Systemic impairment in chronic obstructive pulmonary disease

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Systemic impairment in chronic obstructive pulmonary disease

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Contents

Abbreviations

AA	amino acid
ADP	adenosine diphosphate
ALA	alanine
AMP	adenosine monophosphate
ARG	arginine
ASN	asparagine
ATP	adenosine triphosphate
BMI	body mass index
BW	body weight
C	creatine
CI	confidence interval
CIT	citrulline
COPD	chronic obstructive pulmonary disease
CORT+	COPD patients using prednisolone
CORT-	COPD patients not using oral corticosteroids
CRP	C-reactive protein
CRP+	COPD patients with plasma CRP levels ≥ 10 µg/ml
CRP-	COPD patients with plasma CRP levels < 10 µg/ml
CS	citrate synthase
CT	computed tomography
Ctot	total creatine
DL_{CO}	diffusing capacity for carbon monoxide
DXA	dual-energy X-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
FEV_1	forced expiratory volume in one second
FFM	fat free mass
FFMI	fat free mass index
FM	fat mass
FVC	forced vital capacity
GCS	gamma-glutamylcysteine synthetase
GLN	glutamine
GLU	glutamate
GR	glutathione reductase
GSH	glutathione
GSH-Px	glutathione peroxidase
GST	glutathione S-transferase
HAD	3-hydroxyacyl-CoA dehydrogenase
HK	hexokinase
Ht	height

IL	interleukin
IMP	inosine monophospate
IMP+	COPD patients with detectable muscle IMP levels
IMP-	COPD patients without detectable muscle IMP levels
LBP	LPS-binding protein
LDH	lactate dehydrogenase
LEU	leucine
LPS	lipopolysaccharide
LTOT	long term oxygen therapy
MHC	myosin heavy chain
MRI	magnetic resonance imaging
NAC	N-acetylcysteine
NF-κB	nuclear factor-κB
NH_3	ammonia
NS	not significant
ORN	ornithine
$PaCO_2$	arterial carbon dioxide tension
PaO_2	arterial oxygen tension
PC	phosphocreatine
PFK	phosphofructokinase
Pe-max	maximal expiratory pressure
Pi-max	maximal inspiratory pressure
^{31}P-MRS	^{31}phosphorus magnetic resonance spectroscopy
RBC	red blood cell
REE	resting energy expenditure
ROS	reactive oxygen species
RV	residual volume
SaO_2	oxygen saturation
SD	standard deviation
SEM	standard error of the mean
SOD	superoxide dismutase
SumAA	sum of all amino acids
SumEAA	sum of all essential amino acids
SumNEAA	sum of all non-essential amino acids
TAN	total adenine nucleotides
TLC	total lung capacity
TNF	tumor necrosis factor
U	unit

CHAPTER 1
General introduction

Introduction

In consensus statements of both American Thoracic Society and European Respiratory Society, chronic obstructive pulmonary disease (COPD) is defined as a disease characterized by the presence of airflow obstruction (1,2). This airflow obstruction does not change markedly over several months of observation (1). In these statements, the grading of severity of COPD is solely based on forced expiratory volume in one second (FEV_1), FEV_1 below 50% of the predicted value representing moderate to severe COPD (1,2).

Apart from the expiratory flow limitation, various other lung function abnormalities can be found in COPD, which are a consequence of a number of structural changes in the respiratory system. These lung function abnormalities include a decreased diffusion capacity (DL_{CO}), an increased ventilation to perfusion mismatch and dysfunctioning of the ventilatory pump. Up till now, medical treatment aiming at ameliorating lung function impairment in COPD has proved to be disappointing.

According to definitions of the World Health Organization, chronic diseases are not only characterized by their primary impairments, but also by the disabilities and handicaps resulting from them (3). Two major symptoms which disable COPD patients are dyspnea and exercise limitation. According to a comprehensive review by O'Donnell, the origin of the sensation of breathlessness is complex and involves an imbalance between inspiratory effort and the anticipated ventilatory consequence, caused by impeded inspiratory muscle action due to hyperinflation and muscle weakness (4). Indeed, impaired inspiratory muscle function was found to be associated with increased dyspnea in both cardiorespiratory patients and healthy subjects (5). Furthermore, in COPD patients, exercise performance was found to be related, not only with lung function parameters such as FEV_1 and diffusion capacity, but also with force of the quadriceps femoris muscle and maximal inspiratory mouth pressures (6). Compared to control subjects, force of extremity muscles (7,8) was indeed found to be decreased. Thus, according to these findings, both extremity and respiratory muscle weakness contributes to disabling symptoms in COPD. Muscle weakness in COPD is predominantly associated with loss of muscle mass (7,8). On the other hand, decreased muscle endurance might be associated with altered muscle metabolism. Indeed, the last few years several indications have been found for alterations of muscle energy metabolism in COPD patients. Furthermore, recently, maximum exercise capacity was found to be related to oxidative capacity of the quadriceps femoris muscle in COPD patients (9). Next to morbidity also the prognosis of COPD patients has turned out to be affected by other factors than lung function alone. Both low body weight (10,11,12) and low inspiratory muscle strength (13) were found to be prognostic factors for survival independent of the degree of airflow obstruction.

The abovementioned findings have led to the notion that in addition to local impairment, also systemic disturbances such as weight loss, loss of muscle mass and alterations in muscle metabolism may have major impact on morbidity and mortality of COPD. Further evaluation of systemic impairment in COPD is of importance to position the role of non-pharmacological modulation including nutrition and rehabilitation and to develop new systemic pharmacological strategies.

Body weight and body composition

Subjects were generally considered underweight if they had a body weight of less than 90% of their ideal weight, based on the 1983 Metropolitan Life Insurance tables. Lately, indices of relative weight as the body mass index (BMI) (weight/height^2) are more widely used. In elderly subjects a body weight of less than 90% of ideal approximately corresponds to a BMI of less than 21 kg/m^2. In weight loss, assessment of body composition gives additional information as to which body compartment is depleted. Moreover, body composition can also be disturbed in normal weight. A well known model to assess body composition is the 2 compartment model, dividing body mass in fat mass (FM) and fat free mass (FFM). FFM comprises the intracellular compartment (also known as body cell mass), reflecting muscle tissue and other actively metabolising tissue, and the extracellular compartment representing substance outside the cells. Several techniques are available to assess FFM in clinical practice, including measurement of skinfold thicknesses, deuterium dilution and bioelectrical impedance. Furthermore, dual-energy X-ray absorptiometry (DXA) allows for the differentiation of 3 body compartments: bone, fat and lean tissue. In humans, muscle mass cannot be measured directly. Estimates of regional muscle mass can be obtained using magnetic resonance imaging (MRI), computed tomography (CT) and DXA.

Muscle metabolism

Muscle protein and amino acid metabolism

Skeletal muscle mass comprises approximately 60% of the body cell mass and contains a substantial part of the body's proteins. Therefore, muscle serves as an important reserve system that provides amino acids for protein synthesis and energy metabolism. Of all amino acids that are incorporated into protein, glutamine (GLN) is the most abundant in the human body. It also has the most versatile functions of all amino acids (14). Among other things it serves as a non-toxic ammonia carrier and a fuel for rapidly dividing cells, such as immunocytes and enterocytes. Furthermore, GLN may be involved in the regulation of protein synthesis. Its main production site is muscle tissue. Glutamine is synthesized from the non-essential amino acid glutamate (GLU) and ammonia by the enzyme glutamine synthetase. Except for being the precursor for GLN synthesis, intracellular GLU is a precursor for the antioxidant glutathione and plays an important role in preserving high-energy phosphates in muscle through different metabolic mechanisms (i.e., substrate phosphorylation, replenishment of intermediates of the Krebs cycle). Alanine (ALA), another non-essential amino acid, is an important gluconeogenic amino acid and plays a central role in the glucose-alanine cycle (15). GLU is primarily taken up in muscle, whereas GLN and ALA are released by muscle.

Muscle wasting can be the result of changes in the cell cycle (differentiation, proliferation and apoptosis), but, more often, occurs as the result of a negative protein balance. Protein synthesis and degradation are influenced by factors associated with the level of contractile activity (activity versus disuse), the nutritional

status (insulin, glucagon) and the presence of disease (pro-inflammatory cytokines, glucocorticosteroids). In wasting diseases accompanied by acute metabolic stress such as sepsis or injury, most of the nitrogen lost derives from skeletal muscle sources, and in particular, contractile proteins (16). Muscle amino acid efflux is enhanced, especially efflux of GLN and ALA. These amino acids may then be used for gluconeogenesis and acute phase protein synthesis (17,18). In chronic disease states associated with cachexia, such as COPD, mechanisms leading to muscle wasting are as yet unclear.

Muscle energy metabolism

In all cells, energy is needed for metabolic processes and maintenance of membrane potentials. In muscle cells, energy is further needed for cross-bridge cycling during contraction. Because muscle cell activity ranges from basal metabolic rate to vigorous contractile activity, muscle cells must be capable of large changes in metabolic activity.

Adenosine triphosphate (ATP) is the common energetic currency of the cell. Several strategies are available to muscle cells to ensure that enough ATP is generated under various circumstances. At the onset of contractile activity, energy is derived from a small pool of high energy phosphates. To prevent a substantial decrease in ATP, phosphocreatine (PC) is available to provide phosphate for rephosphorylation of adenosine diphosphate (ADP) to ATP. Although in muscle cells relatively large quantities of PC are available, these stores can only supply energy for a short time. In the mean time, the rates of glycogenolysis and glycolysis accelerate to provide a more sustainable energy source (19, 20). During glycolysis, glucose is metabolized to pyruvate in the cytosol. Pyruvate is either reduced to lactic acid, following an anaerobic pathway, or enters the mitochondrion to be metabolized aerobically. Within the mitochondrion, pyruvate is converted to acetyl CoA, which in turn enters the tricarboxylic acid cycle. In the inner mitochondrial membrane, reducing agents such as nicotinamide adenine dinucleotide, produced during glycolysis and in the tricarboxylic acid cycle, transfer electrons to O_2 to form H_2O. Energy derived from the passage of electrons along this so-called electron transport chain, is used to produce ATP in the oxidative phosphorylation process. In health, the processes of electron transport and oxidative phosphorylation are tightly coupled.

Next to the formation of ATP via PC and the anaerobic glycolysis, the myokinase reaction (2 ADP --> AMP + ATP) is a third pathway for anaerobic formation of ATP (21).

Aerobic metabolism has several important advantages over anaerobic metabolism. Firstly, it produces more energy: 38 ATP per mole of glucose versus 2 ATP per mole glucose in anaerobic glycolysis. Secondly, in aerobic metabolism, except for glucose also lipid can by used as fuel via beta-oxidation (22). These properties are critical for muscle endurance and mitochondrial content is a major determinant of muscle performance during prolonged exercise.

Skeletal muscle contains fiber types with different histochemical and functional characteristics. Using histochemical techniques, fiber types I, IIa and IIb/x can be distinguished (23). Fiber type I has a slow twitch and develops a relatively small tension, but, because it depends mainly on aerobic metabolism, it is fatigue

resistant. In contrast, fiber type IIb/x has a fast twitch and develops large tensions but it is susceptible to fatigue because its energy conversion is based on anaerobic metabolism. Fiber type IIa has intermediate properties in that it also has a fast twitch, develops a moderate tension, is relatively resistant to fatigue and is apt to work under both aerobic and anaerobic conditions (24).

Weight loss and muscle wasting in COPD

The association between weight loss and muscle wasting and COPD has been recognized since the late nineteenth century. Only recently, these problems have been investigated in more detail. Regarding body weight, 17% of stable out-patients (25) and 35% of patients with stable COPD eligible for pulmonary rehabilitation (26) were found to be underweight as indicated by a body weight of less than 90% of ideal. However, this criterium for being underweight might be inappropriate for COPD, as recent studies reported increased mortality rates in patients with severe COPD and BMI less than 25 kg/m^2 (11,12).

By assessment of body composition, different patterns of weight loss can be distinguished (25,26): predominant loss of FM, predominant loss of FFM, or a combination of both. Weight loss predominantly involving loss of FM, occurs when energy expenditure exceeds energy intake. In COPD patients dietary intake can be relatively low because of symptoms such as dyspnea, fatigue and early satiety (27). Recently, systemic inflammation has been suggested to affect appetite and dietary intake, mediated by leptin, a hormone with appetite-regulating properties (28). Moreover, resting energy expenditure (REE) is often elevated in COPD patients. The thermogenic effects of bronchodilating agents may contribute to this elevated REE (29). Furthermore, because levels of acute phase proteins were found to be higher in hypermetabolic COPD patients, an association was suggested between elevated REE and systemic inflammation (30). Indeed, in a recent study, elevated REE was related with plasma TNF-α concentrations (31). Independently of REE, total daily energy expenditure was found to be elevated in COPD (32). It is thought that both an increased oxygen cost of breathing during exercise and the observed loss of efficient aerobic energy metabolism contribute to the increased total daily energy expenditure in COPD patients (32).

It is not exactly known how often predominant FFM depletion occurs in COPD patients. However, in 4% of stable out-patients and in 10% of stable COPD patients eligible for pulmonary rehabilitation, a reduced FFM was found although the patients had a normal body weight (25,26). Furthermore, in probably a larger group of COPD patients, FFM may be reduced disproportionate to body weight loss. FFM comprises total body mass minus FM. It is as yet unknown to what extent FFM depletion in COPD reflects muscle wasting. In an attempt to further evaluate FFM depletion, using DXA, trunk and extremity FFM were compared in COPD patients (33). In that study, extremity FFM was found to be decreased to an even greater extent than trunk FFM. Recently, using MRI and CT, estimates of regional muscle mass were obtained in COPD. This way, the cross-sectional area of calf muscle (34), and of thigh muscles (35), was found to be significantly smaller in patients with severe COPD compared with healthy controls (13 and 24 %, respectively).

A predominant loss of FFM involves an impaired balance between protein anabolism and catabolism. Only limited data are available on protein metabolism in COPD patients. Recently, in weight stable COPD patients with a normal FFM, both whole body protein synthesis and degradation were found to be elevated, indicating an increased protein turnover (36). This study did not provide data on relative contribution of muscle and splanchnic protein metabolism to elevated whole body protein turnover. In an earlier study, in depleted patients with emphysema, Morrison and coworkers (37) found indications for a depressed whole body protein synthesis and a reduced muscle protein synthesis. However, it has been suggested that the latter findings need to be reevaluated (36), because a leucine tracer was used for the measurements, whereas indications have been found that leucine metabolism is disturbed in COPD patients.
Multiple factors such as inactivity, use of corticosteroids, oxidative stress, hypoxemia and systemic inflammation may theoretically contribute to selective FFM depletion in COPD patients. Indeed, parameters of systemic inflammation, were found to be associated with weight loss (38,39) and decreased FFM (30) in COPD. The fact that, in COPD patients, non-response to nutritional therapy is associated with an elevated systemic inflammatory response (40) provides further proof for this supposed relationship. Furthermore, also reduced DL_{CO} (25) which might be associated with intermittent hypoxemia during exercise (41), was found to be related to reduced FFM.

Skeletal muscle energy metabolism in patients with COPD

Energy metabolism in resting muscle has been investigated in biopsy studies, evaluating muscle fiber type distribution, muscle enzyme activities and muscle high energy phosphate content. By histochemical analysis using ATPase staining, a low proportion of type I fibers was found in quadriceps femoris muscle of patients with severe COPD (42), compared with values normally reported in healthy subjects. The proportion of type I fibers was positively related to the arterial PaO_2. In a recent study comparing COPD patients with age-matched healthy control subjects, the reduction in the proportion of type I fibers in quadriceps femoris muscle was confirmed and was found to be accompanied by an increase in the proportion of type IIb fibers (43). Furthermore, in COPD patients the cross-sectional areas of type I, IIa and IIab fibers were smaller as compared with the healthy controls (43). Using a technique which quantifies myosin heavy chain (MHC) isoforms determined by gel electrophoresis, a decreased proportion of slow MHC-I isoform (corresponding with type I muscle fibers) and an increased proportion of MHC-IIa isoform (corresponding with type IIa muscle fibers) was found in quadriceps muscle of COPD patients (44). Because a positive relationship was found between the content of slow MHC and diffusion indices (45), it was hypothesized that a reduced oxygen availability might contribute to muscle alterations in COPD patients. In contrast to these studies in quadriceps femoris muscle, in deltoid muscle Gea and coworkers (46) did not find differences in fiber proportions and sizes comparing COPD patients and healthy controls. In biceps muscle the proportion of type I fibers was similar in COPD patients and controls, although diameters of type I and II fibers were smaller in COPD patients and were

related to the amount of weight loss (47). These findings suggest that changes in muscle fiber type proportions in COPD patients might be reserved for leg muscles.
Further evaluation of energy metabolism in resting muscle of COPD patients comprised assessment of enzyme capacities in biopsies. Two studies investigated oxidative and glycolytic enzyme capacities in resting quadriceps femoris muscle of stable COPD patients. Jakobsson and coworkers (48) found decreased oxidative enzyme capacity as measured by a decreased citrate synthase (CS) capacity, and an increased glycolytic capacity, as measured by an increased phosphofructokinase (PFK) capacity. Maltais and coworkers (49) found a decreased CS and 3-hydroxyacyl-CoA dehydrogenase (HAD) capacity, but found PFK capacity to be unchanged. However, the finding of a decreased oxidative capacity might be muscle specific, since Gea and coworkers (46) found unchanged muscle enzyme capacities in deltoid muscle of COPD patients. Recently, increased cytochrome oxidase capacity and upregulated mitochondrial gene expression of cytochrome oxidase have been reported in resting quadriceps femoris muscle of stable COPD patients with chronic hypoxemia (50). Cytochrome oxidase is a key oxidative enzyme, being the terminal enzyme in the mitochondrial electron transport chain. It is as yet unclear how to interpret the variable results regarding parameters of oxidative metabolism. Further studies, investigating well defined patient groups, and key enzymes of different metabolic pathways, are warranted.
Muscle high energy phosphate content was analyzed in quadriceps femoris muscle comparing stable COPD patients to healthy control subjects (51). Muscle ATP and glycogen were lower and muscle creatine (C) and lactate were increased in COPD patients. In this study COPD patients were mildly hypoxemic (mean PaO_2 7.8 kPa). Comparing stable COPD patients with and without chronic respiratory failure (42) lower muscle ATP, PC and glycogen levels, and higher C levels were found in patients with chronic respiratory failure. Furthermore, PaO_2 was positively and $PaCO_2$ was negatively related with muscle glycogen and PC.
As mentioned above, biopsy studies in resting quadriceps femoris muscle of stable COPD patients found indications for a decreased oxidative capacity. This finding has been confirmed non-invasively using 31-phosphorus magnetic resonance spectroscopy (^{31}P MRS) in exercising lower extremity muscle (34,52). In biopsy studies of upper extremity muscles, no changes in fiber type proportions or enzyme capacities were found. All the same, ^{31}P-MRS studies reported indications for a decreased oxidative capacity in exercising forearm muscle (53,54).

Factors contributing to muscle wasting and alterations in muscle metabolism in COPD

Chronic hypoxia

In studies investigating stable COPD patients, several indications were found for a relationship between hypoxemia and impaired DL_{CO} and parameters of skeletal muscle mass and metabolism. For instance, arterial PaO_2 was positively related to the proportion of type I fibers (42) and to muscle glycogen and PC (42). Furthermore, DL_{CO} was positively related to the content of slow MHC isoform (45) and to FFM (25).

However, hypoxemia is only one of the factors which might contribute to tissue hypoxia. In general, the development of tissue hypoxia is determined by factors associated with oxygen delivery and oxygen utilization such as blood oxygenation, haemoglobin concentration, regional blood flow, tissue capillarity and in case of muscle, myoglobin levels. As for now, it is not possible to measure tissue hypoxia in a clinically applicable way.
In COPD patients, the abovementioned factors associated with the occurrence of tissue hypoxia have only partly been subject of investigation. In studies examining COPD patients with a moderately to severe airflow obstruction and an only slightly impaired PaO_2 and DL_{CO}, in vastus lateralis muscle, myoglobin levels were found to be 25% reduced (55). Muscle capillary to fiber ratio (43) was found to be normal. Also, leg blood flow during exercise was found to be normal at each given submaximal work rate (56,57). Furthermore, overall leg oxygen delivery was comparable between COPD patients and healthy controls (56,57). However, no data are available regarding hypoxemic COPD patients. In the latter patient group a strategy of compensatory mechanisms (such as elevated haemoglobin levels) may (partly) prevent the occurrence of tissue hypoxia.
It is hard to find a good model for evaluating the effects of chronic hypoxia on muscle metabolism in humans. In healthy subjects this has primarily been studied in hypobaric hypoxia of high altitude. Although these data are probably the most relevant human comparison available, it remains doubtful to what extent they can be extrapolated to the situation of COPD patients, because high altitude expeditions are usually accompanied by strenuous physical activity. In high altitude studies a decreased muscle mass was found in healthy subjects (58). However, the precise effect on protein synthesis and degradation is not clearly known. In quadriceps femoris muscle, fiber size was found to be reduced (59). Capillary density (i.e. capillary number per cross-section of muscle tissue) was increased, but capillary to fiber ratio was unchanged (59). A loss of oxidative capacity is found as indicated by a loss of fractional volume of mitochondria in muscle fibers (59) and decreased oxidative enzyme capacities (60). It is thought that chronic hypoxia exerts its negative effects partly through the occurrence of increased oxidative stress during exercise.

Inflammation

Evidence accumulates that inflammation of airways and lung parenchyma is a critical event in the pathogenesis of COPD. An increased presence of CD8 T lymphocytes has been found in biopsies of both central and peripheral airways (61,62). In advanced airflow limitation, elevated amounts of neutrophils were found in the biopsies (63). The mechanisms for these inflammatory responses are not fully known. It is thought that pro-inflammatory cytokines such as interleukin (IL)-8 and TNF-α are involved in the attraction of neutrophils into the lung and the upregulation of adhesion molecules in the endothelium, respectively (64).
It has been shown that an inflammatory response in stable COPD is not restricted to the lungs. In plasma, elevated soluble TNF-α receptor 75 levels were found in a subset of stable COPD patients (30). In COPD patients who were losing weight, elevated plasma TNF-α levels (38) and elevated lipopolysaccharide (LPS) stimulated TNF-α production by monocytes were found (39). Also, in clinically stable COPD patients, elevated plasma

C-reactive protein (CRP) and LPS binding protein (LBP) levels have been found (30). These acute phase proteins are produced in the liver after induction by cytokines like IL-6 and TNF-α and provide an index of systemic inflammatory activity (65).
TNF-α is thought to induce muscle wasting via both indirect and direct mechanisms. TNF-α may alter circulating levels of hormones that regulate muscle growth, such as corticosteroids and insuline, and stimulate production of other catabolic cytokines (66). Recently, it has been shown that TNF-α also promotes nett protein loss by acting directly on muscle cells (67). TNF-α was found to accelerate protein degradation via activation of nuclear factor (NF)-κB, an important transcription factor.
It is as yet unclear if the abovementioned mechanisms also play a role in muscle wasting in stable COPD patients. So far, in COPD patients indications have been found for an association between systemic inflammation and alterations in body composition, in that FFM was decreased in a subset of COPD patients with elevated plasma CRP and elevated plasma LBP, IL-8 and sTNF-R55 and R75 levels (30). Furthermore, a nonresponse to nutritional support was found to be associated with a systemic inflammatory response as indicated by elevated levels of LBP, and soluble TNF receptors 55 and 75 (40).

