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Systemic and Pulmonary Oxidative Stress After Single-Leg Exercise in COPD

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Background: Our aim for this study was to disentangle the contribution of muscular vs pulmonary oxidative stress during endurance exercise in patients with COPD.

Methods: Fifteen COPD patients and 10 healthy age-matched control subjects performed a continuously submaximal single-leg ergometer test (40% of peak workload) for 20 min or until they stopped (muscle endurance [Tlim]). Venous blood, urine samples, and exhaled breath condensate were sampled before, immediately after, and 2 h after exercise.

Results: Tlim was lower in COPD patients than in control subjects ($p < 0.01$). No exercise-induced systemic inflammation (*ie*, no raised levels of interleukin-6 or tumor necrosis factor- α) was found in the groups. Urinary malondialdehyde and uric acid levels ($p < 0.05$) were increased in COPD patients, whereas erythrocyte oxidized glutathione/reduced glutathione levels tended to be increased in COPD patients compared with control subjects after exercise ($p = 0.08$). Despite the relatively low cardioventilatory response to this localized muscle exercise, hydrogen peroxide levels in breath condensate significantly increased in COPD patients ($p < 0.01$). Nuclear factor κ B DNA-binding activity of p50 in peripheral blood monocytes was elevated after exercise in both COPD patients ($p < 0.01$) and control subjects ($p < 0.05$), whereas p65 protein levels were not altered.

Conclusion: COPD patients showed increased pulmonary and systemic oxidative stress after localized leg muscle exercise compared with healthy control subjects, without evidence of increased levels of systemic inflammation. (CHEST 2009; 136:1291–1300)

Abbreviations: EBC = exhaled breath condensate; GSH = reduced glutathione; GSSG = oxidized glutathione; H_2O_2 = hydrogen peroxide; IL = interleukin; MDA = malondialdehyde; MVV = maximum voluntary ventilation; NF = nuclear factor; PASE = physical activity scale for the elderly; PBMC = peripheral blood mononuclear cell; PBS = phosphate-buffered saline; ROS = reactive oxygen species; Tlim = muscle endurance; TNF = tumor necrosis factor; VE = minute ventilation

Peripheral muscle dysfunction is common in COPD patients. The authors of previous studies^{1–3} showed that peripheral muscle strength and

endurance are reduced and more susceptible to fatigue⁴ in patients with COPD compared with healthy subjects. Understanding the underlying mechanisms of peripheral muscle impairment in COPD patients is clinically relevant since skeletal muscle dysfunction is linked to disability and im-

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paired quality of life.⁵ Chronic inflammation and oxidative stress may be implicated in the etiology of peripheral muscle dysfunction.⁶ Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) and antioxidant systems in favor of ROS. Oxidative stress can be due to different mechanisms (*eg*, mitochondrial respiration, xanthine oxidase, and nicotinamide adenine dinucleotide phosphate hydrogen oxidase) and eventually leads to changes in various oxidative stress markers that cannot be related to a specific source of ROS formation. Systemic oxidative stress is indicated by a reduced redox state as measured by erythrocyte oxidized glutathione (GSSG)/reduced glutathione (GSH) ratios, and by increased levels of urinary excretion products of lipid peroxidation such as malondialdehyde (MDA), whereas pulmonary oxidative stress is indicated by increased exhalation of hydrogen peroxide (H₂O₂). Increased systemic and pulmonary oxidative stress has consistently been reported^{7,8} in patients with severe COPD after constant work rate cycle exercise. This type of exercise, however, stresses the whole body and the limitations of the ventilatory system, in COPD patients in particular.⁹ Moreover, under these circumstances, it is difficult to disentangle the relative contributions of lungs vs muscles to the exercise-induced oxidative stress seen in COPD patients.

We assumed that a localized limb exercise may obviate exercise-induced ventilatory and cardiac stress and thus be a useful exercise modality to study the contribution of skeletal muscle to exercise-induced oxidative stress in COPD patients. Couillard and colleagues^{2,10} have provided evidence for localized quadriceps exercise-induced systemic and muscle oxidative stress in patients with COPD, but no data were reported regarding the pulmonary oxidative stress response. Furthermore, in these studies, endurance time was tested on an exercise bench according to the technique of Serres et al.³ In this model, the movement is based on knee extension and flexion on a regular pace with periods of rest in between. A more suitable model to study exercise endurance time is the model of Andersen et al¹¹ in which knee extension is performed continuously. Our aim for this study was therefore to investigate whether, and to what extent, systemic inflammation and oxidative stress as well as pulmonary oxidative stress are differentially triggered by contracting peripheral muscles in COPD patients compared with healthy control subjects, and whether this stress response is associated with peripheral reduced muscle endurance (Tlim) in COPD patients.

