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Systemic inflammatory response to exhaustive exercise in patients with chronic obstructive pulmonary disease

Hanneke A.C. van Helvoort[1], Marjolein H.J. van de Pol[1], Yvonne F. Heijdra, P.N. Richard Dekhuijzen*

Department of Pulmonary Diseases, and Institute for Fundamental and Clinical Human Movement Sciences, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

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Summary Systemic inflammation may be present in patients with chronic obstructive pulmonary disease (COPD). Exercise is known to elicit an inflammatory response. We hypothesized that the systemic inflammatory response to exercise might be exaggerated in COPD patients compared to healthy subjects. Sixteen COPD patients and 11 healthy subjects performed a maximal incremental bicycle test. Before and at maximal exercise arterial blood samples were taken to determine circulating catecholamines, (subsets of) leukocytes, acute phase proteins, creatine kinase and myoglobin. At rest, increased levels of norepinephrine and systemic inflammation were present in COPD. The response of catecholamines to exercise was lower in COPD patients ($P<0.01$), which in part was due to the lower maximal exercise capacity of these patients ($P<0.01$). Exercise-induced leukocytosis showed similar responses in both groups, but occurred at higher levels in COPD. Although patients had increased levels of CRP at rest ($P<0.001$), exercise did not affect acute phase proteins. No systemic signs of muscle damage were found. The present study shows that COPD patients are exposed to systemic inflammation that is intensified by exhaustive exercise. The inflammatory response in COPD is not exaggerated compared to healthy subjects but occurs at a higher level and is observed at lower external workload.

Abbreviations: ANOVA, analysis of variances; BMI, body mass index; CK, creatine kinase; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ECG, electrocardiography; ICS, inhaled corticosteroids; Mb, myoglobin; MVV, maximal voluntary ventilation; NK-cells, natural killer cells; $PaCO_2$, arterial carbon dioxide tension; PaO_2, arterial oxygen tension; PBMC, peripheral blood mononuclear cells; Pred, predicted; TNF-α, tumor necrosis factor-alpha; $\dot{V}_E$, minute ventilation; $\dot{V}_{CO_2}$, carbon dioxide production; $\dot{V}_{O_2}$, oxygen consumption; W_{max}, maximum work capacity

*Corresponding author. Tel.: +31 24 3614579; fax: +31 24 3610324.
E-mail address: R.Dekhuijzen@long.umcn.nl (P.N.R. Dekhuijzen).

[1]First and second author equally contributed in the preparation of this manuscript.

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Introduction

Considerable evidence links chronic obstructive pulmonary disease (COPD) with systemic inflammation,[1,2] including altered numbers[3] and functions of circulating inflammatory cells,[4–6] cytokines,[7,8] acute phase proteins,[3,7] and oxidative stress.[9,10] This indicates that COPD is not restricted to pulmonary disease, but may also affect distant organs, e.g. by inducing substantial skeletal muscle alterations[11] and weight loss.[7] These factors may contribute to exercise limitation[12,13] and reduced quality of life in these patients.[14] Exercise may be beneficial for patients with COPD, in part by improving exercise tolerance (endurance and maximal exercise capacity), and training of muscles.[15] On the other hand, it is known that exercise, if sufficiently intense, leads to a highly stereotyped immune response in healthy subjects, mediated by an interplay of inflammatory cells, hormones, cytokines, neural and hematological factors,[16] that can also affect distant organs.[17] Also, in subjects with cystic fibrosis it was found that low intensity exercise could increase already elevated circulating cytokines.[18] Since COPD patients may show signs of systemic inflammation, elevated levels of circulating catecholamines, marked sympathetic activation[19,20] and muscle wasting[21] at rest, it may be expected that physical activity will further increase these mediators. Indeed, recently it was shown that moderate intensity exercise in COPD abnormally increased plasma tumor necrosis factor-alpha (TNF-α) levels.[22] Moreover, exercise induces systemic[23,24] and muscle[25] oxidative stress in COPD patients. In view of these data, we hypothesized that exercise causes an exaggerated systemic inflammatory response in COPD patients compared with healthy subjects, which consequently might worsen the effects on distant organs.

The purpose of this study was to characterize the effects of a single bout of exhaustive exercise on the systemic responses in COPD patients. Therefore, levels of circulating catecholamines and (subpopulations of) leukocytes as well as serum levels of acute phase proteins (CRP) and the muscle proteins creatine kinase (CK) and myoglobin (Mb) were measured before and after maximal incremental cycle ergometry.

Methods

Subjects

Sixteen (10 males, 6 females) non-smoking COPD patients (age 52–68 years) from our outpatient clinic participated in this study. These patients had moderate to very severe COPD (FEV_1 23–68% predicted) according to global initiative for chronic obstructive lung disease.[26,27] They were free of exacerbations for at least 2 months prior to the study. Exclusion criteria were use of oral corticosteroids, long-term oxygen therapy, and other exercise-limiting diseases. Inhaled corticosteroids (ICS) (if used) were stopped 1 week prior to exercise testing (ten patients). All patients used inhaled bronchodilators (ipratropiumbromide and/or β_2-agonists), and none used theophylline. The patients were recruited before going through a rehabilitation program. For the control group, eleven (9 males, 2 females) non-smoking, sedentary healthy subjects (age 47–64 years) were recruited from the social environment of the patient group. The study was conducted according to the Declaration of Helsinki and approved by the medical ethical committee of our hospital. Written informed consent was obtained from all subjects.

Pulmonary function

Standard pulmonary function tests including spirometry, static lung volumes and diffusing capacity for carbon monoxide (DL_{CO}) were obtained prior to cycle ergometry.

