Does Oxidative Stress Alter Quadriceps Endurance in Chronic Obstructive Pulmonary Disease?

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The role of exercise-induced oxidative stress in the reduced quadriceps endurance of chronic obstructive pulmonary disease (COPD) patients has never been shown. We conducted a randomized, double-blind, and crossover study in which nine severe patients performed localized dynamic quadriceps endurance tests at 40% of maximal strength after oral treatment with the antioxidant, N-acetylcysteine (NAC), and placebo. Venous blood was sampled before, immediately after exercise, and 6 hours later. Endurance time improved by 25% after NAC treatment compared with placebo ($p < 0.05$). Superoxide anion (oxidant) release by stimulated phagocytes decreased after treatment ($p < 0.05$). No change in the antioxidant system was observed. Lipid peroxidation, an index of oxidative stress, was significantly increased 6 hours after exercise in the placebo condition ($p < 0.05$) but not after treatment. Advanced oxidized protein products, another index of oxidative stress, were also increased 6 hours after exercise by 139 ± 27% in the placebo condition but only by 54 ± 19% after treatment ($p < 0.05$). This study shows that NAC treatment in COPD reduced basal disturbance in the prooxidant system, improved endurance time, and prevented exercise-induced oxidative stress. Oxidative stress thus seems to be implicated in the reduced quadriceps endurance of patients with COPD.

Keywords: N-acetylcysteine; antioxidant; placebo; endurance; superoxide anion

Systemic oxidative stress, which can be defined as a disturbance in oxidant/antioxidant systems, has been demonstrated in chronic obstructive pulmonary disease (COPD) patients after strenuous incremental or even light constant cycle exercise (1, 2). However, because general exercise considerably increases ventilation, cardiac output, and related parameters, it has been difficult to determine the source of this stress: lung, peripheral skeletal muscles, both, and/or other sites. The role of peripheral skeletal muscles was recently reported by Couillard and colleagues (3), who used localized quadriceps exercise to minimize cardiac and respiratory responses and showed that this exercise generated systemic oxidative stress in patients with COPD. This work has since been confirmed by biopsy studies showing that peripheral muscles are indeed a potential source of oxidative stress in these patients (4–6). Because oxidative stress can substantially alter muscle function (7) and contribute to its fatigue (8), it may be involved in the peripheral skeletal muscle dysfunction (9) and/or impaired exercise tolerance described in COPD (10, 11). However, although a significant negative correlation was recently found between the increase in indexes of oxidative stress and endurance time in patients with COPD (4), a causal relationship between exercise-induced oxidative stress and reduced quadriceps endurance has not been established.

N-acetylcysteine (NAC), which is used as a mucolytic agent in COPD and other diseases, was also found to be a powerful antioxidant, as a precursor of reduced glutathione, and a potential therapeutic agent in the treatment of diseases characterized by free radical and oxidant damage (12). Numerous studies have suggested that the glutathione antioxidant system may be exceeded during exercise in patients with COPD and thus implicated in the exercise-induced oxidative stress of these patients (13, 14). Bridgeman and colleagues (15) showed that 5 days of pretreatment with oral NAC significantly increased plasma glutathione levels in patients with COPD. NAC thus may be an interesting antioxidant molecule to reduce the oxidant/antioxidant disturbance in patients with COPD.

We hypothesized that if systemic oxidative stress is indeed implicated in the reduced quadriceps endurance of patients with COPD, NAC treatment would diminish it, and as a result, muscle function such as quadriceps endurance would improve. We tested this hypothesis in a randomized, double-blind, and crossover placebo study by investigating the effects of oral NAC treatment on endurance time and the systemic variables of oxidative stress in patients with COPD during localized muscle exercise designed to minimized cardiac and respiratory responses (16). Some of the results of this study have been previously reported in the form of an abstract (17).