MATERIALS AND METHODS

Study Population

Fifteen stable patients who met Global Initiative for Chronic Obstructive Lung Disease criteria for COPD were recruited on admission to the Centre for Integrated Rehabilitation Organ Failure (Horn, the Netherlands).¹² All patients were ex-smokers and had not experienced a respiratory tract infection or exacerbation of their disease for at least 4 weeks prior to the study. Exclusion criteria were rheumatoid arthritis, chronic colitis, diabetes, cancer, cardiovascular diseases, renal diseases, liver diseases, or mental diseases. None of the subjects were receiving therapy with antioxidants or vitamin supplements. All patients received standardized medical treatment according to the Global Initiative for Chronic Obstructive Lung Disease guidelines including anticholinergic agents, β_2 -agonists, and inhaled corticosteroids. Two patients also received theophylline and oral corticosteroids as maintenance therapy. Ten healthy, sedentary, age-matched, nonsmoking control subjects were recruited through newspaper advertisements. Written informed consent was obtained from all participants, and the study was approved by the medical ethics committee of the University Hospital Maastricht.

Subject Characteristics

Body composition,¹³ pulmonary function,¹⁴ and the physical activity scale for the elderly (PASE) questionnaire score¹⁵ were determined as described previously.

Exercise Capacity

Participants performed an incremental cycling exercise test as described previously.¹⁶ A week later, they performed a maximal single-leg ergometer test and 3 days later a submaximal single-leg ergometer test (40% of peak workload) for which subjects were instructed to cycle for 20 min. The exercise equipment and methodology were designed based on the model of Andersen et al.¹¹ The duration time of the exercise test was called "limit time" (*ie*, Tlim). Venous blood, urine samples, and exhaled breath condensate (EBC) samples were obtained at baseline, immediately after, and 2 h after the exercise test was completed. Blood lactate was analyzed (Cobas Mira; Roche; Basel, Switzerland). Dyspnea and muscle fatigue were evaluated by using the Borg scale (0 to 10).

Markers of Systemic Inflammation and Oxidative Stress

Venous blood samples were drawn into ethylenediaminetetraacetic acid-containing tubes (Venoject; Terumo Corp; Leuven, Belgium). Plasma was obtained by centrifugation (800 *g* for 10 min at 4°C) and stored at -80°C until analysis. The volume of the remaining blood was brought to 15 mL with a cold phosphate-buffered saline (PBS) solution and layered on an equal volume of medium (Lymphoprep; Greiner; Alphen a/d Rijn, the Netherlands) in a 50-mL tube with a filter of peripheral blood mononuclear cells (PBMCs). After centrifugation at 800 *g* for 30 min at 4°C, gradient-separated PBMCs were recovered, resuspended in 10 mL of cold PBS solution, and centrifuged again at 250 *g* for 10 min at 4°C. The cells were resuspended in 1 mL of cold PBS solution and centrifuged at 800 *g* for 5 min, and the pellet was used to prepare nuclear extracts for nuclear factor (NF)- κ B determination. Nuclear extracts were prepared as described by Hofmann et al.¹⁷ Urine samples were stored at -20°C until further analysis. Plasma interleukin (IL)-6 and tumor necrosis factor (TNF)- α concentrations were determined by

using a quantitative high-sensitivity enzyme-linked immunosorbent assay kit (R&D Systems; Minneapolis, MN). Urinary MDA and urinary uric acid levels were assessed by using high-performance liquid chromatography.^{18,19} Erythrocyte glutathione was measured in its GSH and GSSG form.²⁰ The GSSG/GSH ratio, another marker of oxidative stress, was calculated. NF- κ B concentrations were determined in nuclear extracts of PBMCs (TransAM NF- κ B p50 and p65 transcription Factor Assay Kit; Active Motif Europe; Rixensart, Belgium).