Protocol cycle ergometry

All subjects performed a maximal, symptom limited, incremental exercise test. They cycled on an electrically braked cycle ergometer (Masterlab, Jaeger, Würzburg, Germany) at a pedaling rate of 60 rotations min^{-1} breathing room air. The workload was increased every minute by 10% of estimated maximum work capacity (W_{max}) until exhaustion. The maximum work capacity was calculated according to the equitation of Jones et al.[28] This maximal value was then adapted to the subject by multiplying it by FEV_1/FEV_1 predicted.[29] The exercise protocol resulted in a test duration of 8–12 min, which meets the exercise testing recommendations.[30] Minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were measured every 30 s breath-by-breath (Oxyconbeta, Mijnhardt/Jaeger, Bunnik, The Netherlands). Electrocardiography (ECG) was conducted throughout the test and saturation was measured using a pulse-oxymeter (Datex, Helsinki, Finland). If ECG-changes or chest pain occurred, or saturation fell below 85%, the test was stopped immediately. Blood pressure was measured every 2 min during the test.

Collection of blood samples

A cannula was inserted into the brachial artery under local anesthesia to obtain arterial blood.

Arterial blood samples were collected at rest, every 3 min during exercise, at W_{max} and 3, 30, 60 and 120 min after the test. Two hours after the exercise testing the arterial cannula was removed. Venous blood was collected via a venapuncture at 6 and 24 h after the test for determination of muscle damage markers (see below). Measurements after exercise were corrected for plasma volume shifts according to Dill and Costill.[31]

Analytical procedures

Blood for determination of hemoglobin, hematocrit, CRP (ELISA, detection limit 1 $\mu g\,mL^{-1}$) and leukocytes was collected (at rest, W_{max}, and 30, 60 and 120 min after exercise) in vacutainers containing EDTA and analyzed immediately according to standard laboratory assays.

To determine CK, Mb, uric acid, and glucose, blood was collected in dry vacutainers and analyzed immediately in serum according to standard laboratory assays.

For blood gas and lactate analysis, arterial blood was collected in special heparinized syringes and analyzed immediately (Gas analyzer Chiron 860). Blood gasses (PaO_2, $PaCO_2$) were measured pre-exercise, every 3 min during the exercise and 3 min after maximal work rate. Lactate levels were determined enzymatically at rest and 3 min after maximal exercise.

Catecholamine measurement

Blood was collected at rest, directly after maximal exercise and after 30 min of recovery in pre-cooled vacutainers containing heparin. Blood samples were put on ice and spun down immediately. Supernatant was stored in tubes containing 0.25 $mol\,L^{-1}$ EGTA and 0.2 $mol\,L^{-1}$ glutathione in distilled water (pH 7.4). Epinephrine and norepinephrine were measured according to Willemsen et al.[32]

Phenotype analysis of peripheral blood mononuclear cells (PBMC)

Blood samples for lymphocyte immunophenotyping by three-color flow cytometry[33] were collected at rest, W_{max} and 30, 60 and 120 min after exercise in heparinized vacutainers. Monoclonal antibodies with a fluorescing label were used to identify the numbers of helper/inducer T-lymphocytes (CD3+/CD4+), B-lymphocytes (CD19+) and natural killer (NK) cells (CD3-/CD56+/CD16+).

Statistics

Differences in baseline values, anthropometric variables and pulmonary function between healthy subjects and patients with COPD were determined with two-sample *t*-tests and Mann–Whitney *U* tests (if the normality assumption (Kurtosis) was not obtained). Repeated measures analysis of variance (ANOVA) was used to analyze all responses to the maximal exercise bout. Between-subject tests were used to compare overall response differences between the control and COPD group across the time points (between-group effect). Single degree of freedom orthogonal polynomials over time were used to characterize possible changes caused by exercise, i.e. linear and quadratic changes across time (time effect). These polynomials were examined for absolute values with all time points and for differences from baseline (for each subject). A difference between the control and the COPD group in the response pattern by exercise was tested using the interaction between each polynomial and the between subject factors (time*group effect). Linear regression analysis was performed to test if exercise capacity, catecholamine response, and lymphocyte response were correlated.

Statistical significance was taken at the $P<0.05$ level. Results are presented as means $\pm$ SE. Data were analyzed with SPSS/PC+, version 12.0 (SPSS, Chicago, IL).

Results

Anthropometric and pulmonary function data

Subjects' characteristics and pulmonary function data are provided in Table 1. Age and body mass indices (BMI) were similar in both groups. The main differences were observed in pulmonary function where the COPD group showed moderate to very severe airflow obstruction. There was no significant difference in arterial oxygen or carbon dioxide tension between the groups. None of the subjects were hypoxemic at rest.

Catecholamines and systemic inflammation at rest

As shown in Fig. 1, there was no significant difference in plasma epinephrine levels at rest

Table 1 Anthropometric and pulmonary function data in healthy subjects and patients with COPD.

	Healthy subjects	COPD patients
N	11	16
Male/female	9/2	10/6
Age, years	56±2	60±2
BMI, kg m^{-2}	27.5±1.3	25.3±1.2
FEV_1, L	3.5±0.2	1.2±0.1***
FEV_1, % pred	110±4	42±3***
FEV_1/VC, %	77±1	39±2***
TLC, % pred	108±4	114±4
Dl_{co}, % pred	109±5	94±11
PaO_2, kPa	11.9±0.4	10.9±0.5
$PaCO_2$, kPa	5.5±0.1	5.3±0.2

Data are expressed as means±SE.
Abbreviations: BMI, body mass index; FEV_1, forced expiratory; volume in first second; VC, vital capacity; TLC, total lung capacity; Dl_{co}, diffusion capacity for carbon monoxide; PaO_2, arterial oxygen tension $PaCO_2$, arterial carbon dioxide tension; pred, predicted value.
***$P<0.001$.