METHODS

Subjects

This study included nine male ex-smokers with stable severe COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease guidelines (18, 19). Exclusion criteria were unstable COPD (i.e., exacerbation in the last 2 months), long-term supplemental oxygen and NAC treatment, neuromuscular disease, cardiac failure, diabetes mellitus, and alcoholism. None of the patients had taken systemic oral steroid medication in the 6 months before the study, and all medication regimens remained unchanged during the study period. Additional data on patient medications are available in the online supplement. The patients were sedentary (scores of less than nine) according to a physical activity questionnaire adapted for an older, retired population and recently used for patients with COPD (4, 10). All were questioned on their dietary habits to exclude supplementation with antioxidants or vitamins. Informed written consent was obtained from all patients, and the research protocol was approved by the local institutional ethics committee and the French Agency of Health Security in Medicine.

Study Protocol

Pulmonary function tests. Measurements included FVC and FEV_1. Tiffeneau's ratio (FEV_1/FVC) was calculated (*see* the online supplement for additional information).

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Localized muscle exercise. The maximal voluntary contraction (MVC) and endurance of the quadriceps were assessed on an exercise bench (Banc de Koch, Genin Medical, Les Angles, France) according to the technique of Andersen and colleagues (16), as modified by Serres and colleagues (20) and recently described by Couillard and colleagues (3). The exercise apparatus consisted of a harness attached to a nonelastic cord, which was connected to a minimal friction system of ball-bearing pulleys and a dynameter (for MVC) or adjustable weights (for endurance). Patient position during exercise was strictly controlled to avoid changes in respiratory responses. The exercises were performed with all patients in a seated position at 90° knee and hip flexion, with arms crossed in front of the chest and the position maintained. To determine MVC, the patients performed three brief (4-second) maximal contractions, each separated by 1 minute of rest. If the values of the contractions, as read on the dynamometer, were reproducible (less than 5% of variability between values), the highest value was defined as MVC. Quadriceps endurance of the dominant leg was then tested on the same exercise bench as previously described (3). This test consisted of repeated knee extensions against weights corresponding to 40% of the MVC at a pace of 12 movements per minute imposed with an audio signal (metronome) until exhaustion. Each dynamic knee extension was performed quickly, immediately followed by passive flexion and then by rest before the next extension. The patients were instructed to release muscles just after maximal extension without maintaining static contraction or resisting when weights were set back. The active part of the movement was thus considered to be the load lift. Rest was always longer than the duration of movement. The test was stopped when the patients could no longer respect the required maximal extension or frequency two consecutive times despite verbal encouragement. The same investigator supervised the endurance tests and gave standardized verbal encouragement to all participants. Neither this investigator nor the patients knew the test condition (NAC or placebo). The duration of the endurance test was called endurance time (expressed in seconds). The sensations of dyspnea and muscle fatigue were measured at rest and immediately after exercise on a 10-cm visual analog scale. Oxygen pulse saturation and heart rate were measured throughout both exercise trials by ear oximetry (Ohmeda Biox II 3740 pulse oximeter; Susquehanna Micro Inc., Red Lion, PA).

NAC supplementation. Each patient was randomly and blindly assigned to NAC-supplemented (NAC) and placebo exercise trials separated by at least 15 days. The supplementation consisted of 1,800 mg per day of NAC or placebo (lactose) for 4 days (3 capsules of 200 mg, three times a day) and a last 600-mg dose (3 capsules of 200 mg) on the day of the test. NAC and placebo doses were prepared and conditioned in blind capsules (Clinical Packaging; Euromedicine Park, Montpellier, France) in accordance with French regulations for clinical trials. Neither patients nor investigators knew the contents of the capsules to ensure double blindness. This was a crossover study, as each patient underwent the two trials (NAC and placebo trials) serving as a self-control to eliminate biological variability in the response to antioxidant supplementation.