Markers of Pulmonary Oxidative Stress

EBC was collected as described previously.²¹ H₂O₂ was measured by means of horseradish peroxidase-catalyzed oxidation of tetramethylbenzidine.²²

Statistical Analysis

Data are expressed as the mean $\pm$ SD unless specified otherwise. Differences between patients and control subjects were tested by using the Student *t* test for independent samples. A paired *t* test was used to test differences between local vs whole-body exercise tests. The Wilcoxon signed rank test was used to evaluate the effect of single-leg exercise on biological markers within the groups. Between-group comparisons were analyzed using the Mann-Whitney *U* test. Nonparametric tests were used because the normality assumption was not obtained. A *p* value of ≤ 0.05 was considered statistically significant. Statistical analyses were analyzed using a statistical software package (SPSS for Windows, version 13.0; SPSS; Chicago, IL).

RESULTS

Subject Characteristics

As shown in Table 1, the COPD group was characterized by moderate-to-severe airflow obstruction with a mean FEV₁ of $46.6 \pm 5.2\%$ predicted. Physical activity assessed by the PASE score was significantly lower in COPD patients compared with control subjects (*p* < 0.001).

Table 1—Characteristics of the Study Population

Characteristics	Control Subjects (n = 10)	COPD Patients (n = 15)
Sex		
Male	4	10
Female	6	5
Age, yr	55.5 $\pm$ 5.8	56.5 $\pm$ 8.9
Smoking history, pack-yr	11.9 $\pm$ 11.1	38.0 $\pm$ 21.7
BMI, kg/m ²	27.6 $\pm$ 4.4	24.6 $\pm$ 4.2
FFMI, kg/m ²	17.3 $\pm$ 2.2	15.9 $\pm$ 1.8
FEV ₁ , % predicted	115.9 $\pm$ 22.0	46.6 $\pm$ 20.2*
FVC, % predicted	126.1 $\pm$ 31.7	112.8 $\pm$ 33.5
FEV ₁ /FVC ratio, % predicted	78.3 $\pm$ 4.8	35.2 $\pm$ 9.6*
PASE score	235.3 $\pm$ 45.2	85.1 $\pm$ 21.2*

Data are presented as No. or mean $\pm$ SD. FFMI = fat-free mass index.

**p* < 0.001 (compared with healthy control subjects).

Table 2—Physiologic Response to Submaximal Single-Leg Ergometer Test

Variables	Control Subjects (n = 10)	COPD Patients (n = 15)
Workload, W	15.6 $\pm$ 5.2	6.4 $\pm$ 3.9*
Tlim, min	19.0 $\pm$ 3.2	11.0 $\pm$ 7.2†
O ₂ consumption, mL/min		
Rest	493.7 $\pm$ 148.8	366.8 $\pm$ 139.4
End exercise	867.0 $\pm$ 201.8	610.6 $\pm$ 198.0
CO ₂ output, mL/min		
Rest	390.0 $\pm$ 163.8	304.4 $\pm$ 135.9
End exercise	770.7 $\pm$ 184.5	560.9 $\pm$ 203.8
HR reserve, %		
End exercise	42.4 $\pm$ 20.2	41.5 $\pm$ 9.0
$\dot{V}_E$ /MVV ratio		
End exercise	0.22 $\pm$ 0.04	0.57 $\pm$ 0.27*
Arterial oxygen saturation, %		
Rest	96.3 $\pm$ 2.9	96.5 $\pm$ 2.4
End exercise	97.0 $\pm$ 2.3	96.8 $\pm$ 1.9
RER		
End exercise	0.89 $\pm$ 0.05	0.91 $\pm$ 0.08
Δ Lactate, mmol/L	0.80 $\pm$ 0.88	0.98 $\pm$ 0.82
Δ Dyspnea Borg score	0.5 $\pm$ 0.8	1.8 $\pm$ 1.6
Δ Leg fatigue Borg score	0.9 $\pm$ 0.8	1.6 $\pm$ 1.3‡

Data are presented as mean $\pm$ SD. HR = heart rate; RER = respiratory exchange ratio.

**p* < 0.001 (compared with healthy control subjects).

†*p* < 0.01 (compared with healthy control subjects).

‡*p* < 0.05 (compared with healthy control subjects).