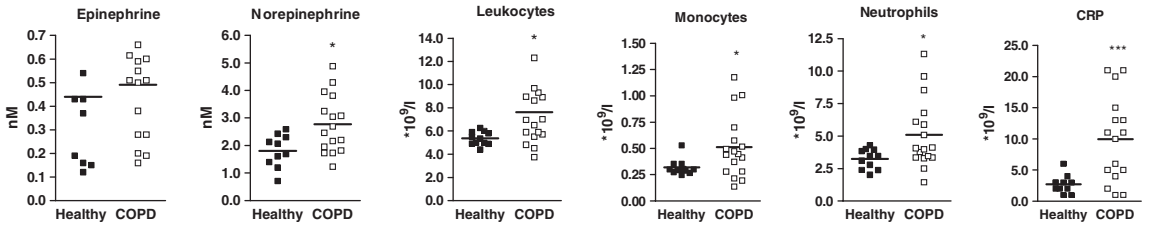

Figure 1 Individual and mean values of baseline epinephrine, norepinephrine, circulating leukocytes, neutrophils, monocytes, and CRP in healthy subjects and patients with COPD. *$P<0.05$, ***$P<0.001$.

between COPD patients and healthy subjects. Baseline norepinephrine levels, however, were significant higher ($P<0.05$) in COPD patients. Systemic inflammation at rest was indicated by increased numbers of total circulating leukocytes ($P<0.05$), neutrophils ($P<0.05$), and monocytes ($P<0.05$) as well as enhanced CRP levels ($P<0.001$) in COPD patients. No significant differences between COPD patients and healthy subjects were measured in numbers of circulating lymphocytes and the subsets NK-cells, T- and B-lymphocytes.

Maximal exercise test

Physiological data

Physiological responses to exercise are shown in Table 2. As expected, W_{max} was significantly lower in COPD patients ($P<0.001$). Also, duration of the exercise test, maximal oxygen uptake (peak $\dot{V}_{O_2}$) and minute ventilation (peak $\dot{V}_E$) were significantly lower in the COPD group. The peak $\dot{V}_E$, however, represented 96±6% of the maximal voluntary ventilation (MVV) in COPD patients and 68±4% in the control group ($P<0.01$). PaO_2 significantly decreased during exercise in COPD patients ($P<0.05$), while $PaCO_2$ increased ($P<0.001$). Six patients became hypoxemic and five became hypercapnic during exercise. No changes in PaO_2 occurred in healthy subjects, while $PaCO_2$ decreased at maximal exercise ($P<0.01$). In both groups, a significant increase ($P<0.01$) in plasma lactate was observed at W_{max}. This increase of lactate levels was significantly lower in COPD patients compared with healthy subjects (Δ lactate; 4.1±0.7 mM vs. 7.3±0.9 mM, $P<0.05$).

Catecholamines

Plasma levels of epinephrine and norepinephrine before and after the exercise test are shown in Fig. 2. Exercise led to significant changes in plasma epinephrine and norepinephrine in both groups ($P<0.001$ for both catecholamines). The response to exercise was significantly lower in COPD patients compared with healthy subjects for both catecholamines ($P<0.05$ for epinephrine, and $P<0.001$ for norepinephrine). The plots in Fig. 3 show that the lower response of catecholamines to exercise in

Table 2 Physiological data after maximal exercise in healthy subjects and patients with COPD.

	Healthy subjects	COPD patients
W_{max}, W	201 ± 18	90 ± 11***
W_{max}, % pred	79 ± 6	57 ± 5**
Endurance, s	729 ± 33	519 ± 32***
Max HR, beats min^{-1}	163 ± 3	128 ± 5***
Max HR, % pred	99 ± 2	80 ± 3***
Peak $\dot{V}_{O_2}$, L min^{-1}	2.4 ± 0.18	1.3 ± 0.12***
Peak $\dot{V}_{O_2}$	30.5 ± 2.7	17.4 ± 1.2***
Peak $\dot{V}_{O_2}$	108 ± 8	65 ± 5***
Peak $\dot{V}_E$, L min^{-1}	84 ± 6	43 ± 4***
Peak $\dot{V}_E$, % MVV	68 ± 4	96 ± 6**
PaO_2, kPa	12.5 ± 0.5	9.7 ± 0.4***
$PaCO_2$, kPa	4.9 ± 0.1	6.0 ± 0.2***
Lactate, mM	8.9 ± 1.0	5.2 ± 0.7**

Data are expressed as means ± SE.
Abbreviations: W_{max}, maximal work capacity; endurance, duration of exercise test; HR, heart rate; peak $\dot{V}_{O2,}$ maximal oxygen consumption; $\dot{V}_E$, minute ventilation; MVV, maximal voluntary ventilation; PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; pred, predicted.
**$P < 0.01$, ***$P < 0.001$.

COPD patients were related the relative lower work intensities they performed. Also, within the COPD group, W_{max} and peak $\dot{V}_{O_2}$ were positively correlated with changes in norepinephrine ($r = 0.60$, $P < 0.05$ and $r = 0.57$, $P < 0.05$, respectively). As described above, basal levels of norepinephrine were significantly higher in COPD patients compared with healthy subjects ($P < 0.01$). To exclude influence of inhaled β_2-agonists on catecholamine levels, a subgroup of six COPD patients was asked to stop the inhalation of β_2-agonists for one week and to perform a second maximal exercise test after this period. Discontinuation of inhaled β_2-agonists in these patients for one week did not affect maximal exercise capacity nor levels of catecholamines at rest (epinephrine, $P = 0.64$; norepinephrine, $P = 0.73$), or at W_{max} (epinephrine, $P = 1.0$; norepinephrine, $P = 0.74$), see Table 3.