Venous Blood Analysis

Antecubital venous blood was sampled in standard, sterile, heparinized, ethylenediaminetetraacetic acid–containing, or clot-activator tubes. Immediately after sampling, serum and plasma were removed by centrifugation (2,500 rpm for 10 minutes at 4°C), put into 500-μl aliquots, and stored at −80°C until analysis for markers of oxidative stress and antioxidants defenses. Superoxide anion production was immediately determined after sampling from a 100-μl aliquot of fresh whole blood.

Markers of systemic oxidative stress. Plasma thiobarbituric acid reactive substances (TBARs) as markers of lipid peroxidation were determined by spectrophotometry (21). The reproducibility calculated as the coefficient of variation (CV) was 4.6%. Plasma levels of protein oxidation were determined (CV = 5.2%) by assessment of advanced oxidation protein products (AOPPs) by spectrophotometry (22). Additional detail on the method for making these measurements is provided in the online supplement.

Determination of superoxide anion release by in vitro–stimulated phagocytes. Superoxide anion release (O_2^-) by *in vitro*–stimulated blood phagocytes was immediately determined after venous sampling from 100 μl of fresh whole blood collected from a heparinized sterile tube according to a method derived from Vachier and colleagues (23). Phorbol-myristate-acetate, a soluble phagocyte stimulant, was added to the tube, and the chemiluminigenic probe, lucigenin, was then used (at a final concentration of 1.5×10^{-4} M) to assess the superoxide anion production. The luminescence was recorded at 37°C with a 125I LKB Wallac luminometer (Wallac Co., Turku, Finland). The reproducibility calculated as the CV was 6.5%. Total and differential counts of white blood cells were determined on fresh blood from a heparinized tube with an automated hematology analyzer (Pentra 120 Retic ABX Diagnostics, Montpellier, France). O_2^- was thus expressed both in absolute value and as normalized by the number of blood phagocyte cells.

Markers of the antioxidant system. Trolox equivalent antioxidant capacity, a quantitative value for general antioxidant levels in biological samples (24, 25), was assayed in plasma with a quantitative colorimetric technique according to the kit supplier's instructions (Kit NX2331; Randox, Mauguio, France). The assay is based on the incubation of a peroxidase and H_2O_2 with 2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate] to produce the radical cation 2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]$^+$. This has a relatively stable blue–green color, which is measured at 600 nm. Antioxidants (albumin, uric acid, ascorbic acid, α-tocopherol, glutathione, β- carotene, etc.) in the sample suppressed 2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]$^+$ color production to a degree proportional to their concentration. The normal range in the European population is between 1.30- and 1.77-mM plasma. The reproducibility calculated as the CV was less than 1%. Plasma cysteine was assessed (CV of less than 5%) using reverse-phase, high-performance liquid chromatography and fluorescence detection (Kit ClinRep, p23000; Recipe, Munich, Germany). Serum uric acid (CV of less than 2%) and albumin (CV of less than 1%) were determined by routine biochemical assay on an AU2700 Olympus analyzer. Plasma vitamin E was measured by high-performance liquid chromatography (CV = 5.6%) according to Cachia and colleagues (26). Plasma triglycerides and cholesterol were assessed using a routine enzymatic method (Boehringer Mannheim, Meyland, France). Additional detail on the method for making these measurements is provided in the online supplement.

Study Design

The patients were instructed to abstain from strenuous physical activity during the study. On the first morning visit, the MVC of the quadriceps was determined for each leg. This established the dominant leg for each patient and was used to calculate the load corresponding to 40% of MVC for the endurance tests. Each patient then received the capsules corresponding to his first randomly assigned trial. Phone calls by the investigator and patient notebooks permitted us to verify patient adherence to the treatment. After NAC or placebo supplementation for 4 days, the patients returned to the laboratory after an overnight fast and without breakfasting at approximately 8 A.M. The last single dose of 600 mg was then taken. One hour later, a resting blood sample (T. rest) was collected, and localized muscle exercise was then performed with the dominant leg after familiarization with endurance test procedures. Blood samples were collected immediately at the end (T. end) of the localized exercise and 6 hours later (T6). After an interval of at least 15 days, the patients received the capsules corresponding to the second randomly assigned trial and then returned to the laboratory for the second endurance test at the same absolute intensity, that is, at 40% of the MVC determined on the first morning visit. The protocol was strictly identical to that of the initial trial.

