IMMUNE RESPONSE TO EXERCISE IN PATIENTS WITH COPD

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de Medische Wetenschappen

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Chapter 1

Introduction and Outline of the Thesis

Introduction and Outline of the Thesis

Chronic Obstructive Disease (COPD) is characterized by progressive and irreversible airway obstruction. It is associated with chronic inflammation in the lungs, which is thought to be initiated by the inhalation of noxious particles and gases such as cigarette smoke.[1;2] The chronic inflammatory response is characterized by cellular infiltrates, as well as production of inflammatory mediators, proteinases, and reactive nitrogen and oxygen species. The intensity and characteristics of the inflammatory process vary as the disease progresses.[3-5]

There is increasing evidence of systemic (i.e. extrapulmonary) effects in patients with COPD (reviewed in [6]). Similarly to the observation in the airways, increased amounts of activated neutrophils have been observed in the circulation of COPD patients in a clinical stable situation.[7] Also, increased activation of circulating lymphocytes, as indicated by enhanced expression of cytochrome oxidase, has been demonstrated.[8] Several studies investigating inflammatory mediators in COPD, reported enhanced concentrations of acute-phase proteins, such as C-reactive protein (CRP), lipopolysaccharide (LPS) binding protein (LBP) and fibrinogen, along with increases in plasma levels of (pro)inflammatory cytokines like IL-6, IL-8 and TNF-α and its receptors.[9-13] Besides inflammation, evidence for systemic oxidative stress in COPD was obtained. Increased production of free radicals or insufficient antioxidant capacity leads to a disbalance between oxidants and antioxidants. An excessive amount of free radicals may be harmful to tissues by inducing functional or structural alterations.[14] Several investigations have shown that markers of oxidative stress (e.g. antioxidant capacity, lipid peroxidation, and peroxidation of arachidonic acid) are increased in patients with COPD and even more pronounced during exacerbations.[15-18]

In addition to systemic inflammation and oxidative stress, muscle wasting and muscle dysfunction are noted in some COPD patients.[19-21] These muscular changes are inversely related to physical performance [22;23], susceptibility to exacerbations and outcome prognosis.[24;25] Causative factors for these muscle disturbances might be multi-factorial, but inflammation and oxidative stress are increasingly recognized to be involved in its pathogenesis.[6]

Besides airflow limitation, muscle wasting and muscle dysfunction have been shown to play a role in dyspnea and exercise limitation, two common complaints in COPD.[26] The interactions between exercise stress and the immune system provide

a unique opportunity to link basic and clinical physiology and to evaluate the role of underlying stress and immunophysiologic mechanisms. 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 [27], that can also affect distant organs.[28] Since COPD patients already show signs of systemic inflammation, elevated levels of circulating catecholamines, marked sympathetic activation [29;30] and muscle wasting [31] 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.[32] Moreover, strenuous exercise induces systemic [15;33;34] and muscle [35] oxidative stress in COPD patients.

It has been postulated that frequent exposure to these effects during daily living might negatively affect tissues (for example skeletal muscles) and thereby play a role in the ongoing systemic inflammation and oxidative stress and progression of COPD. This thesis is focused on the exercise-induced immune response in patients with COPD.

In *chapter 3* the systemic leukocytosis and the stress response to exhaustive exercise in patients with COPD were characterized. The response to a maximal incremental bicycle test was compared between COPD patients and healthy subjects. Circulating leukocytes and their subsets were counted before, during and after the exercise to compare the leukocytosis to exercise between both groups. Plasma levels of catecholamines and cortisol were measured to evaluate the stress response. Creatine kinase and myoglobin were determined in the blood to see if muscle damage could be detected systemically.

The immune response and its possible relation with muscle wasting and dysfunction in COPD was investigated in *chapter 4*. Pulmonary function, body composition, muscle strength, and chronic systemic inflammation and oxidative stress were studied within healthy subjects, patients with COPD, and muscle-wasted patients with COPD. Also, the exercise-induced systemic effects were evaluated after a maximal and a submaximal cycle test. Leukocyte counts, and inflammatory mediators (TNF-α, IL-1RA, IL-6, IL-8) were used for characterization of the inflammatory

process. Free radical production by neutrophils, plasma antioxidant capacity, lipid peroxidation, protein oxidation, and glutathione oxidation were used as markers for oxidative stress.

In *chapter 5,* we investigated whether a probably less intense and more regularly performed physical activity than high intensity cycling, namely 6 minutes of walking, affected the systemic immunology in patients with COPD. The physiologic and systemic immunologic responses to a 6-minute walking test (6MWT) were determined in muscle-wasted COPD patients and compared with the responses to maximal cardiopulmonary exercises testing (CPET). A mobile oxycon was used for characterization of the exercise-physiology during the 6MWT. The exercise-induced immune response was followed using the parameters described in chapter 4.

Several strategies may be able to modulate the inflammatory response and the oxidant/antioxidant balance related to exercise. We have studied the effect of short term oxygen breathing (nasal, $4L \cdot min^{-1}$) on both basal systemic inflammation and oxidative stress (1h treatment with oxygen or compressed air) and exercise-induced systemic effects in muscle-wasted COPD patients (c*hapter 6*). Before and after breathing oxygen for 1h (randomized, placebo controlled) blood samples were taken to analyse markers for inflammation and oxidative stress. Subsequently, before and after cycling (40W, constant work rate) with oxygen or compressed air, the immune response was evaluated.

The most remarkable findings of this thesis are integrated in the summary and conclusions (*chapter 7*). Revealing the consequences and mechanisms of systemic effects of COPD is considered to have high priority in future research to find new targets for therapies and optimize the treatment of these patients.

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Chapter 2

Systemic immunological response to exercise in patients with Chronic Obstructive Pulmonary Disease: what does it mean?

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Abstract

Chronic obstructive pulmonary disease (COPD) is no longer seen as a pulmonary disease, but is increasingly associated with systemic effects with important clinical relevance. Systemic immunological changes in COPD patients are characterized by an increased number of circulating inflammatory cells, functional changes of the inflammatory cells, elevated plasma levels of cytokines, and oxidative stress. Physical exercise induces an abnormal systemic inflammatory and oxidative response in COPD patients, which is seen in both the circulation and the peripheral muscles. Although mechanisms and consequences of these effects are not yet fully understood, they could be harmful in COPD patients by inducing damage or functional changes in, for example, skeletal muscles. Whether these changes of the immune system can also affect the susceptibility to infections in these patients is unknown. The concept of COPD as a systemic rather than only a pulmonary disease also opens new perspectives on the development of new therapeutic interventions. The effects of new anti-oxidative and anti-inflammatory agents are investigated. A better understanding of the complexity of the systemic effects will aid to the development of new therapies and management strategies for patients with COPD.

Introduction

"Chronic Obstructive Pulmonary Disease (COPD) is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases".[1] This definition of COPD focuses exclusively on the lungs. Therefore, it is not surprising that in the diagnosis, staging, prognosis, and therapy of the disease mainly pulmonary variables are considered.[1] Changes are introduced now, since recent studies have provided evidence that COPD is often associated with extra-pulmonary abnormalities (Table 1).[2] Systemic features of COPD are recognized as an important clinical feature of the disease and contribute significantly to decreased exercise capacity, decreased health status and increased mortality.[2;3] For example, a multidimensional grading system was introduced recently to predict the risk of death among patients with COPD.[4] Besides the forced expiratory volume in 1 second and the dyspnea score, this index also contains the 6-minute walking test, and the body mass index. For the first time, both pulmonary and systemic effects of COPD are integrated to stage the severity and estimate prognosis of the disease. Integration of both pulmonary and systemic effects and its consequences may contribute to a better understanding and management of the disease.

Exercise limitation, a common complaint in COPD patients [5] and a significant contributor to the poor quality of life [6;7], has traditionally been explained by the increased work of breathing and dynamic hyperinflation that results from the airflow limitations in COPD patients.[8;9] However, physical activity does not only affect the cardiovascular and respiratory system, but also has effects on the immune system and the skeletalmuscular system.[10;11] The identification of systemic effects, like inflammation, oxidative stress and skeletal muscle wasting in COPD patients, has not only caused a shift in the view of the pathophysiology of COPD, but has also drawn attention to the role of exercise and rehabilitation in these patients.[12] Until now, multiple studies have characterized the exercise-induced immune response in healthy subjects. However, regarding the immune response to exercise in COPD patients, a lot of information is still missing.

In this article, current knowledge regarding basal and exercise-induced systemic immunology, including inflammation and oxidative stress in patients with COPD is

reviewed and potential mechanisms and clinical consequences are discussed. Based on these data, targets for investigation of better clinical management of these patients are suggested.

Table 1. Systemic effects of COPD*

Systemic inflammation
Oxidative stress
Activated inflammatory cells (neutrophils/lymphocytes)
Increased plasma concentrations of cytokines and acute phase proteins
Metabolism and weight
Increased resting energy expenditure
Abnormal body composition
Abnormal amino acid metabolism
Skeletal muscle dysfunction
Loss of muscle mass
Abnormal structure/function
Others
Cardiovascular effects
Nervous system effects
Osteoskeletal effects

* Reprinted with permission from Agusti AGN et al.[2]

Systemic immunological changes in COPD Patients

At rest

Intrapulmonary inflammation and oxidative stress play a key role in the pathogenesis of COPD. Increased numbers of neutrophils, macrophages and T-lymphocytes as well as elevated concentrations of pro-inflammatory cytokines and oxidative stress are characteristic features within COPD.[13] However, recent studies have shown that inflammation and oxidative stress are also present systemically (Table 2).

Table 2. Low-grade systemic inflammation and oxidative stress in patients with COPD

Study	Study groups	Studied Parameters	Important results
Burnett et al. [17]	Emphysema pts Bronchiectasis pts Healthy subjects	chemotaxis and extracellular proteolysis of neutrophils	↑ proteolysis in pts with bronchiectasis, ↑ chemotaxis in pts with emphysema
Calikoglu et al. [26]	26 stable COPD pts 16 exacerbated COPD pts 15 control subjects	serum leptin and TNF-α levels	↑ leptin and TNF-α during exacerbations, ↑ TNF-α and ↓ leptin in stable COPD pts (not significant)
De Jong et al. [21]	42 COPD pts 24 healthy subjects	circulating lymphocytes and subsets	↑ CD8+ lymphocytes, CD4:CD8 ratio correlates with FEV_1 in COPD pts
Dentener et al. [14]	55 stable COPD pts 23 control subjects	circulating leukocyte count, CRP, LBP, sTNF-Rs, sIL-1RII	↑ leukocyte count, ↑ CRP, ↑ LBP, ↑ sTNF-Rs in COPD pts
Di Francia et al. [22]	16 COPD pts with weight loss 14 COPD pts with normal weight	serum TNF-α levels	↑ TNF-α levels in COPD pts with weight loss
Eid et al. [23]	80 stable COPD pts	circulating IL-6, TNF-α, TNF-Rs	↑ IL-6, TNF-α and TNF-Rs in COPD pts with low skeletal muscle mass
Mercken et al. [12]	11 COPD pts 11 healthy controls	free radical-induced DNA damage, MDA, plasma uric acid	↑ urinary MDA in COPD pts
Noguera et al. [15]	23 stable COPD pts 23 healthy controls	circulating neutrophil counts, expression of adhesion molecules	↑ neutrophil count, ↑ Mac-1 expression, ↓ expression of Gαs in COPD pts

Table 2. Continued

Study	Study groups	Studied Parameters	Important results
Noguera et al. [18]	10 COPD pts 8 healthy subjects	in vitro ROS production of neutrophils, expression of adhesion molecules	↑ respiratory burst , ↑ Mac-1 expression in COPD pts
Pratico et al. [28]	38 COPD pts 30 healthy subjects	urinary levels of isoprostane $F_{2\alpha}$-III	↑ levels of isoprostane $F_{2\alpha}$-III in COPD pts
Rahman et al. [29]	18 healthy non-smokers 20 healthy smokers 29 stable COPD pts 20 exacerbated pts	superoxide anion generation, antioxidant capacity, MDA, protein sulfhydryls, protein carbonyls	↑ superoxide anion generation during exacerbation COPD, antioxidant capacity ↓ during exacerbation, ↑ MDA in COPD pts
Sauleda et al. [19]	17 stable COPD pts 8 healthy controls	activity of cytochrome oxidase in circulating lymphocytes	↑ cytochrome oxidase activity in lymphocytes of COPD pts
Schols et al. [24]	30 COPD pts 26 healthy subjects	plasma TNF-α, TNF-Rs, IL-6, IL-8, LBP	COPD pts with ↑ REE and ↓ FFM have ↑ levels of CRP and cytokines
Van Helvoort et al. [16]	16 stable COPD pts 11 healthy subjects	circulating leukocyte count and subsets, plasma levels stress hormones, CRP	↑ leukocytes, ↑ neutrophils, ↑monocytes, ↑ CRP, ↑norepinephrine in COPD pts
Yasuda et al. [25]	20 COPD pts 22 healthy controls	plasma Fas/Apo-1 receptor and Fas ligand, plasma TNF-α, IL-6, CRP	↑ sFas in severe COPD, ↑ plasma TNF-a, IL-6, CRP in all COPD pts

Abbreviations: Pts = patients; FEV_1 = forced expiratory volume in the first second; CRP = C-reactive protein; LBP = lipopolysaccharide-binding protein; sTNF-Rs = soluble TNF receptors; sIL-1RII = soluble interleukin 1 receptor II; MDA = malondialdehyde; ROS = reactive oxygen species; REE, resting energy expenditure; FFM, fat-free mass

For example, increased numbers of circulating leukocytes were found in stable patients with COPD.[14-16] Furthermore, neutrophils harvested from the circulation of COPD patients showed enhanced chemotaxis and extracellular proteolysis.[17] In another study, these cells produced more reactive oxygen species (the so called oxidative burst) compared with those from healthy subjects.[18] Additionally, the expression of several surface adhesion molecules on neutrophils (for example CD11b), that play a role in the attraction and migration of cells, was higher in stable COPD patients than in healthy controls. Other abnormalities described in circulating neutrophils in COPD patients include the downregulation of a G-protein subunit (stimulatory G_{α}) that is indirectly involved in the expression of adhesion molecules and the oxidative burst.[15]

Little is known about the function of circulating lymphocytes in COPD. Sauleda et al.[19] showed that the activity of cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain, was increased in circulating lymphocytes from stable COPD patients compared with healthy non-smoking controls. This abnormality was also detected in other chronic inflammatory diseases [20], suggesting that it may be a non-specific marker of lymphocyte activation. Further, a higher percentage of CD8+ lymphocytes was found in COPD patients [21] compared with healthy subjects. Additionally, a lower CD4+/CD8+ ratio was associated with a decreased pulmonary function. The implication of these findings remains unclear. Because the CD4+/CD8+ ratio is genetically controlled in humans, it could be hypothesized that a smoker with a low CD4+/CD8+ ratio may be more susceptible for the development of COPD.

Next to inflammatory cells, numerous studies have reported increased levels of circulating cytokines and acute phase proteins in the peripheral circulation of patients with COPD.[22-26] Abnormalities include increased concentrations of tumor necrosis factor (TNF)-α, its receptors (TNFR-55 and TNFR-75), interleukin (IL)-6, IL-8, and Fas and Fas ligand, as well as elevated levels of the acute-phase proteins C-reactive protein and lipopolysaccharide-binding protein. These increases were seen in clinically stable patients, but were generally more pronounced during exacerbations of the disease.

Besides inflammation, oxidative stress also plays an important role in the pathogenesis of COPD.[13] Increased production of reactive oxygen species (by neutrophils) or insufficient antioxidant capacity leads to a disbalance between oxidants and

antioxidants. An excessive amount of reactive oxygen species may be harmful to tissues by inducing functional or structural alterations.[27] Different investigations have shown that markers of oxidative stress (antioxidant capacity, lipid peroxidation, and peroxidation of arachidonic acid) are increased in patients with COPD and even more pronounced during exacerbations.[12;28;29] Higher levels of oxidative stress found in smokers with a normal pulmonary function suggests that tobacco smoke plays a role in disturbing the balance between oxidants and antioxidants.

The mechanisms of the above-mentioned systemic immunological changes in COPD patients are unclear, but several mechanisms could be operative (for a review, see ref. [2]). Firstly, tobacco smoke can cause endothelial damage and dysfunction of the systemic vessels [30;31], as well as systemic oxidative stress.[32] A second potential mechanism is that the pulmonary inflammatory process in the lung in COPD patients is the source of the systemic inflammation. Cytokines and oxidants, produced by the inflammatory cells in the lung, can reach the systemic circulation and/or contribute to the activation of inflammatory cells during transit through the pulmonary circulation. A third possibility is that some of the aspects of the systemic immunological abnormalities in COPD patients may be a cause rather than a consequence of COPD. This possibility is based upon the following observations. Only a percentage of all smokers eventually develops COPD [33], suggesting that the participation of other factors, for example genetic factors, is also important in the pathogenesis of COPD.[34] The abnormalities seen in the neutrophils of COPD patients could be the expression of a genetic predisposition that renders these cells more susceptible to the effects of smoking and other pro-inflammatory agents. A more vigorous response to the same degree of stimulation would be the first step in a chain of reactions causing the final systemic inflammation and oxidative stress and their enhanced damaging potential.

After exercise

From literature about exercise and sports it is known that exercise, if sufficiently intense, leads to a highly stereotyped immune response in healthy subjects, mediated by an interplay of metabolic, endocrine and immunological factors.[35] Dependent on type, duration and intensity, exercise induces an increase in plasma concentrations of the stress hormones epinephrine, norepinephrine, β-endorphin, growth hormones, and cortisol.[36] In healthy subjects, arterial plasma concentrations of epinephrine and norepinephrine increase almost linearly with the duration of the dynamic exercise

and exponentially with the intensity, when expressed relative to the individual's $\dot{V}_{O2\text{-}max}$. In contrast, cortisol concentrations increase only in relation to exercise of long duration. The exercise-induced immune response in patients with COPD is poorly investigated. Colice et al.[37] investigated the hormonal response to maximal exercise in COPD patients who became hypoxic during exercise, and reported that the rate of increase in epinephrine (but not norepinephrine) with maximal exercise was smaller compared with healthy subjects. In contrast, the hormonal response to exercise in normoxic COPD patients has been found to be comparable with the response found in healthy subjects.[16] These findings support the idea that hypoxia may interfere with the adrenal medullary response to exercise.

Regarding the interplay of systems involved in the response to exercise seen in healthy subjects and the basal immunological abnormalities seen in COPD patients, the immunological response to exercise in these patients may be very interesting. Despite variation in intensity and duration of the exercise and the fitness level between healthy subjects and COPD patients, several consistent patterns regarding the leukocyte subpopulations in blood were recently described.[16] The response to exercise reflected an early leukocytosis in all subsets in both groups, followed by a second phase during which neutrophils gradually increased and lymphocytes concentrations rapidly fell. Although the response patterns of the inflammatory cells were similar in controls and patients, the whole leukocytosis occurred at an elevated level in the patients because of their low-grade systemic inflammation at rest. The high numbers of these cells and their function may be important in the onset of further inflammatory and oxidative cascades. For example, an increased phagocytosis and decreased oxidative burst of neutrophils in healthy subjects are functional changes that are suggested to be protective against the release of damaging mediators that induce intense inflammatory reactions.[38] However, very recent data show an increase in the oxidative burst of neutrophils of COPD patients in response to high-intensity exercise.[39;40] Although only hypothetical, this response might induce substantial damage to tissues by the release of free radicals. Additionally, the response to exercise involves an increased production and release of circulating inflammatory and pro-inflammatory cytokines. The circulation of high concentrations of TNF-α, IL-1β, IL-6 and IL-1 receptor antagonist after intensive exercise in athletes is even compared with a septic or traumatic response.[41] Although a lot is known about the low-grade increases in basal cytokine concentrations in COPD patients, only two studies have

investigated the effect of exercise on these mediators. Firstly, Rabinovich et al.[42] have shown that submaximal exercise (40% Wmax, 11 min) already induces an abnormal increase in plasma TNF-α levels in COPD patients. Supplementary, in another study [43], levels of IL-6 have been found to increase after both maximal and submaximal (50% Wmax, 30 minutes) exercise in especially muscle-wasted COPD patients compared with healthy subjects.

Besides the above-mentioned hormonal and inflammatory response, oxidative stress also plays a key role in the systemic response to exercise. In healthy subjects, only intense exercise induces glutathione oxidation in blood (reduced glutathioine to oxidized glutathione).[44;45] However, exercise with a relative low external workload already induces glutathione oxidation in COPD patients.[46;47] Within this oxidation, it has been shown that the endogenous enzyme xanthine-oxidase plays an important role in the formation of free radicals.[46] In the above-mentioned studies, oxidation of glutathione in patients with COPD is accompanied by an increase in lipid peroxidation (e.g., malondialdehyde, thiobarbituric acid-reactive substances), a marker for free radical-mediated tissue damage. Furthermore, recently, it has been reported that exercise induces free radical-induced DNA damage in patients with COPD. [12] Not only in the systemic circulation, but also in the peripheral muscles, oxidative stress was elevated after exercise in patients with COPD.[48;49] Together, these data suggest that exercise in patients with COPD induces oxidative stress that is accompanied with tissue damage, which may be involved in the ongoing immunological changes and progression of the disease.

Consequences of systemic inflammation and oxidative stress

During daily life, patients with COPD will frequently perform physical activities at a relatively high percentage of their maximal exercise capacity. Although not yet proven, it seems likely that these activities may influence both the number and function of the immunocompetent cells and the level of systemic oxidative stress. Consequently, these patients may be exposed to repeated bursts of systemic immunological responses, which may affect peripheral tissues and organs [50] by inducing damage or functional changes in, for example, skeletal muscles. Furthermore, it would be relevant to investigate if this exercise-induced immune response also plays a role in the decreased defense against infections in patients with COPD.

Effect on peripheral skeletal muscles

The concept that exercise intolerance in COPD patients is due to dyspnea, in turn caused by increased work of breathing secondary to airflow limitation [8;9], was for the first time challenged by Killian et al.[51] They showed that many patients with COPD stop exercising because of leg fatigue rather than dyspnea. Since then, skeletal muscle dysfunction in COPD is seen as an important systemic effect of COPD and has been extensively studied, but its mechanism is still poorly understood. Exercise intolerance in COPD patients leads to adoption of a sedentary lifestyle, which in turn causes loss of muscle mass, reduction in force generating capacity of the muscles and a decrease in resistance to fatigue.[52] Consequently, the exercise capacity further decreases, resulting in progressive sedentarism. Briefly, patients fall into a vicious circle that worsens their disease state. Exercise training improves condition, muscle function, and muscle mass [53-55], but complete normalization of muscle physiology is often not fully achieved after rehabilitation. Therefore, it is likely that also mechanisms other than inactivity might play a role in skeletal muscle dysfunction in patients with COPD (Table 3). One of the important potential mechanisms is systemic inflammation. The aforementioned low-grade systemic inflammation in patients with COPD and the elevations after exercise may increase levels of inflammation at the level of local organ systems like the liver [56], heart [57;58], or skeletal muscle.[42;59] Cytokines can affect muscle cells in a number of ways. TNF-α, for example, activates the transcription factor nuclear factor-κB and degrades myosin heavy chains through activation of the ubiquitin/proteasome complex.[60] Dysregulation of this complex has been associated with loss of muscle function and muscle mass. Alternatively, TNF-α can activate the leukocytes and induce the expression of several genes that encode for TNF-α itself and many other pro-inflammatory cytokines, which would create a closed loop and contribute to the persistence and amplification of the inflammatory cascade.[60] Finally, TNF-α can induce apoptosis of several cell systems [61], and recently it has been shown that excessive apoptosis of muscle cells occurs in patients with COPD.[62] Moreover, systemic inflammation (overexpression of IL-6) may also be the cause of suppression of the growth hormone/insulin-like growth factor-1 axis, as is seen in chronic diseases [63;64], and is associated with loss of skeletal muscle.[65] According to Table 3, not only systemic inflammation, but also increased levels of systemic oxidative stress may be a potential mechanism in the pathogenesis of skeletal muscle dysfunction in COPD patients. Among other things, oxidative stress causes muscle fatigue [66] and supports proteolysis of muscle cells.[67;68] Furthermore, oxidative stress

contributes to the gradual loss of muscle mass that occurs in the normal process of ageing.[69;70] Whether this process occurs early or is accelerated in COPD patients with muscle wasting has not yet been explored.
Shortly, muscle wasting and dysfunction in patients with COPD are probably caused by multiple mechanisms, including systemic inflammation and oxidative stress. Keeping this in mind, the increased systemic effects in response to exercise might negatively affect the skeletal muscles of these patients.

Table 3. Potential mechanisms of skeletal muscle dysfunction in COPD patients*

Inactivity
Nutritional abnormalities / cachexia
Tissue hypoxia
Systemic inflammation
Skeletal muscle apoptosis
Oxidative stress
Abnormal NO regulation
Tobacco
Individual susceptibility
Hormonal alterations
Electrolyte alterations
Drugs

* Reprinted with permission from Agusti AGN et al.[2]

Risk of infections

Changes within the immune system may influence the defense against infections. Epidemiological data on exercise training and the risk of minor illnesses such as upper airway infections have shown that the relation between physical activity and the risk of infections can be illustrated by the so called (symptom-based) J-curve [71]. Regarding this curve, regular moderate activity will enhance resistance to infections, whereas intense exercise suppresses this resistance in the healthy population.

However, symptoms of infections after exercise are never causally related to exercise-induced changes of the immune system. Whether the risk of infections in patients with COPD resembles this curve is unknown. Firstly, the susceptibility of COPD patients to respiratory infections is much higher than in healthy subjects.[72;73] Therefore, the curve would already start at a higher risk of infections. This higher risk is accompanied with low-grade basal systemic inflammation and oxidative stress as described before, which is even more intensified during exercise in comparison with healthy subjects. Although the function of the immunocompetent cells after exercise is not yet investigated, the intensified levels might be characteristic for a further elevated risk of infections in patients with COPD. To find out whether the relation between physical activity and the risk of infection in patients with COPD can be illustrated by a similar J-curve on a higher level or maybe by a linear or logarithmic relation (Figure 1), further investigations into the exercise-induced effects on the immune system in patients with COPD are needed, as well as causal studies on the relation between these effects and the occurrence of infections.