Systemic inflammation

Numbers of total circulating leukocytes in both COPD patients and healthy subjects at rest, directly after exercise and during recovery are shown in Fig. 4A. Maximal exercise caused an immediate increase of circulating leukocytes in both COPD patients (from 7.6 ± 0.7 to 9.2 ± 0.9* × 10^9 cells L^{-1}) and healthy subjects (from 5.4 ± 0.2 to 7.4 ± 0.2 × 10^9 cells L^{-1}), followed by a rapid decrease during the first 30 min of recovery and a second increase later in the recovery period (time effect: $P < 0.001$). The mean numbers of leukocytes were significant higher in COPD patients compared with healthy subjects ($P < 0.05$), but the response to exercise was comparable in both groups (time*group effect: $P > 0.05$).

The exercise-induced response of leukocytes as caused by its subsets, is also shown in Fig. 4. In all subsets, a significant response to exercise was measured in both COPD patients and healthy subjects ($P < 0.001$). The mean numbers of both neutrophils (Fig. 4B) and monocytes (Fig. 4C) before and after exercise were significantly higher in COPD patients compared with healthy subjects ($P < 0.05$ and $P < 0.01$, respectively), but the responses (absolute and relative) to exercise were not different. As shown in the last panel of Fig. 4 (4D), the absolute response of lymphocytes caused by exercise was significant lower in COPD patients compared with healthy subjects ($P < 0.001$). In addition to this finding, significant correlations ($P < 0.001$) were found between exercise capacity, catecholamine response, and lymphocyte response (Fig. 5). In addition, within the COPD group, peak $\dot{V}_{O_2}$ was correlated with changes in lymphocytes ($r = 0.57$, $P < 0.05$), and changes in norepinephrine were related to the lymphocyte response ($r = 0.54$, $P < 0.05$). Evaluation of the exercise-induced response of the NK-cells and lymphocyte subsets resulted in similar responses for COPD patients and healthy subjects. NK-cells (Fig. 6A) and B-lymphocytes (Fig. 6B) resembled the response of the total lymphocytes, although the reduction in the responses of COPD patients was not significant here. The response of T-lymphocytes immediately after

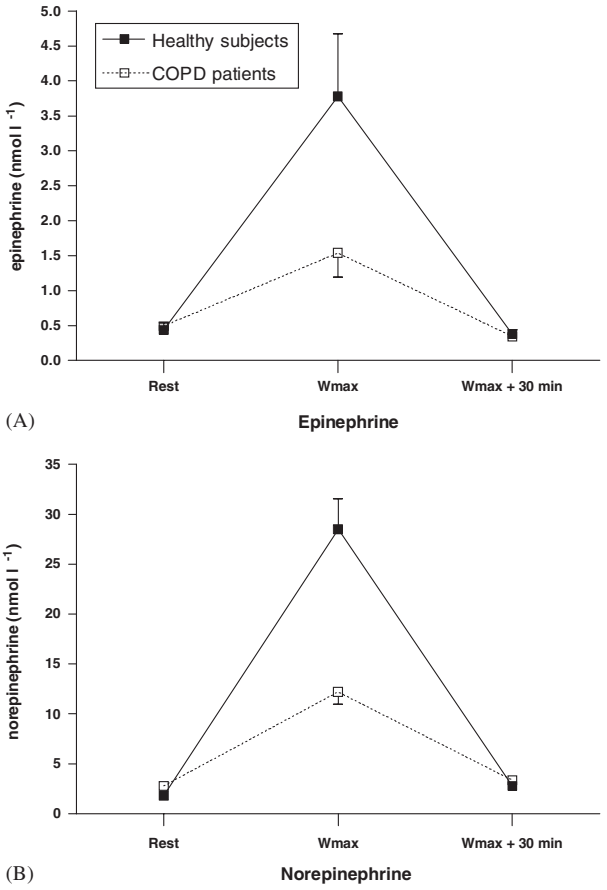

Figure 2 Effects of exercise on plasma concentrations epinephrine (A) and norepinephrine (B) in both healthy subjects and patients with COPD. Exercise induced a significant response of the catecholamines in both groups ($P<0.001$). Both epinephrine and norepinephrine responses were lower in COPD patients ($P<0.05$).

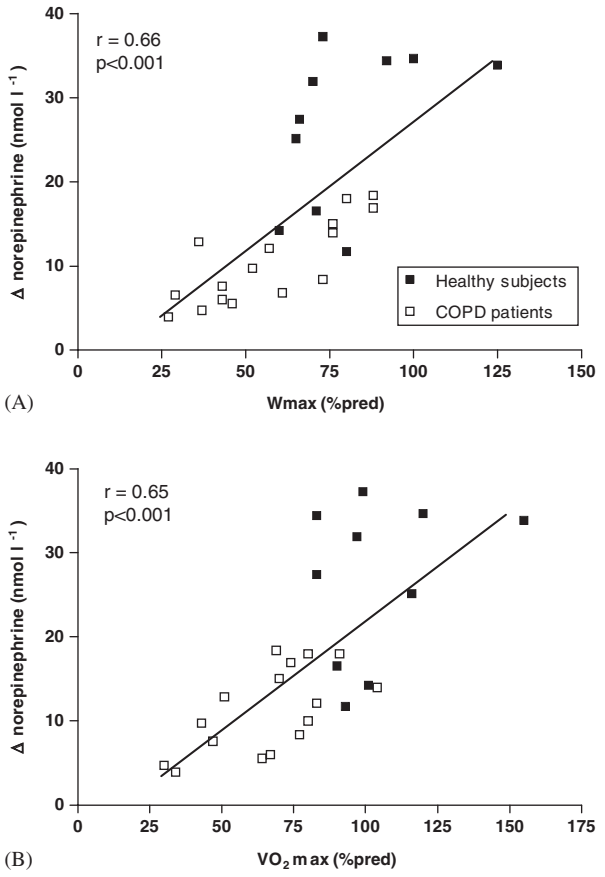

Figure 3 Exercise-induced changes in norepinephrine levels related to maximal workload (A) and peak $\dot{V}_{O_2}$ (B) in healthy subjects and patients with COPD.

maximal exercise was the same in both groups (Fig 6C). The difference occurred after two hours recovery when the T-lymphocytes of COPD patients were still elevated from baseline levels ($P<0.01$).