Oxidant-antioxidant imbalance

Skeletal muscle generates reactive oxidant species (ROS) and production of ROS increases during contractile activity (68). In muscle tissue, ROS are mainly produced in mitochondria, in the electron transport chain (69). In the cytosol, ROS may be produced by xanthine oxidase, which is involved in the degradation of xanthine or hypoxanthine (69). Xanthine and hypoxanthine are degradation products of ATP, which may accumulate during metabolic stress (70). Indications have been found that ROS have a physiological role in maintaining optimal skeletal muscle contractility (71). However, disturbances in pro-oxidant and antioxidant balance in favour of the former, called oxidative stress, is associated with impaired contractile performance (68) and muscle tissue damage (72). ROS are capable of damaging lipids (72), which may result in increased membrane permeability. Because of this, mitochondrial function may be impaired. Protein oxidation by ROS (72) may affect protein function and enhances protein susceptibility to proteinases. Furthermore, ROS may directly activate NF-κB, and may thereby induce NF-κB mediated muscle wasting (67).
To protect myocytes for oxidative stress an extensive pool of antioxidant scavengers and enzymes is present, consisting, among others, of reduced glutathione (GSH), vitamin E, superoxide dismutase, GSH peroxidase and catalase (69). Repeated exposure to oxidative stress, such as occurs during repeated exercise, leads to elevated GSH concentrations and increased antioxidant enzyme capacities (73,74). In case of disuse of muscles, this protective mechanism may be lacking (74), resulting in a low antioxidant status and increased oxidative stress during sudden exercise. Also chronic hypoxia may influence muscle antioxidant status, as hypoxemia was found to inhibit glutathione synthesis (75).
Theoretically, in COPD patients, several mechanisms might contribute to enhanced muscular oxidative stress. As described above, disuse and hypoxia in COPD patients may affect muscle antioxidant status unfavourably. Indeed, recently in quadriceps femoris muscle of stable COPD patients, decreased glutathione concentrations were

found (76). Circulating neutrophils from smokers were found to have an enhanced oxidative burst (77). During exercise, neutrophils are recruited in contracting muscle and may thus contribute to muscle oxidant load in COPD patients, who are still smoking. Furthermore, as described earlier, in subsets of stable COPD patients a systemic inflammatory response was found, including elevated levels of soluble TNF receptors (30,40). Indeed, indications have been found that TNF-α enhances mitochondrial production of ROS (78).
However, as yet, despite interesting experimental studies, it remains to be established to what extent the observed abnormalities in muscle metabolism and function in COPD patients are indeed caused by oxidative stress.

Medication

Of all medications prescribed to patients with COPD, especially oral corticosteroids are under suspicion of impairing muscle metabolism and function. The extent of this impairment seems to be dependent on the type of steroid, the dose, and the duration of treatment. For instance, in experimental studies, fluorinated corticosteroids, such as dexamethasone affect muscle to a greater extent compared with nonfluorinated steroids (79,80). Furthermore, glycolytic muscle fibers seem more susceptible to steroid-induced muscle changes than oxidative muscle fibers (79,81). Both human and experimental studies, investigating high dosages of corticosteroids, found indications for muscle wasting, caused by a decreased protein synthesis and possibly increased protein degradation (79). Moreover, elevated plasma amino acid concentrations have been found (79). It has been hypothesized that corticosteroids stimulate the mobilization of amino acids from muscle (81), supplying the liver with gluconeogenic precursors. Corticosteroids may also affect muscle energy metabolism. Experimental animal studies found alterations in muscular mitochondrial structure, including enlargement (82), aggregation and vacuolation (82,83) of mitochondria. Moreover, quite consistently, elevated muscle glycogen levels have been found caused by an increased glycogen synthetase activity (84,85) and a reduced muscle glycogen phosphorylase activity (86). Conflicting results are available regarding the question if corticosteroids induce overall changes in muscle oxidative or glycolytic metabolism (87,88).
Few studies investigated corticosteroid induced muscle alterations in COPD patients. COPD patients, receiving oral corticosteroids as a maintenance treatment and complaining of severe muscle weakness, were found to have lower quadriceps force and were using higher dosages of oral corticosteroids compared with control COPD patients, who did not complain of muscle weakness (89,90). Furthermore, morphologic examination suggested myopathic changes in the muscle biopsies (90). However, as COPD patients received corticosteroids to treat inflammation, it was difficult to distinguish between steroid-induced effects and the effects of disease exacerbations.

Nutritional depletion

As described earlier in a subset of COPD patients undernutrition occurs, caused by a dietary intake which is inadequate as compared with energy expenditure. Prolonged undernutrition is associated with proportionate reduction of muscle

mass. Although both fiber types are affected, type II muscle fibers were found to be atrophied to a greater extent than type I fibers (91). It is notable that in early undernutrition alterations in muscle function may occur prior to evidence of decreased muscle mass (92). It has been hypothesized that this phenomenon is associated with early alterations in membrane ion transport (92).
Contradictory findings have been reported regarding muscle enzyme activities in undernourished states. In anorexia nervosa patients, glycolytic enzyme activities were found to be decreased to a greater extent than oxidative enzyme activities (91). However, in animal studies different results have been reported, varying from the finding of both decreased oxidative and glycolytic enzyme capacities in undernourished animals (93) to unchanged enzyme capacities (88).

Disuse

The effects of disuse on muscle mass, metabolism and function are mostly studied using models like unloading, microgravity or immobilisation. The results of these studies vary, among others, according to the degree and duration of the imposed disuse and the type of muscle under study. Generally, muscle mass is decreased, caused by reduced protein synthesis (94) and increased protein degradation (95). Myofibrillar proteins are affected to a greater extent than cytoplasmic proteins (96). Atrophy affects both type I and type II fibers, with a clear preference of type I fibers (95). Also the proportion of type I fibers (97) and the capacity of the oxidative enzymes were found to be decreased following muscular disuse (98). As mentioned above, reduced muscular activity has a negative effect on muscle antioxidant status, and may therefore enhance the risk of oxidative damage.
Patients with severe COPD are subjected to varying degrees of deconditioning by reduced physical activity. It remains to be answered to what extent results of animal studies examining effects of (complete) immobilisation can be extrapolated to the situation of reduced activity in humans.

Outline of the thesis

It is as yet clear that systemic abnormalities such as weight loss, loss of muscle mass and alterations in muscle metabolism may occur in COPD patients and contribute significantly to experienced limitations. The presence of these systemic abnormalities is not surprising because in COPD several contributing factors such as hypoxia, inflammation, use of medication and nutritional depletion, have been identified which have a significant systemic impact. In this thesis several systemic abnormalities and influencing factors were investigated in more detail.
In COPD, the relationship between body weight and mortality is well established. However, limited data are available regarding the relationship between body weight and morbidity. Early nonelective readmission can be regarded as a failure of the previous admission, and therefore as a short-term outcome parameter in exacerbated COPD. In **chapter 2,** risk factors for early nonelective readmission were investigated, with special emphasis on body weight on admission and weight changes during hospitalization.

In skeletal muscles of COPD patients various alterations of energy metabolism have been reported. In **chapter 3** high energy phosphate status was analyzed in anterior tibialis muscle of stable COPD patients.
In **chapter 4** a possible association between the observed abnormalities in high energy phosphate metabolism and oxidative and glycolytic enzyme capacities was evaluated in tibialis muscle of COPD patients. Conflicting results are available regarding corticosteroid-induced changes in muscle oxidative or glycolytic metabolism. Furthermore, indications have been found for corticosteroid-induced myopathic changes in COPD patients. Therefore, the effect of prolonged use of low dose prednisolone on muscle energy metabolism and qualitative morphology was investigated.
In COPD patients disturbances in muscle protein metabolism have been suggested. Because amino acids are the currency of protein metabolism, in **chapter 5** muscle and arterial plasma amino acid levels were examined. In an earlier study in COPD patients, FFM depletion was found to be associated with hypermetabolism and systemic inflammation. Therefore, the relationships between amino acid levels and the acute phase response and resting energy expenditure were evaluated.
Several indications of systemic inflammation have been found in stable COPD patients. Inflammatory states are associated with increased oxidative stress. In situations of prolonged oxidative stress, elevated erythrocyte glutathione levels are found. In **chapter 6** the relationship between an acute phase response and erythrocyte glutathione levels was examined in clinically stable COPD patients.

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CHAPTER 2

Early nonelective readmission for chronic obstructive pulmonary disease is associated with weight loss

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Abstract

Aim

To identify risk factors for early nonelective readmission in patients with chronic obstructive pulmonary disease, previously admitted for an exacerbation of their disease. Clinical characteristics were analyzed with special emphasis on body weight on admission and weight changes during hospitalization.

Methods

The computerized hospital database was used to select all hospital admissions in 1994 and 1995 with exacerbation of chronic obstructive pulmonary disease as main discharge diagnosis. Cases were retained if they were nonelectively readmitted within 14 days after prior discharge, and if they had no oedema. Controls were randomly selected from the discharge listing and were not readmitted within 3 months. Cases and controls were matched on several parameters including FEV_1% predicted obtained during a stable phase of the disease. Hospital charts were reviewed for clinical parameters on admission, *discharge and readmission.*

Results

Fourteen cases were retained in the study. On admission lung function, blood gases and parameters describing morbidity and social factors, were not different in cases and controls. The discharge procedure was adequate. During hospitalization the cases lost weight (mean±SD) (-1.6±1.9 kg, p= 0.01), while controls remained weight stable. Using a matched pairs logistic regression analysis, weight loss during hospitalization (p= 0.011) and low BMI on admission (p= 0.046) were related to the increased risk of unplanned readmission.

Conclusion

These findings provide further support for the concept that nutritional status is related to morbidity in COPD.

Introduction

Hospital admissions for exacerbation of chronic obstructive pulmonary disease (COPD) occur frequently and have a major impact on total costs of hospitalization. In chronic diseases in the elderly, unplanned readmission after hospital discharge is common (1), and its frequency of occurrence depends on the time period elapsed since discharge. Factors associated with early nonelective readmission have primarily been analyzed in very large epidemiologic studies, including a variety of hospital discharge diagnoses. These studies quite uniformly found a few factors to be relevant, like the number of hospital admissions in the year preceding the index admission (1,2,3), a comorbidity count (4) and a severity of illness score (1,3). No studies are available which exclusively investigated factors associated with nonelective readmission in COPD patients. Early nonelective readmission can be regarded as a failure of the previous admission and therefore as a short term outcome parameter in exacerbated COPD. Other outcome parameters such as survival and need of mechanical ventilation have been investigated quite extensively in COPD. Forced expiratory volume in one second (FEV_1) (5), functional status (5,6) and blood gases on admission (6,7) have been identified as prognostic factors related to survival. However, apart from these factors, also nutritional parameters were found to be associated with survival (6) as well as with the need of mechanical ventilation in exacerbated COPD patients (8).
The aim of this study was to identify risk factors for early nonelective readmission in COPD patients. Therefore, we retrospectively analyzed COPD patients admitted for an exacerbation of their disease in 1994 and 1995, which were nonelectively readmitted within 14 days after discharge. These cases were compared with a control group admitted in the same period of evaluation, which remained clinically stable after discharge. Clinical characteristics of both groups were compared. In order to assess the role of body weight and changes of it, cases and controls were matched regarding to baseline lung function, age and gender.

Methods

Selection of cases

The computerized hospital database was used to select all hospital admissions with primary discharge diagnosis coded as International Classification of Disease (ICD) code 496 (chronic obstructive pulmonary disease, unspecified) in 1994 and 1995. From this list all cases were selected which were readmitted within 14 days after prior discharge. If one case had multiple early readmissions in the 2 year period, the first occasion was chosen for analysis. Of all selected cases, hospital charts were analyzed. To be certain that the cases actually had COPD and not asthma, cases were only retained in the study, if they were already known in the outpatient clinic with COPD according to the criteria of the American Thoracic Society (9) and if they demonstrated no increase in FEV_1 of more than 10% of the predicted value after inhalation of a bronchodilator. Furthermore, cases were only retained in the study if exacerbation of their COPD was the main diagnosis at discharge. An acute exacerba-

tion was defined as a recent increase in dyspnea, cough and sputum production of sufficient severity to warrant hospital admission. Patients with concurrent diseases which could explain the increase in dyspnea such as pneumonia or left ventricular failure were excluded from the study. The present study meant to analyze COPD patients who experienced a nonelective early readmission caused by respiratory deterioration. Therefore, stable COPD patients, readmitted for other than respiratory problems were excluded. Finally, because analysis of weight changes was one of the objectives in this study, all cases with clinical signs of oedema were excluded.

Selection of controls

For each case a control subject was selected, which did not require hospital readmission within 3 months after discharge. Cases and controls were matched regarding age, gender, and month of admission. Potential controls were randomly selected from the computer derived discharge listing. Subsequently, hospital charts were analyzed, and the same exclusion criteria used for the selection of cases, were used for the selection of controls. Furthermore, control subjects were matched with cases according to their FEV_1% predicted obtained during a stable phase of the disease, in the year prior to admission. If the control subject elected from the computerized list did not apply to all these criteria, the subject was rejected and a next randomly selected control subject entered the selection process.

Data collection

Hospital charts were retrospectively reviewed by a trained research assistant, using a standardized data collection form. The following information was abstracted on admission: age, gender, number of comorbid diseases, number of hospital admissions in the year prior to the index admission, maintenance treatment, use of domiciliary oxygen, smoking status, social factors such as living situation and care arrangements (including home help services and limited home care by a nurse), body weight, height, blood gases breathing room air, lung function parameters and sputum cultures. The discharge procedure was analyzed looking at vital signs, prescription of medication and provision of home care on discharge. In our department, in patients admitted for exacerbation of COPD, FEV_1 and forced vital capacity (FVC) are measured on admission and on discharge. Measurements are performed using a pneumotachograph until 3 reproducible recordings are obtained. Furthermore, 3 times per week body weight is assessed using a digital weighing chair. Therefore, as follow up measurements it was possible to use body weight and spirometric data which were obtained within 3 days before discharge. In the cases, spirometric data, body weight and the diagnosis on readmission were registered.

Therapeutic protocol

All patients were treated according to a standardized protocol, including inhaled bronchodilators, intravenous corticosteroids and aminophylline, nasal oxygen if the PaO_2 breathing room air was less than 8.0 kPa, and antibiotics according to sputum cultures.

Statistical analysis

Cases, controls and follow-up data were compared using the Student's t-test for paired measurements. In case the normality hypothesis was not fulfilled, the Wilcoxon signed ranks test was used. Results were expressed as mean (SD). Frequency data were compared using the McNemar test. The association of five risk factors (number of hospital admissions in the previous year, number of comorbid diseases, FEV_1% predicted on admission, body mass index (BMI) on admission and change of body weight during admission) with early readmission was analyzed using matched pairs logistic regression. Each factor was tested using the likelihood ratio test. Significance was determined at the 5% level. The statistical analyses were performed using the SPSS for Windows statistical package (10).

Results

On computer analysis 659 admissions labelled ICD code 496 were identified in the period between January of 1994 and January of 1996. Thirty-seven times patients were readmitted within 14 days of discharge. After analysis of the hospital charts, 14 patients were excluded from the study for reasons shown in Table 1. Nine out of 23 otherwise eligible patients had oedema and were therefore excluded from the study. Fourteen cases were retained for analysis. On readmission, 7 cases were diagnosed to have an exacerbation of their COPD accompanied by an airway infection, 4 cases had an exacerbation of their COPD without airway infection and 3 cases had pneumonia.

Table 1 - Selection of cases

			Cases
Computer selection on ICD 496			659
Readmitted within 14 days			37
Reasons for ineligibility			
Chart not available		1	
Elective readmission		3	
No previous pulmonary function known		3	
Other diagnosis		7	
Left ventricle failure	3		
Pneumonia	2		
Atelectasis right lung	1		
Insufficient home care	1		
Eligible cases			23
Oedema		9	
Number of cases retained for final analysis			14

The FEV_1 obtained within one year prior to admission, during a stable phase of the disease was 46 (16) versus 49 (15) %predicted (NS) in cases and controls respectively (data not shown). The cases and controls were also well matched regarding age and gender (Table 2). Characteristics on admission are listed in Table 2. Both cases and controls had severe airflow obstruction and moderate hypoxemia with normocapnia. No differences in lung functional parameters, blood gases, body weight, BMI, duration of hospital stay, number of comorbid diseases, number of hospital admissions in the 12 months prior to the index admission were found between cases and controls (table 2). No differences in the use of diuretics (4 cases and 2 controls) and digoxin (4 cases and 2 controls) were found between both groups. During hospital stay, no extra diuretics were added to the regimen. Furthermore, no significant differences in use of domiciliary oxygen (14 versus 0%) and social factors such as living alone (50% versus 50%) and provision of home care (22 versus 28%) were found between cases and controls (data not shown).
Looking at the discharge procedure, no management failure could be detected in cases or controls. Changes in home maintenance treatment occurred as often in cases as in controls (28 versus 21%). Also home care provision at discharge was comparable in both groups (36 versus 43%) (data not shown). In both groups, FEV_1 and FVC on discharge did not significantly differ from data on admission (Table 4). However, in cases weight (mean difference -1.6; 95% Confidence Interval -2.7 to -0.5 kg) significantly decreased during admission, whereas in controls mean body weight did not change (mean difference -0.02; 95% CI -1.3 to 1.3 kg). Individual body weight changes on discharge and on readmission are given in Figure 1. Condi-

Table 2 - Characteristics on admission

		Cases (n= 14)	Controls (n= 14)	p value
Age	(yr)	71 (9)	69 (5)	NS
Gender	(m/f)	8/6	8/6	
FEV_1	(%)	39 (15)	38 (14)	NS
FVC	(%)	78 (24)	68 (18)	NS
PaO_2	(%)	8.6 (1.8)*	8.5 (2.1)†	NS
$PaCO_2$	(kPa)	5.4 (0.9)*	5.3 (0.9)†	NS
Weight	(kg)	56.4 (11.6)	61.1 (16.2)	NS
BMI	(kg/m^2)	21.3 (3.1)	22.4 (5.9)	NS
Sputum culture positive	(y/n)	10/4	8/6	NS
Duration of stay	(d)	12.2 (6.2)	11.4 (4.4)	NS
No. admissions in the previous year#		0.79 (0.89)	0.57 (0.94)	NS
No. comorbid diseases#		0.43 (0.51)	0.43 (0.65)	NS

Data are expressed as mean (SD), *n=11, †n=13, # Wilcoxon signed ranks test

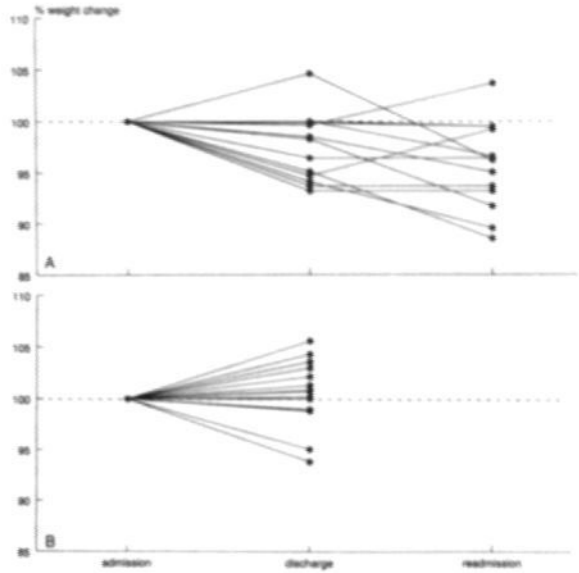

Figure 1
Body weight on discharge and readmission expressed as a percentage of body weight on admission. A= cases (COPD patients admitted for an exacerbation of their disease and readmitted within 14 days after discharge), B= controls (COPD patients admitted for an exacerbation of their disease and not readmitted within 3 months after discharge).

Table 3 - Lung function parameters, weight and body mass index on admission, discharge and readmission

		Admission	Discharge	Readmission
Cases				
FEV_1	(%)	39 (15)	39 (13)	36 (11)
FVC	(%)	78 (23)	75 (21)	74 (29)
Weight	(kg)	56.4 (11.6)	54.8 (10.7)*	53.7 (10.6)†
BMI	(kg/m^2)	21.3 (3.1)	20.7 (3.0)*	20.3 (3.2)†
Controls				
FEV_1	(%)	38 (14)	41 (11)	
FVC	(%)	68 (18)	74 (15)	
Weight	(kg)	61.1 (16.2)	61.1 (14.8)	
BMI	(kg/m^2)	22.4 (5.9)	22.4 (5.2)	

Data are expressed as mean (SD), *p= 0.01 vs admission, †p= 0.005 vs admission

tional on the values of the other variables, it turned out that weight change during hospitalization (p= 0.011) and BMI on admission (p= 0.046) were significantly associated with unplanned early readmission, a greater weight loss and a lower initial BMI being predictive of readmission. Changes in body weight or BMI during admission were not related to the duration of hospital stay.
Furthermore, in the cases, body weight and BMI on readmission tended to be lower than on discharge. FEV_1 and FVC on readmission did not differ from data on discharge or admission.

Discussion

In this study, factors related to nonelective readmission were investigated in COPD patients. Two groups of patients, matched for baseline FEV_1% predicted and admitted for an exacerbation of their COPD without accompanying right heart failure, were compared: cases, which were readmitted within 2 weeks of discharge and controls, which were not readmitted within 3 months of discharge. Parameters describing morbidity, comorbidity, maintenance treatment and social factors were not different between the 2 groups. Also, the severity of the exacerbation as assessed with dynamic lung function and arterial blood gases on admission did not seem different in both groups. No major management problems were identified during hospitalization and on discharge. The two groups only differed from each other with respect to body weight and body weight changes: the cases lost weight during admission, while the controls remained weight stable. Besides these changes in BMI during the hospitalization period, BMI on admission was found to be inversely related to the risk of readmission.
The finding of an association between BMI on admission and the risk of nonelective readmission in COPD patients, stands in line with other studies reporting associations between baseline nutritional parameters and morbidity and prognosis in COPD patients with exacerbated disease. In a recent study examining the outcome of patients hospitalized with an acute exacerbation of severe COPD, survival time was found to be independently related to BMI (6). Malnutrition as assessed by a computed nutritional index, was found to occur more frequently (11) and to be more severe in COPD patients requiring ventilatory support (8,11). Furthermore, nutritional status was found to be a predictive parameter for the outcome of noninvasive mechanical ventilation in exacerbations of COPD (12).
In addition, in the present study an association was found between weight loss during the period of hospitalization for an exacerbation of COPD, and nonelective readmission. At present only very little is known about the course of weight changes in COPD, because most studies regarding body weight and body composition were performed in clinically stable COPD patients in cross-sectional analyses (13,14). It has been hypothesized that weight loss in COPD patients follows a stepwise pattern related to acute disease exacerbations (15). However, in a recent study by Vermeeren et al (16), in a random group of patients admitted for an exacerbation of COPD, no mean weight loss was found during hospitalization. This study also investigated energy balance. During the first days of hospitalization a severely impaired energy balance was found, due to a markedly decreased dietary intake

and an increased resting energy expenditure (REE) (16). In the period prior to discharge, dietary intake gradually increased to values even higher than habitual intake, while REE decreased, resulting in a net restoration of energy balance. In contrast to the patients in the above-mentioned study and in contrast to our controls, our cases lost weight during hospitalization. Therefore, it can be hypothesized that our cases were in a state of ongoing negative energy balance. As we did not measure energy expenditure or intake, it remains unknown which component contributed most to the possible disturbance of the energy balance. Theoretically, although no overt oedema was present, loss of body fluid might have contributed to weight loss during hospitalization. However, in the cases, body weight was even further decreased on readmission. If loss of body fluid would have been an important mechanism explaining the observed weight loss during hospitalization, one would have expected a rise of body weight (due to water retention) after discharge.

