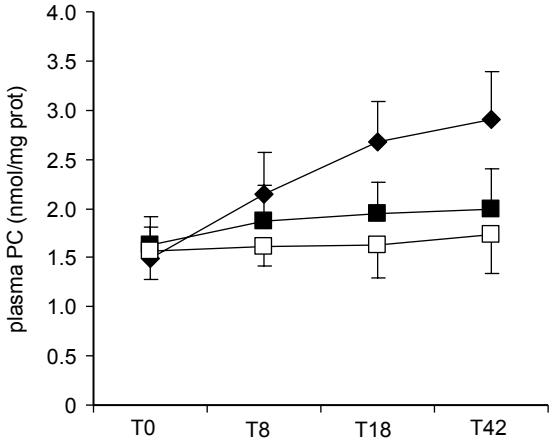


Figure 6. Effect of low flow oxygen administration on plasma oxidized protein levels from controls (O2 + placebo) and treated patients (O2 + NAC 1200 or O2 + NAC 1800) before, during (2 l/min/18 h) and 24 h after oxygen administration. Each group was of 15 patients. Statistical analysis refers to Repeated-ANOVA for the difference intra-group (A) and to ANOVA for the difference between groups (B) In both cases the Tukey-Kramer multi comparison test was applied as post-test. (A) Intra-group (Repeated-ANOVA); $O_2 + placebo$: $F = 52.404$; df = 59; $p < 0.0001$; $T_0$ vs $T_8$, $T_{18}$ and $T_{42}$: $p = 0.001$; $T_8$ vs $T_{18}$ nad $T_{42}$: $p = 0.001$; $O_2 +$ *NAC 1200* mg/day: *ns*; $O_2 +$ *NAC 1800* mg/day: ns; (B) Between group (ANOVA): $T_0$: ns; $T_8$: $F = 9.594$; $p = 0.0004$; df = 44. $O_2$ + placebo vs NAC 1800: $p = 0.001$; $T_{18}$: $F = 34.772$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and vs NAC 1800: $p = 0.001$; NAC 1200 vs NAC 1800: $p = 0.05$; $T_{42}$: $F = 53.334$; $p < 0.0001$; df = 44. $O_2$ + placebo vs NAC 1200 and NAC 1800: $p = 0.001$.

In conclusion, this study confirms that stable state III COPD patients present an unstable redox balance, mainly involving the protein thiol content without affecting GSH status. The administration of low flow oxygen induces blood oxidative stress, which is prevented by NAC administration and that this effect is dose-related. Moreover, we demonstrated that NAC at the dose of 1800 mg completely prevent the erythrocytes protein oxidation. These data support the hypothesis that NAC may be indicated in the treatment of stable COPD patients during oxygen therapy.



## References

[1] Long term domiciliary oxygen therapy in chronic hypoxic cor pulmonale complicating chronic bronchitis and emphysema. Report of the Medical Research Council Working Party. Lancet 1981;1:681–686.

[2] Arkovitz MS, Szabo C, Garcia VF, Wong HR, Wispe JR. Differential effects of hyperoxia on the inducible and constitutive isoforms of nitric oxide synthase in the lung. Shock 1997;7:345–350.

[3] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 1989;6:593–597.

[4] Barnes PJ. Chronic obstructive pulmonary disease. N Engl J Med 2000;343:269–280.

[5] Bearden SE, Cheuvront SN, Ring TA, Haymes EM. Oxidative stress during a 3.5-hour exposure to 120 kPa(a) PO2 in human divers. Undersea Hyperb Med 1999;26:159–164.

[6] Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 1989; 36:127–131.

[7] Carpagnano GE, Kharitonov SA, Foschino-Barbaro MP, Resta O, Gramiccioni E, Barnes PJ. Supplementary oxygen in healthy subjects and those with COPD increases oxidative stress and airway inflammation. Thorax 2004;59:1016–1019.

[8] Chaouat A, Weitzenblum E, Kessler R, Charpentier C, Enrhart M, Schott R, Levi-Valensi P, Zielinski J, Delaunois L, Cornudella R, Moutinho dos SJ. A randomized trial of nocturnal oxygen therapy in chronic obstructive pulmonary disease patients. Eur Respir J 1999;14:1002–1008.