Discontinuation of β_2-agonists in a subset of COPD patients did not affect numbers of (subsets of) leukocytes at rest ($P=0.3$), or after maximal exercise ($P=0.8$), see Table 3.

Plasma levels of CRP after exercise did not differ from rest values in both COPD patients and healthy subjects.

Muscle damage markers

As shown in Table 4, serum levels of the muscle damage markers CK and Mb did not differ between COPD patients and healthy subjects at rest ($P=0.4$) and did not change significantly in response to short exhaustive bicycle exercise.

Discussion

The present study shows that the exercise-induced systemic inflammatory response is not exaggerated in COPD patients compared with healthy subjects. The systemic inflammatory response to exhaustive exercise at relatively low external workload in COPD patients is comparable with the response to high intensity maximal exercise in healthy subjects, but occurs at a higher level in COPD. Therefore, the absolute number of circulating inflammatory cells is intensified to higher levels in patients with COPD, which may stimulate several inflammatory mediators and processes. Owing to lower exercise intensity, catecholamine response was lower in COPD patients. These data indicate that patients with COPD performing strenuous exercise are exposed to an intensified systemic inflammation.

Catecholamines

In response to exercise above 60% of peak $\dot{V}_{O_2}$, plasma concentrations of a number of stress

Table 3 The effect of discontinuation of inhaled β_2-agonists on exercise capacity, catecholamines and circulating leukocytes.

	With β_2-agonists ($n = 6$)		Without β_2-agonists ($n = 6$)	
	At rest	At W_{max}	At rest	At W_{max}
W_{max}, W		114±13		112±14
Peak $\dot{V}_{O_2}$ % pred		81±6		80±8
Epinephrine, nmol L^{-1}	0.39±0.1	2.1±1.0	0.31±0.1	2.1±1.0
Norepinephrine, nmol L^{-1}	2.5±0.5	15.6±2.2	2.2±0.4	17.1±3.9
Total leukocytes, $*10^9 \times L^{-1}$	5.4±0.5	7.3±0.8	6.1±1.0	7.6±1.1

Data are expressed as means ±SE.
Abbreviations: W_{max}, maximal work capacity; peak $\dot{V}O_2$, maximal oxygen consumption.

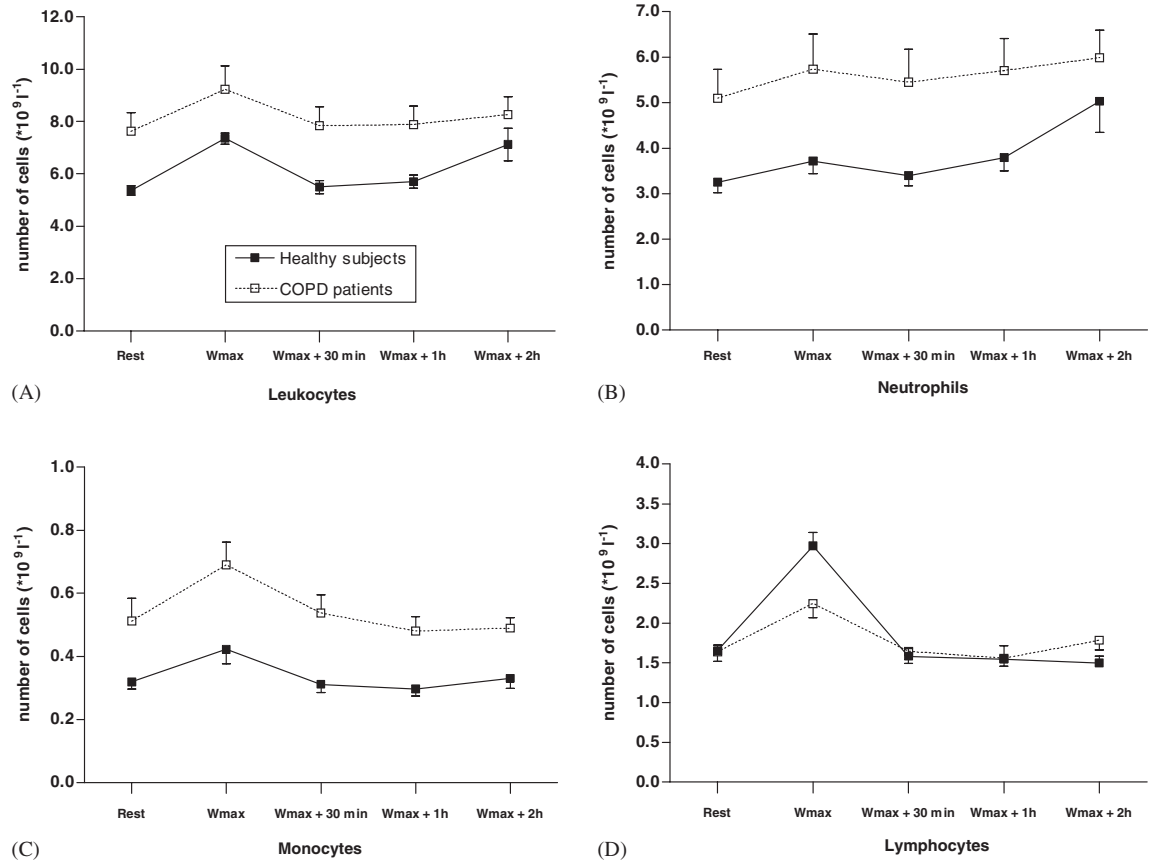

Figure 4 Effects of exercise on circulating leukocytes and subsets in both healthy subjects and patients with COPD. Exercise caused significant responses of total leukocytes (A), neutrophils (B), monocytes (C), and lymphocytes (D) in both groups. Responses of leukocytes and the subsets neutrophils and monocytes were similar in patients and healthy subjects. Lymphocyte response was significantly lower in COPD patients ($P < 0.001$).