Single-Leg Ergometer Test

The results of the submaximal single-leg ergometer test are presented in Table 2. All healthy control subjects except for one reached the 20-min time limit, whereas only five COPD patients were able to complete the entire duration of the test. Workload and quadriceps endurance (*ie*, Tlim) were significantly lower in COPD patients compared with control subjects (*p* < 0.001 and *p* < 0.01, respectively). Peak minute ventilation ($\dot{V}_E$) as a proportion of maximum voluntary ventilation (MVV) was significantly higher in COPD patients compared with control subjects (*p* < 0.001), whereas the heart rate reserve was similar between both groups. Subjective assessment of muscle fatigue showed greater leg fatigue in COPD patients compared with control subjects (*p* < 0.05), whereas perceived breathlessness was not significantly different between the groups.

Comparison Between Whole-Body and Local Exercise

Physiologic responses to the whole-body exercise test (maximal cycle exercise) and the submaximal localized quadriceps exercise (single-leg ergometer test) are compared in Table 3. In COPD patients,

Table 3—Comparison Between Local vs Whole Body Exercise Tests in COPD Patients

Variables	Single-Leg Exercise	Maximal Cycle Exercise
Tlim, min	11.0 ± 7.2	
O ₂ consumption, mL/min	610.6 ± 198.0	1,231.1 ± 428.5*
CO ₂ output, mL/min	560.9 ± 203.8	1,184.4 ± 429.8*
Arterial O ₂ saturation, %	96.6 ± 1.9	92.1 ± 3.8†
HR, beat/min		
Rest	82.6 ± 16.5	85.1 ± 9.6
End exercise	96.4 ± 15.6	128.5 ± 20.0
HR reserve, %		
End exercise	41.5 ± 9.0	21.4 ± 10.3*
VE, L/min		
Rest	15.0 ± 6.1	13.9 ± 3.9
End exercise	29.4 ± 8.4	46.4 ± 14.9
VE/MVV ratio		
End exercise	0.57 ± 0.27	0.95 ± 0.31†
ΔDyspnea Borg score	1.8 ± 1.6	5.0 ± 2.6†
ΔLeg fatigue Borg score	1.6 ± 1.3	3.3 ± 2.6

Data are presented as mean ± SD. See Table 2 for abbreviations not used in the text.

*p < 0.01 (compared with single-leg exercise).

†p < 0.05 (compared with single-leg exercise).

the single-leg ergometer test resulted in significantly lower peak oxygen consumption, peak carbon dioxide output (both $p < 0.01$), and higher oxygen saturation ($p < 0.05$) compared with the results of the whole-body maximal cycle exercise test. Moreover, the $\dot{V}E/MVV$ ratio was significantly lower after the single-leg ergometer test compared with the maximal cycle exercise, whereas the heart rate reserve was more preserved after the single-leg ergometer test ($p < 0.05$ and $p < 0.01$, respectively) in COPD patients. For COPD patients, dyspnea perception ($p < 0.01$) and, to a lesser extent, leg fatigue perception during the single-leg exercise were less when compared with the whole-body maximal cycle exercise.

Markers of Systemic Inflammation and Oxidative Stress

At baseline, only the level of IL-6, a marker of systemic inflammation, was significantly higher in COPD patients compared with control subjects (control subjects, 0.7 ± 0.2 pg/mL; COPD patients, 2.0 ± 0.4 pg/mL; $p < 0.01$). No exercise-induced inflammatory changes were observed in either group (data not shown). Systemic as well as pulmonary oxidative stress markers at baseline did not differ between both groups (data not shown). Urinary MDA levels were determined as a marker of lipid peroxidation. As shown in Figure 1, urinary MDA levels were significantly elevated immediately after exercise in COPD patients, whereas, in control

FIGURE 1. Difference in the concentration of urinary MDA from rest to different time points after submaximal single-leg exercise in healthy control subjects and COPD patients. Values are expressed as the mean ± SEM. * = $p < 0.05$ (significantly different from baseline values within groups; Wilcoxon signed ranks); # = $p < 0.02$ (significantly different between the groups; Mann-Whitney test).