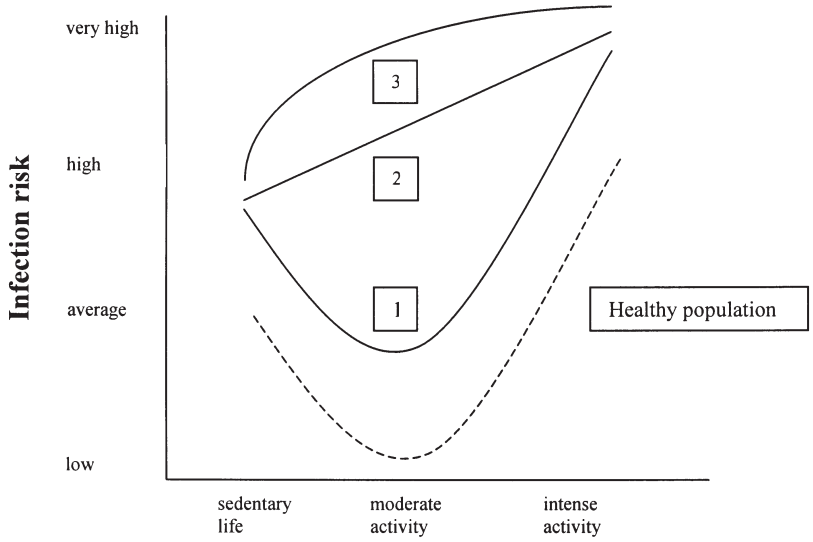

Figure 1. Possible J-curve for patients with COPD.
The relationship between physical activity and infection risk in patients with COPD is unknown. Based on studies about the systemic immunology in these patients, the curve will start at a higher risk of infections than in healthy subjects. For now, it can only be speculated whether the activity level and risk of infection can be illustrated as 1) a J-curve on a higher level, 2) a linear relationship, or 3) a logarithmic relation.

Therapeutic interventions

Pharmacological interventions

Since COPD is recognized as a systemic rather than only a pulmonary disease, the development of new therapeutic interventions has found new targets to manage this difficult disease. The presence of low-grade systemic inflammation and oxidative stress in COPD patients is the scientific rationale for the development of anti-inflammatory and anti-oxidative therapies. Antioxidants play an important role in the protection of tissues against free radical-mediated damage. Supplementation of effective antioxidants may repair the imbalance between oxidants and antioxidants in patients with COPD. A study of Daga et al.[74] showed that supplementation of vitamin E could reduce the level of systemic oxidative stress in patients with COPD. However, very recently, high doses of vitamin E supplements have also been associated with an increased risk of death.[75] Next to the addition of antioxidants, decreasing the production of oxidants would be another possible target to reduce oxidative stress. Heunks et al.[46] showed that xanthine-oxidase plays a role in the exercise-induced radical production in patients COPD. Short treatment with allopurinol (inhibitor of xanthine-oxidase) could prevent the exercise-induced glutathione oxidation and reduced the peroxidation of lipids. Whether allopurinol can also normalize the basal increased level of oxidative stress in COPD patients is unknown. Currently, effective antioxidants for clinical use that do not influence other physiological processes, like microbiological defenses, are now in development.[76-78] Preventing oxidative stress and thereby avoiding free radical-mediated tissue damage in, for example, skeletal muscle might contribute to a better exercise capacity in patients with COPD. However, for now, too little evidence and knowledge about the safety of the use of supplements exists to make a statement about the general use of these therapies.

Additionally, several new treatments targeting the inflammatory process are now in clinical development.[79] A broad spectrum of anti-inflammatory drugs is being investigated, including targets against inflammatory cytokines and cell signaling inhibitors. In the meantime, there is still a lot of discussion about benefits and side effects of both oral and inhalation corticosteroids in the treatment of COPD. For a long time it was suspected that inhalation of corticosteroids could avoid systemic effects. Cameron et al.[80] found a comparable systemic effect on the number of circulating leukocytes (neutrophilia and lymphopenia) with a high dose of inhalation steroids

(beclomethasone dipropionate 1000 µg) and a lower dose of oral steroids (prednisone 2.5 mg). Furthermore, Sin et al.[81] have recently shown that both oral administration and inhalation of fluticasone (30 mg/day and 1000 µg/day, respectively) were effective in reducing serum C-reactive protein levels in patients with COPD.
At this moment, we have to conclude that the basic systemic abnormalities in COPD patients are poorly understood. Because of the complexity and interplay between multiple effects, causes and consequences are still difficult to define, which makes it difficult to find targets for effective and safe pharmacological therapies.

Training

Several large-scale randomized studies have demonstrated that pulmonary rehabilitation improves exercise performance and health status in COPD patients.[82] Additionally, one of the systemic effects, namely peripheral muscle weakness, has shown to improve after rehabilitation. It remains uncertain whether pulmonary rehabilitation affects exacerbation frequency, disease progression, mortality or the systemic effects, like inflammation and oxidative stress. Very recently, promising data about the reduction in exercise-induced oxidative stress after 8 weeks of rehabilitation in 11 patients with COPD were published.[12] However, others [42;83] have reported that, compared with healthy elderly people, patients with COPD showed a reduced ability to adapt to endurance training reflected in a lower capacity to synthesize reduced glutathione and an increased TNF-α expression. The effect of repeated exercises and the addition of pharmacological therapies on these parameters would be interesting. Very recently, Broekhuizen et al.[84] reported that the addition of supplemental polyunsaturated fatty acids (PUFA) to a pulmonary rehabilitation program improved the exercise capacity in COPD patients more than rehabilitation exclusively. Although PUFA can modulate local and systemic cytokine biology, the positive effects of PUFA on the exercise capacity could not be attributed to a decrease in systemic inflammation in this study. A PUFA-induced decrease in local inflammation could not be excluded. Therefore, further research to elucidate the mechanism behind the improved exercise capacity after PUFA intervention is needed. Additionally, it remains to be investigated whether PUFA intervention alone, without exercise training, will have a similar effect on exercise capacity in patients with COPD.

Conclusions

Besides pulmonary involvement, COPD is associated with systemic effects. Low-grade systemic inflammation and oxidative stress are present and abnormally increase in response to exercise. These processes may have harmful effects by inducing damage to local organs and tissues or by negatively influencing the immune system. A better understanding of the complexity of the systemic effects will aid the development of new therapies and management strategies for patients with COPD.

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Chapter 3

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

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Abstract

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 eleven 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.

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 to 68 yr) 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 two 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 one 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, 11 males, 2 females) non-smoking, sedentary healthy subjects (age 47 to 64 yr) 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 (Wmax) 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 to 12 minutes, which meets the exercise testing recommendations.[30] Minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}_{O2}$) and carbon dioxide production ($\dot{V}_{CO2}$) were measured every 30 seconds 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 two minutes 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 minutes during exercise, at Wmax 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 6h and 24h 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 $\mu g \cdot ml^{-1}$) and leukocytes was collected (at rest, Wmax, and 30, 60 and 120 minutes 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 (Pa_{O2}, Pa_{CO2}) were measured pre-exercise, every 3 minutes during the exercise and 3 minutes after maximal work rate. Lactate levels were determined enzymatically at rest and 3 minutes after maximal exercise.

Catecholamine measurement

Blood was collected at rest, directly after maximal exercise and after 30 minutes 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 $mol \cdot l^{-1}$ EGTA and 0.2 $mol \cdot l^{-1}$ 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, Wmax and 30, 60 and 120 minutes 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 ± 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.

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, yr	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
Pa_{O2}, kPa	11.9 ± 0.4	10.9 ± 0.5
Pa_{CO2}, 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; Pa_{O2}, arterial oxygen tension Pa_{CO2}, arterial carbon dioxide tension; pred, predicted value.

*** $p<0.001$ *versus* healthy subjects.

Catecholamines and systemic inflammation at rest

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

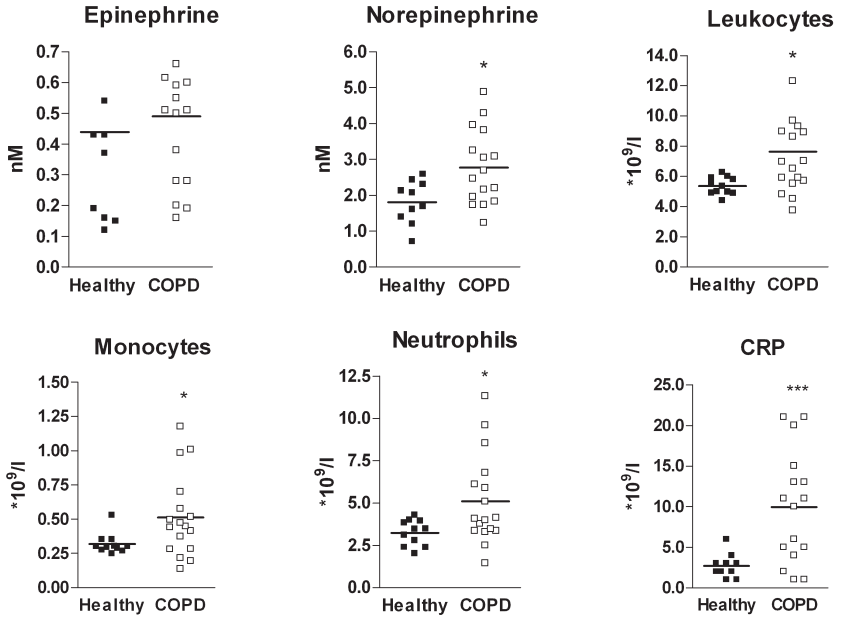

Figure 1. Individual and mean values of baseline epinephrine, norepinephrine, circulating leukocytes, neutrophils, monocytes, and CRP in healthy subjects and patients with COPD.
* p<0.05, *** p<0.001 *versus* healthy subjects.

Maximal exercise test

Physiological data

Physiological responses to exercise are shown in Table 2. As expected, Wmax was significantly lower in COPD patients (p<0.001). Also, duration of the exercise test, maximal oxygen uptake (peak $\dot{V}_{O2}$) 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). Pa_{O2} significantly decreased during exercise in COPD patients (p<0.05), while Pa_{CO2} increased (p<0.001). Six patients became hypoxemic and five became hypercapnic during exercise. No changes in Pa_{O2} occurred in healthy subjects, while Pa_{CO2} decreased at maximal exercise (p<0.01). In both groups, a significant increase (p<0.01) in plasma lactate was observed at Wmax. 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).

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

	Healthy subjects	COPD patients
Wmax, watt	201 ± 18	90 ± 11 ***
Wmax, % pred	79 ± 6	57 ± 5 **
Endurance, sec	729 ± 33	519 ± 32 ***
Max HR, beats min^{-1}	163 ± 3	128 ± 5 ***
Max HR, % pred	99 ± 2	80 ± 3 ***
Peak $\dot{V}_{O2}$, l min^{-1}	2.4 ± 0.18	1.3 ± 0.12 ***
Peak $\dot{V}_{O2}$, ml min^{-1} kg^{-1}	30.5 ± 2.7	17.4 ± 1.2 ***
Peak $\dot{V}_{O2}$, % pred	108 ± 8	65 ± 5 ***
Peak $\dot{V}_{E}$, l min^{-1}	84 ± 6	43 ± 4 ***
Peak $\dot{V}_{E}$, % MVV	68 ± 4	96 ± 6 **
Pa_{O2}, kPa	12.5 ± 0.5	9.7 ± 0.4 ***
Pa_{CO2}, 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: Wmax, 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; Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; pred, predicted.

** $p<0.01$, *** $p<0.001$ *versus* healthy subjects.

Catecholamines

Plasma levels of epinephrine and norepinephrine before and after the exercise test are shown in Figure 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 Figure 3 show that the lower response of catecholamines to exercise in COPD patients was related the relative lower work intensities they performed. Also, within the COPD group, Wmax and peak $\dot{V}_{O2}$ 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 Wmax (epinephrine, p=1.0; norepinephrine, p=0.74), see Table 3.

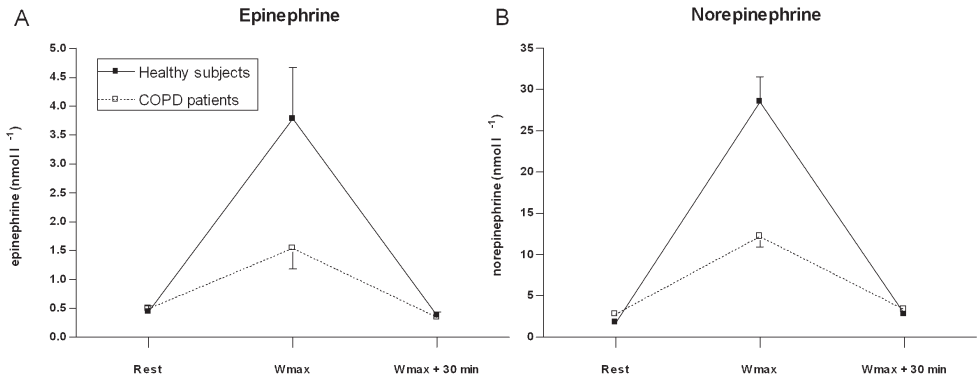

Figure 2. Effects of exercise on plasma concentrations epinephrine *(A)* and norepinephrine *(B)* in both healthy subjects and patients with COPD. Exercise induced a significant response of the catecholamines in both groups (p<0.001). Both epinephrine and norepinephrine responses were lower in COPD patients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

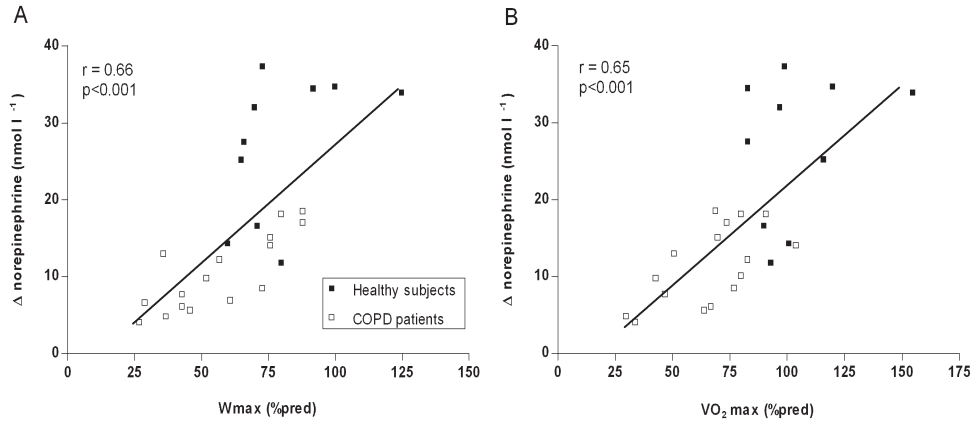

Figure 3. Exercise-induced changes in norepinephrine levels related to maximal workload *(A)* and peak $\dot{V}_{O2}$ *(B)* in healthy subjects and patients with COPD.

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 Wmax*	*At rest*	*At Wmax*
Wmax, watt		114 ± 13		112 ± 14
Peak $\dot{V}_{O2}$, % 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, $\cdot 10^9$ 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: Wmax, maximal work capacity; peak $\dot{V}_{O2}$, maximal oxygen consumption

Numbers of total circulating leukocytes in both COPD patients and healthy subjects at rest, directly after exercise and during recovery are shown in Figure 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 minutes 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 Figure 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 (Figure 4B) and monocytes (Figure 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 Figure 4 (4D), the absolute response of lymphocytes caused by exercise was significant lower in COPD patients compared with healthy subjects ($p<0.001$).

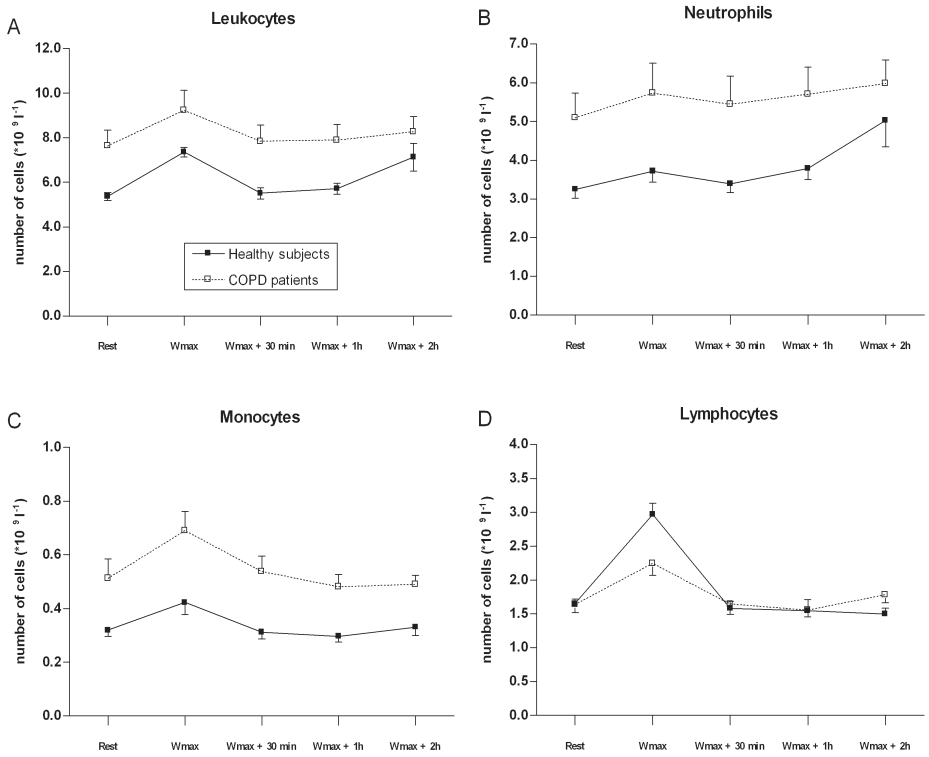

Figure 4. Effects of exercise on circulating leukocytes and subsets in both healthy subjects and patients with COPD. Exercise caused significant responses of total leukocytes *(A)*, neutrophils *(B)*, monocytes *(C)*, and lymphocytes *(D)* in both groups. Responses of leukocytes and the subsets neutrophils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

In addition to this finding, significant correlations ($p<0.001$) were found between exercise capacity, catecholamine response, and lymphocyte response (Figure 5). Furthermore, within the COPD group, peak $\dot{V}_{O2}$ 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 (Figure 6A) and B-lymphocytes (Figure 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 maximal exercise was the same in both groups (Figure 6C).

The difference occurred after two hours recovery when the T-lymphocytes of COPD patients were still elevated from baseline levels ($p<0.01$).

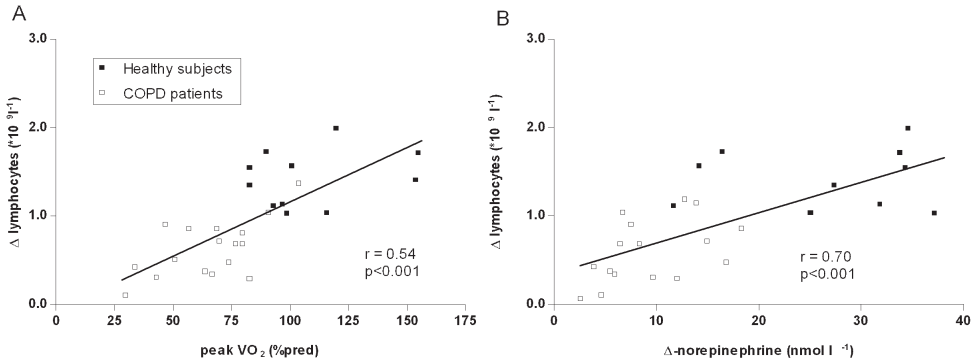

Figure 5. The influence of exercise capacity *(A)* and subsequent changes in norepinephrine *(B)* on lymphocyte response to exercise.

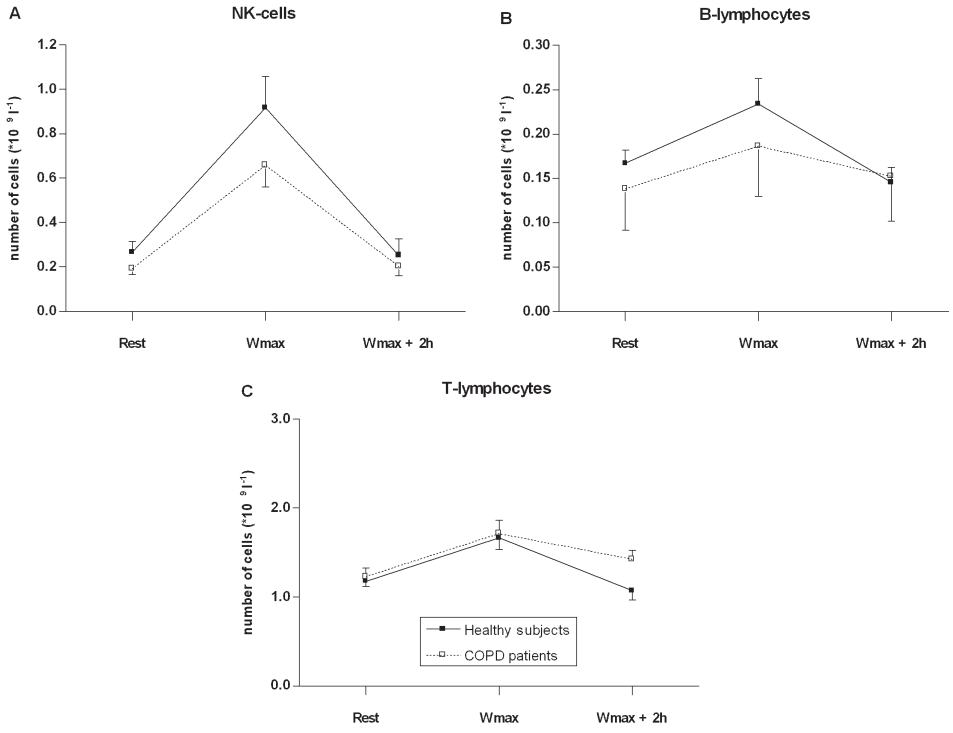

Figure 6. Effects of exercise on circulating NK cells and lymphocyte subsets in both healthy subjects and patients with COPD. The responses of NK-cells *(A)* and B-lymphocytes *(B)* to exercise was comparable in both groups, while the pattern of the response of the T-lymphocytes *(C)* differed between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

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.

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

		At rest	Wmax	Wmax + 1h	Wmax + 2h	Wmax + 6h	Wmax + 24h
CK ($U \cdot l^{-1}$)	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 ($\mu g \cdot l^{-1}$)	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.

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. Due 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 $\dot{V}_{O2}$, plasma concentrations of a number of stress 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 expressed relative to individual's peak $\dot{V}_{O2}$.[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 (Figure 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 $\dot{V}_{O2}$, however, was independent of the increase in workload. Because the catecholamine response was not only correlated with Wmax in the present study, but also with peak $\dot{V}_{O2}$, 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 to 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. Beta2-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 β2-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 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 (Figure 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 2h.

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 minutes uphill (90 % of peak $\dot{V}_{O2}$) or downhill (52% of peak $\dot{V}_{O2}$) running in healthy subjects, Mastaloudis et al.[50] recently demonstrated remarkable increases in CRP in ultra marathon runners (423 minutes, 71% of peak $\dot{V}_{O2}$) . 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 $\dot{V}_{O2}$) 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 $\dot{V}_{O2}$), of long duration (>30 minutes) 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 $\dot{V}_{O2}$ 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 $\dot{V}_{O2}$ 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 Wmax and peak $\dot{V}_{O2}$. 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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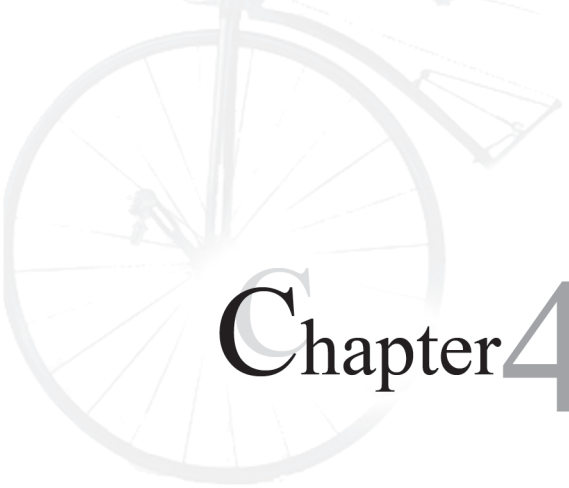

Chapter 4

Exercise-induced systemic effects in muscle-wasted patients with COPD

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Abstract

Physical exercise is known to induce an acute inflammatory response and oxidative stress in healthy subjects and patients with chronic obstructive pulmonary disease (COPD). Increasing evidence associates systemic inflammation and oxidative stress with muscle wasting and muscle dysfunction in COPD. In the present study, it was hypothesized that exercise-induced systemic inflammatory and oxidative responses in muscle-wasted COPD patients are increased compared to non-muscle wasted patients and healthy subjects.

Pulmonary function, body composition, and quadriceps muscle strength were measured in 10 muscle-wasted (fat free mass index (FFMI) <16 $kg \cdot m^{-2}$ (men), <15 $kg \cdot m^{-2}$ (women)) and 10 non-muscle-wasted COPD patients, and 10 healthy subjects. Systemic inflammation (C-reactive protein, CRP; leukocytes; cytokines) and oxidative stress (production of reactive oxygen species (ROS) by neutrophils; plasma antioxidant capacity; protein oxidation; lipid peroxidation; oxidized-to-reduced glutathione ratio, GSSG/GSH) were determined before and after maximal and submaximal (50% of maximal work rate) cycle ergometry.

Low-grade systemic inflammation was significantly ($p<0.05$) elevated in all COPD patients and tended to be highest in muscle-wasted patients. A decreased antioxidant status (plasma antioxidant capacity, $p<0.05$ and GSH, $p<0.05$) and increased protein oxidation ($p<0.001$) reflected increased basal oxidative stress in muscle-wasted COPD patients compared to both other groups. Both maximal and submaximal exercise caused increased inflammatory (IL-6, +1.1 $pg \cdot ml^{-1}$ versus rest, $p<0.05$) and oxidative responses (ROS release by neutrophils, + 32%; GSSG/GSH + 29%; lipid peroxidation, +30% versus rest) in muscle-wasted COPD patients, which were less pronounced or not observed in non-muscle-wasted patients and healthy subjects. These data indicate that both maximal and submaximal exercise induce increased systemic inflammatory and oxidative responses in muscle-wasted COPD patients compared with non muscle-wasted patients and healthy subjects.