hormones, including epinephrine and norepinephrine, increase and return to prevalues shortly after exercise.[34] Discharge from sympathic splanchnic nerves and innervation of the adrenal medulla result in the release of epinephrine and norepinephrine into the plasma immediately after start of intense muscular exercise. These stress hormones have marked physiological effects on heart rate and vasomotor tone, and ultimately on blood flow through lymphoid tissues and leukocyte circulation patterns.[35] In the present study, plasma catecholamine levels indeed increased during maximal exercise in both COPD patients and healthy controls and declined rapidly after exercise. Catecholamines increase almost linearly with the duration of exercise and exponentially with intensity, when it is

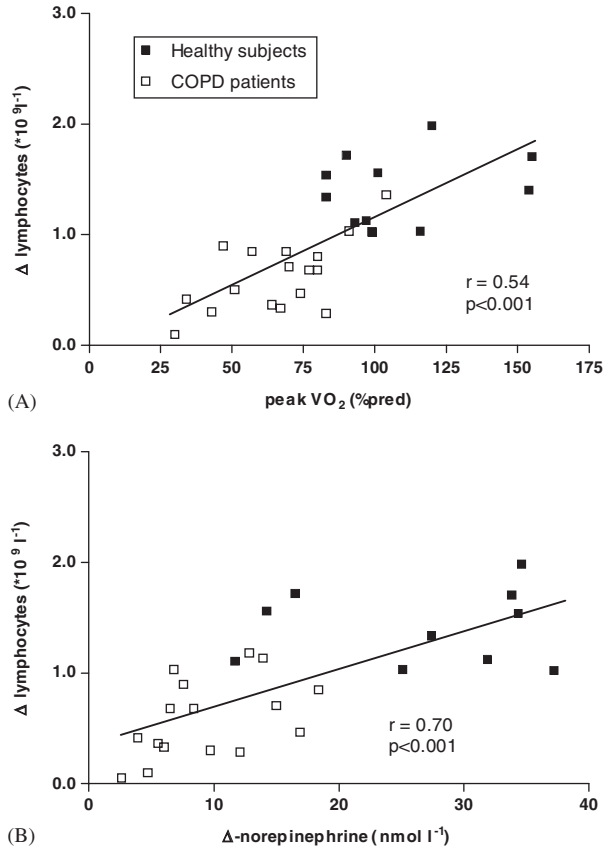

Figure 5 The influence of exercise capacity (A) and subsequent changes in norepinephrine (B) on lymphocyte response to exercise.

expressed relative to individual's peak $\dot{V}_{O_2}$.[36] The lower catecholamine levels of COPD patients at maximal exercise compared with healthy controls in this study can at least partially be explained by the lower exercise capacity (both ventilatory capacity and maximum workload) of the patients or by the shorter duration of their exercise, as is illustrated when exercise capacity is plotted against changes of noradrenaline (Fig. 3). Debigare and coworkers[37] showed that a greater increase in the cycling load during an incremental exercise test resulted in higher achieved peak loads. According to them, peak $\dot{V}_{O_2}$, however, was independent of the increase in workload. Because the catecholamine response was not only correlated with W_{max} in the present study, but also with peak $\dot{V}_{O_2}$, we think that our results were not influenced by the different exercise protocols. Furthermore, the study was not designed to compare the increases of catecholamine levels between the groups at isotime or isoworkload exercise between COPD patients and healthy subjects. If the increases in cycling load had been similar in patients and healthy subjects, probably the exercise recommendations of 8–12 min test duration[30] were not met. Based on our results at maximal exercise, it is suggested that the catecholamine response to exhaustive exercise is not affected by COPD. These results are in disagreement with data of Colice et al.,[38] who reported that the rate of increase of epinephrine (but not norepinephrine) with maximal exercise was smaller in hypoxic COPD patients than in healthy controls. Their findings supported the concept that (chronic) hypoxia interferes with the adrenal medullary response to exercise. In our study, most of the COPD patients remained normoxic during exercise, which may explain the difference between these studies.

Remarkably, plasma levels of norepinephrine at rest were higher in COPD patients compared with healthy subjects in our study. Other investigators also found elevated baseline values of (nor-) epinephrine in (a subgroup of) COPD patients.[38,39] The precise mechanism of these findings is unclear. Beta$_2$-adrenoreceptor agonists, used by many COPD patients, produce a number of metabolic changes.[40] In a study of Malerba et al.,[41] plasma norepinephrine concentrations of COPD patients using systemic β_2-agonists were significantly increased at rest and especially during cardiopulmonary exercise test, due to activation of the orthosympathetic nervous system and anaerobic metabolism. This possible β_2-effect was ruled out in our study because a subgroup of patients performed a second test after discontinuation of their use of inhaled β_2-agonists for one week. Discontinuation of these β_2-agonist caused no differences in catecholamine levels at rest or after exercise testing.