subjects, a significant decrease was found 2 h after exercise (both $p < 0.05$). Additionally, a significant difference was observed in the response to exercise in terms of urinary MDA levels between the groups ($p < 0.02$). Urinary uric acid was assessed as an indicator of xanthine-oxidase activity. Urinary uric acid was significantly increased immediately after exercise in COPD patients ($p < 0.05$) [Fig 2]. Moreover, the exercise-induced uric acid response was significantly higher in COPD patients compared with control subjects ($p < 0.05$). Glutathione was measured in its GSH and GSSG form. GSH is a ubiquitous endogenous antioxidant, whereas GSSG and the GSSG/GSH ratio can be considered as markers of oxidative stress. The data on GSH, GSSG, and GSSG/GSH ratio at rest and after exercise are summarized in Table 4. In control subjects, erythrocyte GSH levels decreased 2 h after exercise ($p < 0.05$) [Fig 3A], whereas, in COPD patients, GSSG levels and the GSSG/GSH ratio tended to be increased immediately after exercise (both $p = 0.08$) [Fig 3B and C]. Exhaled H_2O_2 was determined as an index of exercise-induced pulmonary oxidative stress. In COPD patients, H_2O_2 concentration in EBC was significantly elevated immediately after and 2 h after exercise (both $p < 0.01$) [Fig 4], whereas, for control subjects, no significant effect of exhaled H_2O_2 concentration was observed.

FIGURE 2. Difference in the concentration of urinary uric acid from rest to different time points after submaximal single-leg exercise in healthy control subjects and COPD patients. Values are expressed as the mean $\pm$ SEM. * = $p < 0.05$ (significantly different from baseline values within groups; Wilcoxon signed ranks); # = $p < 0.05$ (significantly different between the groups; Mann-Whitney test).

NF- κ B Nuclear Translocation

The NF- κ B DNA-binding activity of p50 and p65 were determined as markers of exercise-induced NF- κ B transcriptional activation. As shown in Figure 5, basal NF- κ B DNA-binding activity of p50 and p65 in PBMCs did not differ between the groups. Two hours after exercise, the NF- κ B DNA-binding activ-

ity of p50 in PBMCs significantly increased to a similar extent in control subjects and COPD patients ($p < 0.05$ and $p < 0.01$, respectively), whereas exercise did not alter p65 protein levels in both groups.

DISCUSSION

The results of the present study clearly indicate that a local exercise without notable respiratory response in clinically stable COPD patients results in increased systemic and even pulmonary oxidative stress without evidence of systemic inflammation during exercise. Moreover, this study reveals that quadriceps endurance is impaired in these patients compared with healthy control subjects.

Validity of the Local Endurance Test

To study Tlim, we used a local continuous-work quadriceps exercise test based on the model of Andersen et al.¹¹ In COPD patients, the increase in dyspnea, ventilation, and heart rate after this single-leg exercise was of small amplitude, whereas muscle fatigue was the main factor limiting exercise. This response pattern indicates that continuously localized exercise minimizes cardiorespiratory responses and nicely reflects muscle response to exercise. Conversely, during whole-body cycling exercise, the impairment of ventilatory function represented the main limiting factor for exercise capacity. This paralleled the differences in the observed subjective perception of leg fatigue and dyspnea during the different exercise modalities in COPD patients and control subjects.

Local Exercise-Induced Systemic Inflammatory and Oxidative Stress Response

In accordance with a previous study,²³ plasma inflammatory cytokine levels were unchanged by the exercise test in both groups, suggesting that local muscle exercise does not induce systemic inflammation. However, COPD patients showed an increased systemic oxidative stress response after single-leg exercise compared with control subjects, suggesting that exercise did not induce systemic inflammation to the same extent as oxidative stress. Increased urinary MDA levels were observed immediately after exercise in COPD patients, providing evidence for local exercise-induced oxidative stress in these patients. Moreover, urinary uric acid was significantly increased immediately after exercise in COPD patients compared with healthy control subjects, which could point to an increased production of free radicals due to xanthine oxidase activity. Uric acid is the end product of purine metabolism in humans

Table 4—The Effect of Exercise on Erythrocyte GSH, GSSG, and GSSG/GSH Ratio in Healthy Control Subjects and Patients With COPD

Variables	Control Subjects (n = 10)	COPD Patients (n = 15)
GSH, μ mol/g Hb		
Before	4.30 $\pm$ 1.24	3.82 $\pm$ 1.75
After	3.91 $\pm$ 0.88	3.55 $\pm$ 1.56
2 h after	3.80 $\pm$ 1.00*	3.67 $\pm$ 1.53
GSSG, μ mol/g Hb		
Before	0.26 $\pm$ 0.09	0.21 $\pm$ 0.09
After	0.29 $\pm$ 0.14	0.29 $\pm$ 0.08†
2 h after	0.25 $\pm$ 0.09	0.24 $\pm$ 0.06
GSSG/GSH		
Before	0.06 $\pm$ 0.01	0.06 $\pm$ 0.02
After	0.07 $\pm$ 0.03	0.08 $\pm$ 0.02†
2 h after	0.07 $\pm$ 0.01	0.07 $\pm$ 0.02

Data are expressed as the mean $\pm$ SD.