The present study can not elucidate the question if the observed relationships between both low body weight on admission and weight loss during admission and early readmission are causal relationships or if these parameters represent epiphenomena of more severe disease. In the former scenario, low body weight and weight loss during admission might influence morbidity directly, for instance by affecting respiratory muscle function. Attempts to ameliorate energy balance would then be beneficial. In the latter scenario, low body weight on admission and weight loss during admission would be markers for more severe COPD and a more severe exacerbation of COPD, respectively. Although the conventional parameters measured in this study, such as baseline dynamic lung function, lung function and blood gases on admission and duration of hospital stay, were not different in cases and controls, these parameters may not have been sensitive enough to rule out the second possibility. Furthermore, in the cases 10 out of 14 patients were readmitted with infectious complications. Therefore, chronic or recurrent infections might have caused respiratory deterioration necessitating readmission and might, at the same time, have contributed to weight loss through systemic inflammation.

In our study, both in cases and controls, lung function did not change during treatment of the exacerbation. This finding indicates on the one hand, that the patient selection has been thorough, leaving out all asthmatics. On the other hand, this finding confirms that spirometric data are not very useful in assessing the outcome of treatment in patients with exacerbations of COPD.

In contrast to findings in several large epidemiologic studies (1,2,3,4), in the present study no associations were found between comorbidity count or number of previous hospital admissions and unplanned early readmission. The fact that our study had a completely different design compared to the earlier studies, being a small sample disease specific study, might have contributed to this discrepancy.

The present study has some limitations, which need to be discussed. First, the study used data retrospectively derived from hospital charts. Second, the results were based on analysis of a subset of patients admitted for an exacerbation of their COPD, namely, patients already visiting an out patient pulmonology clinic and patients without right heart failure. Third, due to these selection criteria the total number of patients studied has been small, which may have reduced the potential significance of other included variables. On the other hand, the strength of this

study is the fact that, because of the strict selection criteria, the patient group which remained was very homogeneous.

In summary, in this study, early nonelective readmission in COPD patients was found to be associated with weight loss during prior hospitalization and low body weight on prior admission for an exacerbation of their COPD. These findings provide further support for the concept that nutritional status is related to morbidity in COPD and indicate that prospective investigations of metabolic changes during and after exacerbations of COPD are warranted.

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CHAPTER 3

Elevated inosine monophosphate levels in resting muscle of patients with stable COPD

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Abstract

In order to investigate disturbances in energy metabolism in resting muscle of patients with stable chronic obstructive pulmonary disease (COPD), concentrations of adenine nucleotides and related compounds were examined comparing 34 COPD patients with eight age-matched healthy control subjects. Biopsies were taken from the anterior tibialis muscle. Special attention was paid to the muscle content of inosine monophosphate (IMP), a deamination product of adenosine monophosphate (AMP), because IMP formation is thought to reflect an imbalance between resynthesis and utilization of adenosine triphosphate (ATP). The absolute concentrations of high energy phosphate compounds did not differ between patients and control subjects, but the ATP/ADP and the phosphocreatine/creatine (PC/C) ratio were significantly lower in the patients. IMP (detection level 0.06 mmol/kg dry weight), was detected in 25 of 34 patients versus one of eight control subjects ($p= 0.001$). Mean (SD) IMP level in these patients was 0.18 (0.14) versus 0.06 mmol/kg dry weight in the one control subject. Based on the presence of detectable levels of muscle IMP, the patient group was divided into two subgroups. In IMP positive patients, ATP/ADP and PC/C ratios were significantly lower than in IMP negative patients. IMP positive patients were furthermore characterized by a significantly lower DL_{CO}. The results of this study indicate an imbalance between the utilization and resynthesis of ATP in resting muscle of patients with stable COPD.

Introduction

Impaired exercise performance is a frequently occurring problem in patients with chronic obstructive pulmonary disease (COPD) (1). The two dominant symptoms limiting exercise tolerance in COPD, are dyspnea and a sensation of fatigue in leg muscles (1). Recently it has been shown that both skeletal and respiratory muscle weakness contribute to the severity of these symptoms (2) and to reduced exercise tolerance, independent of lung function impairment (2,3).

The cause of muscle weakness in COPD patients is incompletely understood. It has been shown that the development of muscle weakness is frequently associated with loss of body mass and in particular loss of muscle mass (4,5). However, in this group of patients, apart from muscle wasting, also changes in muscle energy metabolism have to be considered as a factor contributing to the impairment of muscle function.

Experimental data on muscle energy metabolism in patients with stable COPD are scarce. In two recent studies, the activity of glycolytic and oxidative enzymes was investigated, in resting quadriceps femoris muscle of patients with stable COPD. Jakobsson and associates (7) found indications for an augmented glycolytic and a decreased aerobic capacity. The latter phenomenon was also observed in the study performed by Maltais and colleagues (8).

The metabolic consequences of the observed changes in enzyme capacities in COPD patients are as yet unclear. Therefore, the first aim of this study was to investigate possible changes in high energy phosphate concentrations in resting muscle of patients with stable COPD in comparison with healthy age-matched control subjects. We hypothesized that, next to measuring concentrations of adenine nucleotides, analysis of concentrations of their degradation products might be useful in detecting subtle alterations in muscular energy status. In particular inosine monophosphate (IMP), a deamination product of adenosine monophosphate (AMP) is of interest in this context. IMP has been primarily studied in healthy volunteers, where an increased IMP formation was universally found during exercise (9). This increased IMP formation during exercise is generally believed to reflect an imbalance between adenosine triphosphate (ATP) utilisation and resynthesis. Under resting conditions, muscle IMP levels are undetectable or very low in healthy subjects.

The second aim of this study was to examine, within the COPD population, the relationship between muscle energy status and lung function parameters, blood gases and nutritional status.

Because it is probable that activity level is reduced in COPD patients compared to healthy control subjects, and since theoretically the anterior tibialis muscle is less influenced by activity level than quadriceps femoris muscle, it was chosen to examine the former muscle.

Methods

Subjects

All patients had COPD according to the criteria of the American Thoracic Society (10) and a forced expiratory volume in one second expressed as a percentage of predicted (FEV_1% pred) < 50%. Only male subjects were included. Patients who demonstrated an increase in FEV_1 of > 10% of the predicted value after inhalation of a bronchodilator (terbutaline, 500 µg) were excluded. Other exclusion criteria were a history of cardiac insufficiency, distal arteriopathy, malignancy, endocrine, hepatic or renal disease, or use of anticoagulant drugs. All patients were stable at the time of the study and did not have any infection or exacerbation of their disease at least 6 weeks prior to the investigation. The patients were compared to a group of healthy age-matched volunteers.
Written informed consent was obtained, and the study was approved by the Medical Ethical Board of the University Hospital Maastricht.

Muscle biopsy analysis

Muscle biopsies were obtained under resting conditions between 9 and 10 hours in the morning, after an overnight fast, while the subjects were breathing room air. As described elsewhere (11), the biopsies were obtained from the anterior tibialis muscle, under local anesthesia, using a conchotome. The biopsies were immediately frozen in liquid nitrogen and stored at -80°C until analysis.
The muscle samples were freeze-dried. After freeze-drying adherent blood and connective tissue were removed and the samples were divided into two portions. One portion was used for determination of adenine nucleotides and related compounds, phosphocreatine (PC), creatine (C), and glucose; the other part was used for determination of muscle glycogen content.
ATP, adenosine diphosphate (ADP), AMP, and IMP were determined with a high performance liquid chromatographic technique using a modified method after Wynants and van Belle (12), as described by Van der Vusse and coworkers (13). In short, the freeze-dried tissue was extracted at -15°C in a mixture of perchloric acid (3.0 M) and dithiothreitol (5 mM). After the tissue was ground in the extraction fluid with a glass rod and after subsequent centrifugation at 4°C at 1,200 x g for 5 min, the supernatant was neutralized with $KHCO_3$. Aliquots of the neutralized supernatant were injected on a reversed-phase column (Lichrosorb RP-18, Merck, Darmstadt, Germany). Stepwise gradient elution using two solvents was applied to separate the compounds of interest. Solvent A was an aqueous buffer of $NH_4(H_2PO_4)$ (150 mM, pH 6.0); solvent B consisted of a 1:1 (vol/vol) mixture of acetonitrile and methanol. Flow rate amounted to 0.8 ml/min. Peaks were detected at 254 nm and were identified by comparing retention times with known standards. LiChroCART 4-4 (Merck) was used as guard column. The detection level for IMP was 0.06 mmol/kg dry weight. Total adenine nucleotides (TAN) was computed adding ATP, ADP, and AMP contents. PC and C were measured fluorometrically (14). Total muscular creatine (Ctot) was computed adding PC and C contents. Free glucose was assayed as described elsewhere (14).

The second part of the freeze-dried tissue was used for glycogen determination. To this end, the tissue specimen was kept at 100°C for 3 h after addition of 1.0 ml of 1 M HCl in order to hydrolyze glycogen. Subsequently, the samples were neutralized with TRIS (0.12 M)-KOH (2.1 M) saturated with KCl. The glucose residues were measured fluorometrically as described elsewhere (14). The values obtained were corrected for the amount of free glucose already present at the time of tissue sampling.

Pulmonary function tests

FEV_1 and forced vital capacity (FVC) were measured, using the pneumotachograph of a constant volume plethysmograph (Masterlab; Jaeger, Wurzburg, Germany) until 3 reproducible recordings were obtained. Highest values were used for analysis. The diffusion capacity for carbon monoxide (DL_{CO}) was measured by the single-breath carbon monoxide method (Masterlab Transfer; Jaeger). Total lung capacity (TLC) and residual volume (RV) were measured by body plethysmography (Masterlab Body; Jaeger). All values were expressed as a percentage of reference values (15).

Blood was drawn by puncture of the radial artery while subjects were breathing room air. Arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) were measured using a blood gas analyzer (ABL 330; Radiometer, Copenhagen, Denmark).

Functional capacity

Inspiratory muscle strength was assessed by maximal inspiratory mouth pressure (Pi-max) according to the method described by Black and Hyatt (16). Exercise performance was evaluated with a 12 minute walking test, performed in a level enclosed corridor according to the methods described by McGavin and colleagues (17). All tests were performed in the afternoon and no encouragement was given. As learning effects have been noticed to occur quickly with repeated walk tests the patients performed one practice test.

Statistical analysis

Statistical analysis was performed using Student's t-test for unpaired measurements. In case the normality hypothesis was not fulfilled, the Mann-Whitney U test was used. Results were expressed as mean (SD). Frequency data were compared using the Chi-square test. Linear regression analysis was used to study relationships between parameters. Significance was determined at the 5% level. The statistical analyses were performed using the SPSS for Windows Statistical package (18).

Results

Characteristics of COPD patients and control subjects

Thirty-four male patients and eight male volunteers were included in the study. Anthropometric and pulmonary function data are listed in table 1. Patients had a severe airflow obstruction (FEV_1% pred 32 (10) %), marked air trapping (RV 205 (74) %), moderate hyperinflation (TLC 126 (20) %), reduced DL_{CO} (62 (29) %), and slightly reduced values of arterial oxygen tension (PaO_2 9.1 (1.3) kPa) in the presence of normocapnia ($PaCO_2$ 5.6 (0.6) kPa). In the control group, all lung function parameters were in the normal range. The body mass index (BMI) was lower in the patient group (mean difference 2.4; 95% confidence interval (95% CI) 0.5-4.4 kg/m²).
All patients used inhalation therapy: beta-2-agonists (n= 34), anticholinergics (n= 29), and steroids (n= 29). Twenty-six patients used theophylline, and 17 patients used acetylcysteine continuously. Furthermore, 17 patients used oral prednisolon as a maintenance treatment (mean daily dose 7.6 (4.4) mg; mean duration of therapy 3.4 (5.3) yr), while three patients used oral bethametasone.

Muscle metabolites in COPD patients and control subjects

The values of the muscle metabolites are summarized in table 2. No significant differences were found between the total COPD group and the control group in ATP, ADP, AMP or TAN. However, the ATP/ADP ratio was significantly lower in the patient group compared with the control group (mean difference 0.5; 95%

Table 1 - Characteristics of COPD patients and healthy control subjects

		COPD (n= 34)	Controls (n= 8)
Age	(y)	65 (6)	64 (8)
BMI	(kg/m²)	23.4 (3.9)*	25.9 (1.9)
FEV_1	(%)	32 (10)*	113 (10)
FVC	(%)	78 (17)*	109 (4)
DL_{CO}	(%)	62 (29)*	118 (20)
RV	(%)	205 (74)*	111 (14)
TLC	(%)	126 (20)*	108 (5)
PaO_2	(kPa)	9.1 (1.3)*	11.6 (1.0)
	(mm Hg)	68 (10)*	87 (8)
$PaCO_2$	(kPa)	5.6 (0.6)*	4.6 (0.5)
	(mm Hg)	42 (5)*	34 (4)

BMI body mass index, RV residual volume, TLC total lung capacity
Data are expressed as mean (SD)
*$p < 0.05$

CI 0.02-0.98). Muscle content of PC, C, and Ctot was not significantly different between both groups, while the PC/C ratio was lower in the patient group (mean difference 0.19; 95% CI 0.02-0.36). IMP could be detected in 25 of 34 patients compared to one of eight control subjects (X^2 = 10.23, p= 0.0014). The mean IMP level in the 25 patients with detectable IMP was 0.18 (0.14), ranging from 0.06 to 0.59 mmol/kg dry weight. The IMP level of the one control subject with detectable IMP was 0.06 mmol/kg dry weight. The lower level of detection of IMP with the present analytic technique was found to be 0.06 mmol/kg dry weight. Muscle contents of glycogen, glucose, and lactate were not significantly different between patients and control subjects.

Characteristics of IMP+ and IMP- patient subgroups

Based on the presence or absence of detectable levels of muscle IMP, the patient group was subdivided into two groups, i.e., an IMP positive (IMP+) and an IMP negative (IMP-) group (Tables 3 and 4). No differences in airflow obstruction or resting arterial blood gases were found between both patient subgroups (Table 3), but DL_{CO} was significantly lower in the IMP+ subgroup (median difference 15%, p= 0.049). No differences in BMI or Pi-max were found between both groups. A

Table 2 - Muscle metabolites in COPD patients and healthy control subjects

	COPD (n= 34)	Controls (n= 8)
ATP	17.5 (3.0)	18.8 (3.8)
ADP	3.1 (0.6)	3.1 (0.5)
AMP*	0.11 (0.04)	0.09 (0.04)
TAN	21.1 (3.9)	22.0 (4.3)
ATP/ADP	5.6 (0.5)†	6.1 (0.6)
PC	79.4 (16.4)	71.5 (10.2)
C	44.5 (10.1)	36.5 (6.5)
Ctot	124 (25)	108 (16)
PC/C	1.8 (0.2)†	2.0 (0.2)
IMP	0.18 (0.14)‡	< 0.06
glycogen§	264 (66)	263 (67)
glucose*	5.7 (3.4)	6.7 (5.8)

ATP adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, TAN = ATP+ADP+AMP, PC phosphocreatine, C creatine, Ctot = PC+C, IMP inosine monophosphate; Values are mean (SD) and are expressed in mmol/kg dry weight of tissue, unless otherwise indicated;
* Mann Whitney U test, † $p < 0.05$, ‡ Mean (SD) of the detectable levels of IMP in 25 patients, detection level was 0.06 mmol/kg dry weight, § Glycogen expressed as mmol glycosyl units/kg dry weight

Table 3 - Comparison of patient characteristics based on the presence of detectable muscle IMP

COPD subgroups		IMP+ (n= 25)	IMP- (n= 9)
BMI	(kg/m^2)	23.0 (4.0)	24.7 (3.6)
FEV_1	(%)	31 (8)	33 (13)
FVC	(%)	81 (17)	75 (21)
DL_{CO}*	(%)	56 (27)†	76 (29)
RV	(%)	198 (65)	226 (94)
TLC	(%)	124 (18)	131 (26)
PaO_2	(kPa)	9.1 (1.3)	9.3 (1.3)
	(mm Hg)	68 (10)	70 (10)
$PaCO_2$	(kPa)	5.5 (0.7)	5.7 (0.7)
	(mm Hg)	41 (5)	43 (4)
Pi-max	(kPa)	7.2 (2.1)	6.9 (2.9)
12 min walking distance	(m)	783 (246)‡§	961 (202)‖

BMI body mass index, RV residual volume, TLC total lung capacity, Pi-max maximal inspiratory mouth pressure
Data are expressed as mean (SD)
* Mann Whitney U test, † $p < 0.05$, ‡ $p < 0.10$, § n= 15, ‖ n= 8

12 minute walking test was performed by 15 of 25 IMP+ and eight of nine IMP- patients, and the 12 minute walking distance tended to be lower (p= 0.09) in the IMP+ group. The patient subgroups did not differ in use of maintenance treatment of oral steroids (14 of 25 in the IMP+, and 6 of 9 in the IMP- patient subgroup; X^2= 0.31, p= NS). Furthermore, no differences were found in daily dose of steroids, or in duration of therapy. Also regarding other drug therapy, no differences were found between both subgroups (data not shown).

Muscle metabolites in IMP+ and IMP- patient subgroups

Muscle metabolites were compared in both patient subgroups (Table 4). No differences in ATP, ADP, AMP or TAN were found between the two subgroups. The ATP/ADP ratio was lower in the IMP+ compared with the IMP- subgroup (mean difference 0.38; 95% CI 0.02-0.73). Although PC, C and Ctot did not differ, the PC/C ratio was significantly lower in the IMP+ patients (mean difference 0.28; 95% CI 0.13-0.43). Muscle glycogen (mean difference 50; 95% CI 2-97 mmol glycosyl units/kg dry weight) and glucose (mean difference 3.0; 95% CI 1.0-4.9 mmol/kg dry weight) were higher in the latter group. However, no significant differences in muscle glycogen were found comparing both patient subgroups separately with the healthy control subjects.

Table 4 - Muscle metabolites in COPD patients; comparison based on the presence of detectable IMP

COPD subgroups	IMP+ (n= 25)	IMP- (n= 9)
ATP	17.7 (2.8)	17.1 (3.9)
ADP	3.2 (0.5)	2.9 (0.6)
AMP	0.11 (0.03)	0.10 (0.05)
TAN	21.0 (3.2)	21.3 (5.7)
ATP/ADP	5.5 (0.5)*	5.9 (0.4)
PC	79.2 (16.5)	80.1 (17.0)
C	46.1 (10.7)	40.0 (7.0)
Ctot	125 (25)	120 (24)
PC/C	1.7 (0.2)†	2.0 (0.2)
glycogen‡	277 (66)*	227 (55)
glucose	6.5 (3.5)†	3.6 (1.8)

ATP adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, TAN = ATP+ADP+AMP, PC phosphocreatine, C creatine, Ctot = PC+C
Values are mean (SD) and are expressed in mmol/kg dry weight, unless otherwise indicated
* $p < 0.05$, † $p < 0.01$,
‡ Glycogen is expressed in mmol glycosyl units/kg dry weight

In the IMP+ patients, a significant negative correlation was found between IMP levels and the ATP/ADP ratio (r= -0.63, p= 0.001) (Figure 1). Correlations between IMP and PC, PC/C ratio, PaO_2, and DL_{CO} did not reach the level of significance.

Discussion

In the present study, elevated muscular IMP levels and decreased ATP/ADP and PC/C ratios were found in patients with stable COPD compared with healthy control subjects under resting conditions. ATP, ADP, AMP, and TAN did not differ between patients and control subjects. Within the patient group, two subgroups could be distinguished: a subgroup with muscular IMP content ≥ 0.06 mmol/kg dry weight (IMP+ patients) and a subgroup with an IMP content < 0.06 mmol/kg dry weight (i.e., below the detection level) (IMP- patients). This division seemed relevant since only one of eight control subjects also had a measurable IMP content. In the IMP+ patients lower ATP/ADP and PC/C ratios were found compared with IMP- patients. Clinically, IMP+ patients differed from IMP- patients, in that they had a significantly lower DL_{CO}.
IMP and ammonia (NH_3) are produced during deamination of AMP (AMP ---> IMP + NH_3). A proportion of the IMP produced, is degraded to inosine which can be further

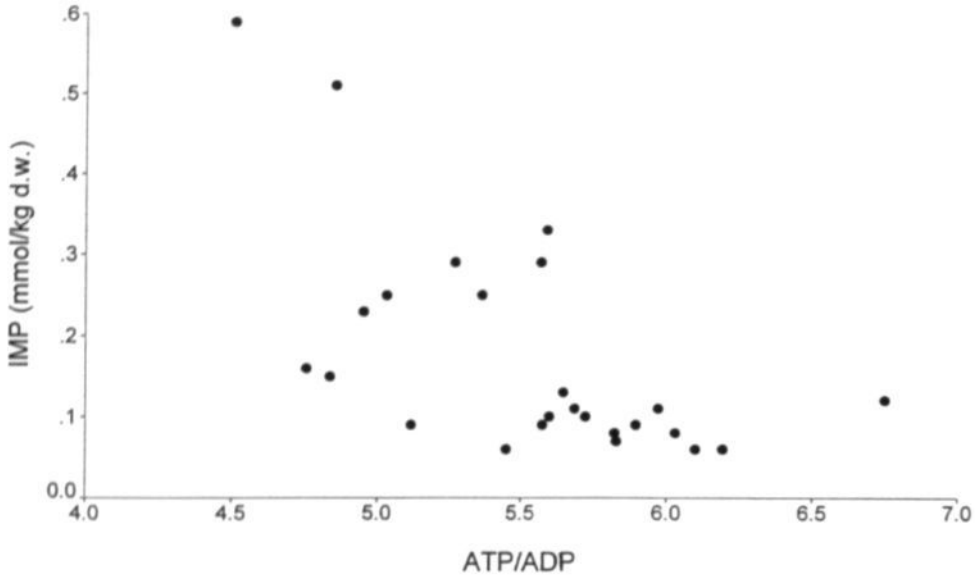

Figure 1. The relationship between ATP/ADP ratio and IMP levels (mmol/kg dry weight muscle) in 25 COPD patients with detectable IMP in resting muscle (r= -0.63, p= 0.001).

catabolized to hypoxanthine and eventually to uric acid. However, IMP can also be reaminated via the purine nucleotide cycle, eventually resulting in ATP resynthesis. AMP is mainly formed by the myokinase reaction, which also produces ATP (2ADP ---> AMP + ATP). AMP formation occurs in times of metabolic stress, when the mitochondrial process for rephosphorylating ADP is unable to maintain a high ATP/ADP level. The preservation of a high ATP/ADP ratio is imperative for adequate cellular function. To ensure an ongoing myokinase reaction in times of metabolic stress, AMP is deaminated to IMP, as outlined above.