Systemic inflammation

The earliest and most consistent observation of the exercise-immune interaction has been the so-called ''leukocytosis of exercise''.[42] Despite variation in type, intensity, duration of the exercise, and fitness level of the subjects, several consistent patterns emerge regarding the leukocyte subpopulations in blood. The leukocytosis in both COPD patients and healthy controls in the present study reflected the previously described patterns: an early leukocytosis characterized mainly by an increase in all subsets, followed by a second phase during which neutrophils gradually increased and lymphocytes concentrations rapidly fell down. This typical inflammatory response of total leukocytes and the subsets neutrophils and monocytes had a similar pattern in COPD patients and healthy

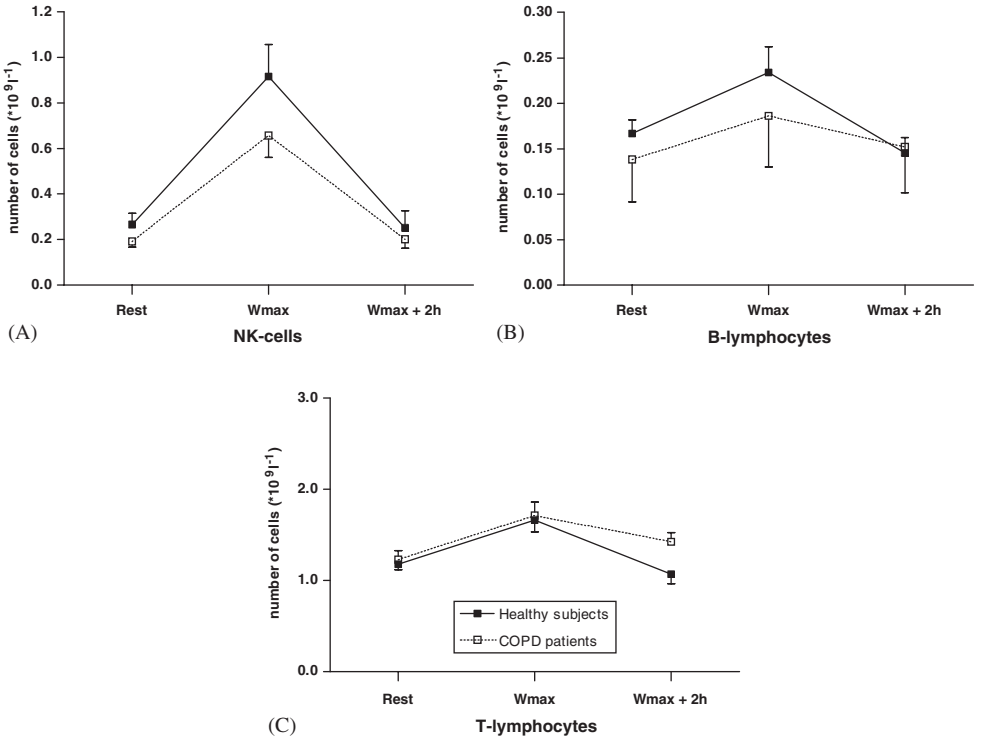

Figure 6 Effects of exercise on circulating NK cells and lymphocyte subsets in both healthy subjects and patients with COPD. The responses of NK-cells (A) and B-lymphocytes (B) to exercise was comparable in both groups, while the pattern of the response of the T-lymphocytes (C) differed between the groups after 2 h of recovery ($P < 0.01$).

Table 4 Muscle damage markers before and after exercise in healthy subjects and COPD patients.

	At rest	W_{max}	W_{max}+1h	W_{max}+2h	W_{max}+6h	W_{max}+24h
CK ($U\,L^{-1}$)						
Healthy subjects	127±15	122±15	127±16	127±17	127±14	126±15
COPD patients	106±13	106±13	98±12	100±12	104±12	100±12
Mb ($\mu g\,L^{-1}$)						
Healthy subjects	30±4	33±5	33±5	31±4	34±4	33±4
COPD patients	30±6	31±6	31±5	32±5	34±6	33±5

Data are expressed as means±SE.
Abbreviations: CK, creatine kinase; Mb, myoglobin.No significant differences between the groups or in response to exercise were observed.

subjects, but occurred on an elevated level in the patients. Acute lymphocytosis also occurred following maximal exercise in all subjects, but the increase in lymphocytes, especially B-lymphocytes, and NK-cells was impaired in COPD patients (only significance in total lymphocytes). Absence of significant differences in B-lymphocytes and NK-cells can partly be attributed to relative large interindividual variability that is known in these cells,[43] which results in large standard errors seen in the present study. According to different models,[35,42,44] catecholamines are responsible for the acute exercise-effects of lymphocyte subpopulations and NK-cells. Finding less increase in levels of catecholamines in COPD patients (due to relative low intensity exercise) supports our results of a slightly decreased lymphocytosis in these patients (Fig. 5). T-lymphocyte response of COPD to exercise

resembled the response of controls, except for the remained elevation of T-lymphocytes of COPD patients after 2 h.

With stopping the use of ICS, if used, one week prior to the study, possible declining effects of ICS on systemic inflammatory markers[45,46] were prevented. Again, any possible effect of β_2-agonists[47] on different leukocyte counts can be ruled out because patients, who stopped the inhalation of these agonists, did not show any difference in leukocyte baseline levels or levels after the maximal exercise test.

Although COPD patients had increased basal levels of CRP compared with healthy subjects, exercise did not change the levels in both groups. Serum levels of acute phase reactants do change with inflammation,[48] but with respect to exercise models, the serum levels of these reactants have not been well characterized. To our knowledge, no data are reported about CRP levels after exercise in COPD patients. While Pyne et al.,[49] published no changes in CRP after 40 min uphill (90% of peak $\dot{V}_{O_2}$) or downhill (52% of peak $\dot{V}_{O_2}$) running in healthy subjects, Mastaloudis et al.,[50] recently demonstrated remarkable increases in CRP in ultra marathon runners (423 min, 71% of peak $\dot{V}_{O_2}$). Additional research on the impact of exercise on acute phase reactants is needed.