Statistical Analysis

Values are reported as mean ± SEM. The endurance times in the NAC and placebo trials were compared using the Wilcoxon test because the normality assumption (Shapiro-Wilkinson test) was not obtained. A paired *t*-test was used to test the effect of treatment on the exercise-induced increase in the sensations of dyspnea and muscle fatigue. A two-way analysis of variance followed by Tukey's pairwise multiple comparison procedure determined the effects of treatment and localized exercise on biological markers. The significance level was set at 0.05. The data were analyzed using the statistical package SigmaStat 1.0 (Jandel Scientific, San Rafael, CA).

RESULTS

Anthropometric and Spirometric Data

Anthropometric and spirometric data and the values for the quadriceps MVC are presented in Table 1. The patients showed severe airflow obstruction with a FEV_1 of 45 ± 3% of predicted values (19). The MVC of the dominant leg (26 ± 2 kg) was in accordance with the values of a previous study on patients with COPD (3). The endurance tests were performed at a mean load of 10.5 ± 1.0 kg (ranging from 6 to 15.5 kg).

Muscle Endurance

In the placebo condition, the endurance time was 167 ± 19 seconds (Figure 1). After NAC treatment, this was improved ($p < 0.05$) by an average of 25% (208 ± 23 seconds; Figure 1). In the placebo condition, the dyspnea sensation increased significantly by 26 ± 4% immediately after exercise compared with the resting value, and the sensation of muscle fatigue increased by 94 ± 11%. No significant difference with these increases was observed after NAC treatment. Pulse oxygen saturation remained constant, and the increase in heart rate was of small amplitude (6 ± 1 heart pulse) during placebo exercise. NAC treatment did not significantly affect these variables.

Oxidant System

A significant increase in blood phagocyte number was observed at T6 compared with the resting value (Figure E1 in the online supplement). The treatment did not affect blood phagocyte number (Figure E1 in the online supplement). Exercise induced a significant increase in O_2^- release by stimulated phagocytes at T6 compared with T. rest (+29.5% in placebo, +11% in NAC; $p < 0.05$; Figure 2). When O_2^- release is normalized by the number of phagocytes in blood sample, the increase observed at T6 is abolished (Figure E2 in the online supplement). After NAC treatment, a significant decrease in O_2^- release by stimulated phagocytes was observed ($p < 0.05$; Figure 2).

Antioxidant System

No increase in baseline plasma cysteine concentration was observed after NAC treatment (Table 2). The nonenzymatic antioxidant levels remained unchanged after exercise and treatment (Table 2).

Systemic Exercise-induced Oxidative Stress

In the placebo condition, a significant increase in plasma TBARs was found at T6 compared with T. rest and T. end ($p < 0.05$; Figure 3). A significant increase in plasma AOPP levels at T6 compared with T. rest (48.0 ± 8.0 vs. 19.6 ± 1.8 μM of chloramine-T equivalents; $p < 0.05$) was also observed in the placebo condition.

After NAC supplementation, TBARs were significantly lower at T. rest, and no increase was observed at T6 ($p < 0.05$; Figure 3). For AOPP levels, no effect of NAC supplementation was observed at T. rest. However, the increase observed at T6 was reduced significantly from 138.9 ± 26.6% in the placebo condition to only 54.3 ± 19.3% after NAC treatment ($p < 0.05$; Figure 4).