In healthy subjects, IMP content in resting muscle is very low. Elevated IMP levels are only found during high intensity exercise (75% of peak oxygen uptake) (9). Several studies in healthy subjects indicated that special conditions, such as hypoxemia (19) or initial shortage of muscular glycogen, induce enhanced IMP accumulation during exercise (20). It is generally believed that, in these acute situations, IMP formation reflects an imbalance between ATP utilization and resynthesis.

In the present study, we hypothesized, based on the previously observed changes in aerobic and glycolytic capacity (7,8), that in patients with stable COPD alterations might occur in muscular high energy phosphate concentrations. Furthermore, we assumed that IMP measurements would enable us to detect subtle changes in muscular energy balance. Indeed, in this study, elevated IMP levels were found that were associated with lower ATP/ADP ratios. The finding of elevated IMP content in resting muscle, is to our knowledge, unique. The observed negative relationship between IMP levels and ATP/ADP ratios seems to be in line with other studies where a direct stimulation of AMP deaminase by low ATP/ADP ratios was suggested (21,22).

Furthermore, this negative relationship suggests that the elevated IMP levels are associated with an imbalance in ATP utilization and resynthesis. Because of the observed relationship between IMP content and ATP/ADP ratio, other explanations for the enhanced IMP content, such as disturbances in pathways catabolizing IMP (such as degradation of IMP to inosine and further) or disturbances in the purine nucleotide cycle (where IMP is reaminated to AMP), seem less relevant.

An imbalance between ATP utilisation and resynthesis can be caused by enhanced ATP utilisation, decreased ATP resynthesis or both. Earlier studies by our group and others, have shown that a significant proportion of COPD patients have increased resting energy expenditure (REE) (23). The cause of this abnormality is incompletely understood. This increased REE might reflect enhanced ATP utilisation in rest. In COPD patients a decreased ATP resynthesis may occur because of hypoxemia or a decreased substrate availability. In our study resting PaO_2 did not differ in IMP+ compared to IMP- patients. However, IMP+ patients had a lower DL_{CO}, and a decreased DL_{CO} is frequently associated with desaturation during exercise (24). Therefore, intermittent hypoxemia might play a role in the observed enhanced IMP content in COPD patients. Regarding the influence of substrate availability on IMP formation, several studies suggested increased IMP formation during exercise in glycogen depleted muscle (20,25). However, during resting conditions, IMP levels were not elevated in glycogen depleted muscle of healthy volunteers (25). In any case, because in our study glycogen was even elevated in resting muscle of IMP+ patients compared to IMP- patients, it was clear that glycogen depletion did not play a role in the elevated IMP levels in COPD patients. The observed higher muscle glycogen and free glucose content in IMP+ patients compared to IMP- patients suggests differences in glucose handling of the skeletal muscle cells between both patient subgroups, the nature of which is presently unknown.

Since medication, physical activity and nutritional status may influence muscle function and metabolism, special attention has been paid to these variables. More than half of the patients used oral corticosteroids as a maintenance treatment. However, no differences were found between the two subgroups of patients in relative number of patients using corticosteroids, daily dose of corticosteroids or duration of treatment. Also, no differences were found between the two patients subgroups in use of other maintenance treatment. All patients were fully ambulatory and there were no apparent differences in training status between both patients subgroups. Nevertheless, 12 minute walking distance tended to be lower in the IMP+ patients, suggesting a functional difference between IMP+ and IMP- patients. Regarding nutritional status, the BMI was significantly lower in the COPD patients compared to the control subjects. Furthermore, there was a trend toward a lower BMI in IMP+ patients compared to IMP- patients, but information on body composition would be essential to fully appreciate these data.

Biopsies were obtained from the tibialis muscle using a conchotome. However, in all the studies quoted here, biopsies were taken from the quadriceps femoris muscle, which has a different fibre type distribution compared to the anterior tibialis muscle (40% and 70% type I fibres, respectively) (26). In healthy subjects during exercise, higher IMP formation has been found in type II fibres compared to type I fibres (9). Therefore, if quadriceps femoris muscle would have been used in this study, results might have been different.

In two other studies high energy phosphate compounds were investigated in stable COPD patients (27,28). In accordance with our study, Möller et al (27) found a decreased ATP/ADP ratio in COPD patients. However, in contrast with our study, both Möller and Jacobsson et al (28) found decreased ATP and glycogen content in resting muscle in COPD patients. Differences in patient selection regarding resting PaO_2, nutritional status and use of corticosteroids might have attributed to these discrepancies, as it is known that ATP levels might be decreased in acute hypoxemia (29), while glycogen concentration might be decreased due to malnutrion (30) and increased during use of corticosteroids (31). Further cause for the discrepancies between the studies may include the fact that both Jakobsson and Möller used quadriceps femoris muscle instead of anterior tibialis muscle.

In summary, in stable COPD patients elevated muscular IMP content was found under resting conditions. IMP levels were negatively related to the ATP/ADP ratio, suggesting an imbalance between ATP utilisation and resynthesis. The fact that these abnormalities were already found under resting conditions, suggests that muscular energy status in COPD patients might even be more compromised during exercise. The cause and consequences of these disturbances in muscle energy metabolism in COPD patients need further exploration.

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CHAPTER 4

Muscle metabolic status in patients with severe COPD with and without longterm prednisolone

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Abstract

Both abnormalities in high energy phosphate metabolism and decreased oxidative enzyme capacity have been reported in skeletal muscle of stable COPD patients. The first aim of this study was to investigate whether these findings are present in anterior tibialis muscle and whether or not they are associated. Abnormalities in mitochondrial structure and function as well as signs of myopathy have been found during corticosteroid treatment. The second aim of this study, therefore, was to investigate whether in COPD patients prolonged use of low dose prednisolone has effects on muscle energy metabolism and qualitative morphology. In a cross-sectional study 15 COPD patients (FEV_1 33 (9) % predicted) who never received maintenance treatment with glucocorticoids (CORT-) were compared with 10 healthy control subjects (HC) and with 14 COPD patients (FEV_1 30 (11) % predicted), which were using oral prednisolone for at least 1 year (CORT+). It was found that ATP/ADP was lower in CORT- compared to HC (5.7 versus 6.2, $p = 0.03$). Inosine monophosphate was detected in 13 of 15 CORT- compared to 3 of 10 HC ($p = 0.004$). However, although indications were found for an imbalance in production and utilization of ATP, comparing CORT- and HC, no differences in oxidative (citrate synthase and 3-hydroxy-acyl-CoA dehydrogenase) and glycolytic (hexokinase, lactate dehydrogenase and phosphophructokinase) enzyme capacities were found. Secondly, comparing CORT+ and CORT- patient subgroups, no differences in the abovementioned parameters of muscle energy metabolism and of muscle qualitative morphology were found.

Introduction

Exercise intolerance and dyspnoea are the most frequently occurring complaints in patients with severe COPD. Weakness of both skeletal and respiratory muscles contributes significantly to these complaints (1). Recently it has been shown that muscle weakness in COPD patients can predominantly be explained by muscle atrophy (2). However, several studies suggested that exercise tolerance might also be impaired by alterations in muscle energy metabolism (3,4,5).

Experimental evidence is accumulating that in COPD patients muscle energy metabolism is already disturbed at rest. Jakobsson and coworkers (6) found a decreased oxidative capacity and an increased glycolytic capacity in quadriceps femoris muscle. Maltais and associates (3) reported a decreased oxidative capacity in quadriceps femoris muscle. In a recent study by our own group (4), in tibialis anterior muscle, indications for a disbalance in adenosine triphosphate (ATP) utilization and resynthesis were found, as suggested by increased inosine monophosphate (IMP) levels, which were negatively related with ATP/ adenosine diphoshate (ADP) ratios. However, in that study oxidative and glycolytic enzyme capacities were not investigated.

In view of the abovementioned findings in skeletal muscle of COPD patients, it might be hypothesized that the observed disturbances in high energy phosphate metabolism in the tibialis anterior muscle are associated with decreased oxidative enzyme capacities. Therefore, the first aim of our study was to investigate both high energy phates and metabolic enzyme capacities in anterior tibialis muscle comparing stable severe COPD patients with healthy control subjects.

In experimental animal studies indications have been found that glucocorticosteroids may affect muscle energy metabolism. Several studies found alterations in muscular mitochondrial structure, including enlargement (8), aggregation and vacuolation (7,8) of mitochondria. Furthermore, indications for impaired muscle glycogenolysis (9) and increased glycogen synthesis (10,11) have been provided. However, virtually no information is available regarding muscle energy metabolism in humans using long term corticosteroid therapy.

In a recent human study histologic abnormalities were observed in biopsies of quadriceps femoris muscle of patients with severe steroid induced myopathy (12). Alterations in qualitative morphology were found, including increased variation of fiber diameters, increased numbers of central nuclei and increased amount of connective tissue in between muscle fibers. In the abovementioned study the doses of corticosteroids administered were quite high. No studies are available investigating muscle morphology in COPD patients using longterm low dose corticosteroids.

It might be hypothesized that treatment with longterm low dose prednisolone leads to altered muscle enzyme capacities, increased glycogen levels and increased occurrence of myopathic features. Therefore, the second aim of this study was to investigate parameters of muscle energy metabolism and muscle qualitative morphology comparing stable severe COPD patients who never received maintenance treatment with oral glucocorticosteroids with COPD patients using low dose oral prednisolone for more than one year.

Methods

Subjects

Fifteen COPD patients who never received maintenance treatment with oral corticosteroids (CORT-) were compared with 10 healthy age-matched volunteers. CORT- patients were also compared with 14 COPD patients who were using oral prednisolone for at least 1 year (CORT+). None of the patients received steroid burst regimens within 3 months prior to the study. Part of the data was reported in an earlier study (4), involving a larger patient group. All patients had COPD (13) and had a forced expiratory volume in one second (FEV_1) expressed as a percentage of predicted of less than 50%. Exclusion criteria were a history of cardiac failure, distal arteriopathy, malignancy, endocrine, hepatic or renal disease or use of anticoagulant drugs. All patients were clinically stable at the time of the study. Written informed consent was obtained and the study was approved by the Medical Ethical Board of the University Hospital Maastricht.

Corticosteroid and concomitant treatment

Daily dose and duration of prednisolone treatment were obtained using a standardized patient questionnaire. The number of steroid burst regimens used in the year prior to examination was noted. All data were checked in available hospital files. COPD patients were treated with inhaled beta-2-agonists (n= 29), inhaled anticholinergics (n= 24), inhaled corticosteroids (n= 25), and oral theophylline (n= 22). None of the patients was using more than 1200µg inhaled steroids per day.

Collection and analysis of muscle biopsies

After an overnight fast muscle biopsies were obtained under resting conditions, while the subjects were breathing room air. Under local anaesthesia, biopsies were taken from anterior tibialis muscle, using a conchotome (14). Samples used for determination of parameters of muscle energy metabolism were immediately frozen in liquid nitrogen and stored at -80°C. The average weight of individual muscle samples was 26 (5) mg. After freeze-drying adherent blood and connective tissue were removed. ATP, ADP, adenosine monophosphate (AMP) and IMP were determined with a high performance liquid chromatographic technique (15). The detection level for IMP was 0.06 mmol/kg dry weight. Phosphocreatine (PC) and creatine (C) were measured fluorometrically (16). Glycogen was determined measuring its glucose residues (16). The values obtained were corrected for the amount of free glucose present at the time of tissue sampling. Oxidative enzymes were represented by citrate synthase (CS), which is responsible for the entry of acetyl CoA into the citric acid cycle and 3-hydroxyacyl-CoA dehydrogenase (HAD) which regulates the beta-oxidation of fatty acids. Glycolytic enzymes were represented by hexokinase (HK), which is involved in glucose phosphorylation, phosphofructokinase (PFK), which is a rate limiting enzyme in glycolysis and lactate dehydrogenase (LDH), which is involved in lactate metabolism. To determine maximal enzyme activities, the frozen tissue sample was homogenized in a SET

buffer, containing (mmol/L): sucrose (25), EDTA (2), TRIS (10, pH 7.4). CS (17), HAD (18), HK (19), PFK (20) and LDH (21) activities were assessed using a Cobas-BIO autoanalyzer (Roche, Basle, Switzerland). Protein content in the homogenates was measured with the micro-BCA method of Pierce (Rockford, IL, USA). For histologic examination each biopsy was oriented longitudinally and was rapidly frozen in isopentane. Samples were stored at -80°C. Cross sections were cut at 8 µm by use of a cryostat kept at -20C. Sections were stained with haematoxylin-eosin and were studied for myopathic changes (22) by a blinded pathologist. The sections were qualitatively scored for presence or absence of the following morphologic features: increased numbers of central nuclei, increased variation of muscle fiber diameters, increased numbers of split fibers, increased numbers of vacuoles, increased numbers of abnormal mitochondria, and increased amount of connective tissue.

Pulmonary function tests

FEV_1 and the forced vital capacity (FVC) were measured (Masterlab, Jaeger, Wurzburg, Germany) until 3 reproducible recordings were obtained. Highest values were used for analysis. Diffusion capacity for carbon monoxide (DL_{CO}) was measured by the single-breath carbon monoxide method (Masterlab Transfer, Jaeger). Total lung capacity (TLC) and residual volume (RV) were measured by body plethysmography (Masterlab Body, Jaeger). Values were expressed as a percentage of reference values (23). Blood was drawn from the radial artery while subjects were breathing room air. Blood gases were measured using a blood gas analyzer (ABL 330, Radiometer, Copenhagen, Denmark).

Anthropometric measures and maximal mouth pressures

Body weight (BW) and height (Ht) were measured standing barefoot. Body mass index (BMI) was computed dividing BW by Ht^2. Fat-free mass (FFM) was determined with a deuterium dilution method (24). FFM index (FFMI) was computed dividing FFM by Ht^2. Fat mass (FM) was computed subtracting FFM from BW. FM was expressed as a percentage of BW. Respiratory muscle strength was determined by maximal inspiratory and expiratory mouth pressures (Pi-max, Pe-max) (25).

Calculations and statistical analysis

Because protein content tended to be lower in CORT+ compared to CORT- patients and control subjects, muscle enzymatic activity was related to protein content. Total adenine nucleotides (TAN) was computed adding ATP, ADP and AMP. Total muscle creatine (Ctot) was computed adding PC and C. On analysis of qualitative morphology presence of a morphologic feature was scored 1 and absence was scored 0. Total amount of morphologic abnormalities was computed adding scores of individual morphologic features. Comparisons between groups were performed using the Mann Whitney-U test. Frequency data were compared using the Chi-square test. Linear regression analysis was used to study relationships between parameters. Significance was determined at the 5% level. Statistical analyses were performed using the SPSS for Windows Statistical Package.

Results

Subject characteristics

Pulmonary function, anthropometric measures and maximal mouth pressures are listed in table 1. CORT- patients had severe airflow obstruction, marked air trapping, moderate hyperinflation, reduced DL_{CO} and slightly reduced values of arterial oxygen with, on average, normocapnia. In the control group, all lung function parameters were in the normal range. Compared with the controls, in CORT- patients the BMI was significantly lower because of a significantly decreased FFMI, whereas the FM was not different from that of the controls. Both Pi-max and Pe-max were decreased in CORT- patients compared with controls. No differences in lung function parameters, anthropometric measures and maximal mouth pressures were found between CORT- and CORT+ patients.

Medical treatment

CORT+ patients were using mean (SD) 7.4 (4.8), ranging from 5 to 17.5 mg prednisolone per day as maintenance treatment. They were using this treatment for 4.8 (5.7), ranging from 1 to 20 years. CORT+ and CORT- patients were treated

Table 1 - Characteristics of COPD patients and healthy controls

		Controls (n= 10)	CORT- (n= 15)	CORT+ (n= 14)
Age	(yr)	66 (8)	65 (6)	66 (8)
FEV_1	(%)	112 (9)	33 (9)*	30 (11)
FVC	(%)	114 (7)	86 (18)*	79 (20)
DL_{CO}	(%)	115 (22)	58 (26)*	57 (36)
RV	(%)	113 (14)	202 (60)*	209 (78)
TLC	(%)	109 (5)	125 (21)*	129 (20)
PaO_2	(kPa)	11.4 (1.5)	9.4 (1.0)*	9.1 (1.0)
$PaCO_2$	(kPa)	4.6 (0.5)	5.2 (0.6)*	5.6 (0.7)
BMI	(kg/m^2)	25.9 (2.2)	22.1 (4.3)*	23.3 (3.0)
FFMI	(kg/m^2)	19.5 (1.2)	15.9 (1.9)*	16.2 (1.8)
FM	(%)	24 (5)	27 (9)	30 (7)
Pi-max	(cmH_2O)	-102 (21)	-74 (23)*	-69 (24)
Pe-max	(cmH_2O)	101 (19)	83 (17)*	85 (19)

CORT- COPD patients not using oral corticosteroids, CORT+ COPD patients using prednisolone, BMI body mass index, FFMI fat-free mass index, FM fat mass as a percentage of body weight, Pi-max maximal inspiratory mouth pressure, Pe-max maximal expiratory mouth pressure. Data are mean (SD). * $p < 0.05$ compared to controls. FFMI and FM are measured in 8 controls, 10 CORT- and 10 CORT+ patients.

Table 2 - Muscle high energy phosphates and related compounds

	Controls (n= 10)	CORT- (n= 15)	CORT+ (n= 14)
ATP	19.2 (3.5)	18.7 (2.5)	16.6 (3.5)
ADP	3.1 (0.5)	3.3 (0.5)	3.0 (0.6)
AMP	0.09 (0.03)	0.12 (0.03)	0.10 (0.03)
ATP/ADP	6.2 (0.6)	5.7 (0.5)*	5.6 (0.5)
TAN	22.4 (3.9)	22.2 (3.0)	19.7 (4.1)
PC	72 (10)	80 (13)	76 (19)
C	36 (7)	45 (8)*	42 (10)
PC/C	2.0 (0.2)	1.8 (0.2)	1.8 (0.4)
Ctot	108 (16)	125 (20)	118 (27)
IMP yes/no	3/7	13/2*	11/3
	0.08 (0.02)	0.14 (0.08)*	0.16 (0.13)
Glycogen	286 (88)	268 (64)	249 (60)

CORT- COPD patients not using oral corticosteroids, CORT+ COPD patients using prednisolone, ATP adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, TAN= ATP+ADP+AMP, PC phosphocreatine, C creatine, Ctot= PC+C, IMP inosine monophosphate. Values are mean (SD) and are expressed as mmol/kg dry weight. Glycogen is expressed as mmol glycosyl units/kg dry weight. * $p < 0.05$ compared to controls.

Table 3 - Muscle enzyme capacities related to muscle protein content

	Controls (n= 10)	CORT- (n= 14)	CORT+ (n= 12)
CS	58 (14)	69 (16)	71 (17)
HAD	38 (12)	41 (19)	40 (17)
HK	3.3 (1.8)	3.0 (1.5)	3.1 (1.3)
PFK	242 (114)	274 (85)	260 (161)
LDH	465 (218)	464 (173)	413 (181)
Protein	151 (30)	141 (31)	128 (35)

CORT- COPD patients not using oral corticosteroids, CORT+ COPD patients using prednisolone, CS citrate synthase, HAD 3-hydroxyacyl-CoA dehydrogenase, HK hexokinase, PFK phosphofructokinase, LDH lactate dehydrogenase. Data are mean (SD) in U/g protein. Protein is expressed as mg/g wet weight.

Table 4 - Abnormalities in qualitative muscle morphology

		Controls (n= 10)	CORT- (n= 14)	CORT+ (n= 11)
Increased numbers of central nuclei		60%	36%	46%
Increased variation in muscle fiber diameters		30%	27%	9%
Increased numbers of split fibers		10%	7%	9%
Increased numbers of vacuoles		10%	0%	0%
Increased numbers of abnormal mitochondria		0%	0%	0%
Increased amount of connective tissue		0%	0%	0%
Sum of all myopathic features	0	30%	57%	55%
	1	30%	29%	27%
	2	40%	7%	18%
	3	0%	7%	0%

CORT- COPD patients not using oral corticosteroids, CORT+ COPD patients using prednisolone

with 2.0 (1.6) (range 0 to 5) and 1.4 (1.1) (range 0 to 3) steroid burst regimens in the preceding year, respectively (NS). Both patient subgroups did not differ in concomitant treatment.

High energy phosphates and related compounds

Values of muscle metabolites are summarised in table 2. No significant differences were found between the CORT- patient subgroup and the control group in ATP, ADP, AMP, or TAN. However, CORT- patients had significantly lower ATP/ADP ratios compared to the healthy control group. IMP could be detected in 13 of 15 CORT- patients compared with 3 of 10 control subjects (X^2= 8.4, p= 0.004). The mean IMP level in the 13 CORT- patients with detectable IMP was 0.14 (0.08), ranging from 0.06 to 0.29 mmol/kg dry weight. The mean IMP level of the 3 control subjects with detectable IMP was 0.08 (0.02), ranging from 0.06 to 0.09 mmol/kg dry weight. In CORT- patients with detectable IMP a significant correlation was found between IMP levels and the ATP/ADP ratio (r= -0.72, p= 0.006). C was significantly higher in CORT- patients compared to controls. Comparing CORT- and CORT+ patient subgroups, no significant differences were found in muscle high energy phosphates or glycogen.

Muscle metabolic enzyme activity

Muscle enzyme activities are listed in table 3. No significant differences in oxidative or glycolytic enzyme activities were found between the CORT- patient subgroup and the control group. In CORT- patients and controls a positive correlation was

found between CS and HAD enzyme capacities (r= 0.68, p= 0.001). No significant relationships were found between enzyme capacities and muscle high energy phosphate contents. Comparing CORT- and CORT+ patient subgroups no differences were found in muscle enzyme activities.

Qualitative morphology in muscle fibers

Qualitative morphologic changes are listed in table 4. In our subjects the sum of all morphologic abnormalities varied between 0 and 3. No significant differences in the occurrence of individual morphologic changes reflecting myopathic alterations, or in the sum of all morphologic changes, were found between the CORT- patient subgroup and the control group. Also comparing CORT- and CORT+ patient subgroups, no differences in qualitative muscle morphology were found.