Introduction

Physical exercise is known to induce an increased production of reactive oxygen species (ROS), and an acute inflammatory response in healthy subjects.[1;2] In patients with chronic obstructive disease (COPD), studies have shown that different types of physical exercise (e.g. cycling and localized quadriceps exercise) result in systemic and muscle oxidative stress.[3-5] Furthermore, two studies have suggested that exercise induces abnormal systemic inflammatory responses in COPD.[6;7] The exact meaning of systemic inflammation and oxidative stress in COPD has not been elucidated, yet and may be multi-factorial, but both processes have increasingly been recognized to play a role in loss of muscle mass and muscle dysfunction.[8] Weight loss and muscle wasting have been reported in approximately 20% of stable COPD patients and have been inversely related to physical performance, susceptibility to exacerbations and outcome prognoses.[9] Increased levels of circulating inflammatory cells, cytokines (interleukin (IL)-6, -8, and tumor necrosis factor-α (TNF-α)), and acute phase reactant protein (C-reactive protein (CRP)) have been reported in stable COPD patients at rest, all reflecting a low grade of systemic inflammation in these patients.[7;10] Different investigations have associated systemic inflammatory mediators and muscle wasting [11;12], supporting the concept of harmful peripheral effects of inflammation in COPD. Furthermore, inflammation may affect peripheral tissues and organs by inducing oxidative stress. Inflammatory mediators reduce the level of antioxidants, and increase generation and release of reactive oxygen species (ROS) by mitochondria and neutrophils.[13] Inversely, oxidative stress itself can enhance inflammation through activation of redox-sensitive transcription factors that regulate the gene expression for inflammatory mediators.[14] Since both oxidative stress and inflammation can induce substantial harmful effects to cells and tissues, they may together be implicated in muscle wasting and –dysfunction. However, no data about basal and exercise-induced systemic inflammation and oxidative stress in especially muscle-wasted COPD patients have been reported, yet.

The present study describes basal and exercise-induced systemic oxidative stress and acute inflammatory response in healthy subjects, non-muscle wasted COPD patients, and muscle-wasted COPD patients, expecting to find increased inflammatory and oxidative responses in the latter group.

Methods

Subjects

Twenty ex-smoking COPD patients [24] and ten non-smoking healthy subjects participated in this study. The patients were recruited from our outpatient clinic and prestratified into two groups: patients with a normal body composition (non-muscle-wasted) and patients with a decreased fat free mass index (muscle-wasted patients: men, FFMI <16 $kg \cdot m^{-2}$; women, FFMI <15 $kg \cdot m^{-2}$).[9] The healthy control group consisted of relatives and friends of the patients, living in the same area. All participants were ex-smokers who stopped at least one year before inclusion (non-muscle-wasted, 47 ± 7 pack years; muscle-wasted 43 ± 5 pack years, and healthy subjects, 39 ± 6 pack years). Exclusion criteria were exacerbations within the last two months prior to the study, the use of oral corticosteroids, long-term oxygen therapy, and other chronic inflammatory or exercise-limiting diseases. The use of inhaled corticosteroids (n=5), antioxidants or vitamins (n=4) was stopped two weeks prior to exercise testing since these therapies may influence the outcome parameters. All patients were on bronchodilator therapy, none used theophylline. None of the patients went through any rehabilitation program before inclusion. The study was conducted according to the Declaration of Helsinki and was approved by the medical ethical committee of our hospital. Written informed consent was obtained from all participants.

Study design

Pulmonary function, body composition, and quadriceps force were assessed as part of the characterization procedure. All subjects performed a maximal, incremental bicycle test until exhaustion. Subjects cycled on an electrical braked cycle ergometer (Masterlab®, Jaeger, Würzburg, Germany). In a subsequent visit (one week later) a submaximal constant work-rate bicycle test of 30 minutes at 50% of their maximal work rate (Wmax) was performed.[15] On both exercise days, a cannula was inserted into the radial artery under local anesthesia to obtain arterial blood before, during and up to 30 minutes after the exercise. During blood sampling, patients were in sitting position for at least five minutes. Measurements after exercise were corrected for plasma volume shifts (hydration status) according to Dill and Costill.[16] Systemic inflammation was characterized by measurements of plasma cytokines, circulating

leukocytes and serum CRP. The oxidative response was reflected by measures of free radical production of neutrophils, plasma total antioxidant capacity, oxidation of proteins (carbonyl formation) and lipids (thiobarbituric acid reactive substances, TBARs), and both the reduced and oxidized form of glutathione (GSH and GSSG, respectively).

Pulmonary function

Static and dynamic lung volumes were measured using a spirometer (Masterlab®, Jaeger, Würzburg, Germany). Diffusion capacity for carbon monoxide (DL_{CO}) and its capacity per unit of alveolar volume (K_{CO}) were determined using the single breath method (Masterlab®, Jaeger, Würzburg, Germany). Maximal inspiratory and expiratory mouth pressures (Pi_{max}, Pe_{max}) were measured to evaluate respiratory muscle function (Masterlab®, Jaeger, Würzburg, Germany).

Assessment of body composition

Besides body height and weight, body composition was assessed by a single frequency bioelectrical impedance analysis (Biostat 1500, Bodystat LTD, Douglas, Isle of Man, British Isles) in the supine position at the left side. Weight parameters were adjusted for body surface area to give body mass index (BMI) and FFMI.

Peripheral muscle strength

Quadriceps muscle function was determined essentially as described previously.[17] The subjects sat on an exercise bench, were strapped at the hips to prevent compensating movements, kept their arms crossed in front of the chest, bent forwards with their upper body and maintained their position during all contractions. A force transducer was strapped at 26 cm from the knee-joint space perpendicularly to the lower leg. The force transducers were connected to an amplifier (Type CA660; Peekel instruments BV, Rotterdam, The Netherlands), linked to a personal computer to display force signals and for off-line analysis. The force during an isometric maximal voluntary contraction (MVC) was determined for the left and right quadriceps muscles. Thereto, the subjects were asked to perform an MVC for three seconds. The maximal force during an MVC was defined as the mean maximal force developed during a three second plateau. The highest value of the dominant leg (< 5% of variability within one leg) was defined as subjects' MVC.

Exercise protocol

First, all subjects performed a maximal, incremental bicycle test until exhaustion, according to the criteria of the American Thoracic Society on cardiopulmonary exercise testing.[18] Subjects cycled on an electrically braked cycle ergometer (Masterlab®, Jaeger, Würzburg, Germany) at a pedaling rate of 60 rotations·min^{-1} breathing room air. After unloaded pedaling for three minutes, the workload was increased every minute by 5-20W until exhaustion. The rate of increase was calculated in order to reach the predicted maximal work rate (Wmax = predicted $\dot{V}O_2$ – basal $\dot{V}O_2$)/10) within 10 minutes of exercise. During exercise, ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}_{CO2}$) were measured breath-by-breath (Oxyconbeta, Mijnhardt/Jaeger, Bunnik, The Netherlands), electrocardiography (ECG) was conducted, and saturation was measured using a pulse-oxymeter (Datex, Helsinki, Finland). Additionally, blood pressure was measured every two minutes during the test and Borg scores (0-10) for dyspnea and leg fatigue were obtained before and at maximal exercise.

One week after the maximal exercise, all subjects performed a submaximal bicycle test at 50% of their Wmax on the same ergometer. After unloaded pedaling for three minutes, the work rate was increased up to the 50% Wmax level within one minute. At this workload, subjects continued cycling for 30 minutes.

Blood gas and lactate analysis

Arterial blood gasses (Pa_{O2}, Pa_{CO2}) were obtained pre-exercise, every three minutes during exercise and three minutes after the end of the exercise in heparinized syringes (Bayer®, Massachusetts, USA). Lactate levels were determined enzymatically at rest and three minutes after exercise. Measurements occurred immediately after sampling (Gas analyzer Chiron 860).

Markers of systemic inflammation

Arterial blood for measurement of plasma cytokines were obtained at rest, Wmax, and 30 min after exercise in ethylene diamine tetra-acetic acid (EDTA)-containing tubes and put on ice. Samples were centrifuged immediately (1000 g, 10 min, 4^{o}C), and plasma was stored at –80^{o}C until analysis. Analyses were performed using quantitative sensitivity and high-sensitivity sandwich enzyme-linked immunosorbent assays (ELISA) in kit form (R&D systems, Minneapolis, USA) according to the

supplier's instructions. Detection limits for TNF-α, IL-1 receptor antagonist (ra), IL-6, and IL-8 were 0.12, 22, 0.039, and 2.5 pg/ml, respectively. Plasma levels of the samples were all above these limits. Inter- and intra-assay precision (coefficients of variances) for these markers were below 7%.

Leukocyte counts and C-reactive protein (CRP) were determined at rest, Wmax, and 30 minutes after exercise. Leukocytes and its differentiation were counted with an automated analyzer (H*3 from Bayer (Tarrytown, New York, USA), and CRP was measured by standard turbidimetric analysis with a detection limit of 3 $\mu g \cdot ml^{-1}$. Assays were performed immediately after sampling.

Markers of the oxidant and antioxidant system

Production of ROS by neutrophil granulocytes in response to stimulation, the so-called oxidative or respiratory burst, was measured in isolated neutrophils. Arterial blood was sampled at rest, Wmax and after 30 minutes of recovery in heparinized tubes. Cell isolation was performed by means of density centrifugation on Percoll immediately after sampling, as described previously.[19] After isolation, cells were resuspended to a final concentration of 2×10^6 cells$\cdot ml^{-1}$ and kept at room temperature. Luminol-enhanced chemiluminescence was measured in 96-well microplates at 37°C for 120 min in an automated LB96V Microlumat Plus luminometer (EG&G Berthold, Bad Wildberg, Germany). Per well 20 µl Luminol (10^{-4} M) was added to 100 µl of neutrophil suspension (2×10^6 cells$\cdot ml^{-1}$) that was either unstimulated or stimulated with 20 µl phorbol myristate acetate (5 $\mu g \cdot ml^{-1}$). Luminescence Peakheight, as a measure for maximum oxygen radical production was expressed in relative light units per second (RLU$\cdot sec^{-1}$).

Total antioxidant capacity was assayed spectrophotometrically by measuring the ferric reducing ability of plasma.[20] Blood samples were taken at rest, immediately after exercise and after 30 minutes of recovery in pre-cooled EDTA-containing tubes. The samples were centrifuged (1000g, 10 min) immediately after sampling and stored at –80°C until analysis. Concentrations of thiobarbituric acid reactive substances (TBARs), as marker for lipid peroxidation, were determined fluorometrically.[21] Levels of protein carbonyls, a marker of protein oxidation, were measured by means of an ELISA.[22] Samples for both protein and lipid oxidation were obtained at rest, immediately after exercise, and after 30 minutes of recovery in EDTA-containing tubes, and put on ice. After centrifugation (1000g, 10 min), supernatant was stored at

–80°C until analysis. Detection limits for carbonyls and TBARs were 0.065 nM and 0.125 μM, respectively. All samples contained protein and lipid oxidation products above these limits.

Glutathione was measured in its reduced (GSH)[23] and oxidized (GSSG)[5] form. The oxidized-to-reduced ratio (%) of glutathione (GSSG/GSH), another frequently used marker of oxidative stress, was calculated.

Statistics

Between-group differences of baseline values and subjects' characteristics were determined with Kruskal Wallis *H*. Repeated measures analysis of variance (ANOVA) was used to analyze all responses to exercise, with effects of time, group and the interaction between the two. Differences in the exercise-induced response patterns were tested using the interaction between each polynomial and the between-subject factors (time*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). Comparisons between groups were only made (with Tukeys correction) if there was significant heterogeneity across groups / over time.

Linear regression analyses (Pearson's correlation or Spearman rank test if normality was not assumed) were performed to test if FFMI, pulmonary function, systemic inflammation, oxidative stress, and responses to exercise were correlated. Differences between the responses to maximal and submaximal exercise were evaluated with paired t-tests or Wilcoxon signed rank tests if normality was not assumed. Statistical significance was taken at the $p<0.05$ level. Results are presented as means ± SE. Sizes of effect are presented as mean difference and its 95% confidence interval (95% CI) Data were analyzed with SPSS/PC+, version 12.0 (SPSS, Chicago, IL).

Results

Subjects

The characteristics of all participants are provided in Table 1.

Table 1: Subjects' characteristics

	Healthy subjects	COPD patients	
		non-muscle-wasted	*muscle-wasted*
n	10	10	10
Male / Female	8 / 2	6 / 4	5 / 5
Age, yrs	59 ± 1	66 ± 2	65 ± 3
Body mass, kg	84.5 ± 3	73.4 ± 5	56.7 ± 2 *** #
BMI, $kg \cdot m^{-2}$	29.1 ± 1.2	26.7 ± 2.0	20.3 ± 0.5 *** ##
FFMI, $kg \cdot m^{-2}$	19.7 ± 0.7	18.6 ± 0.7	14.5 ± 0.2 *** ###
FEV_1, l	3.3 ± 0.2	1.4 ± 0.1 ***	1.3 ± 0.2 ***
FEV_1, % pred	108 ± 4	54 ± 5 ***	53 ± 6 ***
FEV_1/VC, %	75 ± 1	43 ± 3 ***	41 ± 4 ***
FRC, % pred	93 ± 5	132 ± 7 ***	127 ± 7 **
TLC, % pred	103 ± 4	122 ± 6 *	116 ± 5
K_{CO}, % pred	104 ± 5	79 ± 7 *	62 ± 7 ***
Pa_{O2}, kPa	12.1 ± 0.2	9.9 ± 0.3 **	11.0 ± 0.6
Pa_{CO2}, kPa	5.1 ± 0.1	5.2 ± 0.2	5.3 ± 0.3
Pe_{max}, % pred	114 ± 9	89 ± 13	71 ± 6 *
Pi_{max}, % pred	115 ± 10	99 ± 11	83 ± 8
$MVC_{quadriceps}$, N	393 ± 33	346 ± 83	171 ± 27 ** #

Data are expressed as means ± SE.

Abbreviations: BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume in first second; VC, vital capacity; FRC, functional residual capacity; TLC, total lung capacity; K_{CO}, diffusion capacity for carbon monoxide per unit of alveolar volume; Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; Pe_{max}, maximal expiratory pressure of the respiratory muscles; Pi_{max}, maximal inspiratory pressure of the respiratory muscles; $MVC_{quadriceps}$, maximal voluntary contraction of the quadriceps muscle; pred, predicted value.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ *versus* healthy subjects

# $p<0.05$, ## $p<0.01$, ### $p<0.001$ *versus* non-muscle-wasted COPD patients

According to the Global Initiative for Chronic Obstructive Lung Diseases (GOLD)[24], the COPD groups showed mild (GOLD I: FEV_1/VC <70%, and FEV1>80% predicted) to very severe (GOLD IV: FEV_1/VC <70%, and FEV1<30% predicted) airflow obstruction and signs of hyperinflation. Muscle-wasted patients showed significant loss of both respiratory (Pe_{max}<80% predicted, $p<0.05$) and peripheral (quadriceps) muscle strength ($MVC_{quadriceps}$, 171 N vs. 393 N in healthy subjects ($p<0.01$) and 346 N in non-muscle-wasted patients($p<0.05$)). In addition, FFMI and quadriceps strength were strongly correlated within COPD patients ($r = 0.77$, $p<0.01$).

Systemic inflammation and oxidative stress at rest

Between-group differences were observed for basal systemic inflammation and oxidative stress (Table 2). Baseline systemic inflammatory mediators were significantly higher in both muscle-wasted and non-muscle-wasted COPD patients vs. healthy subjects. Baseline inflammatory values did not reach significance between muscle-wasted and non-muscle-wasted patients. Oxidant production at rest did not differ between the groups, while antioxidant capacity was significantly decreased in the muscle-wasted patients, as was reflected by significantly lower total antioxidant capacity ($p<0.05$ vs. both healthy subjects and non-muscle-wasted patients) and reduced GSH levels ($p<0.05$ vs. healthy subjects and $p<0.01$ vs. non-muscle-wasted patients). The imbalance between oxidants and antioxidants in muscle-wasted COPD patients resulted in significantly increased protein oxidation ($p<0.001$) and a tendency towards increased lipid peroxidation ($p=0.06$) and GSSG/GSH ratio ($p=0.07$) compared with the non-muscle-wasted COPD group (Table 2). In addition, basal plasma antioxidant capacity, GSH, GSSG, GSSG/GSH, and IL-8 levels were related with muscle wasting (FFMI) and quadriceps strength (MVC) in patients with COPD (for correlation coefficients, see Table 3). Oxidant production at rest did not differ between the groups, while antioxidant capacity was significantly decreased in the muscle-wasted patients, as was reflected by significantly lower total antioxidant capacity and reduced GSH levels. The imbalance between oxidants and antioxidants in muscle-wasted COPD patients resulted in significantly increased protein oxidation and a tendency towards increased lipid peroxidation ($p=0.06$) and GSSG/GSH ratio ($p=0.07$) in especially this group (Table 2). In addition, basal oxidative status was related to muscle wasting and dysfunction in patients with COPD. Total antioxidant capacity and GSH were both positively correlated with FFMI ($r =0.67$, $p<0.05$, and

r =0.67, p<0.05, respectively), and peripheral muscle strength (r =0.80, p<0.001, and r =0.75, p<0.01). Furthermore, high GSSG levels were associated with low peripheral muscle strength (r = -0.73, p<0.05), and the highest GSSG/GSH ratios were found in COPD patients with the most muscle wasting (r= -0.43, p<0.05).

Table 2: Systemic inflammation and oxidative stress at rest

	Healthy subjects	COPD patients	
		non-muscle-wasted	*muscle-wasted*
CRP, $mg \cdot l^{-1}$	7.3 ± 0.9	10.8 ± 2.8 *	16.0 ± 3.8 **
Leukocytes, $\cdot 10^{9} \cdot l^{-1}$	6.0 ± 0.3	9.2 ± 0.8 **	8.1 ± 0.8 *
IL-6, $pg \cdot ml^{-1}$	1.9 ± 0.6	2.0 ± 0.4	3.6 ± 1.1 $^{*\,\S}$
IL-8, $pg \cdot ml^{-1}$	3.2 ± 0.5	8.7 ± 1.4 *	11.2 ± 2.6 **
IL-1ra, $pg \cdot ml^{-1}$	0.72 ± 0.09	0.79 ± 0.11	0.65 ± 0.11
TNF-α, $pg \cdot ml^{-1}$	0.70 ± 0.13	0.46 ± 0.06	0.39 ± 0.10
ROS production, $RLU*10^{3} \cdot sec^{-1}$	42 ± 6.3	36.8 ± 3.1	34.2 ± 2.8
Total antioxidant capacity, mM	0.93 ± 0.04	0.97 ± 0.04	0.85 ± 0.04 $^{*\,\#}$
GSH, μM	1103 ± 80	1176 ± 121	777 ± 47 $^{*\,\#\#}$
GSSG, μM	52 ± 6.4	51 ± 5.4	49 ± 5.4
GSSG/GSH (%)	5.4 ± 1.0	4.8 ± 0.9	7.1 ± 1.3
Carbonyls, nM	1.2 ± 0.1	1.9 ± 0.2 ***	2.4 ± 0.3 $^{***\,\#\#\#}$
TBARs, μM	0.70 ± 0.1	0.75 ± 0.2	1.05 ± 0.2

Data are expressed as means ± SE.

Abbreviations: CRP, C-reactive protein; IL, interleukin; ra, receptor antagonist; TNF-α, tumour necrosis factor alpha; ROS, reactive oxygen species; RLU, relative light units; GSH, reduced glutathione; GSSG, oxidized glutathione; TBARs, thiobarbituric acid reactive substances.

* p<0.05, ** p<0.01, *** p<0.001 *versus* healthy subjects

§ p=0.05, $^{\#}$ p<0.05, $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001*versus* non-muscle-wasted COPD patients

Table 3: Correlation coefficients between baseline systemic inflammation or oxidative status and muscle function or wasting in patients with COPD

	FFMI	$MVC_{quadriceps}$
Plasma IL-8	-0.51 **	-0.62 **
Plasma antioxidant capacity	0.67 *	0.80 ***
GSH	0.67 *	0.75 **
GSSG	0.41	-0.73 *
GSSG/GSH	-0.60 **	-0.56

Abbreviations: IL, interleukin; GSH, reduced glutathione; GSSG, oxidized glutathione; FFMI, fat free mass index; MVC, maximal voluntary contraction
* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Physiological responses to exercise

Physiological responses to both maximal and submaximal exercise are shown in Table 4. As expected, exercise tolerance was limited in COPD patients. Within COPD patients, a positive correlation was found between FFMI and peak $\dot{V}O_2$ ($r = 0.58$, $p<0.01$). No difference in power output per kilogram muscle (W/kg fat free mass) was found between muscle-wasted and non-muscle-wasted patients. The main reason for stopping the maximal exercise was reported to be dyspnea in all COPD patients, and five of them (all muscle-wasted patients) had also leg muscle pain. In contrast, healthy subjects complained of general fatigue on stopping exercise.

The submaximal exercise at 50% of Wmax corresponded with a steady state $\dot{V}O_2$ of ~70% of the peak $\dot{V}O_2$ reached during the maximal exercise in all groups (healthy subjects, 70 ± 3%; non-muscle-wasted COPD patients, 74 ± 2%; muscle-wasted COPD patients, 72 ± 3%). The lower intensity of the submaximal exercise was also reflected by the smaller changes in lactate levels. Except for two COPD patients, all subjects maintained 30 minutes of cycling at 50% of Wmax. One muscle-wasted patient stopped cycling after 18 minutes because of breathlessness, and one non-muscle-wasted patient only reached 20 minutes of cycling because of general fatigue.

Table 4: Physiological data after maximal and submaximal exercise test

	Subjects	Maximal exercise	Submaximal exercise
Workload, watt	Healthy subjects	206 ± 19	103 ± 10 †††
	Non-muscle-wasted COPD	106 ± 17 **	51 ± 9 ***†††
	Muscle-wasted COPD	82 ± 12 ***	39 ± 6 ***†††
Endurance, min	Healthy subjects	12.4 ± 1	30 †††
	Non-muscle-wasted COPD	8.7 ± 1 ***	29 ± 1 †††
	Muscle-wasted COPD	8.4 ± 1 ***	29 ± 1 †††
HR $^{(a)}$, beats·min^{-1}	Healthy subjects	158 ± 4	126 ± 4 †
	Non-muscle-wasted COPD	134 ± 6 *	118 ± 5 ††
	Muscle-wasted COPD	125 ± 7 ***	107 ± 7 *†
$\dot{V}_{O2}$ $^{(a)}$, ml·min^{-1}	Healthy subjects	2441 ± 262	1648 ± 130 †††
	Non-muscle-wasted COPD	1493 ± 176 **	1103 ± 130 **†††
	Muscle-wasted COPD	1112 ± 80 ***	837 ± 71 ***†††
$\dot{V}_{O2}$ $^{(a)}$, ml·min^{-1}·kg^{-1}	Healthy subjects	29 ± 3.7	20 ± 1.9 ††
	Non-muscle-wasted COPD	19 ± 1.0 **	15 ± 1.6 †
	Muscle-wasted COPD	19 ± 0.8 **	14 ± 0.9 *†††
$\dot{V}_{E}$, % of MVV	Healthy subjects	83 ± 4	44 ± 3 †††
	Non-muscle-wasted COPD	110 ± 7 *	81 ± 6 ***†††
	Muscle-wasted COPD	111 ± 9 *	72 ± 7 **†††
ΔLactate, mM	Healthy subjects	9.3 ± 1.2	1.8 ± 0.8 †††
	Non-muscle-wasted COPD	5.0 ± 0.9 **	0.8 ± 0.3 †††
	Muscle-wasted COPD	6.2 ± 0.8	1.8 ± 0.6 ††

Data are expressed as means ± SE.

Abbreviations: Endurance, duration of exercise test; HR, heart rate; $\dot{V}O_2$, oxygen consumption; $\dot{V}_E$, minute ventilation; MVV, maximal voluntary ventilation; Δlactate, [lactate] post-exercise minus [lactate] pre-exercise.

$^{(a)}$Peak HR and $\dot{V}O_2$ after maximal exercise, steady state HR and $\dot{V}O_2$ during submaximal exercise.

* p<0.05, ** p<0.01, ***, p<0.001 *versus* healthy subjects

†p<0.05, ††p<0.01, †††, p<0.001 submaximal *versus* maximal exercise test

Exercise-induced systemic inflammation and oxidative stress

Exercise induced a significant (time effect, $p<0.001$) leukocytosis in all groups (Figure 1). This leukocytosis had comparable patterns in all groups (time* group effect), but occurred at higher absolute levels in all COPD patients (group effect, $p<0.05$). In healthy subjects, the exercise-induced leukocytosis of the submaximal exercise was significant diminished compared with the response to maximal exercise (maximal, from 6.0 ± 0.3 to $8.8 \pm 0.5 \cdot 10^9$ cells$\cdot l^{-1}$; submaximal, from 5.5 ± 0.2 to $7.1 \pm 0.2 \cdot 10^9$ cells$\cdot l^{-1}$, $p<0.05$). The response to submaximal exercise in COPD patients (both muscle-wasted and non-muscle-wasted), however, was not different from the response to maximal exercise.

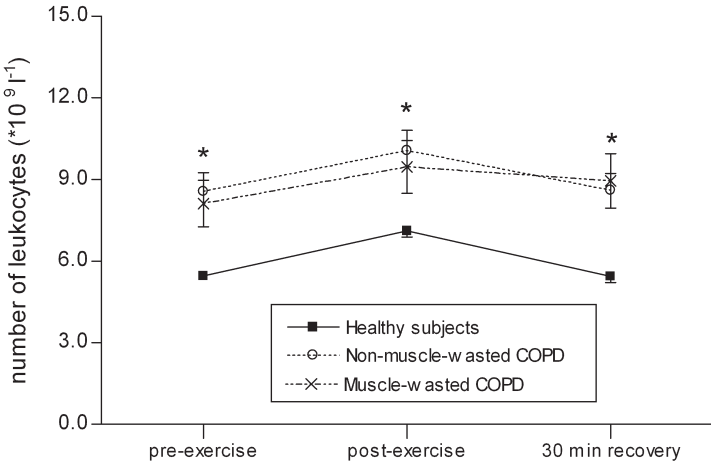

Figure 1. Submaximal exercise-induced leukocytosis in healthy subjects, non-muscle-wasted and muscle-wasted COPD patients. Comparable responses (group*time effect) were observed in all groups, but absolute leukocyte numbers of all COPD patients (muscle-wasted and non-muscle-wasted) were significantly higher compared to healthy subjects (group effects, * $p<0.05$ *versus* healthy subjects).

Plasma IL-6 levels increased immediately after exercise in all groups (time effect, $p<0.05$). IL-6 changes (peak value – rest value) in response to maximal exercise were 0.7 pg$\cdot$ml^{-1} (95% CI 0.17-0.90) in healthy subjects, 0.7 pg$\cdot$ml^{-1} (95% CI 0.22-1.0) in non-muscle-wasted patients , and 1.1 pg$\cdot$ml^{-1} (95% CI 0.35-1.86) in muscle-wasted patients, respectively. Post hoc analysis of these changes revealed increased responses of IL-6 in the muscle-wasted group ($p<0.05$ *versus* both healthy subjects and non-muscle-wasted patients)(Figure 2). Comparing maximal to submaximal

exercise, the increase of IL-6 in healthy subjects was significantly ($p<0.05$) lower after the submaximal exercise (change = 0.37 pg·ml^{-1}, 95% CI 0.08-0.68). In contrast, maximal and submaximal exercises induced comparable increases of IL-6 in non-muscle-wasted COPD patients. The increase of IL-6 in muscle-wasted COPD patients showed a tendency to be even higher after submaximal exercise compared to maximal exercise ($p=0.07$). Levels of CRP, IL-1ra, IL-8, and TNF-α were unchanged (time effect, $p>0.05$) by both exercise protocols in all groups.