TABLE 1. POPULATION CHARACTERISTICS (n = 9)

Age, yr	62 ± 4
Height, cm	168 ± 3
Weight, kg	69.3 ± 5.4
BMI, $kg \cdot m^{-2}$	24.6 ± 1.5
FEV_1, L	1.30 ± 0.12
FEV_1, % predicted	45 ± 3
FEV_1/FVC %	51.1 ± 4.1
MVC, kg	26 ± 2

Definition of abbreviations: BMI = body mass index; MVC = maximum voluntary contraction.

Data presented are mean ± SEM.

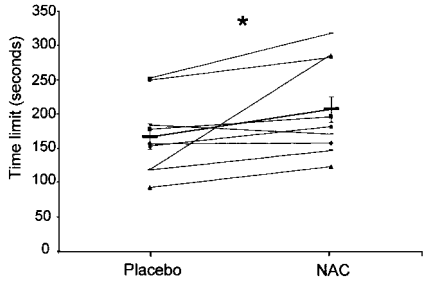

Figure 1. Endurance time during localized quadriceps exercise in placebo and N-acetylcysteine (NAC) conditions for each patient (n = 9). The mean and SEM of the endurance time in each condition is illustrated by a *solid line*. *$p < 0.05$: placebo versus NAC.

DISCUSSION

The major findings of this study were that short-term, high-dose NAC treatment (*1*) improved quadriceps endurance, (*2*) reduced the disturbance in the prooxidant system, and (*3*) prevented exercise-induced oxidative stress. Oxidative stress thus seems to be involved in the diminished quadriceps endurance of patients with COPD.

Methodologic Considerations

NAC treatment. The usual oral dose for NAC as a mucolytic agent and for most other clinical indications is 600 to 1,800 mg daily in three divided doses (12, 15). In this study, we chose a treatment that was shown to act as an effective antioxidant in COPD by Bridgeman and colleagues (15), despite its relatively low bioavailability (27). The NAC treatment was well tolerated: none of the patients complained of any of the side effects associated with high oral doses (e.g., nausea, vomiting, gastrointestinal disturbances, or increased sputum).

Quadriceps endurance. An earlier work discussed the localized exercise used in this study to assess muscle endurance in terms of its reproducibility and specificity to the quadriceps (3). In an experiment consisting of repetitive tests with the same technique, the endurance time was found to be highly reproducible

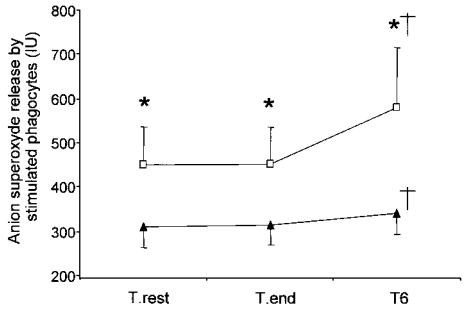

Figure 2. Effect of exercise on superoxide anion release by stimulated phagocytes in placebo condition (*open squares*; n = 9) and NAC condition (*closed triangles*; n = 9). *$p < 0.05$: placebo versus NAC; †$p < 0.05$: T6 versus T. rest.

TABLE 2. LEVELS OF PLASMA CYSTEINE AND ANTIOXIDANTS IN PLACEBO AND N-ACETYLCYSTEINE CONDITION

Antioxidant Plasma Levels	Placebo ($n = 9$)	NAC ($n = 9$)	p Value
Cysteine, μM	222.5 ± 32.0	238.7 ± 17.3	0.36
Total antioxidant capacity, mM	1.42 ± 0.03	1.46 ± 0.04	0.17
Uric acid, μM	301.1 ± 30.8	282.8 ± 29.8	0.46
Albumin, g/L	44.0 ± 1.2	42.4 ± 2.0	0.36
Vitamin E/(TG + CL), μM × 10^{-3}	5.1 ± 0.6	5.1 ± 0.3	0.90

Definition of abbreviations: CL = cholesterol; TG = triglycerides.
Data presented are mean ± SEM.