Discussion

In the present study, in anterior tibialis muscle, oxidative and glycolytic enzyme capacities were not different in CORT- patients and healthy controls. These findings are in accordance with Gea, who did not find abnormalities in enzyme capacities in deltoid muscle (26). However, in studies examining quadriceps femoris muscle, a decreased oxidative capacity as measured by CS (3,6) and HAD (6) and an increased glycolytic capacity as measured by PFK (6) were found. Because patient characteristics seem comparable, other factors seem to be more important in causing the discrepancies between the studies. In the abovementioned studies, 3 different muscles were investigated, which differ greatly in structure and function, tibialis anterior muscle being primarily a postural muscle containing a high percentage of slow, type I muscle fibers (70%), deltoid muscle having both tonic and phasic activity and containing 50 to 60% type I muscle fibers and quadriceps femoris muscle being primarily a locomotor muscle containing only 40% type I muscle fibers (27). In COPD patients both systemic factors such as hypoxia and nutritional depletion and local factors, such as activity level might influence muscle enzyme capacities. At present it is unknown to what extent muscles of different fiber type distribution and function are affected by the combination of these factors. As for the latter factor, it is known that complete immobilisation causes a greater decrease in oxidative capacity in predominantly type I muscle, compared to predominantly type II muscles (28). On the other hand it is probable that the relative inactivity (but not complete immobilisation) that frequently occurs in severe COPD patients affects a locomotor muscle to a greater extent than a non-locomotor muscle. This latter phenomenon might explain the fact that decreased oxidative capacity has been found in quadriceps femoris muscle, but not in deltoid or tibialis anterior muscle.

In CORT- patients compared with the control group elevated IMP levels and decreased ATP/ADP ratios were found in resting anterior tibialis muscle. Furthermore, IMP levels were inversely related with ATP/ADP ratios. These findings have been reported earlier in a larger patient group (4). Based on these findings, it was hypothesized that in COPD patients, an imbalance in ATP utilization and resynthesis

already exists in resting anterior tibialis muscle. It is noteworthy that signs of imbalance in ATP resynthesis and utilization can be found, without accompanying changes in the capacities of the most important oxidative and glycolytic enzymes. This suggests that other factors play a role in the observed disturbances in muscle high energy phosphate metabolism.

The effect of low dose maintenance treatment with oral prednisolone on parameters of muscle energy metabolism was evaluated comparing CORT- and CORT+ patient subgroups. No differences in muscle high energy phosphates levels were found. Based on electron microscopic studies reporting structural changes in mitochondria, it was hypothesized that oxidative metabolism might be impaired during use of corticosteroids. However, in the present study no differences in oxidative and glycolytic enzyme capacities were found between patient subgroups. In accordance with our findings, in rat muscle, no alterations of CS and PFK capacity were found during prolonged use of prednisolone (29). In contrast to our findings, in patients with rheumatoid arthritis using longterm prednisolone (7.5-10mg), decreased CS and HAD capacities were found in quadriceps femoris muscle (30). However, in the latter study only 37.5% type I fibers were found. Because it is unlikely that this decrease was solely caused by the use of corticosteroids, this finding suggests that other factors, such as inactivity might have contributed. In most experimental studies evaluating corticosteroid induced changes in muscle metabolism, elevated muscle glycogen levels were found (9,10,29). This finding has been confirmed in a human study by Fernandez-Sola (11), examining quadriceps femoris muscle of patients with bronchial asthma on chronic treatment with prednisolone (mean daily dose 17.3 mg). Therefore, it is notable that in the present study, no differences in muscle glycogen levels were found comparing CORT- and CORT+ patients. Discrepancies between the abovementioned studies and ours might have been caused by the fact that in the former studies higher dosages of corticosteroids were used. Furthermore, in experimental studies steroid-induced changes were found to be more extensive in type II compared to type I muscle. Therefore, the effect of corticosteroids on anterior tibialis muscle might be less than expected in muscles containing more type II fibers.

In agreement with Fernandez-Sola (11), but in contrast to Decramer (12), in the present study, no differences in parameters of qualitative muscle morphology were found between CORT- and CORT+ patients. Although comparable qualitative morphologic features were assessed, our study cannot readily be compared with the latter study, in which a higher dose of corticosteroids was used (14.2 mg methylprednisolone) and a predominantly type II muscle was analyzed. Furthermore, the subgroup of patients in Decramer's study that showed myopathic morphological features in the muscle biopsy, suffered from severe skeletal and respiratory muscle weakness. Unfortunately, in the present study, skeletal muscle function was not assessed. However, mouth pressures were not different between our patient subgroups. In the present study, some morphologic abnormalities were also found in healthy control subjects. Because an increased number of central nuclei can also be found near a myotendinous junction (22), one might speculate that this phenomenon be partly explained by the location of the sample site. However, because care was taken to obtain biopsies from the belly of the muscle and because no associated increased amount of fibre splitting was present, this explanation seems improbable.

In conclusion, in tibialis anterior muscle of stable COPD patients, no alterations in oxidative and glycolytic enzyme capacities were found, despite of the fact that indications were found for an imbalance between the utilization and resynthesis of muscle ATP. Furthermore, comparing COPD patients who never used maintenance treatment with glucocorticosteroids, with COPD patients using maintenance treatment with low dose prednisolone, no effect of this treatment on the evaluated parameters of muscle energy metabolism and on qualitative muscle morphology was found.

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CHAPTER 5

Plasma and muscle amino acid levels in relation to resting energy expenditure and inflammation in stable COPD

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Abstract

In COPD patients, muscle wasting can occur independently of fat loss, suggesting disturbances in protein metabolism. In order to provide more insight in amino acid metabolism in patients with stable COPD, arterial plasma and anterior tibialis muscle amino acid levels were examined, comparing 12 COPD patients with 8 age-matched healthy control subjects. Furthermore, relationships between amino acid levels, the acute phase response as measured by lipopolysaccharide binding protein (LBP) and resting energy expenditure (REE) were studied. In contrast to findings in acute diseases associated with muscle wasting, in our patient group elevated muscle glutamine (GLN) levels were found (mean (SEM)) (10782 (770) vs 7844 (293) μmol/kg wet weight, $p< 0.01$). Furthermore, muscle arginine, ornithine and citrulline were significantly increased in the patient group, while glutamic acid was decreased. In plasma, the sum of all amino acids (SumAA) was decreased in the patient group (2595 (65) vs 2894 (66) μmol/L, $p< 0.01$), largely because of decreased levels of alanine (254 (10) vs 375 (25) μmol/L, $p< 0.0001$), GLN (580 (17) vs 641 (17) μmol/L, $p< 0.05$) and glutamic acid (91 (5) vs 130 (10) μmol/L, $p< 0.01$). LBP levels were increased in COPD patients (11.7 (4.5) vs 8.6 (1.0) mg/L, $p< 0.05$) and showed a positive correlation with REE ($r= 0.49$, $p= 0.03$), a negative correlation with the SumAA in plasma ($r= -0.76$, $p< 0.0001$), and no correlation with muscle amino acid levels. In conclusion, various disturbances in plasma and muscle amino acid levels were found in COPD patients. A relationship between the observed decreased plasma amino acid levels and inflammation was suggested.

Introduction

Weight loss and muscle wasting occur frequently in COPD patients. Recently, low body weight has been shown to be a negative prognostic factor independent of impaired lung function (1). Furthermore, muscle wasting contributes to muscle weakness and an impaired exercise tolerance in COPD (2,3).
Muscle wasting can occur as part of overall body weight loss, but can also occur isolated in normal weight COPD patients (4). This finding suggests that, besides a negative energy balance, also disturbances in the intermediary metabolism play a role in the development of muscle wasting. Because amino acids are the currency of the intermediary metabolism, analysis of amino acid metabolism seems of interest in COPD patients. Therefore, the first aim of our study was to investigate possible alterations in plasma and muscle amino acid levels in COPD patients as compared with healthy age-matched control subjects.
In a recent study (5), selective depletion of muscle mass was found in stable hypermetabolic COPD patients, who exhibited an acute phase response. This finding suggests that a catabolic response may be present in this subgroup of patients. Therefore, the second aim of our study was to investigate the relationships between plasma and muscle amino acid levels, resting energy expenditure (REE) and the acute phase response as measured by lipopolysaccharide binding protein (LBP).

Methods

Subjects

Twelve patients (mean (SEM) age 66 (2) years) and 8 healthy age-matched volunteers, aged 64 (3) years were included into the study. All patients had COPD according to the criteria of the American Thoracic Society (6) and had a forced expiratory volume in one second (FEV_1) expressed as a percentage of predicted of less than 50%. Because gender differences have been observed in plasma amino acid concentrations (7), only male subjects were included in this study. Patients who demonstrated an increase in FEV_1 of more than 10% of the predicted value after inhalation of the bronchodilator terbutaline (500 μg) were excluded. Other exclusion criteria were: a history of cardiac failure, distal arteriopathy, malignancy, endocrine, hepatic or renal disease or use of anticoagulant drugs. Patients who were using systemic corticosteroids within 3 months of the investigation were also excluded from the study. The patients were clinically stable at the time of the study, defined as an absence of infection or exacerbation of their disease at least 6 weeks prior to the investigation. All patients used inhalation therapy: beta-2-agonists (n= 12), anticholinergics (n= 9) and steroids (n= 10). Eleven patients used theophylline and 4 patients used acetylcysteine. Written informed consent was obtained and the study was approved by the Medical Ethical Board of the University Hospital Maastricht.

Collection and analysis of blood samples

Fasting arterial blood was obtained by puncture of the radialis artery while the subjects were breathing room air. One sample was used for blood gas analysis (ABL 330, Radiometer, Copenhagen, Denmark). The second arterial sample was used for determination of amino acids and was immediately put on ice. Within 15 minutes, centrifugation was performed at 4°C during 5 minutes. After centrifugation, 100 µl of plasma was deproteinized with 4 mg sulfosalicylic acid. Samples were frozen in liquid nitrogen and stored at -80°C until analysis. Amino acids were determined by a fully automated high performance liquid chromatography system as described previously (8). A venous blood sample was drawn from a major draining vein in the cubital fossa for measurement of LBP levels. Samples were collected in evacuated blood collection tubes (Sherwood Medical, St Louis, Missouri, USA) containing 50 units of heparin. Plasma was separated from blood cells by centrifugation at 1000g for five minutes within one hour after collection and plasma samples were stored at -20°C until analysis. LBP levels were measured by ELISA (9). Polyclonal rabbit anti-rh LBP IgG was used as coating for the LBP ELISA and biotin-labelled polyclonal rabbit anti-rh LBP IgG was used for detection of LBP. The standard used was rh LBP. Washing and dilution was performed in buffer containing 40 mM MgCl to prevent disturbance by lipopolysaccharide of LBP recovery in the ELISA. The detection limit of the ELISA was 200 pg/ml.

Collection and analysis of muscle biopsies

After an overnight fast, muscle biopsies were obtained under resting conditions, while the subjects were breathing room air. As described elsewhere (10), under local anaesthesia, the biopsies were taken from the anterior tibialis muscle, using a conchotome. A conchotome is a forceps, primarily used for nasal surgery (10). All biopsies were immediately frozen in liquid nitrogen and stored at -80°C until analysis. To prepare the biopsies for amino acid determination, the frozen tissue was homogenized and deproteinized using a Mini-beater (Biospec products, Bartlesville, U.S.A). Approximately 25 mg tissue was added to 250 µl SSA plus glass beads (1mm). This was put in the mini-beater for 30 seconds. The homogenate was frozen in liquid nitrogen and stored at -80°C until further determinations of the supernatant (8). All determinations of amino acid concentrations in the supernatant were performed in one batch.

Pulmonary function tests

The FEV_1 and the forced vital capacity (FVC) were measured using a pneumotachograph of a constant volume plethysmograph (Masterlab, Jaeger, Wurzburg, Germany) until 3 reproducible recordings were obtained. Highest values were used for analysis. The diffusion capacity for carbon monoxide (DL_{co}) was measured by the single breath carbon monoxide method (Masterlab, Transfer, Jaeger). Total lung capacity (TLC) and residual volume (RV), were measured by body plethysmography (Masterlab body, Jaeger). All values were expressed as a percentage of reference values (11).

Metabolic measures

Body height (Ht) was measured standing barefoot and determined to the nearest 0.5 cm. Body weight (BW) was measured with a beam scale without shoes in light clothing and determined to the nearest 0.1 kg. Body mass index (BMI) was computed dividing BW by Ht^2. Fat-free mass (FFM) was determined by the deuterium dilution method according to the Maastricht protocol (12). Fat mass (FM) was computed by subtracting FFM from BW. Fat-free mass index (FFMI) and fat mass index (FMI) were computed dividing FFM and FM by Ht^2. REE was measured after an overnight fast under standardized conditions (13) by indirect calorimetry using a ventilated hood (Oxycon Beta, Mijnhardt, Bunnik, The Netherlands).

Study protocol

Blood samples and muscle biopsies were obtained on the same day, between 9 and 11 hours in the morning. REE, FFM, BW and lung function tests were obtained within one week after taking the muscle biopsy.

Calculations and statistical analysis

The sum of all amino acids (SumAA) and the sum of all essential amino acids (SumEAA) were calculated by adding the concentration of all the individual amino acids and by adding the concentration of all the essential amino acids, respectively. The sum of all non-essential amino acids (SumNEAA) was calculated by subtracting SumEAA from SumAA. The Student's t-test was used for comparisons between patient and control groups. In cases in which the normality hypothesis was not fulfilled, nonparametric analysis was chosen. Pearson correlation coefficients were calculated for LBP levels, REE/FFM and plasma and muscle amino acids concentrations. Following the simple correlations a linear model was fitted to the data to enable the variables that contributed to the plasma amino acid concentrations to be determined by stepwise regression analysis. Statistical analysis was performed using the SPSS for Windows Statistical Package (14). Significance was determined at the 5% level.

Results

Subject characteristics

Pulmonary function and metabolic measures are listed in table 1 and 2, respectively. Patients had severe airflow obstruction, marked air trapping, moderate hyperinflation, reduced DL_{CO} and slightly reduced values of arterial oxygen tension in the presence of normocapnia. In the control group all lung function parameters were in the normal range. The BMI was significantly lower in the patient group, due to a significantly decreased FFMI, while the FMI was not different. Both REE/FFM and plasma LBP levels were elevated in the patient group. A positive correlation was found between LBP and REE/FFM ($r= 0.49$, $p= 0.03$).

Table 1 - Subject characteristics

		COPD (n= 12)	Controls (n= 8)
Age	(yr)	66 (2)	64 (3)
FEV_1	(%)	32 (2)*	113 (3)
FVC	(%)	83 (5)*	116 (2)
DL_{CO}	(%)	58 (7)*	118 (7)
RV	(%)	216 (19)*	110 (5)
TLC	(%)	129 (6)*	108 (2)
PaO_2	(kPa)	9.2 (0.4)*	11.6 (0.4)
	(mmHg)	69 (3)*	87 (3)
$PaCO_2$	(kPa)	5.3 (0.2)*	4.6 (0.2)
	(mmHg)	40 (1.6)*	34 (1.3)

Data are expressed as mean (SEM)
* significantly different from controls.

Table 2 - Body composition, REE and LBP

		COPD (n= 12)	Controls (n= 8)	p value
Weight	(kg)	63.1 (3.2)	82.6 (2.6)	<0.0001
Height	(cm)	172 (2)	179 (2)	<0.05
BMI	(kg/m^2)	21.5 (1.3)	25.9 (0.7)	<0.05
FFMI	(kg/m^2)	15.9 (0.6)	19.5 (0.4)	<0.0001
FMI	(kg/m^2)	5.8 (0.9)	6.3 (0.5)	NS
REE/FFM	(cal/day.g)	33.5 (0.9)	28.3 (0.8)	<0.01
LBP	(mg/L)	11.7 (1.4)	8.6 (0.3)	<0.05

Data are expressed as mean (SEM). BMI body mass index, FFMI fat free mass index, FMI fat mass index, REE/FFM resting energy metabolism adjusted for FFM, LBP lipopolysaccharide binding protein.

Table 3 - Amino acids concentrations in arterial blood

	COPD (n= 12)	Controls (n= 8)	p value
Glutamic acid	91 (5)	130 (10)	<0.01
Asparagine	48 (2)	58 (4)	<0.05
Serine	120 (5)	121 (5)	NS
Glutamine	580 (17)	641 (17)	<0.05
Histidine	79 (2)	88 (3)	NS
Glycine	242 (11)	253 (20)	NS
Threonine	127 (8)	134 (8)	NS
Citrulline	54 (2)	48 (2)	NS
Arginine	90 (4)	90 (5)	NS
Alanine	254 (10)	375 (25)	<0.0001
Taurine	57 (3)	55 (5)	NS
Tyrosine	56 (3)	56 (3)	NS
Valine	219 (10)	241 (14)	NS
Methionine	26 (1)	28 (1)	NS
Isoleucine	63 (4)	69 (4)	NS
Phenylalanine	53 (1)	53 (1)	NS
Tryptophane	41 (1)	43 (1)	NS
Leucine	122 (6)	136 (5)	NS
Ornithine	74 (6)	61 (2)	NS
Lysine	179 (9)	194 (14)	NS
SumAA	2595 (65)	2894 (66)	<0.01
SumEAA	911 (32)	985 (35)	NS
SumNEAA	1684 (41)	1910 (42)	<0.001

Data are expressed as mean (SEM) in µmol/L. SumAA sum of all amino acids, SumEAA sum of all essential amino acids, SumNEAA sum of all non-essential amino acids

Amino acid concentrations in arterial blood

The total amount of all plasma amino acids was decreased in the patient group, largely due to decreased concentrations of the non-essential amino acids alanine (ALA), glutamine (GLN), glutamic acid (GLU) and asparagine (ASN) (Table 3). In the overall study population (patients and controls) a negative correlation was found between LBP and SumAA ($r= -0.76$, $p< 0.0001$) (figure 1). When excluding the outlier on the right of figure 1, the correlation between LBP and SumAA was -0.48 ($p= 0.04$). Furthermore, in the overall population, negative correlations between LBP and ALA ($r= -0.54$, $p= 0.02$), GLN ($r= -0.65$, $p= 0.003$) and ASN ($r= -0.52$, $p= 0.02$) were found. REE/FFM was found to relate inversely with SumAA ($r= -0.59$, $p= 0.008$), ALA (-0.51, $p= 0.03$) and GLN ($r= -0.63$, $p= 0.004$). On multiple regression analysis

Table 4 - Muscle amino acid concentrations

	COPD (n= 12)	Controls (n= 8)	p value
Glutamic acid	1988 (107)	2610 (197)	<0.01
Asparagine	142 (8)	143 (14)	NS
Serine	352 (24)	281 (18)	NS
Glutamine	10782 (770)	7844 (293)	<0.01
Histidine	232 (16)	197 (8)	NS
Glycine	762 (49)	634 (33)	NS
Threonine	499 (34)	494 (21)	NS
Citrulline	121 (15)	62 (15)	<0.05
Arginine	341 (37)	198 (27)	<0.05
Alanine	1134 (66)	1254 (113)	NS
Taurine	14830 (1200)	15235 (3537)	)NS
Tyrosine	57 (3)	56 (3)	NS
Valine	179 (7)	179 (13)	NS
Methionine	29 (3)	26 (3)	NS
Isoleucine	51 (3)	50 (4)	NS
Phenylalanine	52 (3)	48 (3)	NS
Tryptophane	13 (1)	12 (1)	NS
Leucine	104 (5)	113 (7)	NS
Ornithine	154 (23)	86 (4)	<0.01
Lysine	536 (70)	350 (44)	NS
SumAA	32399 (1741)	29922 (3295)	NS
SumEAA	1695 (114)	1469 (91)	NS
SumNEAA	30705 (1653)	28454 (3301)	NS

Data are expressed as mean (SEM) in µmol/kg wet weight. SumAA sum of all amino acids, SumEAA sum of all essential amino acids, SumNEAA sum of all non-essential amino acids

including all subjects, LBP levels explained 55% of the variation in SumAA and 25% of the variation in ALA, respectively, while REE/FFM was not selected as an independent factor. Furthermore, 39% of the variation in GLN was explained by LBP levels, and 49% of the variation in GLN was explained when REE/FFM ($p< 0.05$) was also added to the analysis.

Muscle amino acid concentrations

Muscle GLN, arginine (ARG), ornithine (ORN), and citrulline (CIT) concentrations were all significantly increased in the patient group. These increases amounted to 137, 172, 179 and 195% of the control values, respectively. Muscle GLU concentration was significantly decreased to 76% of the control value (Table 4). No significant correlations between LBP or REE/FFM and muscle amino acid levels were found.

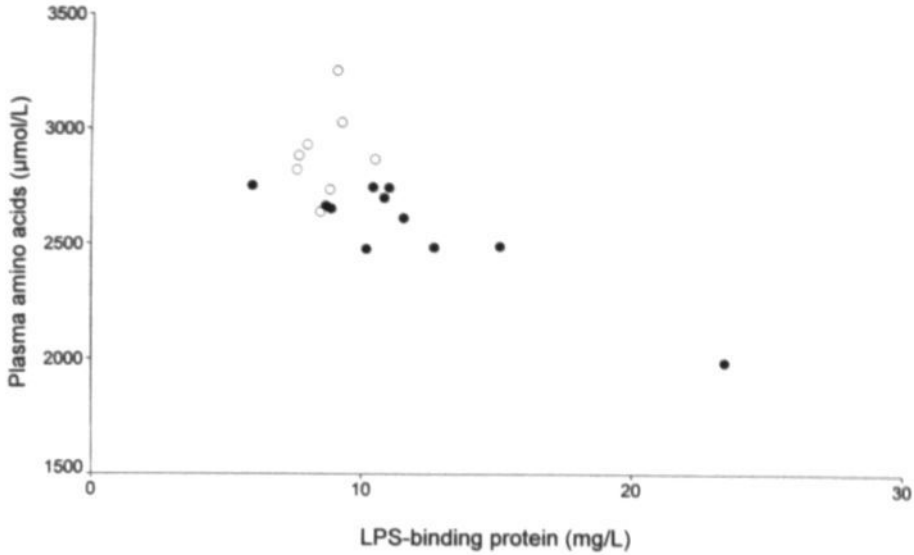

Figure 1. The relationship between lipopolysaccharide (LPS) binding protein and the sum of all plasma amino acids in stable COPD patients (closed circles) and healthy control subjects (open circles) ($r = -0.76$, $p < 0.0001$).

Discussion

In this study, various disturbances in muscle and plasma amino acid levels were found in COPD patients compared to healthy age-matched control subjects. In muscle, elevated GLN, ARG, ORN and CIT levels were found, while GLU levels were decreased. In plasma, GLN, ALA, GLU and ASN were decreased. Although the patients were clinically stable at the time of the study, an acute phase response as indicated by elevated LBP levels and an elevated REE were found. Plasma amino acid levels were inversely related to LBP levels.