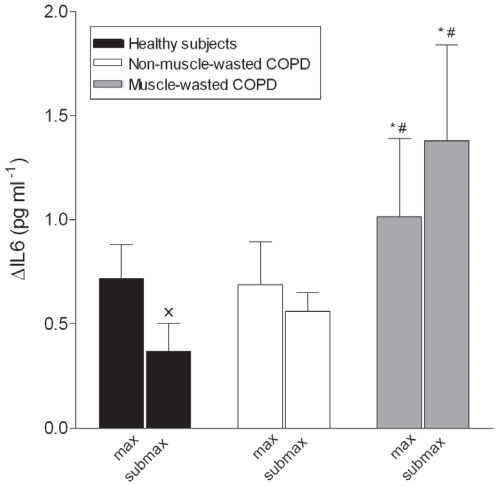

Figure 2. Effect of exercise on plasma IL-6 levels in healthy subjects, non- muscle-wasted and muscle-wasted COPD patients. Data are expressed as ΔIL-6 (peak value – rest value). IL-6 responses to exercise were significantly higher in muscle-wasted COPD patients compared with non- muscle-wasted patients and healthy subjects. In healthy subjects, the response to submaximal exercise was significantly diminished compared to maximal exercise. In COPD patients, however, no differences between the exercises were observed. * $p<0.05$ *versus* healthy subjects; # $p<0.05$ *versus* non- muscle-wasted patients; x $p<0.05$ *versus* maximal exercise.

Production of ROS in response to exercise was different between the groups (time*group effect, $p<0.05$). Neither maximal nor submaximal exercise induced changes of the oxidative burst of neutrophils in healthy subjects (change = 1.5%, 95% CI –11 to 14). In non-muscle-wasted COPD patients, however, production of ROS increased in response to maximal exercise (+15%, CI 1-30), but not in response to submaximal exercise. The highest increase of ROS production (+32%, CI 8-54) was observed in muscle-wasted COPD patients (Figure 3), without differences between maximal and submaximal exercise.

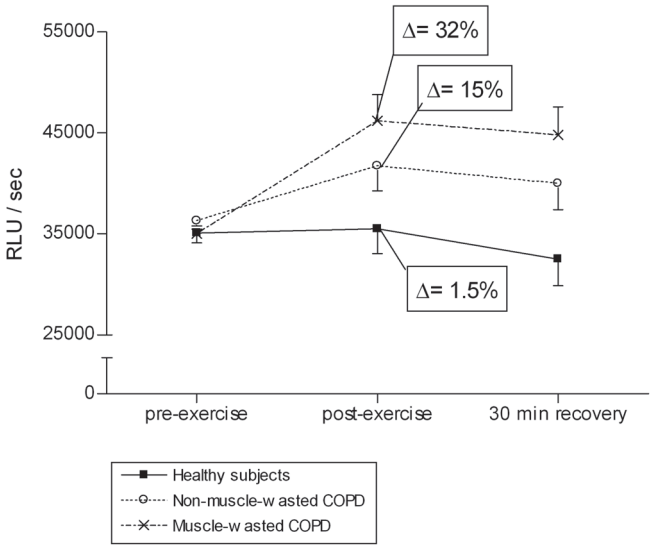

Figure 3. Oxidative burst of neutrophils before and after maximal exercise in healthy subjects, non-muscle-wasted and muscle-wasted COPD patients. Production of ROS in response to exercise was different between the groups (time*group effect, $p<0.05$). Neither maximal nor submaximal exercise induced changes of the oxidative burst of neutrophils in healthy subjects. In COPD patients, however, production of ROS increased in response to maximal exercise (+15%, CI 2-20%) and even further increased (+31%, CI 15-45%) in muscle-wasted COPD patients.

Total antioxidant capacity in plasma decreased independent of exercise intensity. In all groups, both maximal and submaximal exercise caused a small but very consistent (time effect, $p<0.05$) decrease (-5%, 95% CI –6.5 to –1.5) of the antioxidant capacity (Figure 4). After 30 minutes of recovery, the antioxidant capacity rose to levels slightly above baseline (+8%, 95% CI 6-15) in all groups after both exercises. Overall, muscle-wasted COPD patients had lower antioxidant capacity compared with both healthy subjects and non-muscle-wasted COPD patients (group effects, $p<0.05$ *versus* healthy subjects; $p<0.05$ *versus* non-muscle-wasted patients).

Exercise was accompanied with increased lipid peroxidation in muscle-wasted COPD patients (change in TBARs = 0.32 μM, 95% CI 0.1-0.6), but not in the other groups (group effect, $p<0.05$ *versus* both healthy subjects and non-muscle-wasted patients) (Figure 5). No differences in the response to maximal and submaximal exercises were seen. Also, there was a clear inverse relation between FFMI and the response of TBARs to exercise within the COPD patients ($r = -0.59$, $p<0.01$).

Plasma carbonyl levels were not affected by exercise (time effect, $p>0.05$).

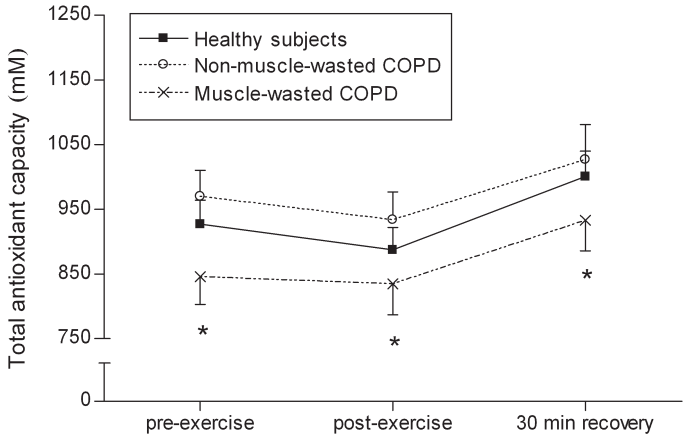

Figure 4. Total antioxidant capacity of plasma before and after maximal exercise in healthy subjects, non- muscle-wasted and muscle-wasted COPD patients. In all groups, exercise induced a significant decrease of the antioxidant capacity directly after exercise, followed by an increase above baseline values during 30 minutes of recovery (time*group effect, $p>0.05$; time effect, $p<0.05$). Overall, muscle-wasted COPD patients had lower antioxidant capacity compared with both healthy subjects and non-muscle-wasted COPD patients (group effects, * $p<0.05$ *versus* healthy subjects and *versus* non- muscle-wasted patients).

Finally, exhaustive exercise caused glutathione oxidation in all groups. GSH levels significantly and similarly decreased in all groups (change GSH = -200 μM, 95% CI –388 to –55) (time effect, $p<0.05$; time*group effect, $p>0.05$, group effect, $p>0.05$)(Figure 6A). Contrary to the decrease of GSH, GSSG levels increased after exercise (Figure 6B). Immediately after exercise, the oxidation of glutathione increased in both COPD groups, but not in healthy subjects. During the 30 min of recovery, oxidation proceeded and increases up to 161% (95% CI 101-221), 173% (95% CI 70-279), and 232% (95% CI 164-300) of rest values were observed in healthy subjects, non-muscle-wasted and muscle-wasted COPD patients, respectively (group effects, $p<0.05$ muscle-wasted patients *versus* healthy subjects, and $p<0.01$ *versus* non-muscle-wasted patients). The exercise-induced changes in reduced and oxidized glutathione concentrations resulted in increased oxidative stress as is shown by highly increased GSSG-to-GSH ratios (Figure 6C). The ratio increased with similar patterns in the groups (time*group effect, $p>0.05$). However, significantly more exercise-induced oxidative stress was seen in muscle-wasted patients (GSSG/GSG = 29%, 95% CI 22-36)(group effects, $p<0.01$ *versus* healthy subjects; $p<0.001$ *versus* non-muscle-wasted COPD patients).

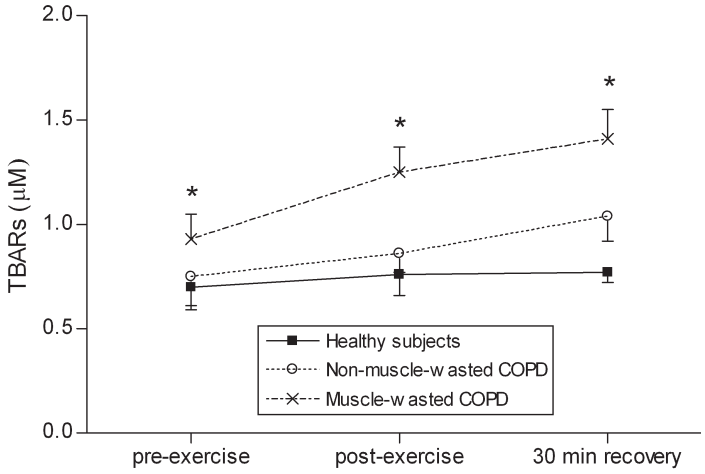

Figure 5. Exercise-induced lipid peroxidation (TBARs) in healthy subjects, non- muscle-wasted and muscle-wasted COPD patients. TBARs were significantly elevated after maximal exercise in muscle-wasted COPD patients, but not in healthy subjects and non- muscle-wasted patients (group effects, * $p<0.05$ *versus* healthy subjects, and versus non- muscle-wasted patients).

Discussion

Our results demonstrate that both maximal and submaximal exercise induce increased systemic inflammatory and oxidative responses in muscle-wasted COPD patients, which are less pronounced or not seen in non-muscle-wasted patients and healthy subjects, respectively. These data support the concept of a role for systemic inflammation and oxidative stress in muscle wasting of COPD patients.

Low-grade basal systemic inflammation and oxidative stress

Different inflammatory mediators that have been associated with increased resting metabolisms and weight loss [9;10], were elevated in COPD patients compared with healthy subjects and the highest levels of inflammation (IL-8, IL-6, CRP) were seen in muscle-wasted COPD patients (*see* Table 2). It has been suggested that increased levels of TNF-α, IL-1, and IL-8 may induce a catabolic response in tissues, triggering muscle proteolysis, with a resulting increase in protein degradation. Since negative feedback mechanisms (e.g. on TNF-α and IL-1) play an important role in the whole cytokine cascade, it remains difficult to identify the specific cytokines that may be involved in the catabolic response.

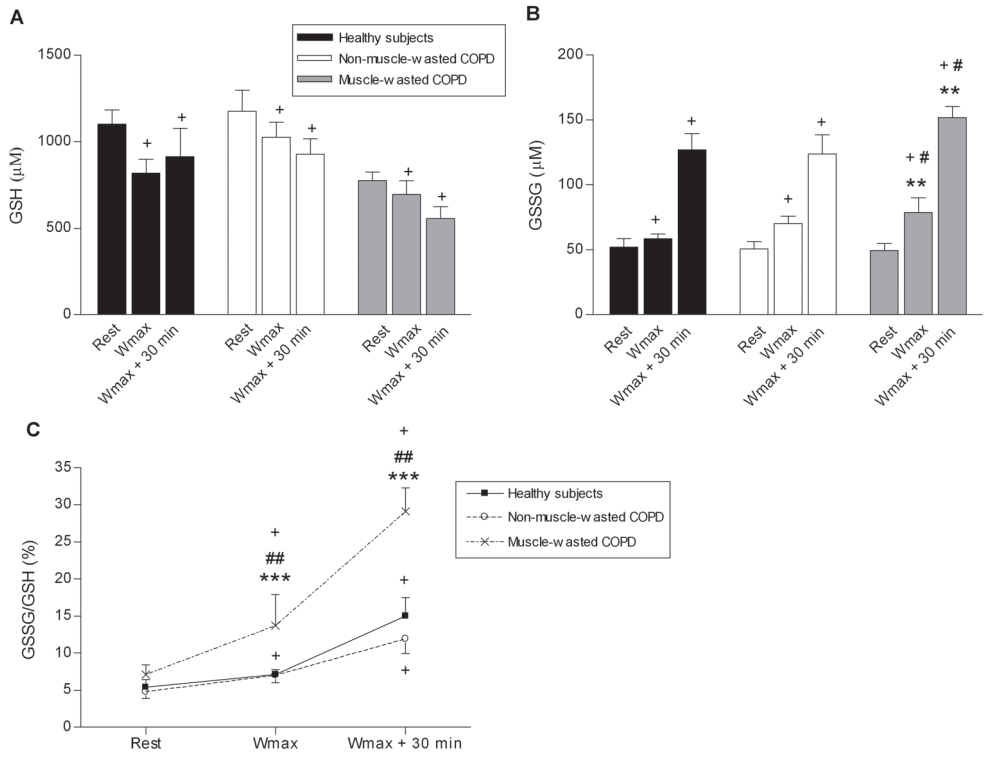

Figure 6. Effect of maximal exercise on plasma levels of GSH *(A)*,GSSG *(B)*, and GSSG/GSH ratio *(C)* in healthy subjects, non- muscle-wasted and muscle-wasted COPD patients. GSH levels significantly decreased in all groups in response to exercise (time effect, $^{+}$ $p<0.05$) while oxidized glutathione levels increased more in muscle-wasted COPD patients (group effects, $^{\#}$ $p<0.05$ muscle-wasted patients *versus* healthy subjects, and ** $p<0.01$ *versus* non-muscle-wasted patients). The exercise-induced changes in reduced and oxidized glutathione concentrations resulted in increased oxidative stress as is shown by highly increased GSSG-to-GSH ratios. The ratio increased with similar patterns in the groups (group*time effect, $p>0.05$). However, significantly more exercise-induced oxidative stress was seen in muscle-wasted patients (group effects, $^{\#\#}$ $p<0.01$ *versus* healthy subjects; *** $p<0.001$ *versus* non-muscle-wasted COPD patients).

To our knowledge, this is the first study showing evidence that muscle wasting in COPD patients is not only accompanied with basal inflammation, but also with less antioxidant capacity and a higher degree of oxidative stress at rest. Total antioxidant capacity and the level of the ubiquitous antioxidant GSH were significantly reduced in muscle-wasted patients, and may have disturbed the oxidant/antioxidant balance, resulting in oxidative stress (lipid peroxidation and protein oxidation). The lack of

increased basal GSSG levels suggests an impaired synthesis of antioxidants rather than an increased oxidation in these patients. Contrary to previous studies [25;26], the release of ROS by neutrophils at rest was not increased in COPD patients. Since the sensitivity of ROS detection is dependent on the method used, this has probably contributed to the contrasting results.

Correlations were found between muscle wasting, muscle dysfunction and different parameters of systemic inflammation, and oxidative/antioxidative status (Table 3), indicating a possible causative effect of systemic inflammation or oxidative stress on muscle wasting and function or vice versa. For now, we cannot distinguish between cause and direct consequence.

Exercise-induced systemic inflammatory and oxidative response

Previously, striking parallels have been observed between exercise and its complex regulatory and counter-regulatory responses in diseases states. Exercise induces changes in circulating leukocyte counts [2] that directly reflect the changes in blood concentrations of (nor)epinephrine and cortisol, which are linearly related to the intensity of exercise. According to this theory, the present study indeed showed that the immediate leukocytosis in healthy subjects is lower after submaximal than after maximal exercise. Contrary, the exercise-induced leukocytosis in patients with COPD seemed to be independent of intensity. Because we showed recently that the (nor)epinephrine response to exercise in COPD patients was not different from the response in healthy subjects [7], this can not explain the discrepancies between the groups.

The relation between intense exercise and circulating inflammatory cytokines is well established. In the current study, both maximal and submaximal exercise resulted in significant IL-6 responses (*see* Figure 2). Remarkably, the increases in muscle-wasted COPD patients exceeded the responses of the other two groups. In COPD patients, the responses were similar after both maximal and submaximal exercise. These results strongly suggest that even moderate exercise further intensifies the ongoing low-grade systemic inflammation in patients with COPD, and may be involved in worsening extra-pulmonary effects like muscle damage. Rabinovich et al.[6] also studied the cytokine response to moderate intensity exercise (11 min, 40% Wmax) in healthy subjects and COPD patients with normal BMI (FFMI not reported). They found an abnormal exercise-induced increase of TNF-α in COPD

patients, but did not observe changes of IL-6 in COPD patients or healthy subjects. Absence of an increase in IL-6 in the latter group seems at least remarkable since appearance of this cytokine in the circulation after exercise is by far the most marked and its appearance precedes that of other cytokines.[27] Maybe the differences in work intensities and duration between the two studies play a role in this discrepancy. Also, evaluation of different time points during exercise might give more insight in the time that is needed to see exercise-induced increases in systemic inflammation.

Other studies have shown that physical exercise can induce systemic or local (e.g. in muscle) oxidative stress in patients with COPD.[3;4] When the formation of free radicals exceeds the antioxidant capacity, the radicals can oxidize lipids, proteins, sugars, and other cell components, and thereby alter normal processes within cells and tissues. The present study showed that both maximal and submaximal exercise cause a significant increase of free radical production in muscle-wasted COPD patients, while antioxidant capacity is decreased. This disbalance might have resulted in the observed lipid peroxidation. Priming of neutrophils is indicated by an augmented response of these cells produce reactive oxygen species without direct stimulatory actions upon the cells themselves. In response to cell stimulation, these primed cells can become activated to release reactive oxygen species and lysosomal enzymes, which in turn destroy cells and tissues. Cytokines are able to cause priming of neutrophils.[28] According to this theory, the increased IL-6 response to cycling in muscle-wasted COPD patients might have been the primer for the neutrophils to produce more ROS. On the other hand, ROS are also known to induce production of inflammatory mediators like IL-6.[29] In that case, ROS can also have been the cause rather than the effect of the acute inflammatory response to exercise.

Beyond the association between oxidative stress, muscle dysfunction and physical performance in COPD, the present data implicate that especially muscle-wasted patients may be frequently exposed to oxidative stress and the resulting tissue damage during daily life activities, which contain low-to-moderate intensity exercises.

Clinical implications

Our results showed that systemic inflammation and oxidative stress coexist in especially muscle-wasted COPD patients, and that these processes are further increased in response to relatively less exercise. The clinical implications of these findings are not yet clear. In general, moderate exercise training is associated with

good or improved states of health, suggesting that factors like IL-6 and oxidants may play a role in the healthy adaptation to exercise. However, even in healthy subjects it is known that the very same process of exercise, if sufficiently intense, can stimulate inflammatory cytokines and lead to a catabolic state.[30] Our finding that relatively less exercise in especially muscle-wasted COPD patients can additionally increase already elevated circulating levels of these mediators suggests that these patients might frequently be exposed to bursts of the chronic inflammatory- and oxidative-catabolic responses during activities of daily life, holding them in a continuous catabolic state which may keep them in a vicious cycle of progression of the disease. Our study only investigated the systemic effects of acute exercise in stable patients with COPD, not including possible effects (e.g. adaptation) of chronic exercise. The investigation of Rabinovich and coworkers [6] has shown that 8 weeks of training could not modify the abnormal systemic inflammatory response in patients with COPD. Theoretically, the beneficial effects of exercise training in these patients, e.g. increase of physical performance, skeletal muscle mass and function, may be neutralized by the increased inflammatory and oxidative responses to exercise. On the other hand, Mercken et al.[31] reported very recently that 8 weeks of pulmonary rehabilitation was associated with reduced exercise-induced oxidative stress in patients with COPD, suggesting that the body adapts to chronic exercise. Further studies are needed to evaluate possible adaptation mechanisms to decline the exercise-induced inflammation and oxidative stress in patients with COPD.

In view of both the resting catabolic state and possible influence on the effect of exercise training, it would be of interest to know if any intervention can modify the basal or exercise-induced systemic inflammation and/or oxidative stress. Previously, it has been shown that exercise-induced oxidative stress can be (partially) prevented by antioxidants.[3] In addition, Vassikopoulos et al.[29] found a blunted cytokine response in healthy subjects after antioxidant therapy. Recently, however, it has also been shown that antioxidants did not prevent muscle damage in response to exhaustive exercise in healthy subjects.[32] Effects of other therapies are under investigation now. Our own group has recently shown that exercise-induced oxidative stress in a group of muscle-wasted COPD patients could be prevented by supplemental oxygen.[33] Although clinical benefits of this prevention need to be elucidated, yet, these findings invites further research in this area.

Finally, our data implicate that special attention is needed for the group of muscle-

wasting COPD patients. We think it would be a major clinical advancement if we were able to predict who will be a muscle waster and who will not. Early identification of potential muscle wasting allows for more intensive therapy and possible mitigation of the disease.

In conclusion, the present study shows that exercise-induced systemic inflammatory and oxidative response are increased in response to both maximal and submaximal exercise in muscle-wasted COPD patients compared to non muscle-wasted patients and healthy subjects. These findings support the concept of an association between inflammation, oxidative stress and muscle wasting or dysfunction. Frequent exposure to elevated levels of inflammation and oxidative stress might hold muscle-wasted patients in a continuous catabolic state, keeping them in a vicious cycle of progression of the disease. Further studies are needed to evaluate 1) the exact mechanisms and direct consequences of systemic inflammation and oxidative stress in COPD; 2) if daily life activities and/or training also intensifies systemic inflammation and oxidative stress; 3) whether or not muscle wasting in COPD can be prevented by diminishing systemic inflammation and oxidative stress.

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Chapter 5

Six-Minute Walking-Induced Systemic Inflammation and Oxidative stress in Muscle-Wasted COPD Patients

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Abstract

Systemic inflammation and oxidative stress are potential mechanisms for muscle wasting in chronic obstructive pulmonary disease (COPD). The six-minute walking test (6MWT) has been suggested as simple and valid exercise test in COPD, which is well tolerated, and reflective of activities of daily living. The present study investigated physiologic and systemic immunologic responses to a 6MWT in muscle-wasted COPD patients and compared them with maximal cardiopulmonary exercise testing (CPET).

10 muscle-wasted COPD patients were included (Fat Free Mass Index, FFMI; males <16 $kg \cdot m^{-2}$, females <15$kg \cdot m^{-2}$). A 6MWT and CPET were carried out in random order. The physiologic response was followed by a mobile oxycon. Arterial blood was obtained at rest and after exercise to measure blood gases and markers of systemic inflammation and oxidative stress.

In these patients (FEV_1 55 ± 4 % pred), the 6MWT was a submaximal, albeit intense, exercise as reflected by $\dot{V}O_2$, $\dot{V}_{E,}$ heart rate, and lactate values. Leukocytosis was less intense after 6MWT compared to CPET. Contrary, the increase in IL-6, free radical release by neutrophils, oxidation of proteins and lipids, and the reduction in antioxidant capacity were similar after both exercises. FFMI was inversely related to 6MWT-induced increases in protein and lipid peroxidation.

This study shows that a 6MWT induces a systemic immunologic response in muscle-wasted COPD patients, which is comparable to CPET-induced responses. The correlation between systemic oxidative stress and the degree of muscle wasting supports a possible causal relation between systemic inflammation, oxidative stress and muscle wasting.

Introduction

Chronic obstructive pulmonary disease (COPD) is defined currently by the ATS/ERS as a disease characterized by airflow limitation, which is not fully reversible and produces significant systemic consequences.[1] This systemic involvement in COPD has become extremely important since it seems conceivable that exploring the presence of systemic biomarkers and their relation to systemic manifestation of the disease, will help us in developing and applying novel strategies that will improve outcome of our patients. Based on the current understanding of the pathobiology of COPD, most notably suggested markers are the inflammatory cells and their products that are believed to be the proximate causes of tissue destruction in patients with COPD.[2] The exact meaning of these biomarkers are not elucidated yet, and may be multifactorial, but both systemic inflammation and oxidative stress have been associated with loss of muscle mass and muscle dysfunction.[3-6] Very recently, moderate and high intensity cycle ergometry has shown to increase systemic inflammation and oxidative stress [7], especially in the subgroup of muscle-wasted COPD patients.[5] Although strong evidence is lacking, frequent exposure to these effects might play a role in the ongoing muscle wasting and its consequences in these patients. Additionally, it was shown that cycling-induced systemic inflammation in these muscle-wasted patients could be attenuated and oxidative stress be prevented by supplemental oxygen during exercise.[8] Based on these results, we postulated that daily life activities, which can be classified as moderate intense for these patients, can cause frequent bursts of systemic inflammation and oxidative stress which may be involved in muscle wasting. Patients with COPD, however, are relatively inactive and they will not perform cycle exercises regularly. The six-minute walking test (6MWT) has been suggested as a simple and valid exercise test in COPD, which is well tolerated, and reflective of activities of daily living.[9] The physiologic responses to 6MWT in patients with COPD has been studied previously and described as maximal [10] or submaximal [11] sustainable exercise in these patients.

The current study was designed to characterize both the physiologic and systemic immunologic responses to a 6MWT in muscle-wasted COPD patients and compare them with the responses to maximal cardiopulmonary exercises testing (CPET).

Methods

Subjects

Ten (6 males) ex-smoking, muscle-wasted COPD patients (Fat Free Mass Index, FFMI; males <16 $kg \cdot m^{-2}$, females <15$kg \cdot m^{-2}$)[12] participated in this study. The patients were recruited from our outpatient clinic and had moderate to severe COPD according to the GOLD classification.[1] All had been free of exacerbations for at least two months prior to the study, and had stopped smoking at least 6 months before inclusion. Exclusion criteria were the use of oral corticosteroids, long-term oxygen therapy, and other chronic inflammatory or exercise-limiting diseases. The use of inhaled corticosteroids (n=6) and antioxidants (N-acetylcysteine, n=3) was discontinued one week prior to exercise testing. All patients were on bronchodilator therapy, none used theophylline. The study was conducted according to the Declaration of Helsinki and was approved by the medical ethical committee of our hospital. Written informed consent was obtained from all subjects.

Study design

As part of the characterization procedures, resting pulmonary function, bioelectrical impedance analysis (Biostat 1500, Bodystat LTD, Douglas, Isle of Man, British Isles), and peripheral muscle strength (as described in [5]) were carried out in all patients. Weight parameters were adjusted for body surface to give body mass index (BMI) and FFMI. On two different study days (separated by one week), two exercise protocols were carried out in random order: 6MWT and CPET. During both exercises, the physiologic response was followed in all participants. In all patients arterial blood was obtained from a catheter in the radial artery to measure blood gases, lactate concentrations and systemic inflammation and oxidative stress at rest and in response to the exercises.

Exercise testing

A portable breath-by-breath system (Mijnhardt/Jaeger, Bunnik, The Netherlands) a pulse-oxymeter (Datex, Helsinki, Finland), and a polar belt were used to monitor oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation ($\dot{V}_E$), and heart rate (HR) on-line during exercise. Validation of this new system, the mobile oxycon, is described in the equipment

section below. Before exercise, a catheter was inserted into the radial artery to obtain arterial blood before, during and directly after (within 15 sec) exercise.