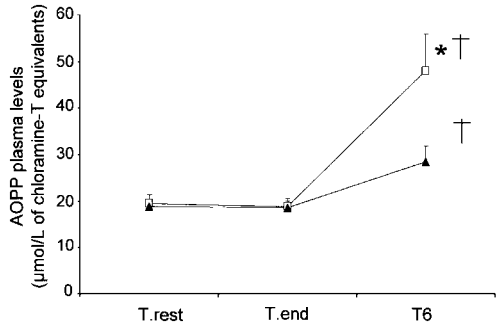

Figure 4. Effect of exercise on plasma advanced oxidized protein products (AOPPs) in placebo condition (*open squares*; n = 9) and NAC condition (*closed triangles*; n = 9). *p < 0.05: placebo versus NAC; †p < 0.05: T6 versus T. rest.

(28). In this study, we randomized and double-blinded the endurance tests to avoid learning effects. After the study, we learned that four of the nine patients with COPD had received NAC treatment before the placebo. Using this exercise protocol, electromyographic recordings taken from different muscles in addition to exercising quadriceps indicated that only the quadriceps was active during the local exercise (16). In this study, the postexercise increase in dyspnea sensation, as an index of ventilation, was of small amplitude. In contrast, the increase in the sensation of muscle fatigue was high. Finally, oxygen saturation remained constant throughout the exercise trials, and the increase in heart rate was of small amplitude. This suggests that the localized exercise required substantial work from the quadriceps muscle and minimized cardiac and respiratory responses.

Markers of oxidative stress. A global method was first used to assess oxidant-mediated lipid damage: lipid peroxidation by measurement of plasma TBARs, which is a nonspecific oxidative stress index that should be used with caution (29). We used a fluorometric method, however, that was sufficiently sensitive and reproducible to provide a valid estimation of oxidative stress (21) as previously discussed (3, 4). As no one method best assesses oxidative stress, the recommendation is to use several markers (25). The detection of oxidized amino acids in biological systems also provides a fingerprint of oxidative damage and is strong evidence of oxidative stress (25). To assess oxidant-mediated protein damage, we chose to measure diotyrosine-containing protein cross-linking products reflected by plasma AOPP levels (22). Numerous recent studies have used AOPP as a reliable marker of oxidant-mediated protein damage after oxidative stress (30, 31).

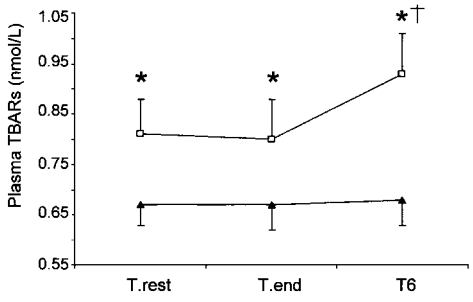

Figure 3. Effect of exercise on plasma thiobarbituric reactive substances (TBARs) in placebo condition (*open squares*; n = 9) and NAC condition (*closed triangles*; n = 9). *p < 0.05: placebo versus NAC; †p < 0.05: T6 versus T. rest.

NAC Treatment and Quadriceps Endurance of Patients with COPD

NAC treatment is claimed to have clinical beneficial effects in patients with COPD, and this has been illustrated by a reduction in symptoms, the duration, and rate of exacerbations (32); however, none of these studies addressed the question of whether NAC's clinical benefits in COPD derive specifically from antioxidant action. This study is the first to demonstrate that NAC treatment (1,800 mg/day for 4 days) was able to improve localized exercise endurance time significantly in patients with COPD (25%) and that this was most likely due to its action on the oxidant/antioxidant system. This is an essential observation because the peripheral muscle abnormalities in COPD are associated with poor health-related quality of life (33) as well as a poor prognosis for survival (34, 35).