Among the observed disturbances in muscle amino acid levels, the elevated GLN level was most striking. GLN is a special amino acid, in that it is the most abundant in the human body and has the most versatile functions of all amino acids (15). It serves as a non-toxic carrier for NH_3 and is the major fuel for rapidly replicating cells, such as immune cells and enterocytes. A whole body of literature is available describing GLN metabolism in acute disease states associated with muscle wasting, such as injury and sepsis. In these disease states, muscle GLN concentration is decreased (16,17,18,19). Several studies even suggested a relationship between low muscle GLN concentrations and decreased protein synthesis in acute disease states (20). A decreased protein synthesis has indeed been shown previously in patients with emphysema, by Morrison et al (21). Therefore, the present finding of elevated muscle GLN concentrations in COPD patients was quite unexpected. However, as stated before, previous studies dealt with acute disease conditions, while very little is known about the muscle free GLN pool in chronic disease states associated

with muscle wasting. Why muscle GLN concentration is elevated in COPD patients remains unknown but deserves further analysis. Based on the combined finding of an elevated muscle GLN and a decreased plasma GLN concentration resulting in an increased muscle to plasma ratio (data not shown), both an impaired outward GLN transport and an altered intracellular GLN metabolism might be hypothesized. The former suggestion would be in accordance with the finding of Morrison et al (21), of a decreased GLN muscle efflux in COPD patients.

Further disturbances in muscle amino acid levels included decreased GLU and increased ARG, ORN and CIT levels. GLU is a non-essential amino acid which can be synthesized in muscle by combining the carbon skeletons of any two amino acids and can give rise to GLN in the glutamine synthetase reaction. However, the observed increase in GLN is disproportionate to the decrease in GLU levels. Based on an unchanged muscle to plasma ratio (data not shown) GLU membrane transport appears intact. An important function of ARG, ORN and CIT consists of their participation in the urea cycle in which ammonia is disposed of as urea. Increased ammonia production may be hypothesized associated with a previously reported increase in inosine monophosphate in resting muscle of part of the COPD patients (22). However, the urea cycle operates mainly in the liver. Furthermore, these 3 amino acids are normally not metabolized in muscle. Therefore, further studies are needed to explain the substantial increase in intracellular concentrations of these related amino acids.

In arterial blood the total amount of amino acids was decreased due to decreased concentrations of the non-essential amino acids ALA, GLN, GLU and ASN. Circulating amino acids serve as substrates for protein synthesis, gluconeogenesis, ureagenesis and oxidative catabolism. Because of the extensive movement of amino acids between tissues and the plasma compartment, plasma amino acid levels are difficult to interpret. In healthy subjects, plasma amino acid levels remain stable. In general, in acute disease states associated with muscle wasting a plasma hypoaminoacidemia is found which pattern is aspecific (17,23,24). Several studies examined venous amino acid levels in COPD patients (21,25,26). Both Morrison and Schols reported decreased plasma ALA, GLN, GLU, leucine (LEU) and valine levels in COPD patients, while Hofford found normal plasma amino acid levels except for an elevated GLN and a decreased LEU level. Our results in arterial plasma largely confirm the findings of Morrison and Schols in venous plasma. The reason for the discrepancies between the findings of these studies and the findings of Hofford are not immediately clear. Probably these discrepancies are caused by differences in the composition of the patient groups. Overall, the patients in the present study were characterized by a selective depletion of fat free mass, an elevated REE and indications for an inflammatory response. It is not known to what extent patients in the study by Hofford et al met these descriptions.

Because in COPD patients, muscle depletion was found to be associated with hypermetabolism and systemic inflammation (5), in the present study, we aimed to explore relationships between muscle and plasma amino acid levels, REE and the acute phase response as measured by plasma LBP. In our patient group REE was elevated. The occurrence of hypermetabolism in COPD patients is well established (5,13). As mentioned before, evidence was recently presented that in some COPD patients, REE is related to systemic inflammation (5). The presently observed relationship between REE and LBP levels confirm those earlier findings.

LBP is one of the type 1 acute phase proteins and an established marker for the acute phase response (27). In our study, LBP levels were moderately elevated in spite of the fact that all patients showing any sign of infection, such as fever, or increased or purulent sputum production, were excluded from the study. This finding is in accordance with recent studies, which found an acute phase response (5), elevated tumor necrosis factor (TNF) α levels (28) or elevated soluble TNF receptor levels (5) in stable COPD patients without signs of infection.

In animal studies, analyzing acute inflammatory processes, indications have been found that amino acids are redirected from muscle to the liver for acute phase protein synthesis and gluconeogenesis (29,30). Furthermore, GLN released from muscle may be used as fuel for immune cells (31). As mentioned above, in our study, muscle GLN levels were found to be increased in COPD patients as opposed to decreased muscle GLN levels found in acute inflammatory processes. No relationships were found between muscle GLN or other muscle amino acid levels and LBP. On the other hand, fairly consistent negative relationships were found between several plasma amino acid levels and REE and LBP. The observed relationship between arterial plasma GLN levels and REE confirm earlier findings in venous plasma (26). On regression analysis most of the observed relationships were found to be independently determined by LBP levels. Only in the case of GLN an additional independent influence of REE was observed. At this moment, it is unclear how to explain these relationships. Possibly an increased need for amino acids for acute phase protein synthesis and as fuel for immune cells cannot be met by efflux from muscle. Further studies are needed to elucidate the relationship between inflammation and amino acid metabolism and its potential therapeutic implications in COPD patients.

In conclusion, in stable severe COPD patients indications were found for disturbances of the intermediary metabolism as reflected by various alterations in plasma and muscle amino acid levels. In COPD, muscle amino acid alterations follow a different pattern compared to diseases associated with acute muscle wasting. Our findings suggested a relationship between the observed decreased plasma amino acid levels and inflammation.

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CHAPTER 6

An acute phase response is associated with elevated erythrocyte glutathione levels in patients with stable COPD

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Abstract

In stable COPD patients indications have been found for systemic inflammation and oxidative stress. Red blood cells (RBC) can be viewed as circulating antioxidant carriers. The nonenzymatic antioxidant glutathione (GSH) in RBC may be enhanced upon exposure to inflammatory mediators or oxidative stress. In this study the relationship between RBC antioxidant status and systemic inflammation as reflected by an acute phase response, was investigated in stable COPD patients (mean (SD)) (FEV_1%pred 38 (12)). In 28 out of 50 patients detectable C-reactive protein (CRP) levels were found (median 6.5, ranging from 1 to 68 μg/ml). RBC GSH levels were higher in COPD patients with CRP ≥ 10 (CRP+), compared with patients with CRP < 10 μg/l (CRP-) (9.2 (1.6) versus 7.2 (1.6) μmol/g Hb, respectively, p= 0.001). No differences in RBC antioxidant enzyme capacities were found between CRP+ and CRP- patients. In the 28 COPD patients with detectable CRP, RBC GSH levels were significantly related with CRP levels (r= 0.50, p= 0.007). No relationships were found between RBC GSH levels and lung function, use of medication or smoking status. It was hypothesized that the relationship between RBC GSH levels and plasma CRP levels represents an adaptive response of RBC to oxidative stress caused by the inflammatory state.

Introduction

Evidence accumulates that both inflammation and oxidative stress are involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). The ongoing inflammatory process in the lungs seems to be related to an increased influx of lymphocytes and macrophages in both central and peripheral airways, whereas a contributing role of neutrophils is hypothesised in the progression of airflow limitation (1). In addition, enhanced levels of the pro-inflammatory cytokine tumour necrosis factor (TNF)-α and of interleukin (IL)-8 have been observed in the sputum of COPD patients (2). Except for local abnormalities, several indications were found for a systemic inflammatory response in stable COPD. Elevated plasma TNF-α (3) and soluble TNF-α receptor 75 (4) levels were found in subsets of stable COPD patients. Furthermore, in weight-losing COPD patients, LPS stimulated TNF-α production was found to be elevated in monocytes (5). Also, in clinically stable COPD patients, elevated plasma C-reactive protein (CRP) (4) and lipopolysaccharide binding protein (LBP) levels have been found (4,6). These acute phase proteins are produced by the liver after induction by cytokines like IL-6 and TNF-α. They provide an index of systemic inflammatory activity (7).

Oxidative stress occurs when exposure to oxidants exceeds antioxidant capacity. Cigarette smoke and the release of reactive oxygen species (ROS) from circulating neutrophils and macrophages may cause oxidant injury. In smokers and COPD patients, an increased oxidant burden has been demonstrated in airspaces, breath, blood and urine (8). The finding of a fall in plasma antioxidant capacity after acute smoking and during exacerbations of COPD (9) also supports the assumption that oxidative stress reaches the circulation in these patients.

Red blood cells (RBC) are important antioxidant carriers, which provide protection against oxidant attacks both in the lungs (10) and systemically. The major nonenzymatic antioxidant in the RBC is the tripeptide, L-g-glutamyl-L-cysteinylglycine, or glutathione (GSH). RBC further contain high activities of antioxidant enzymes which catalyse reactions in the GSH system such as GSH reductase, GSH peroxidase and GSH transhydrogenase and antioxidant enzymes such as superoxide dismutase (SOD) and catalase.

Indications are found that RBC GSH levels undergo adaptive changes upon exposure to oxidative stress (10,11,12). Furthermore, experimental studies have shown that inflammatory mediators may directly induce increased intracellular GSH levels (13). Therefore, the aim of our study was to investigate the relationship between systemic inflammation, as reflected by an acute phase response, and RBC GSH levels and antioxidant enzyme capacities, in stable COPD patients. Furthermore, the influence of potential confounding circumstances, such as use of medication and smoking status, on RBC antioxidant status was evaluated.

Methods

Patients

A total of 50 patients, 41 men and 9 women, consecutively admitted to a pulmonary rehabilitation centre, were evaluated. All patients had COPD according to the criteria of the American Thoracic Society (14) and were clinically stable at the time of the study. Information regarding smoking and drug use was obtained using a standardized patient questionnaire. Thirteen patients were current smokers, and 34 patients were former smokers. Smoking was not allowed on the morning prior to the blood collection (see below). All patients were treated with inhaled bronchodilators (beta-2 agonists and/or anticholinergics). Twenty-four patients were using oral N-acetylcysteine (NAC) 600 mg once daily, and 3 patients were using NAC 600 mg twice daily. Twenty-three out of 50 patients were treated with inhaled corticosteroids and 25 were treated with oral corticosteroids as maintenance treatment. The mean (SD) daily dose of oral corticosteroids in these 25 patients was 8.0 (4.7) mg. Oral medication was not given on the morning prior to the blood collection. Written informed consent was obtained and the study was approved by the Medical Ethical Board of the University Hospital Maastricht.

Pulmonary function tests

The FEV_1 and the forced vital capacity (FVC) were measured, using the pneumotachograph of a constant volume plethysmograph (Masterlab, Jaeger, Wurzburg, Germany) until 3 reproducible recordings were obtained. Highest values were used for analysis. The diffusion capacity for carbon monoxide (DL_{CO}) was measured by the single-breath carbon monoxide method (Masterlab Transfer, Jaeger). Total lung capacity (TLC) and residual volume (RV) were measured by body plethysmography (Masterlab Body, Jaeger). All values were expressed as a percentage of reference values (15).
Blood was drawn by puncture of the radial artery while subjects were breathing room air. Arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) were measured using a blood gas analyzer (ABL 330, Radiometer, Copenhagen, Denmark).

Anthropometric measures

Body height (Ht) was measured standing barefoot and determined to the nearest 0.5 cm. Body weight (BW) was measured with a beam scale without shoes in light clothing and determined to the nearest 0.1 kg. Body mass index (BMI) was computed dividing BW by Ht^2.

RBC GSH levels and antioxidant enzyme activities

Between 9 and 10 hours in the morning, after an overnight fast, venous blood was collected in EDTA-coated tubes (Sherwood Medical, Balleymoney, N-Ireland). Blood was immediately stored at 4°C after collection and processed within 1 hour.

Subsequently blood was centrifuged for 10 min., 4000 rpm at 4°C. The buffy coat and plasma were removed and RBC were washed with 10mL phosphate buffered saline. Afterwards RBC were centrifuged again for 10 min, 3000 rpm at 4°C. This procedure was repeated 3 times. Subsequently, samples were frozen in aliquots of 1 mL at -70°C until analysis.
Total GSH (reduced plus oxidized) and glutathione S-transferase (GST) were determined as described previously (16,17). Haemoglobin was determined according to the method of van Kampen and Zijlstra (18). Glutathione-peroxidase (GSH-Px), superoxide dismutase (SOD) and glutathione reductase (GR) activity were determined as described by Spooren and Evelo (19). For GSH-Px the selenium dependent form was determined using 1.5 mM H_2O_2 as substrate. Catalase activity was determined as described by Aebi (20) measuring decomposition of H_2O_2 at 240 nm for 30 s.

Plasma C-reactive protein

Concurrently with the collection of blood for measurement of RBC antioxidant status, venous blood was collected for measurement of CRP levels. Evacuated blood collection tubes (Sherwood Medical, St Louis, Missouri, USA) containing 50 units of heparin (Leo Pharmaceutical Products BV, Weesp, The Netherlands) were used for this purpose. Plasma was separated from blood cells by centrifugation at 1000 g for five minutes within one hour after collection. Plasma samples were stored at -20°C until analysis. CRP was measured by turbidimetric analysis.

Calculations and statistical analysis

Differences between groups were tested using the Mann-Whitney U-test. Spearman's correlation coefficients were calculated to test relationships between pairs of continuous variables. To be able to test the influence of dichotomous clinical variables on GSH levels, a linear regression analysis was performed, including both dichotomous variables such as gender, smoking behaviour and use of maintenance treatment with oral NAC or corticosteroids, and continuous variables. Significance was determined at the 5% level. Statistical analyses were performed using the SPSS for Windows Statistical Package.

Results

Subject characteristics

Age, gender, pulmonary function and BMI are listed in table 1. The patients had a severe airflow obstruction, marked air trapping, moderate hyperinflation, reduced DL_{CO} and slightly reduced values of arterial oxygen, with, on average, normocapnia. In 28 out of 50 patients detectable CRP levels were found. In the patients with detectable CRP, the median CRP level was 6.5, ranging from 1 to 68 µg/ml. Twelve patients had a CRP level greater than or equal to 10 µg/ml (CRP+ patients), 38 patients had a CRP level smaller than 10 µg/ml (CRP- patients). Age, gender, pulmonary

Table 1 - Clinical characteristics of stable COPD patients

		CRP- (N= 38)	CRP+ (N= 12)
M/F		32/6	9/3
Age	(yr)	66 (8)	64 (8)
FEV_1	(%)	39 (11)	35 (13)
FVC	(%)	87 (20)	82 (17)
DL_{CO}	(%)	59 (25)	53 (17)
RV	(%)	191 (53)	208 (72)
TLC	(%)	123 (17)	127 (26)
PaO_2	(kPa)	9.6 (1.4)	9.4 (1.8)
$PaCO_2$	(kPa)	5.7 (0.9)	5.8 (0.8)
BMI	(kg/m^2)	23.3 (3.8)	22.6 (3.7)

CRP C-reactive protein, CRP- COPD patients with plasma CRP levels < 10 µg/ml, CRP+ COPD patients with plasma CRP levels ≥ 10 µg/ml, data are mean (SD)

Table 2 - Erythrocyte antioxidant status in stable COPD patients

		CRP- (N= 38)	CRP+ (N= 12)
Total GSH	(µmol/g Hb)	7.2 (1.6)	9.2 (1.6)*
GSH-Px	(U/g Hb)	15.7 (2.5)	16.7 (3.1)
GR	(U/g Hb)	5.8 (0.8)	5.8 (0.8)
GST	(U/g Hb)	3.0 (1.3)	3.1 (1.2)
Catalase	(K/g Hb)	146 (20)	147 (29)
SOD	(U/mg Hb)	4049 (471)	4358 (585)

CRP C-reactive protein, CRP- COPD patients with plasma CRP levels < 10 µg/ml, CRP+ COPD patients with plasma CRP levels ≥ 10 µg/ml, GSH glutathione, GSH-Px glutathione peroxidase, GR glutathione reductase, GST glutathione S-transferase, SOD superoxide dismutase, data are mean (SD), * p= 0.001

function and BMI were not different between CRP+ and CRP- patient subgroups (Table 1). No differences in smoking behaviour, use of oral corticosteroids and use of NAC were found between CRP+ and CRP- patient subgroups: 3 out of 10 CRP+ versus 10 out of 37 CRP- patients were current smokers (X^2= 0.04, NS), 4 out of 11 CRP+ versus 21 out of 36 CRP- patients were using oral corticosteroids (X^2= 1.6, NS) and 5 out of 12 CRP+ versus 22 out of 38 CRP- patients were using NAC (X^2= 0.9, NS).

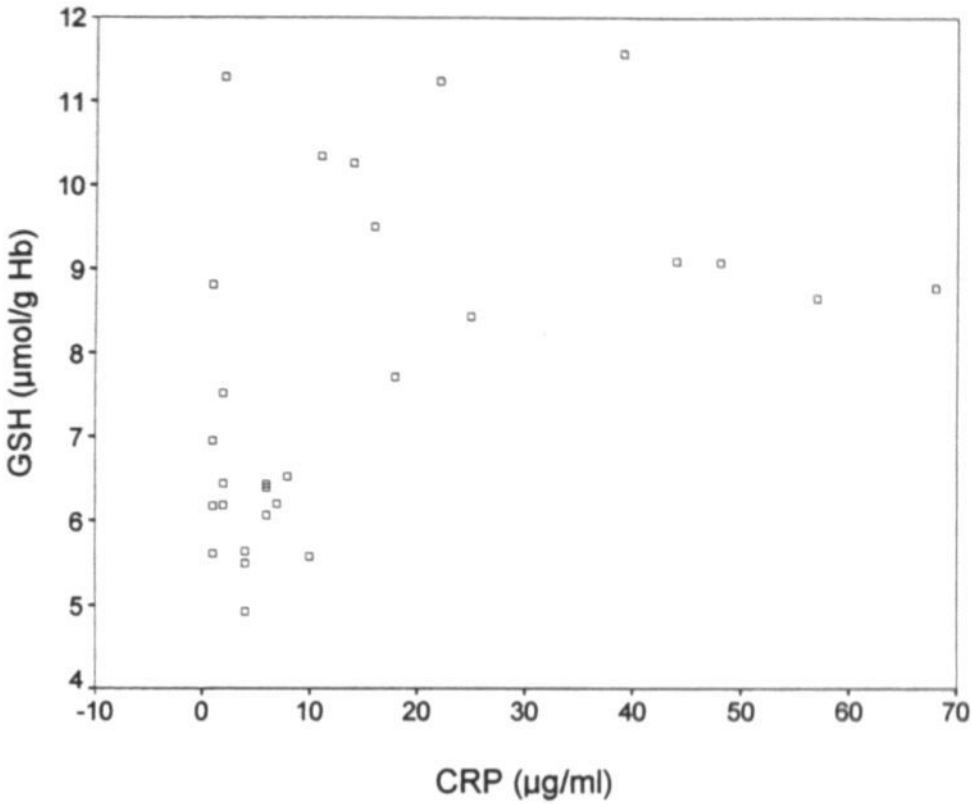

Figure 1. Relationship between plasma levels of C-reactive protein and total glutathione levels in erythrocytes of stable COPD patients with detectable CRP levels (r= 0.50, p= 0.007).

RBC GSH levels and antioxidant enzyme activities

In the whole group mean (SD) erythrocyte GSH levels were 7.5 (1.8) µmol/g Hb. RBC GSH levels were significantly higher in CRP+ COPD patients compared to CRP- patients, 9.2 (1.6) versus 7.2 (1.6) µmol/g Hb respectively (p= 0.001). No differences in antioxidant enzyme activities were found between CRP+ and CRP- patient subgroups (Table 2). Comparing patients using oral corticosteroids with patients not using oral corticosteroids and patients using NAC with patients not using NAC, no differences in RBC antioxidant status were found between patient subgroups (data not shown). Also comparing current smokers with former smokers, no differences in RBC antioxidant status were found (data not shown).

No significant simple relationships were found between RBC GSH levels and age, lung functional parameters or BMI. In the 28 COPD patients with detectable CRP, GSH levels were found to be positively related with CRP levels (r= 0.50, p= 0.007) (Figure 1). On multiple regression analysis, including both dichotomous variables such as gender, smoking behaviour and use of maintenance treatment with oral

NAC or corticosteroids and CRP greater or lower than 10 μg/ml, and continuous variables such as age, BMI, and lung function parameters, the only factor which was independently related to RBC GSH levels, was CRP ≥10 μg/ml ($r = 0.54$, $p < 0.0001$). Including the absolute value of CRP in the regression analysis instead of the factor CRP ≥ 10, absolute CRP levels were also independently related to GSH levels ($r = 0.39$, $p = 0.007$).

Discussion

In the present study, RBC GSH levels were found to be elevated in stable COPD patients who displayed an acute phase response as measured by plasma CRP levels ≥ 10 μg/ml. Furthermore, positive relationships were found between RBC GSH levels and plasma CRP levels, expressed in both absolute and relative terms. No relationships were found between RBC GSH levels and lung functional parameters, use of medication or smoking status. No differences in RBC antioxidant enzyme activities were found between CRP+ and CRP- patient subgroups.

In accordance with an earlier study by Schols and coworkers (4), in the present study moderately elevated CRP levels were found in a subset of clinically stable COPD patients. A hepatic acute phase response in stable COPD patients has found to be associated with several systemic metabolic derangements, including a decreased fat free mass (4), an increased resting energy expenditure (4,6), decreased total plasma amino acid levels (6) and an absence of weight gain following nutritional support (21). Elevated plasma CRP levels have also been found in clinically stable patients with other chronic disorders, such as chronic heart failure. In the latter disorder elevated CRP levels have been associated with poor outcome (22).

In the present study, in COPD patients with low plasma CRP levels, a wide range of RBC GSH levels is found (Figure 1). This is in accordance with data in healthy subjects, showing a large interindividual variation of RBC GSH levels (23). However, in patients with elevated CRP levels, RBC GSH levels are consistently high, while both parameters are positively related. At present it is not possible to fully elucidate the basis for the observed relationship between RBC GSH levels and plasma CRP levels. However, it is thought that inflammatory states are associated with increased oxidative stress (24). Recently, in an in vitro study, a direct effect of TNF-α on intracellular GSH levels has been established (13). Exposure of human alveolar epithelial cells to TNF-α resulted in increased GSH levels concomitant with an increase in gamma-glutamylcysteine synthetase (GCS) activity. GCS is the rate limiting enzyme in GSH synthesis. GCS activity was found to be upregulated through transcriptional induction of the GCS heavy subunit gene (13). In the last decade already, numerous studies reported increased GSH levels in various cell types, in response to other prolonged or repeated oxidant stresses, for instance radiation (25,26). Also in these studies, upregulation of GCS was found. It is thought that the response to repeated oxidative stress differs from the response to an acute oxidant stress, which may temporarily induce GSH depletion (11,13).