The 6MWT was performed according to the ATS guidelines.[13] All patients were familiar with the test. A straight walking course of 30m was used and two cones marked the turnaround points. Before the 6MWT, the patient sat in a chair for at least 30 minutes to measure stable physiologic and immunologic baseline values. After starting the exercise, participants were encouraged every minute with standardized phrases. Patients were allowed to stop and rest during the six minutes of exercise, but none of the participants in this study stopped walking before finishing the complete 6 minutes.

A maximal, symptom limited, incremental bicycle test was carried out on an electrical braked cycle ergometer (Masterlab®, Jaeger, Würzburg, Germany) according to the ATS guidelines.[14]

Equipment

Using the mobile oxycon and the belonging software, we were able to evaluate the physiological response to the 6MWT with the 9-panel plots.[15] The portable system consisted of a face mask, polar belt, battery and transmitting unit (containing the O_2 and CO_2 gas analyzers), and a receiving unit. The transmitting unit with battery pack and face mask with tubing (total weight 0.5 kg) was attached to the individual with a harness, and the receiving unit was connected to a personal computer anywhere within 500 m of the transmitting unit. The face mask contained a turbine for measurement of ventilation as well as a capillary gas sampling port within the turbine's housing. The expired gas was sampled at a rate proportional to ventilation into a microchamber containing O_2 and CO_2 electrodes. O_2 and CO_2 analyzers were thermostated and compensated for the variations of barometric pressure and humidity of the environment. Calibration of the turbine by use of a 3-liter syringe and a two-point calibration of the gas analyzers by use of gas mixtures from tanks of standard gas were performed before each test.

Before the study, the agreement between the mobile oxycon and the breath-by-breath (Oxyconbeta, Mijnhardt/Jaeger, Bunnik, The Netherlands) systems in assessing pulmonary physiological parameters was verified in four healthy subjects at different exercise levels of cycle-ergometer exercise: 1) incremental test until exhaustion, and 2) moderate constant work rate tests at 25% and 75% of maximal workload (Wmax)

achieved during test 1. Subjects performed each protocol on both the mobile oxycon and breath-by-breath systems. The test order was randomized. Values of $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ did not differ significantly between mobile oxycon and breath-by-breath, either at peak or at submaximal level of exercise. With the Bland-Altman test [16], the mean $\dot{V}O_2$ difference between the two methods was 10.1 ml·min^{-1}, and the limits of agreement (means ± 2 SD) were + 171.8 to –151.7 ml·min^{-1}. The biases in $\dot{V}CO_2$ and $\dot{V}_E$ were comparably small. Furthermore, two walking tests of 5 minutes on a treadmill (5 km/h) were performed with the mobile oxycon. In this way an intratest variability of <11% was measured.

Analysis

Arterial blood gases (oxygen tension, Pa_{O2}; carbon dioxide tension, Pa_{CO2}) and lactate levels were determined immediately after sampling (Gas analyzer Chiron 860). Plasma cytokines were before and after the exercises using quantitative sensitivity and high-sensitivity sandwich enzyme-linked immunosorbent assays (ELISA's) in kit form (R&D systems, Minneapolis, USA) according to the supplier's instructions. Leukocyte counts and CRP were determined at rest and immediately after exercise, following standard laboratory assays.

Production of reactive oxygen species (ROS) by neutrophil granulocytes in response to stimulation with phorbol myristate acetate (PMA), the so-called oxidative burst, was measured in isolated neutrophils. Cell isolation was performed by means of density centrifugation on Percoll immediately after sampling, as described previously.[17] Luminol-enhanced chemiluminescence was measured with an automated LB96V Microlumat Plus luminometer (EG&G Berthold, Bad Wildberg, Germany) as described by Wanten et al.[18] Luminescence Peakheight, as a measure for maximum oxygen radical production was expressed in relative light units per second (RLU/sec). Exercise-induced changes in luminescence were expressed relatively to luminescence values obtained at rest.

Total antioxidant capacity was assayed spectrophotometrically by measuring the ferric reducing ability of plasma, as described by Benzie et al.[19]

Concentrations of thiobarbituric acid reactive substances (TBARs), as marker for lipid peroxidation, were determined fluorometrically using the method described by Conti et al.[20] Levels of protein carbonyls, a marker of protein oxidation, were measured by means of an ELISA as described by Buss et al.[21], and modified by

Zusterzeel et al.[22]
Measurements after exercise were corrected for plasma volume shifts according to Dill and Costill.[23]

Statistical analyses
Group data are expressed as mean values ± SE. Comparisons between rest and exercise values were made using Student paired t tests (or Wilcoxon signed-rank test if normal distribution was not assumed). When the response to exercise was expressed as percentage change compared to baseline, a one sample t test (or Mann Whitney U test) was used. Comparisons between 6MWT and CPET were made using Student paired t tests (or Wilcoxon signed-rank test). One way analyses of variance (ANOVA) for repeated measurements were carried out to examine physiologic profiles at the different exercise protocols. Spearman Rank tests were used to evaluate the correlations between physiological response, immune response, and muscle wasting. The level of statistical significance was set at $p<0.05$.

Results

The study group (Table 1) showed moderate-to-severe airflow obstruction (FEV_1, 1.55 ± 0.20 L; 55 ± 4 % predicted, range 36 to 74 % predicted) without arterial hypoxemia or hypercapnia at rest. Mean FFMI was 14.1 $kg \cdot m^{-2}$ in females and 14.8 $kg \cdot m^{-2}$ in males.

Physiologic responses
Figure 1 displays $\dot{V}O_2$ and $\dot{V}_E$ profiles during 6MWT and CPET in the same patients. Both $\dot{V}O_2$ and $\dot{V}_E$ showed a steady-state profile during the last 4 minutes of 6MWT, while they gradually increased until exhaustion during CPET. Comparing $\dot{V}O_2$ and $\dot{V}_E$ after 6 min of walking and cycling (isotime), no significant differences were seen. In contrast, peak $\dot{V}O_2$ and $\dot{V}_E$ were significantly higher (both $p<0.01$) than steady state 6MWT (6^{th} min) values. Also, heart rate and lactate values were significantly lower after 6MWT compared with CPET ($p<0.01$ and $p<0.001$, respectively). Detailed information on physiological responses is provided in Table 2.

Table 1. Subject characteristics

Subjects, n	10
Male/Female	6/4
Age, yrs	64 ± 4
BMI, $kg \cdot m^{-2}$	20.8 ± 0.5
FFMI, $kg \cdot m^{-2}$	
Male	14.8 ± 0.3
Female	14.1 ± 0.3
FEV_1, L	1.55 ± 0.2
FEV_1, % pred	55 ± 4
FEV_1 post bronchodilation , % pred	57 ± 3
FEV_1/FVC, %	45 ± 4
RV/TLC, % pred	49 ± 3
FRC, % pred	138 ± 11
K_{CO}, % pred	69 ± 8
Pa_{O2}, kPa	10.3 ± 0.4
Pa_{CO2}, kPa	5.0 ± 0.1
Pi max, % pred	90 ± 8
Pe max, % pred	72 ± 3
$MVC_{quadriceps}$, N	162 ± 15

Values are represented as mean ± SE

Abbreviations: BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; FRC, functional residual capacity; K_{CO}, diffusion capacity for carbon monoxide per unit of alveolar volume; Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; Pi max, maximal inspiratory mouth pressure; Pe max, maximal expiratory mouth pressure, $MVC_{quadriceps}$, maximal voluntary contraction of the quadriceps muscle; pred, predicted value.

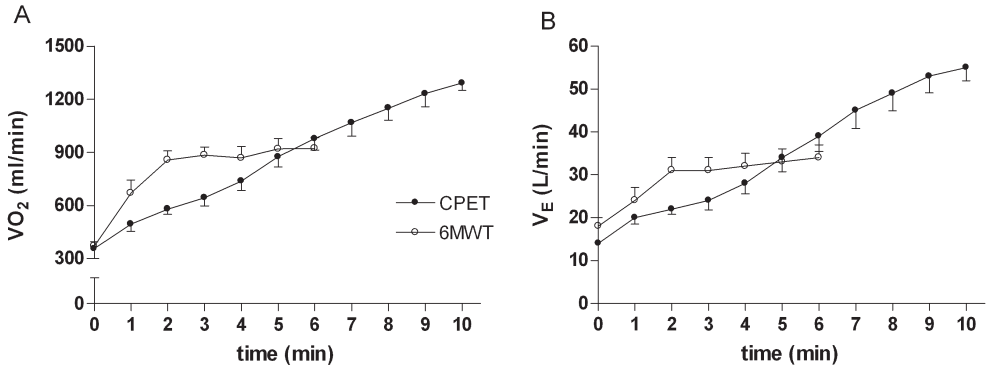

Figure 1. Physiologic responses during exercise. Mean data ± SE for oxygen uptake ($\dot{V}O_2$) *(A)*, and minute ventilation ($\dot{V}_E$) *(B)* during CPET and 6MWT.

Table 2. Physiologic responses to cardiopulmonary exercise testing (CPET) and during the last minute of a 6-min walking test (6MWT)

	CPET	6MWT (6^{th} min)
Work rate, W	89 ± 10	
6MWD, m		462 ± 32
$\dot{V}_{O2}$, L·min^{-1}	1.12 ± 0.09	0.90 ± 0.05 *
$\dot{V}_{CO2}$, L·min^{-1}	1.26 ± 0.11	0.83 ± 0.07 **
RER	1.11 ± 0.04	0.90 ± 0.03 **
$\dot{V}_E$, L·min^{-1}	50 ± 5	37 ± 3 **
BR, L·min^{-1}	2 ± 2	15 ± 4 **
Bf, min^{-1}	36 ± 2	29 ± 1 *
$\dot{V}_E/\dot{V}_{CO2}$ †	40 ± 2	45 ± 2 *
HR, beat·min^{-1}	133 ± 6	107 ± 5 **
O_2 pulse, ml·beat $^{-1}$	8.2 ± 0.4	8.7 ± 0.4
Pa_{O2}, kPa	9.6 ± 0.6	10.1 ± 0.6
Pa_{CO2}, kPa	5.4 ± 0.2	5.2 ± 0.4
$P(_{A-a})O_2$, kPa	5.02 ± 0.73	3.20 ± 0.80 **
V_D/V_T	40 ± 2	40 ± 2
Lactate, mM	7.5 ± 0.9	1.8 ± 0.3 ***

Values are represented as mean ± SE.

Abbreviations: 6MWD, 6-minute walking distance; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; BR, breathing reserve; Bf, breathing frequency; HR, heart rate; Pa_{O2}, arterial O_2 tension; Pa_{CO2}, arterial CO_2 tension; P(A-a)O_2, alveolar-arterial P_{O2} pressure difference; V_D, physiologic dead space; V_T, tidal volume; pred, predicted value.

† $\dot{V}_E/\dot{V}CO_2$ during CPET at anaerobic threshold, during 6MWT at 6th min of walking (lack of anaerobic threshold).

* p<0.05, ** p< 0.01, *** p<0.001 6MWT *versus* CPET.

Immunologic responses

A significant leukocytosis occurred after both CPET ($p<0.001$) and 6MWT ($p<0.01$), which was caused by increases in all subsets. Compared to the response to CPET, the increase of the circulating inflammatory cells was less intense after the 6MWT (Figure 2), with significantly lower changes in total leukocytes (CPET, +20%; 6MWT,+10%, $p<0.05$) and lymphocytes (CPET, +38%; 6MWT,+17%, $p<0.01$).

Plasma levels of IL-6 were significantly increased after CPET (from 5.62 ± 1.1 $pg \cdot ml^{-1}$ to 6.33 ± 1.4 $pg \cdot ml^{-1}$, $p<0.05$). and 6MWT (from 5.95 ± 1.3 $pg \cdot ml^{-1}$ to 6.77 ± 1.5 $pg \cdot ml^{-1}$, $p<0.05$), as shown in Figure 3. The mean increase after walking was not different from the response to CPET (0.82 ± 0.3 $pg \cdot ml^{-1}$ *versus* 1.0 ± 0.4 $pg \cdot ml^{-1}$, $p>0.05$).

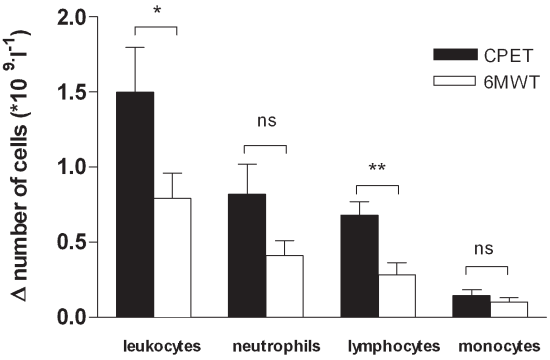

Figure 2. Leukocytosis to exercise. Comparison of increases in leukocytes and subsets after CPET and 6MWT. * $p<0.05$, ** $p<0.01$ 6MWT *versus* CPET; ns, not significant.

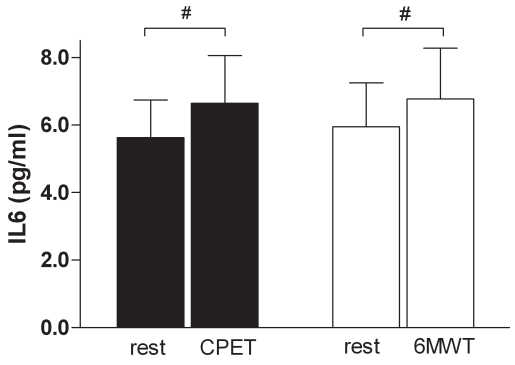

Figure 3. IL-6 response to exercise. Both CPET and 6MWT induced a significant increase of IL-6 levels compared to rest values. The IL-6 response was comparable between the two different exercises. # $p<0.05$ *versus* rest.

Exercise induced free radical production by neutrophils (Figure 4A). Isolated neutrophils produced significantly more ROS after walking than at rest (change = 28 ± 8% in stimulated cells, $p<0.01$). Comparable changes in neutrophil ROS production were observed after CPET (change = 29 ± 11%, $p<0.01$). While the production of oxidants increased, plasma antioxidant capacity significantly decreased in response to exercise (Figure 4B). A relatively small but consistent decrease of ~8% after 6MWT resembled the response to CPET.

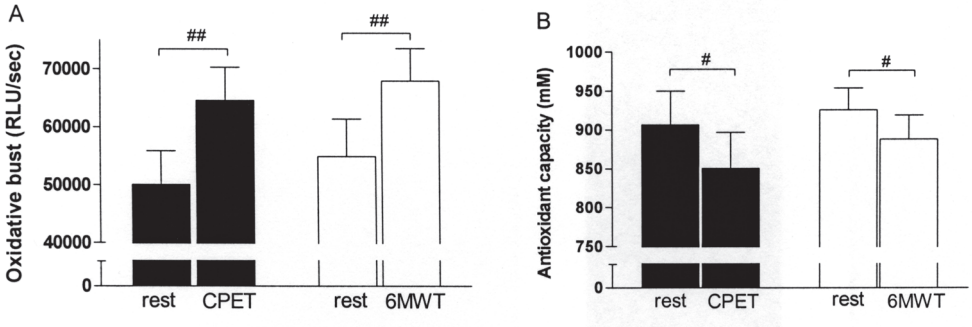

Figure 4. Production of reactive oxygen species (ROS) by PMA-stimulated neutrophils *(A)*, and plasma total antioxidant capacity *(B)* before and after CPET and 6MWT. Exercise-induced oxidative burst was comparable between cycling and walking. RLU/sec, relative light units per second (chemiluminescence). Antioxidant responses were not different between the two exercises. $^{\#}$ $p<0.05$ *versus* rest, $^{\#\#}$ $p<0.01$ *versus* rest.

As markers of free radical-induced tissue or cell damage, both plasma levels of carbonyls and TBARs were significantly elevated after 6MWT ($p<0.05$ and $p<0.01$, respectively) (Figure 5). Also after CPET, levels of TBARs were increased ($p<0.05$), whereas carbonyls remained unchanged.

Finally, the magnitude of the systemic oxidative stress response was related to the degree of muscle-wasting. Figure 6 shows that increases in both plasma carbonyls and TBARs were significantly correlated to FFMI ($r = 0.83$, $p<0.01$, and $r = 0.75$, $p<0.05$, respectively). Contrary, systemic oxidative stress response was not correlated with BMI ($p>0.05$).

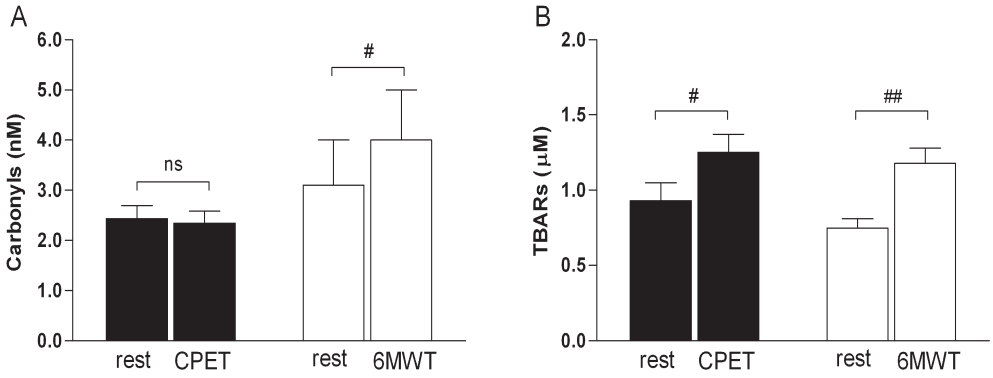

Figure 5. Oxidative stress in response to CPET and 6MWT. Plasma levels of carbonyls (protein oxidation) *(A)* and TBARs (lipid peroxidation) *(B)* at rest and after CPET and 6MWT. # p<0.05, ## p<0.01 *versus* rest; ns, not significant.

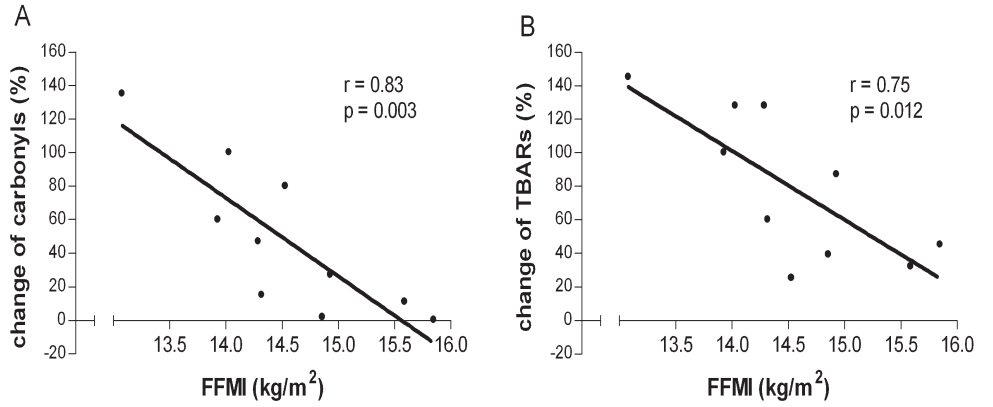

Figure 6. Relation between muscle-wasting and magnitude of exercise-induced oxidative stress. FFMI was inversely correlated with 6MWT-induced increases in both plasma carbonyls (r = 0.83, p<0.01) and TBARs (r = 0.75, p<0.05).

Discussion

The most important finding of the current study in muscle-wasted COPD patients is the increased systemic inflammation and oxidative stress after a 6MWT. Both the IL-6 and oxidative response to 6MWT are comparable to CPET induced responses. Furthermore, the degree of muscle-wasting was correlated with the increase of oxidative stress. Physiologically, the 6MWT is a submaximal, albeit intense, exercise

in these patients, as reflected by lower $\dot{V}O_2$, $\dot{V}_E$ and lactate values after walking compared to CPET.

Physiologic response

A clear steady state $\dot{V}O_2$ was shown in the last 4 minutes of the 6MWT as has also been described by others.[10;11] Although the patterns of the physiologic response to both exercises were different, at isotime (6 minutes of exercise), ventilatory requirements (lower $\dot{V}O_2$, $\dot{V}_E$) were similar between walking and cycling. In contrast with findings of Troosters et al.[10], who found identical $\dot{V}O_2$ and HR after 6MWT and CPET, the physiological burden imposed on the system, in terms of both ventilatory and cardiovascular response, was lower after 6MWT than at peak cycling in the present study. Discrepancies between the two studies may be due to differences in patient characteristics. Our patients showed slightly higher FEV_1, less hyperinflation, and more muscle wasting compared to the patients in the study by Troosters et al. Furthermore, 6MWD was considerably higher in the patients of Troosters et al, although maximal work rates during cycling were similar.

In the present study $\dot{V}O_2$ during 6MWT (3^{rd}-6^{th} min) was lower compared with peak $\dot{V}O_2$ after CPET. It is worth noting, however, that all patients were capable of walking for 4 minutes at >80% of their maximal $\dot{V}O_2$. Half of these patients even finished their 6MWT without any breathing reserve. Together with the lower lactate levels after 6MWT compared to CPET, this study supports earlier work[10] that suggests that the 6MWT generates high, albeit submaximal, metabolic and cardiovascular requirements.

Systemic immunologic response

Despite the fact that the leukocytosis of exercise [24] is affected by type, intensity and duration of the exercise, several consistent patterns emerge regarding the leukocyte subpopulations in blood. The current study showed that a 6MWT already induces a typical systemic leukocytosis, as a result of an increase in all leukocyte subpopulations. Immediately after start of muscular exercise, epinephrine and norepinephrine are released into the plasma. 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.[25] Catecholamines increase almost linearly with the duration of exercise and exponentially with intensity, when it is expressed

relative to individual's peak $\dot{V}O_2$.[26] In line with the observation that the 6MWT is another type of exercise, less intense and a little shorter than CPET, the leukocytosis was less pronounced after 6MWT. In contrast, the plasma IL-6 response to exercise was similar between 6MWT and CPET. IL-6 precedes the appearance of other inflammatory mediators [27] and is related to fat-, protein- and muscle depletion.[28] Inflammatory markers have indeed been found in skeletal muscles of patients with COPD.[29] Whether IL-6 indeed plays a role in the ongoing and progressing systemic inflammation and its consequences in COPD, needs to be elucidated further.
Regarding the oxidative response, earlier results have shown that exercise in COPD leads to a disturbance of the oxidant/antioxidant balance, which may result in free radical mediated tissue damage.[5;30-33] Following CPET and submaximal ergometry [5;6], the current data showed that the oxidative burst of neutrophils increased, and total antioxidant capacity of plasma decreased after 6MWT, resulting in an increase of systemic oxidative stress. Although the 6MWT has been shown to be a submaximal, but relatively intense exercise for patients with COPD, it is also suggested to reflect activities of daily life.[9;34] Consequently, this would support our concept that patients with COPD are regularly exposed to bursts of oxidative stress. In this respect, additional research to the exposure to oxidative stress and inflammation during daily activities is needed. Also, the question arises whether the described responses are specific for muscle-wasted COPD patients and thereby support the concept of possible negative consequences, like muscle wasting or damage. Previously, we showed that the systemic responses to high and moderate cycling indeed were specific for muscle-wasted COPD patients and not seen in non-muscle wasted patients and healthy subjects.[5] Our present finding that the degree of muscle wasting was correlated with the magnitude of the systemic oxidative response, further supports a possible causal relation between systemic inflammation, oxidative stress and muscle wasting.

Some limitations of the present study and suggestions for future research deserves discussion. A relatively small number of patients was included in the study. The results, however, were very consistent within these 10 patients and thereby seem to be representative for these group of patients. Furthermore, muscle wasting is usually seen in GOLD stage 3 and 4 of the disease. Our study group had less severe COPD (GOLD 2 and 3). Diminished exercise capacity (maximal workload of 89W) and clear respiratory and peripheral muscle weakness, however, supported the wasted

condition in these moderate to severe COPD patients.

Finally, attention should be paid to the clinical relevance of increased oxidative stress. Different mechanisms are known to be involved in the generation of free radicals, e.g. mitochondrial electron transport chain, activated neutrophils, ATP degradation, aldehyde oxidase, arachidonic acid cyclo-oxygenase pathway, and nitric oxide synthase. Free radical release from contracting skeletal muscles have been shown, but using in vivo models, it is impossible to exclude other sources, including liver, lung , small intestine, or circulating cells. There is good evidence that overproduction of free radicals is associated with muscle dysfunction. On the other hand, it is also known that free radicals are needed for optimal muscle function. To find out the clinical relevance of the oxidative burst, we support future research to markers of muscle damage and mechanistic studies to the source of oxidative stress and its consequences in these patients. Recently, it was shown that pulmonary rehabilitation was associated with reduced exercise-induced oxidative stress in COPD.[33] On the other hand, patients with COPD showed a reduced ability to adapt to endurance training compared to healthy subjects, reflected in lower capacity to synthesize the antioxidative GSH.[35] This decreased glutathione synthesis may be of great concern in muscle-wasted COPD patients. Furthermore, supplemental oxygen has been show to attenuate exercise-induced free radical production by neutrophils and ATP degradation, resulting in the prevention of exercise-induced oxidative stress in muscle-wasted COPD patients.[8] Additionally, supplemental oxygen seemed to interfere in one of the inflammatory pathways, resulting in a decreased exercise-induced IL-6 response. Understanding the mechanisms of the systemic disease COPD will help us understanding the disease and finally optimize the treatment strategies for these patients.

In conclusion, the present study has shown that a 6MWT induces a systemic immunologic response in muscle-wasted COPD patients, which is comparable to CPET induced responses. Since walking is regularly performed during daily life, it might be postulated that muscle-wasted patients with COPD are regularly exposed to bursts of systemic inflammation and oxidative stress. The correlation between systemic oxidative stress and the degree of muscle wasting further supports a possible causal relation between systemic inflammation, oxidative stress and muscle wasting.

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Chapter 6

Supplemental oxygen prevents exercise-induced oxidative stress in muscle-wasted patients with chronic obstructive pulmonary disease

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Abstract

Although oxygen therapy is of clear benefit in patients with severe chronic obstructive pulmonary disease (COPD), recent studies have shown that short-term supplementary oxygen may increase oxidative stress and inflammation within the airways. We investigated whether systemic inflammation and oxidative stress at rest and during exercise in patients with COPD are influenced by supplemental oxygen. Nine normoxemic, muscle-wasted patients with moderate to very severe COPD were studied. Plasma markers of systemic inflammation (leukocyte counts, interleukin 6 (IL-6)) and oxidative stress (lipid peroxidation, protein oxidation, antioxidant capacity) were measured after treatment with either supplemental oxygen (nasal, 4 $L \cdot min^{-1}$) or compressed air, both at rest (1 h treatment) and after submaximal exercise (40 W, constant work rate). In addition, free-radical production by neutrophils and ATP-degradation products were determined before and after exercise.