* $p < 0.05$ (significantly different).

† $p = 0.08$ (a tendency towards significance from baseline values within groups).

FIGURE 3. Difference in the concentration of erythrocyte GSH (A), GSSG (B), and GSSG/GSH ratio (C) from rest to different time points after submaximal single-leg exercise in healthy control subjects and COPD patients. Values are expressed as the mean $\pm$ SEM. * = $p < 0.05$ (significantly different); § = $p = 0.08$ (a tendency toward significance from baseline values within groups; Wilcoxon signed ranks).

and is an indicator of xanthine oxidase activity, which is seen as an important source of ROS.^{24,25} In healthy control subjects, the levels of the ubiquitous antioxidant GSH decreased 2 h after exercise. Previously, studies^{1,26} have shown that limb skeletal muscle mass of COPD patients is reduced compared with that of control subjects. Moreover, the duration of the exercise test was shorter in COPD patients. Therefore, it is feasible that less GSH is needed during exercise in comparison with healthy control subjects. Although statistical significance was not reached, GSSG and the GSSG/GSH ratio slightly increased in COPD patients after exercise, which indicates an increased oxidative stress response after localized exercise in COPD patients. This finding is in agreement with previous studies^{7,27} that have reported increased GSSG/GSH ratio after exhaustive whole-body exercise. These results suggest that in patients with COPD the antioxidant system is not able to cope with the higher rate of exercise-induced ROS

production, thus leaving skeletal muscle more susceptible to oxidative stress. As a localized exercise minimizes cardiorespiratory responses, we suggest that the contracting muscle was, at least in part, the source of the exercise-induced systemic oxidative stress in COPD patients. However, it cannot completely rule out that the increased oxidative stress response in COPD patients originated from sources other than the quadriceps (*eg*, the liver or heart).

NF- κ B is a transcription factor that plays a pivotal role in a diversity of cellular processes, including inflammation, survival, proliferation, and differentiation. Sustained activation of NF- κ B by oxidative stress has been suggested to play a central role in the etiology of the systemic features of COPD.²⁸ In particular, a growing body of evidence from experimental models²⁹ supports the involvement of NF- κ B in the pathogenesis of muscle wasting. NF- κ B is constituted by homodimers or heterodimers of the Rel family of proteins, which include p50, p52, RelA

FIGURE 4. Difference in the concentration of H₂O₂ measured in EBC from rest to different time points after submaximal single-leg exercise in healthy control subjects and COPD patients. Values are expressed as the mean $\pm$ SEM. * = $p < 0.01$ (significantly different from baseline values within groups; Wilcoxon signed ranks).

(p65), c-Rel, and Rel B. A main transcriptional activated form of NF- κ B is the heterodimer p65/p50, while the homodimer p50/50 is transcriptionally repressive. The heterodimer p50-p65 is activated by proinflammatory stimuli (eg, TNF- α and lipopolysaccharide), which in turn activate the transcription of inflammatory genes. In the present study, different patterns were observed for exercise-induced NF- κ B DNA-binding activity of p50 and p65 subunits in PBMCs but no differences between the two groups. Only DNA-binding activity of the p50 subunit was significantly elevated 2 h after exercise in both COPD patients and control subjects. This indicates that after a local exercise the induced NF- κ B DNA binding consists mostly of p50 homodimers. It is well known that p50 homodimers can act as suppressors of NF- κ B-dependent inflammatory cytokine transcription.^{29,30} This is in agreement with the present finding that plasma inflammatory cytokine levels were unchanged by the exercise test in both groups.

Remarkably, even despite the low cardioventilatory responses, we observed that the H₂O₂ concentration in the EBC of patients with COPD was significantly elevated immediately after and 2 h after local exercise, whereas, in control subjects, no significant increase was observed, indicating an enhanced production of ROS in the airways of these patients. Previously, we showed that H₂O₂ concentration was increased in COPD patients after maximal whole-

FIGURE 5. NF- κ B DNA-binding activity of p50 (A) and p65 (B) was determined in PBMCs in healthy control subjects and COPD patients 2 h after submaximal single-leg exercise. Values are expressed as the mean $\pm$ SEM. * = $p < 0.05$; ** = $p < 0.01$ (significantly different from baseline values within groups; Wilcoxon signed ranks).