In COPD patients, data on GSH metabolism are scarce and difficult to interpret. In induced sputum, increased GSH concentrations were found in COPD patients compared to healthy control subjects (27). In lung epithelium, enhanced expression

of GCS heavy subunit was found in smokers with COPD compared to smokers without COPD (28). These findings may indicate enhanced GSH synthesis as an adaptive mechanism to increased oxidative stress. However, in quadriceps femoris muscle decreased GSH levels were found, which were in turn related to decreased muscle glutamate levels (29).

RBC differ from other cells in that they lack DNA and RNA synthesis as well as oxidative energy metabolism (11). However, also in RBC, GSH levels were found to be increased by repeated oxidative stress. Toth and coworkers found increased RBC GSH levels in chronic smokers compared to nonsmokers. In the latter study, RBC from cigarette smokers provided increased protection to hydrogen peroxide in vitro (10). Furthermore, Siems and coworkers found increased RBC GSH levels after repeated exposure to short-term whole body cold exposure (12).

Based on the abovementioned findings it can be hypothesized that the increase of RBC GSH levels in COPD patients exhibiting an acute phase response, is an adaptive response to oxidative stress caused by the inflammatory state. However, the mechanism described above, of transcriptional induction of the GCS heavy subunit gene, cannot be responsible for the observed increased RBC GSH levels in the present study. Therefore, other mechanisms should be considered, such as increased availability of substrate, diminished feedback inhibition of GCS by GSH or decreased GSH export out of the cell (11). Uptake of intact GSH from the extracellular environment and intracellular degradation of GSH have not been found in RBC (11,30).

Recently, Rahman and coworkers failed to find a relationship between plasma antioxidant capacity and lung function parameters in COPD patients (31). In line with these data, no relationships were found between RBC GSH levels and lung function parameters in the present study.

As mentioned above, in healthy smokers RBC GSH levels were found to be elevated (10). In the present study, this effect of chronic smoking could not be reproduced in COPD patients. In line with the present data, Rahman and coworkers did not find differences in plasma antioxidant status comparing COPD patients who stopped smoking with COPD patients who were still smokers (31). Also in studies investigating RBC antioxidant status in patients with coal miner's pneumoconiosis no such relationship has been found (32).

In the present study, no differences in RBC GSH levels were found between patients subgroups with and without maintenance treatment with oral corticosteroids. Only few studies examined the effect of corticosteroids on GSH metabolism on a cellular level. Recently, in a study by Rahman and coworkers (13), dexamethasone was found to decrease GSH levels in human alveolar cells by downregulating the transcription of the GCS heavy subunit gene. However, as mentioned earlier, such a mechanism cannot exist in RBC because DNA is lacking. Indications have been found that treatment with NAC increases levels of plasma cysteine, which is a precursor of GSH (33). However, it remains to be established if NAC treatment is actually able to elevate intracellular GSH levels (28). In the present study, oral NAC treatment did not result in elevated RBC GSH levels. This finding is in line with a study by Witschi and coworkers in which GSH levels in lymphocytes were not affected in patients with AIDS treated with NAC (34). It should be remarked that the data presented here are cross-sectional. Therefore, randomized trials are

needed to confirm the observed lack of effect of the medication under study on RBC GSH levels.

In conclusion, in stable COPD patients, RBC GSH levels were found to be related to plasma CRP levels. It was hypothesised that this relationship represents an adaptive response of RBC to oxidative stress caused by the inflammatory state.

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CHAPTER 7
General discussion

This thesis describes a range of systemic abnormalities in COPD patients. New findings regarding weight loss, muscle metabolism, systemic inflammation and systemic oxidative stress, as well as therapeutic perspectives are discussed below.

Weight loss and the risk of early readmission in COPD

Low body weight has been identified as an independent risk factor for mortality in COPD (1,2,3) and was found to be related to the risk of hospitalization for acute exacerbation of COPD (4). Furthermore, it has been demonstrated that body weight is an important factor in longterm (survival) (5) outcome of hospital admissions for exacerbations of COPD. The importance of weight loss in the outcome of COPD is underscored by the finding that appropriate therapy can reverse the negative effects of low body weight in some of the patients with COPD (2). At present only little is known about the course of weight changes in COPD, because most studies regarding body weight and body composition were performed in clinically stable COPD patients in cross-sectional analyses. In **chapter 2**, low body weight was identified as an independent risk factor for early nonelective readmission after prior admission for exacerbated COPD. Moreover, nonelective readmission was found to be associated with weight loss during hospitalization. This finding is in line with the finding that weight loss during hospitalization for general medical or surgical diagnoses is associated with early nonelective readmission in elderly patients (6).
Because of its retrospective design, in the present study the cause of weight loss during hospitalization cannot be clarified. It seemed improbable that loss of body fluid contributed to the observed weight loss, because patients with overt oedema were excluded, no diuretics were prescribed and body weight was even further decreased on readmission. In a recent study evaluating nutritional parameters during exacerbated COPD, dietary intake was found to be decreased and energy expenditure was found to be elevated during the first days of hospitalization (7). In the period prior to discharge these parameters ameliorated, resulting in a net restoration of the energy balance and stable body weight. In the latter study, relations between nutritional parameters and outcome of COPD were not investigated. Further studies are warranted, investigating the relationships between energy balance, body composition and both short term outcome of exacerbations COPD, as well as longterm prognosis of COPD.

Muscle energy metabolism in COPD

In COPD patients, in several peripheral muscles, indications have been found for decreased oxidative metabolism at rest as well as during exercise. To investigate disturbances in muscle energy metabolism in COPD patients in more detail, in **chapters 3 and 4**, both high energy phosphate status and oxidative and glycolytic enzyme capacities were investigated in anterior tibialis muscle. In **chapter 4**, no differences in oxidative and glycolytic enzyme capacities were found in anterior tibialis muscle comparing stable COPD patients with healthy control subjects. This

result is in line with findings in deltoid muscle (8), but in contrast with findings in quadriceps femoris muscle of COPD patients (9,10). These discrepancies may be related to differences in muscle function and fiber distribution. Notwithstanding this finding, in **chapter 3**, various disturbances in muscle high energy phosphate levels were found. The ADP/ATP ratio was significantly lower in COPD patients, compared with healthy controls. Although PC levels were unchanged, the PC/C ratio was decreased. Furthermore, in COPD patients, elevated muscle IMP levels were found, which were inversely related with ATP/ADP levels. These results suggest an imbalance between ATP utilisation and synthesis in resting muscle of stable COPD patients.

In healthy subjects, decreased ATP/ADP levels and increased IMP levels in muscle are only observed when they are in metabolic stress and PC concentrations are substantially decreased (lower than 50% of baseline) (11). However, in **chapter 3**, such changes in high energy phosphate levels were already found in resting muscle while PC levels were unchanged. Also in the study by Jacobsson and coworkers, decreased muscle ATP levels were reported in COPD patients, although PC levels were only 20 to 30 % decreased (12). Apparently, in COPD patients, depletion of muscle ATP levels may occur at higher PC levels.

In chapter 1, several factors have been proposed, which may contribute to alterations in muscle energy metabolism. However, the relevance of each of these factors for muscle energy metabolism in COPD has, as yet, not been determined. In a study by Jakobsson, a positive correlation was found between PaO_2 and both muscle PC levels and the proportion of type I muscle fibers (13). However, in the latter study, nearly half of the patients were included because they had chronic hypoxemia. The fact that the reported relationship between PaO_2 and muscle PC levels was not confirmed in our study, may have been caused by these differences in patient selection regarding PaO_2. A decreased diffusion capacity may be associated with intermittent hypoxemia during exercise (14). In **chapter 3**, COPD patients with elevated muscle IMP levels were found to have a lower diffusion capacity. Moreover, diffusion capacity was found to be positively related to the content of slow MHC isoform (15). Taken together, these findings suggest a role for tissue hypoxia. Unfortunately, as for now, tissue hypoxia cannot be measured in a clinically applicable way. Moreover, other factors known to influence tissue oxygen tension, such as haemoglobin concentration, tissue capillarity and myoglobin levels, are largely unstudied in hypoxemic COPD patients.

The effects of another factor, namely oral glucocorticosteroid treatment, on muscle energy metabolism, was investigated in **chapter 4**. Previous studies reported structural alterations in mitochondria (16,17), impaired muscle glycogenolysis (18) and increased glycogen synthesis during use of glucocorticosteroids (19,20). Therefore, an impaired muscle oxidative enzyme capacity and elevated glycogen levels were hypothesized in our study. However, comparing COPD patients who never used maintenance treatment with oral glucocorticosteroids and COPD patients using maintenance treatment with low dose prednisolone, no differences in muscle enzyme capacities, nor muscle glycogen levels were detected. Also, no differences in muscle high energy phosphate levels were found. The lack of changes in the observed parameters might be explained by the fact that lower dosages of corticosteroids were used in the current study. It has been suggested

that corticosteroid-induced changes are more extensive in type II fibers (21,22). Therefore, the effects of corticosteroids on anterior tibialis muscle, containing predominantly type I fibers, might be less than expected in muscles containing more type II fibers.

Not only the mechanisms but also the consequences of the observed alterations in muscle energy metabolism are as yet unclear. Elevated muscle IMP levels related to ATP/ADP ratios (as reported in **chapter 3**) suggest an increased usage of the myokinase pathway in resting muscle in COPD. In healthy subjects similar findings are only observed during metabolic stress, such as intensive exercise (11). Xanthine and hypoxanthine are degradation products of IMP. Degradation of xanthine and hypoxanthine is catalyzed by xanthine oxidase, an enzyme known to generate ROS. Based on these findings it can be hypothesized that muscle xanthine oxidase capacity is increased in COPD patients and contributes to oxidative stress. The finding that allopurinol, a xanthine oxidase inhibitor, attenuates exercise-induced oxidative stress in COPD patients (23), provides indirect support that this pathway is active in COPD patients. Overproduction of ROS in skeletal muscle may impair contractility (24) and may induce tissue damage by lipid and protein oxidation (25). As yet, only limited data are available regarding the effects of the observed alterations in muscle energy metabolism on functional performance. Theoretically, the observed decreased oxidative capacity in limb muscle may lead to decreased endurance of limb muscles. Indeed, the endurance of quadriceps femoris muscle was found to be significantly decreased in COPD patients compared to healthy control subjects (26). However, in the latter study muscle enzyme capacities were not investigated (26). Examining relationships between oxidative enzyme capacities and maximal whole body exercise performance, Maltais and coworkers found a significant association between the capacity of muscle citrate synthase and peak oxygen uptake (27). Moreover, after a 12 week exercise training program, both peak workload, peak oxygen uptake and muscle oxidative enzyme capacities increased (28), whereas a negative relationship was found between changes in oxidative enzyme capacity and arterial lactic acid during exercise. More research is needed to unravel mechanisms of altered muscle energy metabolism and its consequences for exercise performance in COPD patients.

Plasma and muscle amino acids in COPD

In COPD, depletion of FFM may occur independent of loss of FM. This finding suggests that intermediary metabolism is disturbed. In acute disease states associated with muscle wasting, decreased muscle GLN levels are mostly accompanied by decreased GLU levels (29,30,31). Furthermore, it has been suggested that GLN supplementation has an anabolic effect on muscle protein economy (32). Therefore in **chapter 5**, plasma and muscle amino acid levels were investigated, with emphasis on GLN and GLU levels. In arterial plasma, the sum of all amino acids was found to be decreased, largely because of decreased levels of alanine (ALA), GLN and GLU. Plasma amino acid levels are difficult to interpret because the pool of free plasma amino acids is very small compared to the intracellular pool of free amino acids and the protein-bound amino acid pool. Interpretation is further hampered by the fact that amino

acids are subject of extensive inter organ exchanges. However, the presently observed decreased plasma ALA, GLN and GLU levels were confirmed in two (33,34) of four studies investigating plasma amino acids in COPD (33,34,35,36). In the present study, the levels of the sum of all amino acids and ALA and GLN were inversely related to plasma LBP levels. However, in the study by Engelen no indications for an acute phase response were found, as measured by plasma CRP levels (36). In the other studies, no data on inflammatory parameters were available. Therefore, it remains questionable if patient groups were comparable between the studies. Comparison might be further hampered by the fact that in three (34,35,36) of the four studies, patients using oral corticosteroids were not excluded from the study. It is thought that corticosteroids have a profound effect on protein and amino acid levels, as they may induce a negative nitrogen balance and induce a nett amino acid efflux from muscle and influx in other organs (i.e. liver) (22).

Chapter 5 further describes alterations in amino acid levels in tibialis anterior muscle. Compared to healthy control subjects, muscle GLN level was found to be increased and muscle GLU level was found to be decreased. In view of the abovementioned findings in acute disease states associated with muscle wasting (29,30,31), the finding of increased muscle GLN levels was unexpected. Based on the finding of an increased muscle to plasma GLN ratio it can be hypothesized that an impaired outward GLN transport contributes to elevated muscle GLN levels in these patients. This suggestion would be in accordance with the finding of Morrison (33), of a decreased GLN muscle efflux in COPD patients. A second possibility which might contribute to the observed elevated muscle GLN levels, is increased GLN production in the glutamine synthetase reaction: Glutamate + ATP + NH_3 ---> Glutamine + ADP. This mechanism may also contribute to the observed decreased muscle GLU levels. Since the concentration of GLU in muscle is decreased, the most probable reason for increased GLN production by this reaction is an elevated muscular NH_3. Firstly, the NH_3 could arise from the glutamate dehydrogenase reaction: Glutamate + NADP + H_2O ---> 2-oxoglutarate + NADPH + NH_3. However, in the presence of decreased GLU levels, this source of NH_3 formation seems unlikely. Alternatively, the NH_3 could arise from the deamination of AMP: AMP ---> IMP + NH_3. Since IMP levels in tibialis muscle are elevated in this patient group (as described in **chapter 3**) the latter source for NH_3 production seems more probable. In fact, the only other disease state, associated with elevated muscle GLN levels is acute liver failure, in which GLN synthesis is increased due to the presence of hyperammonia (37). A third mechanism which might contribute to the observed elevated muscle GLN level is increased muscular protein degradation or decreased protein synthesis. Likewise opposite changes in protein synthesis and degradation may contribute to decreased muscle GLU levels. Furthermore, changes in intracellular transaminase reactions may contribute to decreased muscle GLU levels. However, in explaining decreased muscle GLU levels, altered GLU membrane transport seems to be of no importance because muscle to plasma ratio for GLU was unchanged.

In a recent study by Engelen and coworkers (36) in COPD patients, decreased GLU levels were also found in quadriceps femoris muscle. In the total group of patients, muscle GLN levels were comparable with healthy controls. However, it is difficult to compare this study with ours because biopsies were taken from a muscle with different fiber type distribution and function and use of oral corticosteroids

was not excluded. As for GLN metabolism, indications have been found that corticosteroids increase muscle glutamine synthetase activity (38) and increase net muscle glutamine release (39). Although both studies are not readily comparable, it is striking that also in the study by Engelen, elevated muscle GLN levels were found in a subgroup of patients, namely patients without significant emphysema.
The abovementioned studies clearly show that the intermediary metabolism is disturbed in resting muscle of COPD patients. Further studies are necessary to investigate the clinical consequences of these findings. In this line, a recent study already pointed out a relationship between decreased muscle GLU levels and decreased muscle GSH, an important antioxidant (40).

Metabolic disturbances in different skeletal muscles in COPD

At present, in COPD, muscle metabolism has been investigated in four different peripheral skeletal muscles: the lower extremity muscles quadriceps femoris (9,10,12,13,41,42) and tibialis anterior (present thesis), and the upper extremity-rib cage muscles biceps (43) and deltoid (8). These four muscles differ substantially in structure and function, quadriceps femoris muscle being primarily a locomotor muscle containing only 40% of slow, type I muscle fibers, tibialis anterior muscle being primarily a postural muscle containing 70% type I muscle fibers, biceps brachii muscle containing 40-50% type I muscle fibers and deltoid muscle containing 50-60% type I muscle fibers (44). As described earlier, quadriceps femoris muscle has been investigated most extensively. Using several techniques, indications for decreased oxidative capacity have been found in this muscle. The proportion (13,42) and cross-sectional area (42) of type I fibers, the proportion of MHC-I isoform (41) and the capacity of oxidative enzymes (9,10) were all found to be decreased. Furthermore, in mildly hypoxemic patients decreased muscle ATP levels were found (12). Also in tibialis anterior muscle (**chapter 3**) indications for a reduced energy charge were found, whereas oxidative and glycolytic enzyme capacities were unchanged. However, the observed elevated IMP levels may be viewed as indicators for an increased anaerobic metabolism in this muscle. In lower extremity calf muscle indications for a decreased oxidative capacity were found during exercise using ^{31}P-MRS (45,46). Using this technique, also in upper extremity muscles indications for a decreased oxidative capacity have been found during exercise (47,48). However, in upper extremity-rib cage muscles unchanged fiber type proportions (8,43) and unchanged oxidative but elevated glycolytic enzyme capacities (8) were found. As for estimates of muscle mass, the cross-sectional areas of both calf muscle (45) and thigh muscle (49) were found to be significantly smaller in COPD patients compared to healthy controls. Furthermore, the cross-sectional area of both type I and II fibers were found to be smaller in biceps muscle of COPD patients (43).
To date, it is questionable if in COPD patients, metabolic disturbances observed in one muscle can be extrapolated to other muscles or if disturbances are muscle specific. Theoretically, it seems probable that disturbances may vary according to muscle function and fiber type distribution. For instance systemic factors such as hypoxia, nutritional depletion, inflammation, aging and medication

may affect all muscles, whereas inactivity may primarily affect muscles with locomotor function. Furthermore, factors may primarily affect type I or type II fibers. For instance it is thought that immobilization primarily affects type I fibers (50) and corticosteroids primarily affect type II fibers (21,22). Aging predominantly affects type II fibers (51). In this respect it is interesting that in contrast to peripheral muscles, a shift from type II fibers to type I fibers has been reported in diaphragm of COPD patients, who were undergoing lung-volume-reduction surgery (52). In COPD the diaphragm is probably not disused and it has been hypothesized that a kind of endurance training effect may contribute to the observed shift from type IIx to type I fibers (53). However, muscle disuse is clearly not the only factor at stake. In a recent study in hamsters, the induction of emphysema was associated with a reduction in the oxidative capacity of several peripheral muscles, but an opposite effect on the diaphragm (54). These differences could not be attributed to reduction in physical activity level, because activity level was documented to be similar in emphysematous and control hamsters.

Systemic inflammation in COPD

Evidence is accumulating that in COPD patients chronic inflammation is not restricted to airways and lung parenchyma, but may also be present systemically. In subsets of stable COPD patients, elevated soluble TNF-α receptor 55 (55) and 75 levels (56), elevated plasma TNF-α levels (57,58) and elevated levels of acute phase proteins CRP (55,56) and LBP (55,56) were found as indications for systemic inflammation. In **chapters 5 and 6**, the finding of elevated plasma acute phase proteins in subsets of clinically stable COPD patients was confirmed.

In COPD patients the presence of a systemic inflammatory response has found to be associated with several metabolic derangements. In hypermetabolic stable COPD patients, plasma CRP and LBP levels were found to be increased compared to normometabolic patients (56), whereas plasma TNF-α levels were found to be related to increased REE (58). Both plasma TNF-α levels (57) and LPS stimulated TNF-α production by monocytes (59) were elevated in weight-losing COPD patients.

Furthermore, systemic inflammation in COPD seems to be related to loss of FFM, in that FFM was decreased in a subset of COPD patients with elevated plasma CRP, LBP, IL-8, and sTNF-R55 and R75 levels (56) and soluble TNF-R55 levels were related with FFM index (55). Moreover, a nonresponse to nutritional support was found to be associated with elevated levels of LBP and sTNF-R55 and sTNF-R75 (60).

In line with the abovementioned results (56,58), in **chapter 5**, plasma LBP levels were found to be positively related to REE. Furthermore, LBP levels were found to be negatively related to the sum of all plasma amino acids. Decreased plasma GLN and ALA levels were primarily responsible for this relationship. Plasma LBP levels were not related to muscle amino acid levels. As yet, it is not exactly clear how to explain the observed relationship. In animal studies of acute inflammatory processes, indications have been found that amino acids are redirected from muscle to the liver for acute phase protein synthesis and gluconeogenesis (61,62). It was hypothesized that the observed decreased concentration of total plasma amino

acids and its relationship with LBP levels be explained by an increased need for amino acids for synthesis of acute phase proteins and for fuel of immune cells, which cannot be met by efflux of amino acids from muscle.
In **chapter 6**, plasma CRP levels were found to be positively related to GSH levels in erythrocytes. It was hypothesised that this relationship represents an adaptive response of erythrocytes to oxidative stress caused by the inflammatory state.
In summary, in subsets of clinically stable COPD patients indications are found for systemic inflammation. This systemic inflammatory state was found to be related with various metabolic alterations. Longitudinal studies are warranted, evaluating systemic inflammatory parameters in relation to parameters of oxidative stress, in stable and exacerbated COPD. Furthermore, research should focus on determining consequences of systemic inflammation in COPD patients regarding muscle mass and strength.

Therapeutic perspectives

Conventional treatment in COPD such as bronchodilator and anti-inflammatory agents aims at improving lung function impairment. Unfortunately, only modest reductions in airway obstruction are achieved. As described earlier, symptoms and prognosis of COPD patients are not only related with local impairment, but also with systemic impairment such as loss of body weight (2,3), lean body mass (63,64), muscle strength (65,66) and muscle metabolic status (27).
This knowledge has urged development of new treatment modalities in COPD. Furthermore, effects of conventional treatment on systemic impairment are now under survey.

Training

In healthy subjects it is obvious that training improves muscle endurance, strength or both, depending of the type of training and provided that the intensity of the training stimulus is adequate. Because it has been suggested that disuse contributes to the observed changes in limb muscle in COPD patients, it has been proposed that training may ameliorate muscle performance in COPD. Indeed, several studies found indications for altered muscle metabolism and performance following programs for endurance or strength training.
Following a lower limb endurance training program, reduced exercise induced lactate acidosis and CO_2 production (67,68) were found. Also, cross-sectional areas of oxidative fibers (42) and oxidative enzyme capacities (28) were found to be increased in quadriceps femoris muscle. Furthermore, both quadriceps femoris muscle endurance (69) and strength (69,70) were found to be increased after multimodality endurance exercise training. Regarding overall exercise capacity it has been found that both submaximal (71,72,73,74) and maximal exercise capacity (72,73) were increased after endurance training in COPD patients. Interval training may resemble the daily life activity pattern in severe COPD patients better than continuous endurance training. However, comparing both training modalities in COPD patients, no significant differences in exercise capacity were reported (75).