Short-term oxygen breathing at rest did not influence systemic low-grade inflammation and oxidative stress. The IL-6 response to exercise was attenuated during cycling with supplemental oxygen. Exercise-induced lipid and protein oxidation were prevented by treatment with supplemental oxygen. This was associated with both decreased free-radical production by neutrophils and reduced formation of (hypo)xanthine and uric acid. Short-term supplementary oxygen does not affect basal systemic inflammation and oxidative stress but prevents exercise-induced oxidative stress in normoxemic, muscle-wasted patients with COPD, and attenuates plasma IL-6 response. Inhibition of neutrophil activation and ATP degradation appears to be involved in this effect.

Introduction

Long-term oxygen therapy is one of the few treatments with clear benefits for hypoxemic patients with chronic obstructive pulmonary disease (COPD). It prolongs survival, reduces the frequency of hospitalization and development of pulmonary hypertension, and improves exercise performance and quality of life.[1–3] Although benefits are less pronounced, supplemental oxygen has been shown to reduce ventilation, dynamic hyperinflation, and dyspnea during exercise in normoxemic patients with COPD and in those with mild hypoxemia and COPD.[4;5]

Despite the proven benefits of oxygen therapy in COPD, recently published data may shed another light on the effects of this therapy. Philips and coworkers [6] reported that breathing of 28% oxygen at 2.0 $L \cdot min^{-1}$ via nasal prongs for 30 min while resting resulted in an increase of breath methylated alkane contour in healthy subjects, suggesting increased oxidative stress in exhaled breath. In addition, Carpagnano and coworkers [7] investigated the effects of short-term supplementary oxygen on markers of oxidative stress and inflammation in exhaled breath condensate in both healthy subjects and patients with COPD. Exposure to increased inspiratory oxygen fraction (Fi_{O2}, 0.28) for 1 h resulted in enhanced concentrations of interleukin 6 (IL-6) and 8-isoprostane in exhaled breath condensate of patients with COPD and healthy subjects. It is unknown if supplemental oxygen as used in clinical practice alters systemic markers of oxidative stress and inflammation. In healthy subjects, there is some evidence that markers of oxidative stress (i.e., lipid peroxidation and superoxide dismutase activity) in plasma are increased after hyperbaric oxygen therapy.[8;9] Data on the effects of increasing Fi_{O2} on these markers in patients with COPD are not known to the best of our knowledge.

Markers of oxidative stress and inflammation are known to be increased by intense exercise in healthy subjects. Oxidative stress and inflammation are now recognized to play an important role in the pathogenesis of COPD [10;11], and an increased response to exercise has also been described in patients with COPD.[12;13] The effect of oxygen on exercise-induced systemic oxidative stress and inflammation in patients with COPD is largely unknown. Vina and colleagues [14] showed in five patients with very severe COPD (FEV_1, 0.79 ± 0.07 L) with advanced hypoxemia (Pa_{O2}, 7.5 ± 0.1 kPa) that exercise-induced (constant work rate, 40 W) blood glutathione oxidation was partially reduced by supplemental oxygen (2–3 $L \cdot min^{-1}$), indicating that supplemental oxygen

decreased exercise-induced oxidative stress. Mechanistically, it is unknown which of the three major sources of intracellular free-radical generation (e.g., neutrophils, mitochondrial electron transport chain, and the xanthine oxidase pathway [12;15;16]) are affected by supplemental oxygen.

We investigated the effect of short-term supplemental oxygen at rest and during exercise on markers of systemic inflammation and oxidative stress in normoxemic patients with moderate to very severe COPD. In a crossover design, plasma markers of inflammation (circulating leukocytes, concentration of IL-6) and oxidative stress (lipid peroxidation and oxidation of proteins) were measured in nine patients with COPD during treatment with either compressed air (nasal, 4 $L \cdot min^{-1}$) or supplemental oxygen (nasal, 4 $L \cdot min^{-1}$), both at rest (after 1 h treatment) and after a bout of cycle exercise (40 W, constant work rate). To improve insight in the possible mechanisms involved in these systemic effects, both ATP-degradation products [12;17] and neutrophil activation [15;16] were measured. In the present study, we selected muscle-wasted patients with COPD to evaluate the systemic effects of supplemental oxygen, because of the increasing evidence for a relation between systemic inflammation, oxidative stress, and muscle wasting in COPD [11; 18; 19], and the importance of any intervention on these features in this specific subgroup of patients with COPD.

Methods

Subjects

Nine (five males) muscle-wasted patients with COPD (fat-free mass index: males < 16 $kg \cdot m^{-2}$, females < 15 $kg \cdot m^{-2}$) [20] participated in this study. The patients were recruited from our outpatient clinic and had moderate to very severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification.[21] All had been free of exacerbations for at least 2 mo before the study, and had stopped smoking at least 6 mo before inclusion. Exclusion criteria were the use of oral corticosteroids, long-term oxygen therapy, respiratory insufficiency (Pa_{O2} < 8 kPa, Pa_{CO2} > 6.7 kPa), and other chronic or exercise-limiting diseases. The use of inhaled corticosteroids (n = 4), antioxidants (n = 1), and supplemental vitamins was discontinued 1 wk before exercise testing. All patients were on bronchodilator therapy; none used theophylline. The study was conducted according

to the Declaration of Helsinki and was approved by the medical ethical committee of our hospital. Written, informed consent was obtained from all subjects.

Study Design

Pulmonary function, body composition, and a maximal, symptom-limited, incremental bicycle test were assessed as part of the characterization procedure. In two subsequent visits (separated by 1 wk), the systemic effects of supplemental oxygen were evaluated in a double-blind, randomized, and placebo-controlled crossover design (Figure 1). At rest, subjects breathed either supplemental oxygen or compressed air via nasal prongs for 1 h at a flow rate of 4 $L \cdot min^{-1}$. After a wash-out period of 2 h breathing ambient air, a submaximal constant work rate bicycle test at 40 W was performed with either supplemental oxygen or compressed air (nasal, 4 $L \cdot min^{-1}$, again randomized and double blind). Oxygen supplementation of 4 $L \cdot min^{-1}$ was chosen because this flow rate is commonly used in training programs for patients with COPD.[3; 22] A work rate of 40W was used to produce an energy expenditure of approximately 3 metabolic equivalents, which is equivalent to the power output required to walk in usual activities during daily life.[23] Patients were instructed to cycle as long as possible but for a maximum of 30 min. One week later, the protocol was repeated with the other interventions (crossover). At this second visit, patients were instructed to cycle exactly as long as at the previous visit, if possible. Because of the nasal prongs for supplementation of oxygen or compressed air, we were not able to measure ventilatory parameters breath by breath during cycling. At both visits, five arterial blood samples (20 ml/sample) were taken. Samples A1 and A2, and A4 and A5, were used to evaluate the effects of supplemental oxygen compared with compressed air at rest and during exercise, respectively. Sample A3 was used to control wash-out values with baseline values. Measurements after exercise were corrected for plasma volume shifts according to Dill and Costill.[24] Changes in systemic inflammation and oxidative stress in response to supplemental oxygen compared with compressed air were measured after 1 h at rest and after constant work rate bicycle exercise.

Blood Gas and Lactate Analysis

Arterial blood gasses (Pa_{O2}, Pa_{CO2}) and lactate concentrations were measured using Gas analyzer Chiron 860 (Bayer, Massachusetts, USA).

Systemic Inflammation

Plasma IL-6 concentrations were measured using quantitative sensitivity and high-sensitivity sandwich ELISAs in kit form (R&D Systems, Minneapolis, MN) according to the supplier's instructions (detection limit, 0.039 $pg \cdot ml^{-1}$). Leukocyte counts were determined following standard laboratory assays.

Oxidative Stress

Concentrations of thiobarbituric acid–reactive substances (TBARs) were determined fluorometrically.[25] Levels of protein carbonyls were measured by means of an ELISA.[26;27]

To investigate the role of free-radical generation by neutrophils, production of reactive oxygen species by isolated neutrophils (stimulated with phorbol myristate acetate) was measured by chemiluminescence.[28;29] To evaluate the role for ATP degradation, plasma levels of xanthine, hypoxanthine, and uric acid were measured by a standard HPLC method with photodiode array detection, essentially as described previously.[30] Total antioxidant capacity was assayed spectrophotometrically by measuring the ferric-reducing ability of plasma.[31]

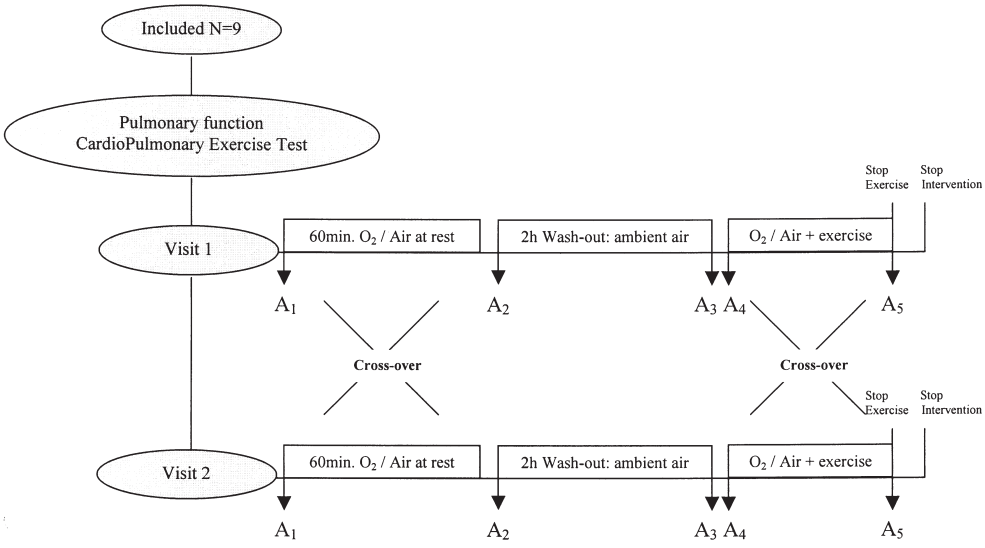

Figure 1. Study Design. A_1 = arterial blood sample at baseline; A_2 = arterial blood sample after 60 min intervention at rest; A_3 = arterial blood sample after wash-out period (control); A_4 = arterial blood sample after 3 min unloaded cycling with intervention; A_5 = arterial blood sample after constant work rate cycling with intervention.

Statistics

Results are presented as means ± SE if the variables were normally distributed. Otherwise, median values were presented. Responses were expressed as absolute values and/or as changes compared with baseline. Paired *t* tests and Wilcoxon signed-rank tests were used to evaluate the responses to 1 h supplemental oxygen and compressed air, and also to compare the exercise-induced effects between supplemental oxygen and compressed air. Statistical significance was taken at the $p<0.05$ level. Data were analyzed with SPSS/PC+, version 12.0 (SPSS, Inc., Chicago, IL).

Results

Subjects showed moderate to very severe airflow obstruction (Table 1). During the incremental bicycle test, none of the patients reached their predicted $\dot{V}O_2$max, but all achieved their maximal voluntary ventilation ($\dot{V}_E$/MVV = 98 ± 6%) as a consequence of their ventilatory limitation, whereas heart rate reserve was preserved.

Effects of Oxygen Breathing at Rest

The effects of treatment with either compressed air or supplemental oxygen on blood gas tensions, systemic inflammation, and oxidative stress at rest are shown in Table 2. As expected, arterial blood gas tensions were not affected after 1 h treatment with compressed air. Treatment with supplemental oxygen, however, induced a significant increase of Pa_{O2}, whereas Pa_{CO2} was not affected. No differences in markers of systemic inflammation and oxidative stress were observed after 1 h breathing supplemental oxygen or compressed air at rest (Table 2).

Supplemental Oxygen during Constant Work Rate Cycling

After the wash-out period, an arterial blood sample was taken as control (A3 in Figure 1). Blood gas tensions as well as markers of inflammation and oxidative stress after the wash-out were similar to baseline values (data not shown).

Median cycle time at 40 W was 14 min with supplemental oxygen (range, 4–20 min) and 15 min with compressed air (4–20 min; $p = 0.71$). Individual exercise durations of both exercise tests are shown in Table 3.

Table 1. Subject characteristics

Subjects, n	9
Male/Female	5/4
Age, yrs	61 ± 4
BMI, $kg \cdot m^{-2}$	20.2 ± 1.1
FFMI, $kg \cdot m^{-2}$	
Male	14.1 ± 0.5
Female	14.3 ± 0.4
Respiratory function	
FEV_1, L	1.11 ± 0.13
FEV_1, % pred	41 ± 4
FEV_1/VC, %	38 ± 4
RV/TLC. %	51 ± 3
K_{CO}, % pred	74 ± 12
Pi_{max}, % pred	90 ± 3
Pe_{max}, % pred	71 ± 5
Pa_{O2}, kPa	10.0 ± 0.6
Pa_{CO2}, kPa	5.0 ± 0.1
CPET peak values	
Work rate, W	76 ± 13
$\dot{V}O_2$, $L \cdot min^{-1}$	1.06 ± 0.07
$\dot{V}O_2$, % pred	73 ± 3
$\dot{V}_E$, $L \cdot min^{-1}$	41 ± 3
$\dot{V}_E$, % MVV	98 ± 6
HR, % pred	83 ± 2
Pa_{O2} end-exercise, kPa	8.7 ± 0.7
Pa_{CO2} end-exercise, kPa	6.0 ± 0.2
Δlactate, mM	4.6 ± 0.3

Abbreviations: BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume in first second; VC, vital capacity; TLC, total lung capacity; FRC, functional residual capacity; K_{CO}, diffusion capacity for carbon monoxide per unit of alveolar volume; Pi_{max}, maximal inspiratory mouth pressure; Pe_{max}, maximal expiratory mouth pressure; CPET, cardiopulmonary exercise testing; $\dot{V}O_2$, oxygen uptake; $\dot{V}_E$, minute ventilation; MVV, maximal voluntary ventilation; HR, heart rate; Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; Δlactate , [lactate] at Wmax – [lactate at rest]; pred, predicted value. Values are presented as mean ± SE.

Table 2. Blood gas tensions, systemic inflammation, and oxidative stress pre and post treatment with supplemental oxygen or compressed air **at rest**

	Pre Air †	Post Air ‡	Pre Oxygen †	Post Oxygen ‡
Blood gas tensions				
Pa_{O2}, kPa	9.5 ± 0.4	9.5 ± 0.3	9.9 ± 0.3	17.8 ± 1.0 ***
Pa_{CO2}, kPa	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.3	5.4 ± 0.6
Inflammation				
Leukocytes, $\cdot 10^9$ cells$\cdot l^{-1}$	8.7 ± 1.0	8.5 ± 1.1	8.2 ± 0.6	8.1 ± 0.8
Neutrophils, $\cdot 10^9$ cells$\cdot l^{-1}$	6.6 ± 0.8	6.5 ± 1.0	5.9 ± 0.6	6.0 ± 0.8
Lymphocytes, $\cdot 10^9$ cells$\cdot l^{-1}$	1.4 ± 0.2	1.2 ± 0.2	1.6 ± 0.2	1.5 ± 0.1
Monocytes, $\cdot 10^9$ cells$\cdot l^{-1}$	0.45 ± 0.06	0.46 ± 0.08	0.44 ± 0.05	0.47 ± 0.05
IL-6, pg$\cdot$ml^{-1}	5.5 ± 2.5	5.2 ± 2.4	5.4 ± 2.4	5.5 ± 2.6
Oxidative stress				
TBARs, µM	1.1 ± 0.2	1.2 ± 0.2	0.97 ± 0.03	1.0 ± 0.07
Carbonyls, nM	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.4	3.2 ± 0.4
Antioxidant capacity, mM	989 ± 47	980 ± 44	984 ± 54	990 ± 55

Abbreviations: IL, interleukin; TBARs, thiobarbituric acid reactive substances.
Values are presented as mean ± SE.
† baseline sample (A_1)
‡ sample after 1h intervention at rest (A_2)
*** $p<0.001$ versus pre treatment

Table 3. Individual exercise duration of the two exercise trials

	Exercise duration (min)	
Subject	Air	Oxygen
1	20	20
2	20	20
3	15	12
4	20	20
5	7	4
6	7	10
7	14	14
8	20	20
9	4	5.5

At the second visit, four of the nine patients were exhausted before the aimed endurance was reached. Two of them (Subjects 6 and 9) received supplemental oxygen during cycling at the first visit and compressed air at the second visit. The other two patients (Subjects 3 and 5) received compressed air during the first cycle test and could not reach the aimed cycle time with supplemental oxygen during exercise. Physiologic responses to exercise are presented in Table 4. Similar duration and intensity of the exercise resulted in comparable increases of heart rate and arterial lactate concentrations.Pa_{O2}, however, changed differently during cycling with compressed air and supplemental oxygen. Although Pa_{O2} slightly decreased to levels less than 9 kPa (mild hypoxemia) during cycling with compressed air, it decreased from hyperoxemic to normoxemic tensions during the exercise with supplemental oxygen. In contrast, Pa_{CO2} similarly increased during exercise with compressed air and supplemental oxygen.

Table 4. Physiological responses to submaximal exercise with supplemental oxygen or compressed air

	Air		Oxygen	
	Pre exercise †	Post exercise ‡	Pre exercise †	Post exercise ‡
Endurance, min		15 (range 4-20)		14 (range 4-20)
Heart rate, bpm	91 ± 6	122 ± 5 **	95 ± 4	121 ± 5 **
Pa_{O2}, kPa	9.9 ± 0.4	8.7 ± 0.4 *	17.4 ± 0.6	11.7 ± 0.6 ***
Pa_{CO2}, kPa	5.0 ± 0.1	5.6 ± 0.2 *	5.2 ± 0.1	5.8 ± 0.2 *
Lactate, mM	1.5 ± 0.3	4.5 ± 0.9 **	1.5 ± 0.1	4.5 ± 0.7 **

Values are presented as mean ± SE, except for endurance time (median).

† sample after 3 min unloaded cycling with intervention (A_4)

‡ sample after constant work rate cycling with intervention (A_5)

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus pre exercise.

Exercise-induced leukocytosis did not differ between the two treatments. With compressed air, the number of leukocytes increased from 8.1 ± 0.6 to $9.4 \pm 0.9 \cdot 10^9\ l^{-1}$ ($p < 0.01$), and with supplemental oxygen, the number of cells rose from 8.2 ± 0.8 to $9.2 \pm 0.9 \cdot 10^9\ l^{-1}$ ($p < 0.01$). These increases were caused by similar elevations of the differential leukocyte subsets (i.e., neutrophils, lymphocytes, monocytes; data

not shown) after treatment with supplemental oxygen and compressed air. Plasma levels of IL-6 significantly increased after exercise with both interventions ($p < 0.01$ with compressed air, $p < 0.05$ with supplemental oxygen; Figure 2). The increase in IL-6 during cycling with supplemental oxygen (ΔIL-6 = 0.2 ± 0.1 pg·ml^{-1}), however, was significantly lower ($p < 0.05$) compared with the increase after cycling with compressed air (ΔIL-6 = 0.9 ± 0.1 pg·ml^{-1}). Post-exercise concentrations of IL-6 were significantly higher after cycling with compressed air compared with cycling with supplemental oxygen ($p < 0.05$).

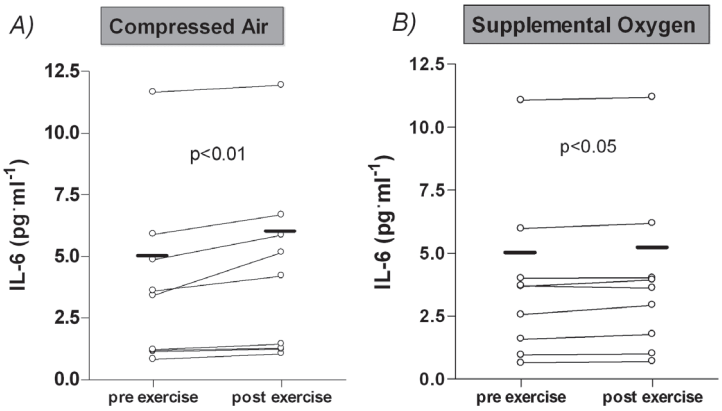

Figure 2. Plasma interleukin (IL)-6 concentrations before and after cycle exercise (40W) with compressed air *(A)* or supplemental oxygen (nasal, 4 L•min-^{1}; *B*) in muscle-wasted patients with chronic obstructive pulmonary disease (COPD). $p<0.01$, $p<0.05$ for differences between mean IL-6 concentrations (horizontal bars) between pre and post exercise.

Constant work rate exercise resulted in free-radical–mediated tissue damage, as indicated by increased lipid peroxidation (Figure 3A). Oxygen supplementation prevented the exercise-induced lipid peroxidation (Figure 3B). Changes in TBARs were significantly different between cycling with compressed air (ΔTBARs = 0.8 ± 0.2 μM) and cycling with supplemental oxygen (ΔTBARs = 0.3 ± 0.1 μM; $p<0.01$). Formation of protein carbonyls was significantly increased after exercise with compressed air ($p < 0.05$; Figure 3C). When the exercise was performed with supplemental oxygen, however, protein oxidation remained unaffected (Figure 3D). Changes in plasma carbonyls were significantly different between exercise with compressed air (Δcarbonyls = 0.6 ± 0.2 nM) and supplemental oxygen (Δcarbonyls

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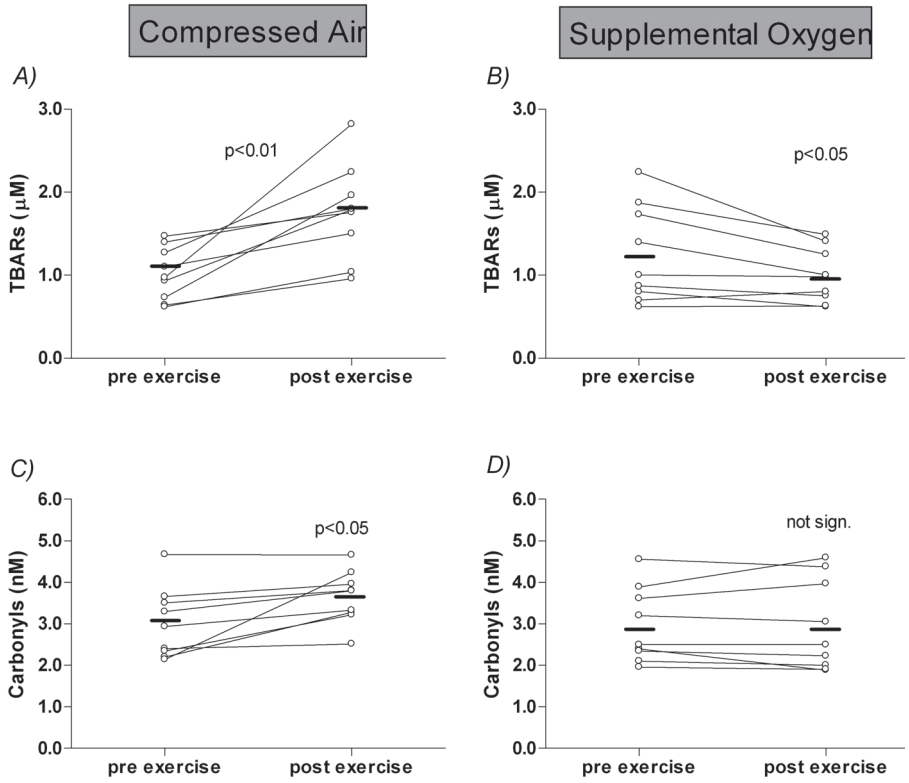

Figure 3. Plasma levels of thiobarbituric acid-reactive substances (TBARs) and protein carbonyls before and after cycle exercise (40W) with compressed air *(A, C)* or supplemental oxygen (nasal, 4 L•min^{-1}; *B, D*) in muscle-wasted patients with COPD. $p<0.01$, $p<0.05$ for differences between mean TBARs and carbonyl concentrations (horizontal bars) between pre and post exercise. Not sign. = not significant.

In response to exercise with compressed air, production of reactive oxygen species by neutrophils significantly increased ($p < 0.001$; Figure 4A). When the exercise was performed with supplemental oxygen, however, production of reactive oxygen species did not increase (Figure 4B). The increase in radical production (Δrespiratory burst) was significantly higher after exercise with compressed air than after exercise with supplemental exercise ($p< 0.01$). Elevated levels of (hypo)xanthine ($p < 0.05$) and uric acid ($p < 0.01$) after exercise with compressed air suggest ATP degradation. The absence of an increase of these purines after exercise with supplemental oxygen pointed to attenuated ATP degradation (Figure 5). Changes of xanthine, hypoxanthine, and uric acid were significantly different between the two exercise protocols ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively).

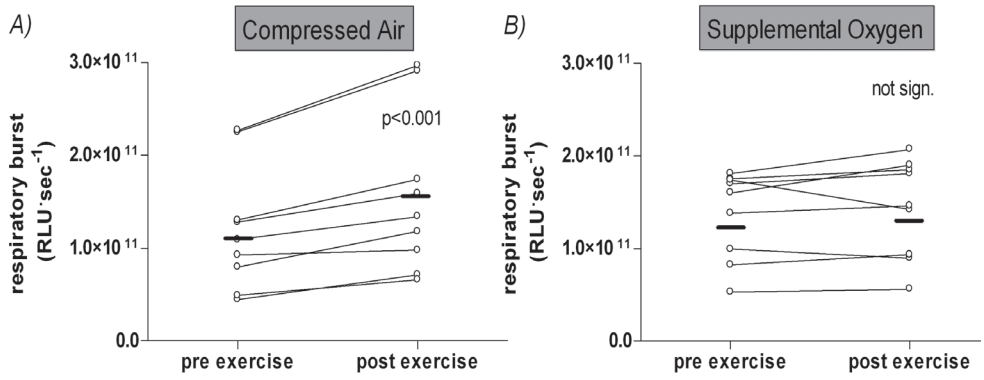

Figure 4. Production of reactive oxygen species by isolated neutrophils before and after cycle exercise (40W) with compressed air *(A)* or supplemental oxygen (nasal, 4 L•min^{-1}; *B*) in muscle-wasted patients with COPD. RLU, relative light units. p<0.001 for differences between mean respiratory burst (horizontal bars) between pre and post exercise. Not sign. = not significant.