NAC Treatment and Basal Oxidant/Antioxidant Disturbance in Patients with COPD

Clear evidence of a higher susceptibility to O_2^- release from stimulated phagocytes has been previously reported in patients with COPD (3, 4, 36, 37). The results of this study showed that localized muscle exercise enhanced this susceptibility and that it could be attributed to an exercise-induced increase in blood phagocyte number. The most interesting result, however, was that NAC treatment was able to reduce O_2^- release by *in vitro*–stimulated phagocytes, suggesting that NAC may be an effective means to reduce COPD susceptibility to reactive oxygen release. Because our NAC treatment did not significantly affect blood phagocyte number, an NAC effect on the activity of NADPH oxidase, which is the membrane-associated enzyme complex of the lysosome-yielding superoxide anions, might be one mechanism, as suggested by the *in vitro* work of Janiszewski and colleagues (38).

In contrast, NAC treatment failed to increase cysteine levels in our study, and no improvement in blood antioxidant levels was observed. Nevertheless, these plasma antioxidant pathways do not necessarily reflect the major antioxidant pathways of muscle, one of which is the thioredoxin system. We thus cannot exclude the possibility that the disturbance in the muscle antioxidants observed in COPD (13, 15) would be reduced by NAC treatment, for example, by supplying cysteine or sulfhydryl groups for muscle glutathione biosynthesis (12, 36).

NAC Treatment and Oxidative Stress in Patients with COPD

In placebo condition, the significant increase in plasma lipid peroxidation (18%) and AOPPs (139%) 6 hours after local exercise

indicated exercise-induced oxidative stress. The rise in lipid peroxidation agrees with that noted by Couillard and colleagues (3) and confirms that patients with COPD are susceptible to this stress; however, the interesting result was in the NAC condition, 6 hours after exercise, there was a significant decrease in both TBARs and AOPP levels. This suggests that NAC treatment reduced the local exercise-induced systemic oxidative stress in the patients. In this study, kinetics of TBARs production paralleled that of superoxide anion from phagocytes, suggesting its pathway as a systemic source of TBARs generation. Neutrophils and phagocytes appear thus to be critical cells in the pathogenesis of COPD systemic oxidative stress. The increase of plasma proinflammatory cytokines reported in patients with COPD can also stimulate systemic reactive oxygen species (ROS)-producing systems (39, 40).

Reduced Quadriceps Endurance and Oxidative Stress in COPD

The improvement in COPD quadriceps endurance after an NAC treatment that reduced exercise-induced oxidative stress demonstrates, for the first time, the role of oxidative stress in the development of the altered quadriceps endurance of patients with COPD. Reid and colleagues were the first to demonstrate that pretreatment with intravenous NAC increases force output of the tibialis anterior in healthy humans when electrically stimulated to fatigue at low frequencies (41). The NAC dose used in this study is the lowest ever found to improve exercise endurance in humans, pointing out the COPD susceptibility to oxidative stress. The major findings on the NAC treatment effects are illustrated in Figure 5, which was inspired by Reid's model (8). Indeed, the steady state of endogenous ROS appears to be essential for muscle function: the deleterious effect of oxidants on muscle function was noted only beyond a critical set point of excessive accumulation (point A). Under normal basal conditions (dashed line), the endogenous ROS levels are less than optimal, and thus, the organism can handle an increase in ROS (as demonstrated during walking, cycling, force, and/or endurance tests) without experiencing the deleterious effects of oxidative stress. In patients with COPD, the basal ROS release by stimulated phagocytes was above normal (basal state of oxidant production rightward along the curve, near point A), suggesting a lower capacity of adaptation to an increase in ROS. Given this condition, a stimulus such as the performance of a muscle task of even low intensity was able to act as a trigger of oxidative stress in these patients, thereby inducing muscle alteration and fatigue, as seen in point B. NAC treatment, by decreasing the basal susceptibility to systemic ROS release (basal state of oxidant production leftward along the curve, near point A'), increased the capacity to adapt to the rise in ROS induced by exercise. Exercise-induced oxidative stress was thus lower, as was the degree of muscle alteration and fatigue (B'), which may explain the improvement in quadriceps endurance time that we observed.