Limitations

In the present study, the gender distribution is different between the two study groups. Although there is no consensus in the literature, some data[51] suggest that there may be gender-based differences in muscle damage, inflammation and oxidative stress after exercise, possibly caused by estrogen. Because all females included in the presented study were postmenopausal, the possible effect of estrogen can be minimized. In addition, Moyna et al.,[52] showed that the alterations in the numbers of circulating leukocytes during and following an acute progressive incremental exercise test (3 periods of 6 min cycling at 55%, 70%, and 85% of peak $\dot{V}_{O_2}$) are independent of gender. Therefore, the exercise-induced inflammatory responses found in this study are probably not affected by gender differences.

Leukocyte source

The source of the leukocytes mobilized during exercise and what mechanisms are involved in the mobilization were not subject of this study but are of considerable interest. It is clear that the immediate mobilized cells are derived from the marginated or non-circulating leukocyte pool(s), while the delayed (second) increase of leukocytes after two hours (especially neutrophils) can also be influenced by release of (immature) cells from the bone marrow. Less clear is the place in the body where the pool for the immediate response is located. The spleen, the lungs, and the peripheral blood vessels seem important candidates.[16,42] Whether the source(s) of exercise-induced leukocytosis or the mechanism(s) involved in this mobilization differs between healthy subjects and COPD patients, is not known yet. Speculatively, the lungs might play a more important role in the leukocytosis of COPD patients compared to healthy subjects because pulmonary inflammation causes increased levels of neutrophils and lymphocytes in the lungs.

Markers of muscle damage

Muscular exercise commonly results in injury to fibers in the active muscles, particularly when the exercise is relatively intense ($>60\%$ of peak $\dot{V}_{O_2}$), of long duration (>30 min) and/or includes eccentric contractions.[11,53] One of the clinical symptoms associated with muscle injury includes elevated plasma levels of muscle proteins (e.g. CK and Mb). In the presented study, no evidence of muscle damage was found systemically in COPD patients or in healthy subjects after a short bout of maximal exercise. Both groups did not show changes in circulating levels of CK and Mb after exercise. The mechanism of metabolic stress, characterized by disturbances in cellular metabolism, has been proposed to explain muscle damage after prolonged (>30 min) high intensity or exhaustive exercise.[11] Metabolic stress not only induces muscle damage, but also activates the hormonal and inflammatory response to repair tissue damage. Several explanations for not finding evidence of muscle damage are possible. Firstly, changes in plasma levels of muscle proteins are related to membrane (sarcolemmal) damage or permeabilization. Absence of changes in these proteins does not exclude injury of sarcomere or other organelles (e.g. mitochondria). Using muscle proteins in blood as marker for muscle damage has been criticized by many authors.[54] Some of them even conclude that muscle damage can never be correctly estimated by any marker in circulating blood because these markers are always a reflection of the difference between release and uptake by other tissues.[55] Secondly, we did not assess muscle injury or damage directly. And finally, the

duration of the exercise test, especially in COPD patients could have been too short to induce markedly muscle damage. For these reasons, definite conclusions about muscle damage cannot arise from the present findings.

Clinical relevance

Mechanisms and consequences of systemic inflammation are still poorly understood. So far, it is thought that systemic inflammation may be associated with nutritional abnormalities, weight loss, skeletal muscle dysfunction and subsequently exercise tolerance in patients with COPD.[1] Increased numbers of leukocytes and also pre-activation of these cells, especially monocytes and neutrophils, have been reported in earlier studies with stable COPD patients.[2] Changes in the numbers and functions of these cells may be of relevance for the normal process of neutrophils clearance by macrophages from inflamed tissues.[4,56,57] High levels of circulating leukocytes will also affect production of inflammatory mediators and reactive oxygen species, which together play an important role in the regulation of systemic inflammatory response and the possible effects on distant organs. Circulating lymphocytes have been less well studied than circulating neutrophils in patients with COPD. There are some indications of abnormal lymphocyte function in these patients,[58] but whether these issues influence patient's defense against inflammation, remains to be resolved.

Although the present study shows that the exercise-induced leukocytosis in COPD patients is not different from healthy subjects, two important aspects have to be kept in mind. Firstly, the patterns of the leukocytosis are not different between the groups, but the absolute numbers of the inflammatory cells in COPD patients rise to rather high levels. These levels probably also affect other parts of the inflammatory cascade, which together contribute to a further increase of systemic inflammation. A second concern is the comparison of exercise tests of healthy subjects and ventilatory limited patients. Unlike healthy subjects, patients with COPD become exhausted at low external workload; for instance during daily life activities, which therefore may result in a more frequent exposure to systemic inflammation. During daily life activities, patients with COPD reach a $\dot{V}_{O_2}$ of 8–10 mL kg^{-1} min^{-1}.[59,60] In the present study, however, a standardized maximal exercise protocol was used, resulting in a mean peak $\dot{V}_{O_2}$ of 17 mL kg^{-1} min^{-1} in the COPD patients. Therefore, further studies are needed to investigate if submaximal exercises and daily life activities also leads to an increase of systemic inflammation as shown in the present study.

In summary, the present study shows that exercise-induced systemic inflammatory response is not exaggerated in COPD compared with healthy subjects, but occurs on higher levels in COPD. Unlike healthy subjects, COPD patients become easily exhausted during daily life by using a relative high percentage of their W_{max} and peak $\dot{V}_{O_2}$. Recurrent exhaustion and fatigue may therefore result in frequent exposure to intensified levels of systemic inflammation that may also affect several distant organs. In these patients, an association exists between systemic inflammation, metabolic derangement,[61] oxidative stress and skeletal muscle dysfunction.[1] These processes further enhance the extrapulmonary effects of COPD by positive feedback mechanisms and autocrine functions and, thus, may maintain the vicious cycle of COPD, systemic effects and inactivity. Future investigations are needed to further clarify the exercise-induced inflammatory response in COPD patients and its (systemic and local) clinical consequences in view of treatment of these patients.

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