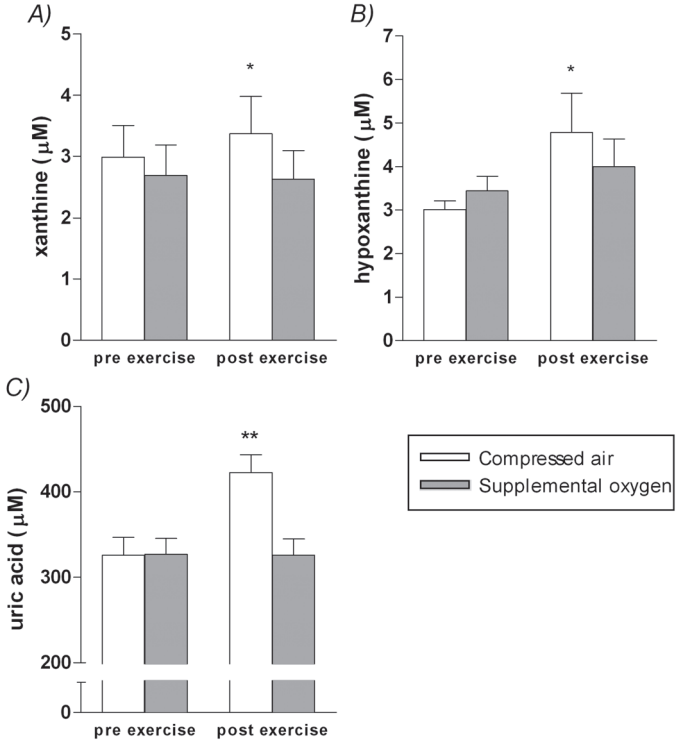

Figure 5. ATP degradation. Plasma levels of xanthine *(A)*, hypoxanthine *(B)*, and uric acid *(C)* before and after cycle exercise (40W) with compressed air or supplemental oxygen (nasal, 4 L•min^{-1}) in muscle-wasted patients with COPD. *p<0.05, **p<0.01 *versus* pre exercise.

Plasma antioxidant capacity decreased during exercise ($p < 0.05$) without changes ($p = 0.10$) between breathing compressed air and supplemental oxygen (Figure 6).

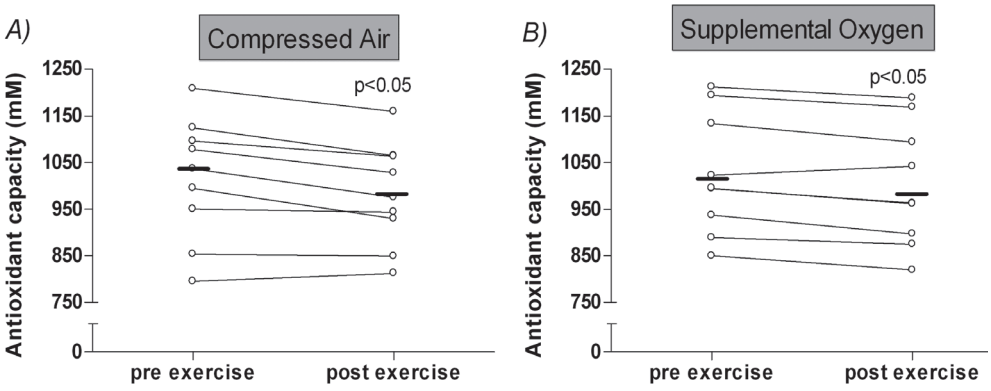

Figure 6. Plasma antioxidant capacity before and after cycle exercise (40W) with compressed air *(A)* or supplemental oxygen (nasal, 4 L•min^{-1}; *B*) in muscle-wasted patients with COPD. $p<0.05$ for differences between mean antioxidant capacity (horizontal bars) between pre and post exercise.

The effect of cycle time on the oxidative stress response was evaluated to exclude any possible effect of a shorter exercise endurance (not significant). No correlation with difference in cycle time between compressed air and supplemental oxygen and the difference in oxidative stress between the two exercise trials was found. Furthermore, even the two patients with a longer cycle time during intervention with supplemental oxygen showed a decreased oxidative stress response compared with compressed air.

Discussion

Our results demonstrate that short-term oxygen breathing (nasal, 4 L·min^{-1} for 1 h) does not affect basal systemic inflammation and oxidative stress in normoxemic, muscle-wasted patients with moderate to very severe COPD. Second, supplemental oxygen prevents exercise-induced systemic oxidative stress, and attenuates the response of IL-6 in these patients. Free-radical generation by neutrophils and also formation of purines are reduced after exercise with supplemental oxygen, suggesting a mechanistic role for both neutrophils and ATP degradation in the oxidative response to exercise.

Oxygen Therapy at Rest in Normoxemic Patients with COPD

Oxygen therapy prolongs survival, reduces the frequency of hospitalization and development of pulmonary hypertension in hypoxemic patients with COPD [1;2], and has been shown to improve exercise performance in both hypoxemic and normoxemic patients.[3–5] Recently, interesting data about the effects of hyperoxia on markers of oxidative stress and inflammation in exhaled breath condensate of patients with mild to severe hypoxemic COPD and healthy subjects were presented.[6;7] Short-term (30–60 min) breathing of supplemental oxygen (Fi_{O2}, 0.28) increased 8-isoprostane, breath methylated alkane contour, and IL-6 concentrations in exhaled breath condensate. One of the aspects important for the clinical relevance of these findings [32] is whether oxygen administration exerted similar systemic effects in these patients. Within healthy subjects, 2 to 3.5 h exposure to very high Fi_{O2} (Fi_{O2}, 1.0, and P_{O2} of 120 kPa, respectively) was associated with increased oxidative stress in both the airways and plasma, as measured by lipid peroxidation, and superoxide dismutase activity.[8;9] In our study, increasing Pa_{O2} to approximately 17 kPa did not affect markers for systemic inflammation or oxidative stress in normoxemic patients with moderate to very severe COPD at rest. This suggests that the increase of markers of inflammation and oxidative stress after short-term supplemental oxygen [7] in a concentration applied to patients with COPD is confined to the airways. The markedly higher fractions and longer exposure time in the studies with healthy subjects [8;9] compared with our study may have contributed to the different findings.

Supplemental Oxygen during Exercise

During exercise training in both normoxemic and hypoxemic patients with COPD, supplemental oxygen can enhance training intensity [3;4] and relieve dyspnea. Only a few studies about the systemic effects of supplemental oxygen during exercise have been published. In hypoxemic patients with COPD, oxygen inhalation partially improves the impaired muscle oxidative metabolism during exercise, as indicated by less intracellular acidosis, lower Pi/PCr ratio, and an increased PCr resynthesis compared with breathing air.[33] In the same study, no effects of supplemental oxygen on muscle oxidative metabolism during exercise were found in healthy subjects.

Markers of oxidative stress and inflammation, both systemically and locally (e.g., in muscle), are known to be increased by exercise in both healthy subjects (reviewed in reference [34]) and patients with COPD.[12;14;35] Very recently, the effects of supplemental

oxygen on oxidative stress during training in healthy subjects were reported for the first time.[36] In the latter study, supplemental oxygen used in conjunction with high-intensity interval training at altitude resulted in improvement in exercise performance without inducing additional oxidative stress. Our study shows that exercise-induced oxidative stress in normoxemic patients with COPD was prevented by supplemental oxygen. These findings in normoxemic patients are in line with the results of Vina and colleagues [14], who reported partial prevention of exercise-induced glutathione oxidation by oxygen therapy in five hypoxemic patients with COPD. Besides prevention of systemic oxidative stress, the present study shows that the exercise-induced increase of plasma IL-6 in normoxemic patients with COPD was attenuated by supplemental oxygen. Plasma cytokines are known to increase during exercise.[37] The appearance of IL-6 in the circulation is by far the most marked, and its appearance precedes that of the other cytokines.[38] IL-6 is produced by many different cells, but the main sources *in vivo* are stimulated monocytes/macrophages, fibroblasts, and vascular endothelial cells [39], indicative of its role in the modulation of the immune system. Furthermore, other cells have the ability to express IL-6 in response to proinflammatory stimuli, including keratinocytes, osteoblasts, T cells, B cells, neutrophils, eosinophils, mast cells, smooth muscle cells, and skeletal muscle cells.[40] Hypoxia induces IL-6 in cultured endothelial cells [41], and hypoxia *in vivo* elevates serum IL-6 in humans.[42] Thus, prevention of low Pa_{O2} using supplemental oxygen during exercise is a potential explanation for the diminished IL-6 response in the present study. Furthermore, cytokines (i.e., IL-6) can cause priming of neutrophils.[43–45] Priming of these cells results in an augmented response of these cells to produce reactive oxygen species without direct stimulatory actions on the cells themselves. In response to cell stimulation, these primed cells can become activated to release reactive oxygen species and lysosomal enzymes, which in turn may destroy cellular membranes, induce DNA damage, or affect protein functions by modifying the structures.[43–45] Within the present study, neutrophils produced increased reactive oxygen species after exercise with compressed air, but not after exercise with supplemental oxygen. After exercise, circulating neutrophils have a higher potential of producing highly toxic oxidants due to activation of the myeloperoxidase pathway.[15] Without influencing the number of cells, supplemental oxygen seems to reduce the activation of neutrophils and thereby prevents exercise- induced free-radical production. According to the abovementioned theory, the diminished IL-6

response, and the resulting lower post-exercise IL-6 concentrations after cycling with supplemental oxygen, might have been the absent primer for the neutrophils. On the other hand, oxidative stress has also been shown to be a major stimulus for exercise-induced cytokine production.[46] It has been suggested that oxidative stress precedes the acute inflammatory response to exercise.[47] In this way, the prevention of exercise-induced oxidative stress by supplemental oxygen might also have been the cause rather than the result of diminished IL-6 production.

Preventing the production of reactive oxygen species by neutrophils with supplemental oxygen may have contributed to the absence of exercise-induced oxidative stress as was indicated by the absence of oxidation of proteins and lipids. Besides neutrophils, the mitochondrial electron transport chain and the xanthine oxidase pathway have been identified as major sources of intracellular free-radical generation during exercise.[12;15;16] The elevated hypoxanthine, xanthine, and uric acid levels observed in the present study after cycling with compressed air indeed suggest a role for the latter mechanism. Exercise increases the demand for energy production. The ATP requirements of skeletal muscles must be met by the metabolic processes that are available to regenerate ATP from ADP. When energy requirements exceed the ability of the cell to (re)synthesize ATP, net ATP degradation occurs. Degradation of ATP leads to the release of purine metabolic intermediates (i.e., adenosine, inosine, hypoxanthine, and xanthine). In the presence of xanthine oxidase activity, hypoxanthine and xanthine are converted to superoxide and uric acid. It has been proposed that generation of superoxide by xanthine oxidase is substrate (ATP-degradation products) and not enzyme activity limited.[48] Supplemental oxygen is likely to stimulate ATP formation from ADP, resulting in reduced ATP degradation. The decreased levels of ATP degradation products and uric acid found after cycling with supplemental oxygen strongly suggest the involvement of this pathway in the prevention of exercise-induced oxidative stress.

Regarding a possible role for hypoxia in the exercise-induced inflammatory and oxidative response, it seems interesting that oxygen supplementation reduced oxidative stress after exercise even in the absence of marked decline of Pa_{O2}. We did not find a correlation between the magnitude of oxygen desaturation and the reduction in exercise-induced oxidative stress with supplemental oxygen. This may at least be partially explained by the small number of patients included in the study and by the small differences of Pa_{O2} decline between the subjects. In addition to our

study, it would be useful to investigate whether the effects of supplemental oxygen are comparable or even more pronounced in a group of hypoxemic patients with COPD.

Clinical Implications and Future Directions

Repetitive exercise during daily life, or more specifically during exercise training, in patients with COPD may cause frequent exposure to systemic oxidative stress and inflammation, which may result in functional changes (i.e., modification of contractile amount and/or protein function). Indeed, we have recently shown evidence for impaired diaphragm contractile protein function in patients with COPD.[49] An interesting hypothesis to be tested is that exercise-induced systemic inflammation and oxidative stress, as shown in the present study, contribute to impaired (respiratory) muscle function in these patients.

Furthermore, the current study has indicated two mechanisms that are involved in exercise-induced systemic oxidative stress, which both can be influenced by treatment with supplemental oxygen. Better understanding of the mechanisms of the systemic effects in COPD may improve insight into the consequences of these effects and may offer new targets for therapies. Diminishing or preventing bursts of systemic inflammation and oxidative stress after physical activities in patients with COPD might be beneficial, but data showing a causal relation among reductions in inflammation and/or oxidative stress and improvement of exercise tolerance, outcomes of rehabilitation, or quality of life have not yet been published. Before we can recommend oxygen supplementation during exercise, it needs to be evaluated whether (*1*) the effects of supplemental oxygen as shown here are specific for normoxemic, muscle-wasted patients with COPD; (*2*) the effects remain when patients exercise frequently with oxygen; and (*3*) there are clinical consequences of oxygen supplementation.

In conclusion, this study demonstrates that supplemental oxygen during exercise diminishes the increase of plasma IL-6 and prevents systemic oxidative stress in normoxemic, muscle-wasted patients with COPD. Neutrophil activation and ATP degradation are both associated with these findings. Oxygen breathing at rest, however, has no effect on the low-grade systemic inflammation and oxidative stress in muscle-wasted patients with COPD. These data suggest that normoxemic, muscle-

wasted COPD patients, a subgroup with clear systemic consequences of COPD, can be prevented from exposure to bursts of oxidative stress and inflammation by supplemental oxygen during exercise. Whether this will also result in improvement of exercise tolerance, outcomes of rehabilitation, or quality of life needs to be evaluated.

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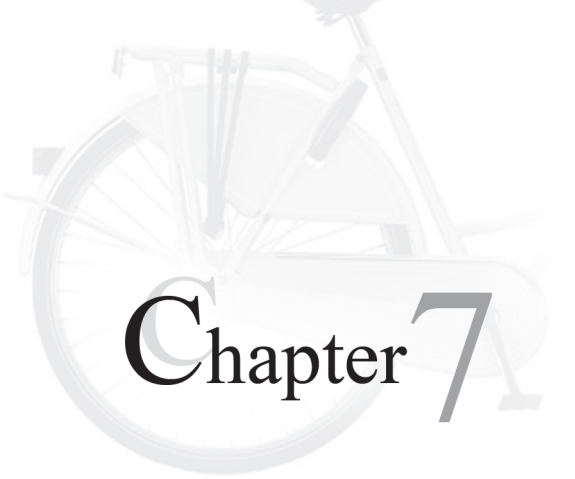

Chapter 7

Summary and General Discussion

Summary

Chronic Obstructive Pulmonary Disease (COPD) is increasingly being recognized as systemic rather than only a pulmonary disease. Increasing amounts of activated inflammatory cells, inflammatory mediators and oxidative stress are not restricted to the local compartment- including airways, lung parenchyma, and pulmonary vasculature – but are also present in the circulation and even more pronounced during exacerbations. The origin and consequences of the systemic inflammation and oxidative stress present in COPD patients remains still to be elucidated. The interactions between exercise stress and the immune system provide a unique opportunity to link basic and clinical physiology and to evaluate the role of underlying stress and immunophysiologic mechanisms. The immune response to exercise has been studied in healthy subjects, especially highly trained athletes, and has shown to be mediated by an interplay of inflammatory cells, hormones, cytokines, neural and hematologic factors. Since COPD patients already show signs of systemic inflammation, elevated levels of circulating catecholamines, and marked sympathetic activation at rest, it may be expected that exercise will affect these mediators. Hypothetically, increased exposure to inflammation and oxidative stress might negatively affect tissues, e.g. skeletal muscles, and thereby play a role in the ongoing and progressive systemic effects of COPD. This thesis aimed to investigate the effects of exercise on systemic inflammation and oxidative stress in patients with COPD.

In the **third chapter** of this thesis systemic leukocytosis and the stress response to exhaustive exercise were compared between healthy subjects and patients with COPD. Our data revealed that the response of catecholamines (both epinephrine and norepinephrine) to intense exercise was lower in COPD patients than in healthy subjects, which could at least partly be contributed to the lower maximal exercise capacity of these patients. The amounts of leukocytes in patients with COPD indicated a low-grade systemic inflammation in these patients. Additionally, the exercise-induced leukocytosis showed similar patterns in both groups, but occurred at higher levels in COPD. No signs of muscle damage in response to this exercise were found systemically. It was concluded that COPD patients are exposed to systemic inflammation that is intensified by exhaustive exercise. The inflammatory response is not exaggerated compared to healthy subjects, but occurs at a higher level and is already observed at low external workload.

Systemic inflammation and oxidative stress are two potential mechanisms that may play a role in the commonly seen muscle dysfunction and muscle wasting in patients with COPD. The aim of **chapter 4** was firstly to investigate coexistence of systemic inflammation and oxidative stress in muscle-wasted COPD patients, and secondly to study whether especially these patients are exposed to increased bursts of systemic inflammation and oxidative stress in response to exercise. Maximal and submaximal cycle exercises were performed by muscle-wasted COPD patients, non muscle-wasted patients, and healthy subjects. Inflammation and oxidative stress were measured systemically both at rest and after the exercises. The results showed that basal levels of CRP, leukocyte counts, IL-8, and IL-6 were elevated in all COPD patients and tended to be highest in muscle-wasted patients. A decreased antioxidant status and increased protein oxidation reflected systemic oxidative stress in muscle wasted COPD patients. Also, both peripheral and respiratory muscle strength was decreased in this group. Both high and moderate intensity exercise caused increased inflammatory and oxidative responses in muscle-wasted patients, which were not observed in non muscle-wasted patients and healthy subjects. These data indicated that muscle-wasted COPD patients are chronically exposed to systemic inflammation and oxidative stress, which further increase after both high and moderate intensity exercise.

Due to dyspnea and muscle dysfunction, patients with COPD are relatively inactive during daily life. **Chapter 5** describes a study with the aim to investigate whether a probably less intense and more regularly performed physical activity than intense cycling, namely 6 minutes of walking, also affected the systemic immunology in patients with COPD. Because in chapter 4 the highest immune responses to exercise were observed in muscle-wasted COPD patients, this study was performed in this subgroup of patients. Firstly, this type of physical activity was characterized with physiologic parameters. The 6-minute walking test (6MWT) was found to be submaximal, albeit relative intense, exercise in muscle-wasted patients with COPD. Although walking was a submaximal exercise, both the IL-6 and the systemic oxidative response were comparable between the 6MWT and a maximal cycle test. These findings support the concept that in particular the subgroup of muscle-wasted patients with COPD might frequently be exposed to systemic inflammation and oxidative stress during activities of daily life.

Oxygen therapy is one of the few treatments with clear benefits for hypoxemic COPD patients. It prolongs survival, reduces the frequency of hospitalisation and development of pulmonary hypertension, and improves exercise performance and quality of life. It is also known, however, that oxygen can be very toxic. Within the lungs, increased inflammation and oxidative stress have been found after short term breathing of supplementary oxygen. The aim of the study in **chapter 6** was to investigate whether supplemental oxygen also exaggerates systemic inflammation and oxidative stress in patients with COPD. No effects of 1h supplemental oxygen on systemic inflammation and oxidative stress were found when muscle-wasted patients were treated at rest. Giving them supplemental oxygen during a constant work rate cycle test, however, diminished the IL-6 response to exercise and prevented exercise-induced systemic oxidative stress. Additional mechanistic measurements showed that this could be explained by 1) decreased free radical production by neutrophils and 2) reduction of ATP degradation (decreased plasma concentrations of the ATP degradation products xanthine and hypoxanthine and their reaction product uric acid after exercise with supplemental oxygen). These findings suggested that oxygen reduces the stimulus for neutrophil activation, and diminishes metabolic stress of tissues and thereby prevents ATP degradation and the following production of free radicals.

General Discussion

COPD is defined currently by the ATS/ERS as a disease characterized by airflow limitation, which is not fully reversible and produces *significant systemic consequences*."[1] Conventionally, the severity of COPD has been graded on the basis of the FEV_1.[1-3] However, COPD is associated with a range of clinical manifestations not closely related to the severity of airflow limitation, such as a worsening dyspnea, reduction in exercise capacity, pulmonary hypertension, peripheral muscle weakness, and malnutrition.[4;5] Furthermore, several large studies have shown that the FEV_1 is not the only determinant of mortality and a number of other risk factors have now been identified, including hypoxemia, hypercapnia, the timed walking distance [6;7], and a low body mass index.[5] Therefore, grading COPD solely on the basis of the FEV_1 does not reflect the clinical manifestations of the disease and its ultimate

prognosis. The concept of a single global marker has the attraction of simplicity and convenience, but may not be appropriate to a complex multicomponent disorder, such as COPD. Additional markers and outcomes are needed to provide a more comprehensive and clinically meaningful assessment and so provide a more informed basis for treatment decisions.[8;9] Exiting is the increasing number of studies documenting the presence of systemic abnormalities associated with the disease. This systemic involvement of COPD is extremely important because it may become the object of therapeutic interventions that could influence outcomes independent of the capacity to modify lung function. It seems conceivable that exploring the presence of systemic biomarkers in COPD and their relation to systemic manifestations of the disease, will help us in developing and applying novel strategies that will improve the outcome of our patients.[10] Current understanding of the pathobiology of COPD suggests a number of markers as potential candidates. These include most notably the inflammatory cells and their products that are believed to be the proximate causes of tissue destruction in patients with COPD. The studies presented in this thesis clearly focused on systemic inflammation and oxidative stress in COPD. In concordance with others, this thesis describes the presence of low-grade systemic inflammation and oxidative stress in stable patients with COPD. Increased plasma and serum levels of inflammatory cells, CRP, IL-6, and IL-8 were shown in COPD patients compared to healthy subjects, as well as decreased antioxidant capacity and elevated oxidation of proteins. The exact meaning of these biomarkers are not elucidated yet, and may be multifactorial, but both systemic inflammation and oxidative stress have increasingly been associated with loss of muscle mass and muscle dysfunction in patients with COPD.[11;12] One of the important aspects described in this thesis is the difference in low-grade systemic inflammation and oxidative stress between COPD patients with and without muscle wasting. The systemic effects as found in non-muscle wasted COPD patients compared with healthy subjects, were even further elevated in COPD patients with muscle wasting. Correlations were found between muscle wasting, muscle dysfunction and different markers of systemic inflammation and oxidative stress, indicating a possible causative effect of inflammation and oxidative stress on muscle wasting and function or vice versa. From these results, immediately several questions arise. For example, what is the nature of these systemic effects? Issues such as whether markers of systemic inflammation and oxidative stress are a “spill-over” from a site primarily in the lungs, are markers of systemic

consequences that cause damage in the lungs, or the result of secondary processes in other organs (e.g. muscles) resulting from primary disease in the lungs, need to be elucidated. Another additional and returning question is the unresolved problem of cause and consequence (chicken and egg story). Do systemic inflammation and oxidative stress cause muscle destruction or are they a consequence of muscle breakdown? It has been suggested that increased levels of cytokines may induce a catabolic response in tissues, triggering muscle proteolysis, with an increase in protein degradation. Furthermore, an excessive amount of free radicals may also be harmful to tissues like muscle proteins by inducing functional and/or structural alterations.[13] Indeed, our group has recently shown evidence for impaired diaphragm contractile protein function in patients with COPD.[14] An interesting hypothesis to be tested is that systemic inflammation and oxidative stress contribute to impaired muscle function in these patients. To answer this question the need for another group of systemic markers becomes clear. If some novel markers for specific protein damage (e.g. skeletal muscle troponin I), breakdown (e.g. ubiquinated proteins / proteasome activity) and/or synthesis can be detected systemically, studying these effects will become easier compared with for example muscle biopsies. Additionally, intervention studies (e.g. proteasome inhibitors) would be simplified if important outcomes could be measured systemically.

The other side of the chicken and egg story might implicate muscle wasting as cause. Exercise intolerance in patients with COPD is known cause adaptation of a sedentary life style, which in turn causes loss of muscle mass, reduction in force generating capacity of the muscles and a decrease in the resistance to fatigue.[15] Although strong evidence lacks, breakdown of proteins due to inactivity, might lead to the release of e.g. cytokines and inflammatory cells from the destructed muscle cells. If this hypothesis is true, however, then two other questions arise. Since airflow limitation of the muscle-wasted and non-muscle wasted COPD patients in our study was not different, it is unlikely that this has resulted in less exercise tolerance and thus more inactivity in the muscle wasted group. Why then becomes one COPD patient muscle wasted and the other not? Furthermore, exercise training improves condition, muscle function, and muscle mass [16-18], but complete normalization of muscle physiology is often not fully achieved. Therefore, it is likely that also other mechanisms than inactivity might play a role in skeletal muscle dysfunction in patients with COPD.[19]

The possible interaction between exercise intolerance, muscle wasting / dysfunction, and systemic inflammation and oxidative stress provides the unique opportunity to link basic and clinical physiology and to evaluate the role of underlying stress and immunophysiologic mechanisms in patients with COPD. 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, that can also affect distant organs.[20] Since patients with COPD already show signs of systemic inflammation and oxidative stress at rest, it was hypothesized that exercise would further increase these mediators. The main part of this thesis describes the exercise-induced systemic immune response in patients with COPD. Intensifying systemic inflammation and oxidative stress by exercise might be harmful for tissues. Especially in COPD patients with muscle wasting frequent exposure to these effects might play a role in the pathogenesis and ongoing progression of the disease. The studies in this thesis revealed that both maximal (high-intensity) and submaximal (moderate-intensity) cycle exercise increased systemic inflammatory and oxidative responses in muscle-wasted patients, which were not observed in non muscle-wasted patients and healthy subjects. Based on these results, it was postulated that daily life activities, which can be classified as moderate intense for these patients, can cause frequent bursts of inflammation and oxidative stress which may be involved in muscle wasting. Indeed, in an additional study it has been shown that only 6 minutes of walking was able to induce similar increases of systemic inflammation and oxidative stress as intense cycling in muscle-wasted patients. These findings support the concept that especially these patients may be exposed to bursts of inflammation and oxidative stress during activities of daily life. Determination of the immune response during activities like climbing the stairs, washing the dishes, or sweeping the floor can confirm or reject this hypothesis. Also here, the importance for systemic markers of protein damage or breakdown comes forward to link the systemic effects with ongoing muscle wasting. Since muscle wasting has already been associated with poor prognosis [21], this would be an important step in the pathophysiology of COPD.

Showing these findings and hypothesize about the possible negative effects of exercise-induced systemic inflammation and oxidative stress on muscles often amazes the listening public. A justified question is if we should not describe exercise to patients with COPD? First of all, this thesis emphasizes the need to independently take care

of different subgroups of COPD. Secondly, no direct evidence of negative effects of exercise on muscle wasting has been given. In contrast, in the light of inactivity and deconditioning, exercise improves health status of patients with COPD. Therefore, we would not recommend inactivity in order to prevent bursts of inflammation and oxidative stress. Another way of preventing these effects should be considered.