Limitations of the Study

The specificity of the exercise protocol used in this study strengthened the hypothesis of peripheral muscle as a source of ROS (mitochondria, xanthine oxidase, inflammation, etc.) in the systemic exercise-induced oxidative stress of patients with COPD. We could not, however, confirm it in this study because of the scientific and method limitation inherent to using plasma levels to infer changes occurring at the muscle level. The direct or indirect cellular mechanism(s) by which our NAC treatment may have altered muscle sources of ROS and/or enhanced muscle antioxidant systems thus remains to be elucidated. These fundamental issues should be addressed in muscle biopsy studies to understand better the altered quadriceps endurance of patients with COPD.

Conclusions

This study demonstrates, for the first time, that an antioxidant treatment that (*1*) reduced the systemic disturbance in the prooxidant/antioxidant balance in patients with COPD, (*2*) prevented systemic exercise-induced oxidative stress, and also (*3*) significantly improved the endurance time during localized muscle exercise in these patients. This suggests that oxidative stress may be involved in the diminished quadriceps endurance of patients with COPD and points to the importance of future development of potent antioxidant therapies with good bioavailability.

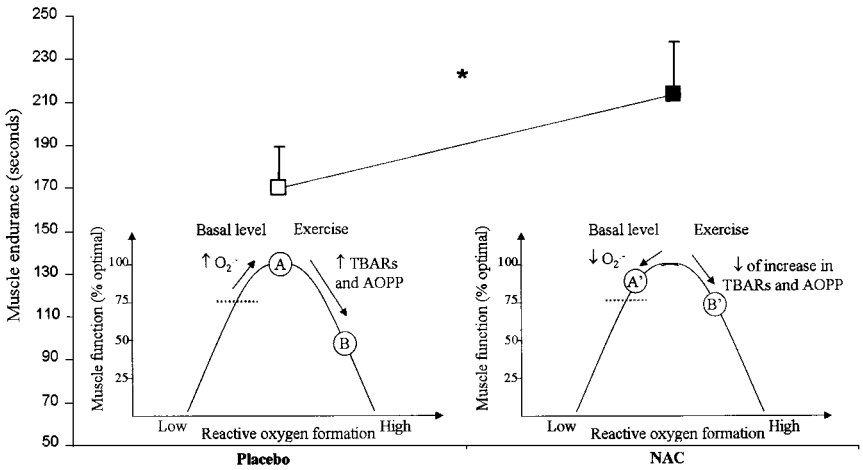

Figure 5. A proposed model of the relationship between quadriceps endurance and reactive oxygen formation in chronic obstructive pulmonary disease (COPD) patients, according to the results of the present study and inspired by Reid and colleagues (8). The effect of NAC treatment on quadriceps endurance (*upper panel*). The effect of NAC treatment on biological markers of oxidative stress (*lower panel*). The *dashed line* illustrates the normal basal endogenous reactive oxygen species levels. In the placebo condition, (*A*) the basal state of patients with COPD with an increase in oxidants exposure, as reflected by the increased burden of superoxide anion originating from phagocytes, and (*B*) a decrease in muscle function after excessive reactive oxygen species exposure because of exercise. In NAC condition, the decrease in superoxide anion production drove the COPD basal state leftward along the curve (*point A'*). Given this condition, exercise-induced oxidative stress was lower and thus so were the degree of muscle alteration and fatigue (*point B'*), which would explain the improvement in quadriceps endurance time that we observed.

Conflict of Interest Statement: C.K. has no declared conflict of interest; A.C. has no declared conflict of interest; D.S. has no declared conflict of interest; J.P.C. has no declared conflict of interest; H.B. has no declared conflict of interest; M.H. has no declared conflict of interest; C.P. has no declared conflict of interest.

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