[9] Crockett AJ, Cranston JM, Moss JR, Alpers JH. Domiciliary oxygen for chronic obstructive pulmonary disease. Cochrane Database Syst Rev CD001744 2000;.

[10] Fariss MW, Reed DJ. High-performance liquid chromatography of thiols and disulfides: Dinitrophenol derivatives. Methods Enzymol 1987;143:101–109.

[11] Freeman BA, Crapo JD. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. J Biol Chem 1981;256:10986–10992.

[12] Gorecka D, Gorzelak K, Sliwinski P, Tobiasz M, Zielinski J. Effect of long-term oxygen therapy on survival in patients with chronic obstructive pulmonary disease with moderate hypoxaemia. Thorax 1997;52:674–679.

[13] Grattagliano I, Vendemiale G, Sabba C, Buonamico P, Altomare E. Oxidation of circulating proteins in alcoholics: Role of acetaldehyde and xanthine oxidase. J Hepatol 1996; 25:28–36.

[14] Holdiness MR. Clinical pharmacokinetics of N-acetylcysteine. Clin Pharmacokinet 1991;20:123–134.

[15] Levine RL, Wehr N, Williams JA, Stadtman ER, Shacter E. Determination of carbonyl groups in oxidized proteins. Methods Mol Biol 2000;99:15–24.

[16] Li XY, Donaldson K, Rahman I, MacNee W. An investigation of the role of glutathione in increased epithelial permeability induced by cigarette smoke *in vivo* and *in vitro*. Am J Respir Crit Care Med 1994;149:1518–1525.

[17] MacNee W. Oxidants/antioxidants and COPD. Chest 2000;117:303S–317S.

[18] Mantell LL, Lee PJ. Signal transduction pathways in hyperoxiainduced lung cell death. Mol Genet Metab 2000; 71:359–370.

[19] Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther 1991;51:155–194.

[20] Menzel DB. Antioxidant vitamins and prevention of lung disease. Ann NY Acad Sci 1992;669:141–155.

[21] Morcillo EJ, Estrela J, Cortijo J. Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants. Pharmacol Res 1999;40:393–404.

[22] O'Donohue WJ, Jr, Plummer AL. Magnitude of usage and cost of home oxygen therapy in the United States. Chest 1995; 107:301–302.

[23] Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur J Clin Pharmacol 1988;34:77–82.

[24] Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. Am J Respir Crit Care Med 2002;166: S31–S37.

[25] Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease

(GOLD) Workshop summary. Am J Respir Crit Care Med 2001;163:1256–1276.

[26] Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83:346–356.

[27] Rahman I, MacNee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease. Thorax 1996;51:348–350.

[28] Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med 1996;154:1055–1060.

[29] Rahman I, Swarska E, Henry M, Stolk J, MacNee W. Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic obstructive pulmonary disease? Thorax 2000;55:189–193.

[30] Repine JE, Bast A, Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 1997;156:341–357.

[31] Santini MT, Straface E, Cipri A, Peverini M, Santulli M, Malorni W. Structural alterations in erythrocytes from patients with chronic obstructive pulmonary disease. Haemostasis 1997;27:201–210.

[32] Straface E, Matarrese P, Gambardella L, Forte S, Carlone S, Libianchi E, Schmid G, Malorni W. N-Acetylcysteine counteracts erythrocyte alterations occurring in chronic obstructive pulmonary disease. Biochem Biophys Res Commun 2000;279:552–556.

[33] Tarpy SP, Celli BR. Long-term oxygen therapy. N Engl J Med 1995;333:710–714.

[34] Vacchiano CA, Osborne GR, Tempel GE. 8-ISO-PGF2alpha production by alveolar macrophages exposed to hyperoxia. Shock 1998;9:266–273.

[35] Vina J, Servera E, Asensi M, Sastre J, Pallardo FV, Ferrero JA, Garcia-De-La-Asuncion J, Anton V, Marin J. Exercise causes blood glutathione oxidation in chronic obstructive pulmonary disease: prevention by $O_2$ therapy. J Appl Physiol 1996;81: 2198–2202.