body cycling exercise,⁸ which could be explained by the increased ventilatory responses during this exercise modality. However, in the present study, the immediate increase of H₂O₂ concentration in the EBC of COPD patients after local exercise is likely to be derived from a different mechanism since a local exercise is attended by less pronounced increased ventilatory responses. Until now, authors of other studies have not investigated the effect of exercise on H₂O₂ concentration in EBC. However, basal differences have been attributed to an enhanced activity of xanthine oxidase in the lungs of

COPD patients.^{31,32} Additionally, an increased number of inflammatory cells has been found in the BAL fluid of stable COPD patients compared with healthy nonsmokers.³³ Still, it could be speculated that xanthine oxidase activity in the epithelial cells of COPD patients is increased after exercise compared with control subjects, leading to the immediate increase in H_2O_2 concentration in COPD patients observed in this study. Moreover, it could be hypothesized that the pulmonary inflammatory response is intensified in COPD patients after exercise. The immediate increased H_2O_2 concentration may also originate from enhanced and/or inappropriate nicotinamide adenine dinucleotide phosphate hydrogen oxidase activation (for a review, see van der Vliet³⁴). Further research will have to elucidate whether these or other mechanisms also play a role in the exercise-induced H_2O_2 concentration.

It has been suggested that oxidative stress plays a role in peripheral muscle dysfunction in patients with COPD. However, no correlations were observed between the levels of markers of oxidative stress and quadriceps endurance in COPD patients. This is in contrast with a previous study² showing an inverse relationship of muscle lipid peroxidation and oxidized protein levels with quadriceps endurance in COPD patients.

Systemic Inflammation and Oxidative Stress at Rest

In the present study, resting values of plasma IL-6 were increased in clinically stable COPD patients compared with control subjects,³⁵ indicative of a low-grade systemic inflammation in these patients. However, no differences were observed in basal plasma TNF- α levels between both groups. Conflicting results have been reported^{23,36} regarding the proinflammatory cytokine TNF- α levels in the plasma of COPD patients. Contrary to previous studies, no differences in oxidative stress markers (urinary MDA, urinary uric acid, and H_2O_2 in breath condensate) were noted in COPD patients compared with control subjects.^{8,22} These discrepancies may be attributable to a difference in disease severity in terms of an altered oxidant/antioxidant balance, with the patients of the present study less severely affected than those of our previous study.⁸ Additionally, no differences between reduced GSH and oxidized GSSG levels were observed between both groups, which are in agreement with the results of previous studies.^{7,37} Moreover, no differences in NF- κ B DNA-binding activity of p50 and p65 were found between the two groups. Data regarding DNA-binding activity of both subunits in PBMCs of COPD were not previously available.

Limitations of the Study

We acknowledge several limitations to the interpretation of our data. The study involved a relatively small number of subjects studied. Yet, despite the relatively small number of subjects studied, we found consistent results in terms of increased systemic exercise-induced oxidative stress in COPD patients compared with healthy control subjects. We were also able to detect between-group comparisons of the exercise response. The exercise response of urinary MDA and urinary uric acid was significantly different between COPD patients and healthy control subjects. Patients who had received long-term therapy with oral corticosteroids and/or theophylline were not excluded in this study. However, the exclusion of these patients did not alter any of the outcomes of the statistical comparisons, indicating the medication did not confound our results. Another limitation of the current study is that we were not able to verify that the source of systemic oxidative stress markers was really the quadriceps. However, the use of a quadriceps exercise protocol in this study strengthened the hypothesis of peripheral muscle as a source of ROS in the systemic exercise-induced oxidative stress of patients with COPD since cardiorespiratory responses are minimized.

In summary, the results of this study strongly suggest that COPD patients have a more pronounced exercise-induced systemic oxidative stress response to localized leg muscle exercise without evidence of increased levels of systemic inflammation compared with healthy control subjects when tested at a similar relative intensity. Moreover, despite the low cardioventilatory responses after a localized exercise, COPD patients showed a greater susceptibility to local pulmonary oxidative stress response than control subjects. This suggests that healthy control subjects are able to tolerate localized exercise more effectively than COPD patients. The authors of future studies are encouraged to study the effects of antioxidant modulation on the acute exercise-induced oxidative stress response as well as the long-term effects of nutritional or pharmacologic modulation as an adjunct to exercise training on skeletal Tlim capacity.

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