Targeting the systemic effects of COPD has created new opportunities for pharmacological agents in the treatment of this disease. Only limited data are available on the effects of specific intervention on systemic inflammation and oxidative stress in COPD. It has been reported that polyunsaturated fatty acids can modulate local and systemic cytokine biology.[22] A recent trial with polyunsaturated fatty acids failed to demonstrate any decrease in systemic inflammatory parameters in patients with COPD.[23] Several pharmacologic agents with demonstrated cardioprotective activities appear to reduce CRP levels. Of these, the findings for statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are the most robust.[24] The role of these drugs in modulation of systemic effects and outcomes in patients with COPD is not studied yet. Recently reported pilot data suggest that inhaled corticosteroids may modulate the level of systemic inflammation in COPD.[25] Further studies are needed to strengthen these early observations. The concept of overflow of the local inflammation in the lungs to the systemic compartment in patients with stable COPD may challenge this hypothesis .[26] Antioxidant therapies may play a role in targeting both the local and systemic component of COPD. In this area of specific intervention on systemic inflammation and oxidative stress in COPD and thereby revealing the pathophysiology of the disease, also oxygen therapy has been investigated. Oxygen therapy is one of the few treatments with clear benefits for patients with COPD, but recent investigations have shown that short term supplemental oxygen increased inflammation and oxidative stress in exhaled breath condensate.[27;28] The next question was what the influence of oxygen therapy on systemic effects of COPD would be. In this thesis, it was shown that short term oxygen therapy did not affect basal systemic inflammation and oxidative stress in patients with COPD. However, it was also shown that exercise-induced oxidative stress was prevented by supplemental oxygen during exercise and the systemic IL-6 response was attenuated. Inhibition of neutrophil activation and ATP degradation appeared to be involved in these effects. These and future findings are needed to explore the mechanisms underlying improvement of lack of improvement in clinical outcomes (health status, exercise tolerance, dyspnea,

hospitalization, etc.) and prognosis based on firm insights of the biological processes involved and modulated by the intervention.

Finally, COPD is a complex, multicomponent disease, comprising a number of pathophysiologic processes that interact with each other. Systemic inflammation and oxidative stress are two of these processes that seem especially important in a subgroup of COPD patients, those with muscle wasting. Further investigations are needed to explore cause and consequences of the systemic effects. This will contribute to new therapeutic targets based on mechanistic insights that will finally end in a better treatment and improved outcomes and prognosis for patients with COPD.

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Chapter 8

Nederlandse samenvatting

Nederlandse samenvatting

COPD

"Chronic Obstructive Pulmonary Disease", ofwel COPD, is de Engelstalige benaming voor chronisch obstructief longlijden. COPD is een chronische aandoening van de longen, die zich meestal op een later leeftijd uit in een geleidelijk en grotendeels onomkeerbare verslechtering van de longfunctie. Patiënten met COPD hebben longemfyseem, chronische bronchitis of een combinatie van beide ziektebeelden. Roken wordt gezien als de belangrijkste risicofactor voor de ontwikkeling van COPD. Echter, slechts 10-20% van alle rokers ontwikkelt in de loop der jaren COPD. Er lijken dus ook andere, nog onbekende factoren (bijv. genetisch) een rol te spelen bij het ontstaan van COPD.

ONTSTEKINGSREACTIES IN COPD

Het is duidelijk geworden dat chronische ontstekingsprocessen in de longen – inclusief de luchtwegen, longblaasjes en bloedvaten - een belangrijke rol spelen bij de vermindering van de longfunctie en in het ontstaan van de klachten (benauwdheid, hoesten, moeheid, steeds terugkerende infecties) bij patiënten met COPD. In de afgelopen jaren is ook ontdekt dat deze ontstekingsreacties zich niet beperken tot de longen, maar ook aanwezig zijn in de bloedbaan (systemische ontsteking). De oorzaken en precieze gevolgen van deze ontstekingsreacties in de bloedbaan van COPD patiënten zijn echter nog niet duidelijk.

INSPANNING BIJ PATIËNTEN MET COPD

Snelle vermoeidheid bij inspanning is één van de vaakst voorkomende klachten bij patiënten met COPD. Er is aangetoond dat dit enerzijds te maken heeft met de benauwdheid van deze patiënten door hun chronische vernauwing van de luchtwegen, maar anderzijds speelt ook een verminderde spierfunctie een rol. Verlies van spierfunctie en spiermassa zijn twee van de zogenoemde systemische effecten van COPD, die dus niet in de longen, maar daar buiten optreden. Hoe deze effecten ontstaan is onbekend, maar de bovengenoemde systemische ontstekingsreacties

lijken een goede kandidaat.
Inspanningsonderzoek bij gezonde personen heeft laten zien dat inspanning niet alleen een beroep doet op het hart (bijv. snellere hartslag), de longen (bijv. diepere ademhaling), circulatie (bijv. toename van bloeddruk) en de spieren, maar dat ook het afweersysteem hierbij betrokken is. In gezonde personen is aangetoond dat een zware inspanning gepaard gaat met o.a. een toename van de systemische ontstekingsreacties. Omdat COPD patiënten al in rust tekenen van systemische ontsteking laten zien, is te verwachten dat lichamelijke activiteit deze reacties verder doet toenemen. Hierbij kunnen ontstekingscellen, mediatoren en vrije radicalen vrijkomen die een schadelijke werking op bijvoorbeeld spieren kunnen hebben.

DOEL VAN HET ONDERZOEK

Het doel van het onderzoek is om meer inzicht te krijgen in de systemische effecten van COPD. Hiertoe hebben we onderzoek gedaan naar:

- Het voorkomen van systemische ontstekingreacties bij COPD patiënten en een mogelijke relatie met spiermassa en spierfunctie verlies
- Het optreden van een toegenomen ontstekingsreactie na lichamelijke inspanning bij gezonde personen en patiënten met COPD
- Het beïnvloeden van de systemische ontstekingsreactie bij patiënten met COPD door toediening van extra zuurstof.

RESULTATEN VAN HET ONDERZOEK

In **hoofdstuk 3** werd het aantal ontstekingscellen in de bloedbaan van COPD patiënten en gezonde personen onderzocht voor en na een maximale fietstest. Tevens werd de hormonale respons (adrenaline en noradrenaline) op inspanning bestudeerd. De hormonale respons op inspanning was lager in COPD patiënten dan in gezonde personen wat in elk geval gedeeltelijk verklaard kon worden door de lagere belasting die deze patiënten konden fietsen. Het aantal ontstekingscellen in patiënten met COPD liet inderdaad een systemische ontsteking zien. Tijdens inspanning vertoonden de ontstekingsreacties van gezonde personen en COPD patiënten eenzelfde patroon, maar de reactie in COPD patiënten vond plaats op een hoger absoluut niveau (meer ontstekingscellen). Op basis van deze resultaten werd er geconcludeerd dat COPD

patiënten blootstaan aan systemische ontsteking, welke geïntensifieerd wordt door uitputtende inspanning. De ontstekingsreactie is niet groter in COPD patiënten, maar vindt plaats op een hoger niveau en al bij een relatief lage belasting.
Systemische ontsteking is één van de potentiële mechanismen die een rol kan spelen bij de regelmatig voorkomende spierdysfunctie en spiermassa verlies in patiënten met COPD. Het doel van **hoofdstuk 4** was om de systemische ontstekingsreactie in patiënten met spiermassa verlies te karakteriseren en om te zien of inspanning in deze groep leidt tot verhoogde blootstelling aan systemische ontsteking. Een maximale en submaximale (50% van maximaal) fietstest werd gedaan bij COPD patiënten met spiermassa verlies, COPD patiënten zonder spiermassa verlies en gezonde controles. Voor en na de fietstesten werd bloed afgenomen om de systemische ontstekingsreacties te meten. De resultaten lieten zien dat basale waarden van het aantal ontstekingscellen en ontstekingsmediatoren verhoogd waren in alle COPD patiënten en het hoogste leken in de patiënten met spiermassa verlies. Een daling van het aantal antioxidanten (welke vrije radicalen moeten wegvangen om schade te voorkomen) en verhoogde eiwitschade toonden dat er ook sprake was van systemische oxidatieve stress (disbalans tussen oxidanten (vrije radicalen) en antioxidanten) in de patiënten met spiermassa verlies, die leidde tot weefselschade. Tevens was zowel de perifere spierkracht (benen) als de ademhalingsspierkracht in deze patiënten duidelijk afgenomen. Zowel de maximale als de submaximale inspanning veroorzaakte een verhoogde systemische ontstekingsreactie en toegenomen oxidatieve stress in patiënten met spiermassa verlies, maar niet in gezonden en COPD patiënten zonder spiermassa verlies. Deze resultaten suggereren dat COPD patiënten met spiermassa verlies chronisch blootstaan aan systemische ontsteking en oxidatieve stress, welke nog verder toenemen door inspanning. Speculatief zou dit de buiten de longen optredende effecten van COPD (bijv. spierdysfunctie) kunnen verergeren.

Door kortademigheid en spierfunctie verlies zijn patiënten met COPD relatief inactief tijdens hun dagelijkse leven. **Hoofdstuk 5** beschrijft een studie met het doel te onderzoeken of een waarschijnlijk minder zware en vaker uitgevoerde lichamelijke activiteit dan fietsen, namelijk 6 minuten lopen, ook een effect op de systemische ontstekingsreactie heeft in patiënten met COPD. Allereerst werd, aan de hand van fysiologische parameters gekeken wat voor soort inspanning de 6-minuten looptest is voor COPD patiënten met spiermassa verlies. Er werd gevonden

dat de 6-minuten looptest een submaximale inspanning was voor deze patiënten. De efficiëntie van het ademhalen was tijdens lopen minder dan tijdens fietsen. Doordat er tijdens lopen meer spieren gebruikt worden dan tijdens fietsen, zouden er meer prikkels naar de hersenen kunnen gaan om de ventilatie sterker te verhogen tijdens lopen. Hoewel lopen een submaximale inspanning bleek, was de systemische respons van de ontstekingsmediatoren en de oxidatieve respons vergelijkbaar na een looptest en een maximale fietstest. Deze bevindingen ondersteunen het idee dat COPD patiënten, vooral de subgroep met een verminderde spiermassa, regelmatig blootstaan aan toegenomen systemische ontsteking en oxidatieve stress tijdens hun dagelijks leven.

Zuurstof therapie is een van de weinige behandelingen met duidelijke voordelen voor COPD patiënten. Het verbetert de kans op overleving, vermindert het aantal ziekenhuisopnamen en de ontwikkeling van pulmonale hypertensie en verbetert de inspanningscapaciteit en kwaliteit van leven. Het is echter ook bekend dat zuurstof erg toxisch kan zijn. In de longen werden verhoogde ontstekingsreacties en oxidatieve stress gevonden na kortdurende behandeling met extra zuurstof. Het doel van **hoofdstuk 6** was om te onderzoeken of extra zuurstof ook effect heeft op systemische ontsteking en oxidatieve stress. Eén uur toediening van extra zuurstof in rust had geen effect op de systemische effecten in COPD patiënten met een verminderde spiermassa. Toediening van zuurstof tijdens een fietstest met constante belasting, echter, verminderde de inspanningsgeïnduceerde respons van de systemische ontstekingsmediatoren en voorkwam systemische oxidatieve stress. Twee mechanismen bleken hier een rol in te spelen. Allereerst werden de ontstekingscellen minder gestimuleerd om vrije radicalen te produceren. Ten tweede werd er, waarschijnlijk als gevolg van minder “stress” in de weefsels, minder ATP (substraat voor de vorming van purines welke weer omgezet kunnen worden tot vrije radicalen) gedegradeerd bij de behandeling met zuurstof. Tijdens het fietsen met zuurstof werd minder xanthine en hypoxanthine (ATP degradatie producten) gevonden, waardoor minder urine zuur en vrije radicalen (reactie producten) gevormd weren en geen oxidatieve stress ontstond.

Discussie

In de meest recente criteria van de American Thoracic Society en de European Respiratory Society wordt COPD gedefinieerd als niet geheel reversibele luchtweg obstructie met belangrijke systemische consequenties.[1] Conventioneel werd de ernst van COPD gegradeerd aan de hand van de hoeveelheid lucht die in de eerste seconde kan worden uitgeademd (FEV_1).[1-3] Patiënten met COPD hebben echter een verscheidenheid aan klachten die niet in lijn zijn met de ernst van de luchtweg obstructie, zoals kortademigheid, afgenomen inspanningscapaciteit, pulmonale hypertensie, perifere spierzwakte, en ondervoeding.[4;5] Verschillende studies hebben ook aangetoond dat de FEV_1 niet de enige determinant van mortaliteit is en andere factoren zijn inmiddels geïdentificeerd; hypoxemie, hypercapnie, loopafstand [6;7] en BMI (body mass index).[5] Graderen van COPD alleen op basis van FEV_1 reflecteert de klinische presentatie van de ziekte en de uiteindelijke prognose voor de patiënt niet. Het concept van een enkele, globale marker heeft het voordeel van eenvoud en gemak, maar zal niet afdoende zijn in een multi-component ziekte als COPD. Additionele markers en uitkomstparameters zijn nodig om een meer omvattende en klinische belangrijke evaluatie mogelijk te kunnen maken en ook om een goede basis te vormen voor behandelingsbeslissingen.[8;9] Opvallend is het stijgende aantal studies dat aanwezigheid van systemische abnormaliteiten bij COPD beschrijft. Deze systemische component bij COPD is uitermate belangrijk, omdat het een van de doelen voor nieuwe therapeutische interventies zou kunnen worden met effect op uitkomstmaten anders dan longfunctie.[10] Huidige kennis van de pathobiologie van COPD suggereert een aantal potentiële kandidaten als markers voor systemische abnormaliteiten. De belangrijkste zijn de ontstekings cellen en hun producten, waarvan gedacht wordt dat ze de rol spelen bij weefselschade in patiënten met COPD. De studies in dit proefschrift zijn dan ook gericht op systemische ontsteking en oxidatieve stress (disbalans tussen oxidanten – ofwel vrije radicalen, en antioxidanten) in COPD. Overeenkomstig met andere auteurs, beschrijft deze studie de aanwezigheid van chronische ontsteking en oxidatieve stress in stabiele patiënten met COPD. Vergeleken met gezonde controle patiënten, werden verhoogde plasma en serum concentraties ontstekingscellen en hun mediatoren (CRP, IL-6, IL-8) gevonden in patiënten met COPD, als ook verminderde antioxidant capaciteit en verhoogde oxidatie van eiwitten. De precieze betekenis van dit soort mediatoren is nog niet

duidelijk, en zou wel eens multifactorieel kunnen zijn, maar zowel systemische ontsteking als oxidatieve stress worden meer en meer geassocieerd met verlies van spiermassa en spierdysfunctie in patiënten met COPD.[11;12] Een van belangrijke aspecten uit dit proefschrift is het verschil in systemische ontsteking en oxidatieve stress tussen COPD patiënten met en zonder spiermassa verlies. De systemische effect zoals ze gevonden werden in COPD patiënten met een normale spiermassa, waren nog verder verhoogd in patiënten met spiermassa verlies. Correlaties werden gevonden tussen spiermassa verlies, spierdysfunctie en verschillende markers van systemische ontsteking en oxidatieve stress, duidend op een mogelijk causaal effect van ontsteking en oxidatieve stress op spiermassa verlies and dysfunctie of vice versa. Deze resultaten wekken meteen verschillende vragen op. Waar komen deze effecten vandaan? Zijn deze systemische markers een "spill-over" vanuit de longen, zijn het markers van systemische consequenties die schade veroorzaken in de longen, of zijn het markers van secundaire processen in andere organen (bijv. de spieren)? Een andere additionele en vaak terugkerende vraag is het onopgeloste probleem van oorzaak en gevolg (verhaal van het kip en het ei). Veroorzaken systemische ontsteking en oxidatieve stress spierafbraak of zijn ze het gevolg van spierafbraak? Het wordt gesuggereerd dat toegenomen concentraties cytokines een katabole reactie in weefsels kunnen veroorzaken; verminderde spieraanmaak en toegenomen eiwitafbraak. Ook vrije radicalen kunnen schadelijk zijn voor weefsels zoals de spieren doordat ze functionele en/of structurele veranderingen teweeg kunnen brengen.[13] Onze onderzoeksgroep heeft inderdaad aangetoond dat de functie van contractiele eiwitten in het diafragma van patiënten met COPD verminderd is.[14] Een interessante hypothese is dat systemische ontsteking en oxidatieve stress zouden bijdragen aan de verminderde spierfunctie van deze patiënten. Om deze vraag te kunnen beantwoorden, wordt ook de behoefte aan een andere groep systemische markers duidelijk. Indien het mogelijk zou zijn om specifieke markers voor eiwit schade (bijv. skeletspier troponine I), eiwit afbraak (bijv. geubiquitineerde eiwitten / proteasoom activiteit) en/of eiwit synthese systemisch te detecteren, zou het bestuderen van deze effecten makkelijker worden vergeleken met bijvoorbeeld spierbiopsies. Tevens zouden interventie-studies (bijvoorbeeld met proteasoomremmers) eenvoudiger worden als dergelijke uitkomsten systemisch gemeten zouden kunnen worden.

De andere kant van het kip en ei verhaal impliceert spiermassa verlies als oorzaak. Van inspanningsintolerantie bij patiënten met COPD is bekend dat het adaptatie aan

een sedentaire levensstijl veroorzaakt, waardoor spiermassa en spierkracht verloren gaan en er sneller vermoeidheid optreedt.[15] Hoewel sterke bewijzen ontbreken, zou afbraak van eiwitten als gevolg van inactiviteit, het vrijkomen van bijvoorbeeld cytokines en ontstekingscellen uit de afgebroken spiercellen kunnen induceren. Als deze hypothese waar zou zijn, komen twee andere vragen op. Omdat de luchtweg obstructie van de patiënten met COPD in onze studie niet verschillend was tussen de patiënten met en zonder spiermassa verlies, is het onwaarschijnlijk dat dit heeft geresulteerd in minder inspanningstolerantie en dus meer inactiviteit in een van beide groepen. Waarom gaat bij de ene COPD patiënt dan wel en bij de andere geen spiermassa verloren? Verder is ook bekend dat training positieve effecten heeft op conditie, spierfunctie en spiermassa [16-18], maar dat de spierfysiologie vaak niet geheel terugkomt op het oude niveau. Daarom lijkt het waarschijnlijk dat ook andere mechanismen dan inactiviteit een rol spelen bij skelet spierdysfunctie bij COPD.[19]

De mogelijke interactie tussen inspanningstolerantie, spiermassa verlies en dysfunctie en systemische ontsteking en oxidatieve stress biedt ons de mogelijkheid om basale en klinische fysiologie aan elkaar te koppelen en de onderliggende mechanismen te bestuderen in patiënten met COPD. Uit de literatuur is bekend dat zware inspanning een typische immuunrespons veroorzaakt in gezonden, gekenmerkt door verschillende ontstekingscellen, hormonen, cytokines, neurale en hematologische factoren die effect kunnen hebben op weefsels en organen op afstand.[20] Omdat patiënten met COPD al tekenen van systemische ontsteking en oxidatieve stress in rust vertonen, hypothetiseerden we dat inspanning deze mediatoren verder zou verhogen. Het grootste deel van dit proefschrift beschrijft de inspanningsgeïnduceerde systemische immuunrespons in patiënten met COPD. Toegenomen ontsteking en oxidatieve stress als gevolg van inspanning zou schadelijk kunnen zijn voor weefsels. Vooral in patiënten met spiermassa verlies zou blootstelling aan deze effecten een rol kunnen spelen in de pathogenese en progressie van de ziekte. De studies in dit proefschrift lieten zien dat zowel zware als matig zware inspanning op de fiets (hometrainer) een toegenomen systemische ontstekingsreactie veroorzaakte die niet of in mindere mate gezien werd bij gezonde personen en patiënten met COPD zonder spiermassa verlies. Gebaseerd op deze resultaten werd verondersteld dat activiteiten van het dagelijks leven, die bij patiënten met COPD geclassificeerd kunnen worden als matig zware inspanning, regelmatige blootstelling aan systemische ontstekingsreacties

kunnen veroorzaken die mogelijk een rol kunnen spelen bij het spiermassa verlies. Een additionele studie liet inderdaad zien dat slechts 6 minuten lopen bij patiënten met COPD en spiermassa verlies eenzelfde systemische ontstekingsreactie kon veroorzaken als een maximale inspanning op de fiets. Deze bevindingen ondersteunen het idee dat vooral deze groep patiënten tijdens dagelijks leven bloot zou kunnen staan aan deze systemische reacties. Het meten van zulke systemische effecten tijdens traplopen, de afwas doen of de vloer dweilen kan deze hypothese bevestigen of verwerpen. Ook hier komt het belang van systemische markers voor eiwit afbraak naar voren om de link tussen spiermassa verlies en systemische effecten aan te tonen. Omdat spiermassa verlies gerelateerd is aan een slechte prognose [21], zou dit een belangrijke stap in de pathofysiologie van COPD kunnen betekenen.

Het presenteren van bovenstaande resultaten en speculeren over mogelijk negatieve effecten van inspanningsgeïnduceerde systemische ontsteking op spieren, verbaast het publiek vaak. Een terecht vraag is of we inspanning voor patiënten met COPD dan zouden moeten afraden? Ten eerste laat dit proefschrift duidelijk zien dat er speciale aandacht zou moeten zijn voor subgroepen patiënten met COPD. Ten tweede is er geen direct bewijs van spiermassa verlies door inspanning. In tegendeel, in het licht van inactiviteit en deconditie, verbetert inspanning juist de gezondheidsstatus van patiënten met COPD. Daarom zouden we geen inactiviteit willen aanbevelen ter voorkoming van systemische ontsteking, maar lijkt een andere manier van preventie van deze effecten nodig.

Het vinden van systemische effecten van COPD heeft nieuwe mogelijkheden voor therapeutische interventies gecreëerd. Slechts beperkte data over de effecten van specifieke interventie van systemische ontsteking bij COPD zijn beschikbaar. Het is beschreven dat meervoudig onverzadigde vetzuren locale en systemische cytokine productie kan moduleren.[22] In een recent onderzoek met meervoudig onverzadigde vetzuren werd geen verlaging van systemische ontstekingsparameters bij patiënten met COPD gevonden.[23]

Verschillende farmacologische middelen met beschermende werking tegen hart- en vaatziekten hebben een reducerende werking op CRP. Binnen deze middelen zijn de bevindingen voor statines (cholesterol verlagende werking) het sterkst.[24] De rol voor statines in de regulatie van systemische effecten en uitkomsten bij patiënten met COPD is nog niet onderzocht.

Andere recente data suggereren dat geïnhaleerde corticosteroïden ook een rol kunnen

spelen in de modulatie van systemische ontsteking bij COPD.[25] Meer onderzoek in dit veld is nodig om deze data te bevestigen. Het idee van overloop van locale ontsteking naar het systemische compartiment speelt een rol in deze hypothese.[26]
Naast ontstekingsremmende therapie, zouden ook specifieke antioxidanten aan kunnen grijpen op zowel de lokale als de systemische componenten van COPD.
In het aandachtsgebied van de specifieke interventie van systemische effecten van COPD en de daarbij horende pathofysiologie van de ziekte, is ook het effect van zuurstof therapie onderzocht. Zuurstof therapie is een van de weinige behandelingen waar patiënten met COPD profijt van hebben. Echter, recent onderzoek liet zien dat zuurstof therapie een toename van ontsteking in uitgeademde lucht veroorzaakte.[27;28]
De volgende vraag was wat het effect van zuurstof op de systemische ontsteking in COPD zou zijn. In dit proefschrift werd aangetoond dat kortdurende zuurstof therapie geen effect had op de basale ontstekingsreactie bij patiënten met COPD. Maar, er werd ook gezien dat inspanningsgeïnduceerde oxidatieve stress voorkomen kon worden met zuurstof therapie tijdens de inspanning en dat ook de ontstekingsreactie hierdoor geremd kon worden. Remming van activatie van een van de ontstekingscellen (neutrofiel) en remming van de afbraak van ATP (een energiedrager) bleken betrokken bij deze effecten. Deze en toekomstige bevindingen zijn nodig om de mechanismen die ten grondslag liggen aan verbetering of gebrek aan verbetering van klinische uitkomsten and prognoses te kunnen verklaren.

Tot slot is COPD dus een complexe, multicomponent ziekte, die diverse pathofysiologische processen omvat met onderlinge interactie. Systemische ontsteking en oxidatieve stress zijn twee van die processen die vooral in een specifieke subgroep van patiënten met COPD belangrijk lijken. Verder onderzoek is nodig om de kip en het ei binnen de systemische effecten aan te kunnen wijzen. Dit zal er toe leiden dat nieuwe therapeutische opties, gebaseerd op mechanistische inzichten, ontwikkeld kunnen worden en uiteindelijk resulteren in een verbeterde behandeling, uitkomst en prognose voor patiënten met COPD.

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Curriculum Vitae

Hanneke van Helvoort werd op 26 januari 1980 geboren te Berlicum en groeide aldaar op. Na het behalen van het VWO-diploma aan het R.K. Gymnasium Beekvliet te Sint Michielsgestel in 1998, studeerde zij van 1998 tot 2002 Biomedische Gezondheidswetenschappen aan de Katholieke Universiteit Nijmegen in Nijmegen. Zij volgde de afstudeerrichting toxicologie en de bijvakken geneesmiddelenonderzoek en reproductie. In het kader van deze studie werden drie wetenschappelijke stages afgerond: de eerste aan de faculteit voor Bètawetenschappen (Radboud Universiteit Nijmegen), afdeling organismale dierfysiologie, getiteld "Effects of cortisol on the hypothalamus-pituitary-adrenal axis in tilapia (Oreochromis mossambicus)" (Dr. P.P.L.M. Pepels); de tweede bij de afdeling Longziekten van het UMC St Radboud, getiteld "Het gebruik van vernevelaars in de thuissituatie" (Prof. Dr. P.N.R. Dekhuijzen); en de derde op de afdeling Biochemische toxicologie van het Institute for Risk Assessment Sciences (Universiteit van Utrecht), getiteld "The use of sandwich-cultured rat hepatocytes to determine biotransformation parameters *in vitro*" (Dr. N. Treijtel).

Aansluitend aan haar afstuderen startte zij met het in dit proefschrift beschreven onderzoek. Dit onderzoek werd uitgevoerd van oktober 2002 tot oktober 2005 op de afdeling Longziekten van de Radboud Universiteit Nijmegen (hoofd: Prof. Dr. P.N.R. Dekhuijzen). Tijdens haar promotie onderzoek, beoefende Hanneke Acrogym op topsportniveau en wist zij in juni 2005 voor de 2e keer in haar sportcarrière Nederlands kampioene te worden.

Sinds 1 oktober 2005 is zij werkzaam als wetenschappelijk onderzoeker binnen het aandachtsveld klinische fysiologie van de afdeling longziekten van de Radboud Universiteit Nijmegen en is zij tevens aangesteld als hoofd van het longfunctielaboratorium op de locatie Dekkerswald.

Hanneke is getrouwd met John Lemmens.

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