Because peripheral muscle weakness contributes to exercise limitation in patients with lung disease (66,76), strength training seems to be a rational component of exercise training in these patients. However, to date, only few studies addressed this training modality in COPD. In a study by Simpson and coworkers (77) a 16 to 40% increase in muscle strength was found after strength training, depending on the muscle group evaluated. The increase in muscle strength was associated with an improvement in endurance to fatigue during cycling at a submaximal level (77). Bernard and coworkers (74) evaluated the effect of addition of strength training to aerobic training. A significantly greater increase in muscle strength and cross-sectional area was found in COPD patients completing the combined aerobic and strength training program compared to patients completing the aerobic training program. However, the addition of strength training did not provide additional improvement in exercise capacity.
As mentioned earlier disuse may lead to increased oxidative stress during exercise. Therefore, theoretically, training may have another positive effect, in that it may improve muscular antioxidant status and thereby diminish oxidative stress during sudden exercise.
Due to the overall favourable results, exercise training has become an essential component of a pulmonary rehabilitation program. Further work is required to assess the role of different exercise programmes and the particular category of patients for whom they might be beneficial.

Oxygen therapy

There is little doubt that acute administration of oxygen improves exercise capacity in COPD patients (78,79). Mechanisms involved, probably include reduced ventilatory requirement (80), a relief of pulmonary vasoconstriction (81) and a reduced feeling of dyspnea (78), the latter being an important determinant of exercise limitation. Regarding energy metabolism in peripheral muscles, indications have been found that acute supply of oxygen during exercise enhances peripheral muscle oxidative metabolism in hypoxemic COPD patients. Using ^{31}P-MRS, Pi/CP, pH and CP recovery were found to improve during acute oxygen suppletion (46).
Long-term oxygen therapy (LTOT) is known to improve survival and quality of life of COPD patients (82,83). Theoretically, LTOT may be able to induce alterations in skeletal muscle metabolism in COPD patients other than the abovementioned temporary changes induced by acute oxygen supply. Mechanisms may include increased physical activity and improved appetite and meal related symptoms during LTOT. Indeed, Jacobsson and coworkers (12) found increased CP/CP+C ratios in 4 hypoxemic COPD patients breathing room air after 8 months of LTOT. However, PaO_2 breathing room air improved in the study period. The latter finding may have contributed to the ameliorated muscle energy index. No changes in muscle glycogen concentrations and oxidative or glycolytic enzyme activities were found by the same group (12). No studies are available on the effects of LTOT on exercise tolerance in patients with resting hypoxemia. However, in normoxemic COPD patients who desaturated during exercise, supplementation of oxygen during the training was not found to augment the effects of training in room air (79).

In summary, the abovementioned findings suggest that LTOT may at most partly reverse intrinsic alterations in muscle metabolism in hypoxemic COPD patients.

Nutrition

As stated in chapter 1, weight loss and low BMI are important determinants of mortality and morbidity in COPD patients. Furthermore, muscle wasting in COPD has found to be related with muscle weakness (49,84), peak exercise capacity (85), and quality of life (63).
Nutritional suppletion aims at increasing body mass, preferably by restoring FFM. Substantial changes in body composition and physiological function can only be expected if the nutritional suppletion offered consists of an adequate energy intake and is actually consumed by the patient. During adequate nutritional suppletion, muscle function has been shown to improve prior to increases in muscle mass (86). Early restoration of muscle electrolyte status may contribute to this finding (86). However, for increases in muscle mass and further improvement of function an anabolic stimulus such as exercise seems necessary.
Several large placebo-controlled studies investigating the efficacy of oral nutritional supplementation in depleted COPD, reported increased body weight and FFM and improved limb and respiratory muscle function (87,88,89). However, not all studies reported favourable effects (90). Discrepancies may partly be caused by failure of the intervention (see above). In studies where adequate nutritional support was given, nonresponse seemed to be related with underlying disease-specific problems. In a recent study, nonresponse to nutritional suppletion was found to be associated with ageing, a lower baseline dietary intake relative to REE and an elevated systemic inflammatory response (60).
The clinical relevance of nonresponse to nutritional support is emphasized by a recent study reporting weight gain during nutritional therapy as a significant, independent predictor of the mortality rate in patients with COPD (2).

Anabolic hormone supplementation

In addition to nutritional supplementation and training, anabolic endocrinological therapeutic options are worth to take into account for the treatment of wasting in COPD. An argument in favour of this treatment might be the fact that low testosterone levels are found in part of the patients with COPD (91).
Anabolic steroids act on muscle by inducing protein anabolic effects mediated by the androgen receptor and by inhibiting protein catabolic processes via interaction with the glucocorticoid receptor (92). In depleted COPD patients, a placebo controlled trial was conducted, evaluating the effects of oral stanazol treatment during 6 months (93). No nutritional suppletion was offered in this trial. An increased body mass and FFM was found, whereas no improvements in respiratory muscle strength or endurance exercise capacity could be detected. Besides this longterm trial evaluating oral anabolic treatment, two short term placebo-controlled trials evaluated the effects of addition of nandrolone decanoate i.m. to nutritional intervention, in COPD patients participating in a pulmonary rehabilitation program (89,94). Patients using nandrolone obtained an enhanced FFM

(89,94), an improved respiratory muscle strength (89,94) and a greater improvement in health status (94) compared to patients merely treated with nutritional support. In the study by Creutzberg and coworkers, treatment with nandrolone appeared to be especially favourable in COPD patients using maintenance treatment with oral corticosteroids. In these patients, not only inspiratory mouth pressures but also exercise capacity improved to a greater extent after treatment with nandrolone compared with placebo (94). It was suggested that the positive effect of nandrolone treatment in patients using corticosteroids could be explained by competitive binding of anabolic steroids to the corticosteroid receptor, neutralizing the deleterious effects of corticosteroids (92).

Growth hormone is known to induce lipolysis, protein anabolism and muscle growth, either directly or via insulin growth factor 1. The effects of administration of recombinant human growth hormone in patients with COPD attending a pulmonary rehabilitation program has been evaluated in two controlled studies (95,96). Although FFM (95,96) increased in the patients using recombinant human growth hormone, no improvement of muscle strength (95) or exercise tolerance (95,96) was found.

In animal studies, beta-2-adrenergic drugs have been shown to induce muscle protein gain, increase fiber size and improve muscle strength (97). Studies in humans reported varying results. In healthy humans, FFM was found to be unchanged, whereas the strength of several limb muscles and inspiratory mouth pressures were found to be increased during treatment with oral salbutamol (98). The effects of inhaled salbutamol on body composition and muscle strength in COPD patients, has only been investigated in a nonrandomized study comparing high and standard dose salbutamol (99). In this study, no differences in anthropometric measures such as triceps skinfold and midarm circumference, and handgrip strength were found between both groups.

Antiinflammatory and antioxidant therapy

As described in chapter 1, COPD is characterized by inflammatory changes in airways and lung parenchyma (100). Furthermore, in subsets of stable COPD patients a systemic inflammatory response is found (56,57,59,101). Therefore, theoretically, antiinflammatory treatment might be of benefit in COPD. In asthma, corticosteroids are remarkably effective in controlling local inflammation and respiratory symptoms. Although many COPD patients are also treated with inhaled or oral corticosteroids, the efficacy of this treatment in COPD is strongly under debate (102,103). In a recent study, in induced sputum of stable COPD patients, no changes in neutrophil counts, granule proteins or inflammatory cytokines after use of inhaled and oral corticosteroids (104). It is as yet unclear if corticosteroids have any effect on the systemic inflammatory response in COPD. However, it is known that oral corticosteroids may produce serious side effects, such as osteoporosis. Furthermore, it is known that high dose corticosteroids may cause muscle weakness (105), caused by muscle atrophy or myopathy. It is not exactly known if these changes are also found during treatment with low dose corticosteroids. Recently, treatment with low dose oral corticosteroids in COPD patients, was found to be associated with a diminished response to pulmonary rehabilitation, with respect to improvement of

respiratory muscle function and exercise capacity (94). Furthermore, in a retrospective study in severe COPD patients, indications were found for a relationship between use of oral corticosteroids and increased mortality (2). However regarding muscle energy metabolism, in the present thesis in **chapter 4**, no alterations in muscle high energy phosphate levels and oxidative and glycolytic enzyme capacities were found in COPD patients using low dose oral prednisolone (101).

In conclusion, in view of the disputable effects and the potentially serious side effects, oral corticosteroids are better avoided in stable COPD and search for better antiinflammatory treatment is warranted. Recently, new antiinflammatory agents have been suggested to be of use in COPD (106). These involve among others, IL-10, transcription factor inhibitors and phosphodiesterase inhibitors. Also antagonists against inflammatory mediators like leukotriene B4, TNF-α and prostaglandins are under investigation (106). Antiinflammatory modulation may also be achieved by use of polyunsaturated fatty acids. Indeed, fish-oil supplementation reduced inflammatory mediators and had an anticachectic effect in patients with cancer (107). Polyunsaturated fatty acids may have additional effects in lung disease in that they may induce a decreased production of bronchoconstrictive leukotrienes by shunting away the eicosanoid production from the arachidonic acid pathway (108). Furthermore, in animals exposed to endotoxins, administration of polyunsaturated fatty acids resulted in decreased lung tromboxane levels and decreased pulmonary vascular resistance (108).

Evidence is increasing that oxidative stress contributes to impairment in muscle function and damage of muscle tissue (109,110). As described earlier, oxidative stress may be of special importance in COPD patients, because unfavourable factors such as disuse, hypoxemia, smoking and systemic inflammation frequently play a role in this patient group. In humans, biochemical markers of exercise-induced oxidative stress were found to be decreased by chronic supplementation with antioxidant nutrients (selenium, vitamin C, vitamin E) (111). However, no improvement of exercise performance was found (112). Recently, the xanthine oxidase inhibitor allopurinol was found to inhibit exercise induced GSH oxidation and lipid peroxidation in COPD patients (23). However, as yet it remains unclear whether allopurinol affects exercise performance and what the effects of chronic administration of allopurinol are. Acute administration of high dose N-acetylcysteine has been shown to inhibit certain types of muscle fatigue in humans (113), but therapeutic relevance may be limited by side effects of the drug and needs to be explored in COPD patients.

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CHAPTER 8
Summary

The last few years it has become clear that in addition to local lung impairment, also systemic disturbances such as weight loss, loss of muscle mass and alterations in muscle metabolism are related to morbidity and mortality of patients with chronic obstructive pulmonary disease (COPD). The presence of these systemic abnormalities is not surprising because in COPD various contributing factors such as hypoxia, inflammation, and oxidative stress, have been identified which have a significant systemic impact. In this thesis several systemic abnormalities and influencing factors were investigated in more detail.

The clinical significance of systemic impairment was described in **chapter 2**. Early nonelective readmission can be regarded as a failure of the previous hospital admission, and therefore as a short-term outcome parameter in exacerbated COPD. In **chapter 2,** risk factors for early nonelective readmission were investigated, with special emphasis on body weight. Two groups of patients were compared: cases readmitted within 2 weeks after discharge and controls not readmitted within 3 months of discharge. Parameters describing morbidity, comorbidity, maintenance treatment and social factors were not different between the two groups. Also, the severity of the exacerbation as assessed with lung function and blood gases did not differ. The two groups only differed from each other with respect to body weight and body weight changes: cases lost weight during hospitalization, while controls remained weight stable. Furthermore, both weight loss during hospitalization and low body weight on admission were found to be associated with early nonelective readmission. These findings provide further support for the concept that nutritional status is related to morbidity in COPD.

In several peripheral muscles of COPD patients, indications have been found for a decreased oxidative metabolism at rest as well as during exercise. Furthermore, decreased oxidative capacity was found to be related to decreased exercise capacity. To investigate skeletal muscle energy metabolism in more detail, in **chapter 3**, high energy phosphate status was analyzed in resting anterior tibialis muscle. Comparing stable COPD patients with healthy control subjects no differences in absolute concentrations of muscle high energy phosphate compounds such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and phosphocreatine (PC) were found. However, ATP/ADP and PC/C ratios were significantly lower in COPD patients. In healthy subjects, muscle concentrations of inosine monophosphate (IMP), a deamination product of AMP, are very low and elevated IMP levels are only found during high intensity exercise. However, in COPD patients, IMP levels were already found to be elevated in resting muscle. Furthermore, IMP levels were found to be inversely related to muscle ATP/ADP ratios. Therefore it was suggested that the results of this study indicate an imbalance between the utilization and resynthesis of ATP in resting muscle of patients with stable COPD. Comparing COPD patients with elevated IMP levels, with COPD patients without detectable IMP levels, diffusion capacity was lower in the former patient subgroup. Because a decreased diffusion capacity may be associated with hypoxemia during exercise, it was suggested that intermittent hypoxemia contributes to the observed enhanced muscle IMP levels in COPD patients.

As stated above, a decreased oxidative capacity has been reported in quadriceps femoris muscle of COPD patients. In contrast to these findings, in **chapter 4**, comparing COPD patients with healthy control subjects, no differences in oxidative and glycolytic enzyme capacities were found in tibialis anterior muscle. It was hypothesized that this discrepancy might be caused by functional differences between tibialis anterior and quadriceps femoris muscle.
Corticosteroids are known to influence muscle protein and amino acid metabolism. Furthermore, it has been suggested that corticosteroids alter mitochondrial metabolism and induce myopathic changes. However, in **chapter 4**, a comparison between COPD patients using maintenance treatment with low dose oral prednisolone, and COPD patients who never used maintenance treatment with glucocorticosteroids, showed no effect of this treatment on parameters of muscle energy metabolism and on qualitative muscle morphology.

In COPD, muscle wasting can occur independently of fat loss, suggesting disturbances in protein metabolism. Because amino acids are the currency of protein metabolism, in **chapter 5**, muscle and plasma amino acid levels were investigated. In an earlier study in COPD patients, fat free mass depletion was found to be associated with hypermetabolism and systemic inflammation. Therefore, also relationships between amino acid levels, and the acute phase response as measured by lipopolysaccharide-binding protein (LBP) levels, and resting energy expenditure were evaluated. In contrast to findings in acute diseases associated with muscle wasting, in stable COPD patients, increased muscle glutamine (GLN) levels were found. Furthermore, muscle glutamate (GLU) levels were decreased. Both an impaired outward GLN transport and altered intracellular GLN metabolism were proposed as mechanisms. Regarding the latter possibility it can be hypothesized that the ammonia (NH_3) needed to produce GLN might come from the AMP deaminase reaction, as IMP levels have been reported to be elevated in tibialis anterior muscle in stable COPD patients (**chapter 3**).
In plasma, the sum of all amino acids was decreased in the patient group, largely because of decreased levels of alanine, GLN and GLU. LBP levels were increased in COPD patients as compared with controls and showed a positive correlation with resting energy expenditure. Furthermore, LBP levels were found to be negatively related to the sum of all plasma amino acids. Decreased plasma GLN and alanine levels were primarily responsible for this relationship. Plasma LBP levels were not related to muscle amino acid levels. As yet, it not exactly clear how to explain the observed relationship. In animal studies of acute inflammatory processes, indications have been found that amino acids are redirected from muscle to the liver for acute phase protein synthesis and gluconeogenesis. It was hypothesized that the observed decreased concentration of total plasma amino acids and its relationship with LBP levels can be explained by an increased need for amino acids for synthesis of acute phase proteins and as fuel for immune cells, which cannot be met by efflux of amino acids from muscle.

Several indications of systemic inflammation have been found in stable COPD patients. Red blood cells (RBC) can be viewed as circulating antioxidant carriers.

The nonenzymatic antioxidant glutathione (GSH) in RBC may be enhanced upon exposure to inflammatory mediators or oxidative stress. In **chapter 6** the relationship between an acute phase response (measured by C-reactive protein (CRP)) and RBC GSH levels was examined in clinically stable COPD patients. In more than half of the COPD patients detectable CRP levels were found. In patients with detectable CRP levels, RBC GSH levels were significantly related with CRP levels. No relationships were found between RBC GSH levels and lung function, use of medication or smoking status. The relationship between RBC GSH levels and plasma CRP levels was interpreted as an adaptive response of RBC to oxidative stress caused by the inflammatory state.

Chapter 7 comprises the general discussion of this thesis, including therapeutic possibilities aiming at systemic impairment in COPD.

CHAPTER 9
Samenvatting

De afgelopen jaren is het duidelijk geworden dat naast lokale pulmonale stoornissen, ook systemische afwijkingen zoals gewichtsverlies, afname van spiermassa en veranderde spierstofwisseling gerelateerd zijn aan de morbiditeit en mortaliteit van patiënten met chronisch obstructief longlijden (COPD). Het is niet onverwacht dat systemische afwijkingen worden gevonden bij patiënten met COPD, omdat bij deze aandoening verschillende factoren, zoals hypoxie, ontsteking en oxidatieve stress een rol spelen, die allen ook systemische effecten hebben. In dit proefschrift werden een aantal systemische afwijkingen en factoren die een rol spelen bij COPD nader onderzocht.

In **hoofdstuk 2** werd het klinisch belang van systemische afwijkingen bij COPD beschreven. Vroege niet-electieve heropnames kunnen beschouwd worden als een ongewenst beloop na de voorafgaande ziekenhuis opname en kunnen daarom gebruikt worden als korte termijns uitkomstparameter van COPD exacerbaties. In **hoofdstuk 2** werden risicofactoren met betrekking tot vroege niet-electieve heropname onderzocht, met speciale aandacht voor het lichaamsgewicht. Er werden twee groepen patiënten met elkaar vergeleken: patiënten die binnen 2 weken na ontslag opnieuw moesten worden opgenomen (cases) en patiënten die minstens 3 maanden na ontslag niet opnieuw opgenomen hoefden te worden (controls). Beide groepen verschilden niet ten aanzien van parameters betreffende morbiditeit, comorbiditeit, onderhoudsbehandeling en sociale factoren. Bovendien bleek de ernst van de exacerbatie, zoals bepaald met longfunctie en bloedgassen niet verschillend. De twee groepen verschilden alleen van elkaar wat betreft lichaamsgewicht en gewichtsveranderingen: de "cases" verloren gewicht tijdens de opname, terwijl het lichaamsgewicht van de "controls" niet veranderde. Bovendien bleken gewichtsverlies tijdens opname en een laag uitgangsgewicht bij opname geassocieerd te zijn met het voorkomen van vroege niet-electieve heropnames. Deze bevindingen ondersteunen het concept dat bij COPD patiënten de voedingsstatus is geassocieerd met de morbiditeit.

Bij onderzoek in verschillende perifere spieren van COPD patiënten zijn aanwijzingen gevonden voor een verminderde oxidatieve stofwisseling, zowel in rust als gedurende inspanning. Deze verminderde oxidatieve capaciteit bleek gerelateerd te zijn aan verminderde inspanningscapaciteit. Om het energie metabolisme in de spier in meer detail te bestuderen, werd in **hoofdstuk 3** de energierijke fosfaat status bepaald in de anterior tibialis spier in rust. Er werden in dit onderzoek geen verschillen gevonden tussen stabiele COPD patiënten en gezonde controle personen voor wat betreft absolute concentraties van energierijke fosfaten, zoals adenosine trifosfaat (ATP), adenosine difosfaat (ADP), adenosine monofosfaat (AMP) en creatine fosfaat (PC) in de spier. De ATP/ADP en PC/C ratios waren wel significant verlaagd bij de patiënten met COPD. Inosine monofosfaat (IMP) wordt geproduceerd tijdens deaminatie van AMP. Bij gezonde personen is de IMP concentratie in rustende spieren altijd erg laag. Verhoogde IMP concentraties worden alleen gevonden tijdens zware inspanning. Opvallend was dat bij de patiënten met COPD al verhoogde IMP concentraties werden gevonden in de rustende spier. Bovendien bleken IMP concentraties omgekeerd evenredig gerelateerd te zijn aan de ATP/ADP ratio in de spier. Op grond van deze resultaten werd

gesuggereerd dat er bij patiënten met stabiel COPD sprake is van onevenwichtigheid tussen verbruik en aanmaak van ATP in de rustende tibialis anterior spier. COPD patiënten met een verhoogde IMP concentratie in de spier bleken, vergeleken met COPD patiënten waarbij geen IMP in de spier kon worden aangetoond, een verminderde diffusie capaciteit voor koolmonoxide te hebben. Omdat een verminderde diffusie capaciteit geassocieerd is met het optreden van hypoxemie tijdens inspanning, werd gesuggereerd dat intermitterend optredende hypoxemie bij zou kunnen dragen aan de verhoogde IMP concentraties in de spier bij COPD patiënten.

Zoals boven vermeld werd in eerder onderzoek bij COPD patiënten een verminderde oxidatieve capaciteit gevonden in de quadriceps femoris spier. In **hoofdstuk 4** werden oxidatieve en glycolytische enzym capaciteiten bepaald in de tibialis anterior spier. De enzym capaciteiten bleken bij stabiele COPD patiënten niet te verschillen van die van gezonde proefpersonen. Het werd verondersteld dat het verschil met eerdere bevindingen werd veroorzaakt door functionele verschillen tussen de tibialis anterior en de quadriceps femoralis spier.
Het is bekend dat corticosteroiden de eiwit en aminozuur stofwisseling beïnvloeden. Bovendien is in eerder onderzoek gesuggereerd dat corticosteroiden invloed kunnen hebben op de mitochondriale stofwisseling en bovendien myopathische veranderingen kunnen induceren. In **hoofdstuk 4** werden daarom twee groepen patiënten met ernstig COPD met elkaar vergeleken: patiënten die een onderhoudsbehandeling met laag gedoseerde orale prednisolon gebruikten en patiënten die nooit een onderhoudsbehandeling met corticosteroiden hebben gebruikt. Het bleek dat een onderhoudsbehandeling met laag gedoseerd prednisolon geen effect had op metabole parameters betreffende de energie stofwisseling in de spier. Ook werd bij COPD patiënten geen effect van deze behandeling op de kwalitatieve morfologie van de spier gevonden.

Onafhankelijk van het feit of er ook sprake is van verlies aan vetmassa, kan zich bij COPD patiënten verlies van spiermassa voor doen. Deze bevinding suggereert een stoornis in de eiwit stofwisseling. In **hoofdstuk 5** werd het aminozuurprofiel in plasma en in de tibialis anterior spier bestudeerd. Uit eerder onderzoek is gebleken dat vermindering van vet-vrije massa bij COPD patiënten is geassocieerd met een verhoogd rustmetabolisme en systemische ontsteking. In verband hiermee werden ook de verbanden tussen aminozuur concentraties, de acute fase respons uitgedrukt als concentratie van het lipopolysaccharide binding protein (LBP), en het energie verbruik in rust onderzocht. In tegenstelling tot bevindingen bij acute ziekten die gepaard gaan met spiermassa verlies, werd bij patiënten met stabiel COPD een verhoogde concentratie glutamine (GLN) in de spier gevonden. De concentratie glutamaat (GLU) in de spier was verlaagd. Zowel een gestoord transport van GLN de cel uit, als een veranderde intracellulaire GLN stofwisseling zouden kunnen bijdragen aan deze bevindingen. Wat betreft de laatste mogelijkheid zou het zo kunnen zijn dat ammoniak nodig om GLN aan te maken, wordt verkregen uit de AMP deaminase reactie. Zoals in **hoofdstuk 3** is beschreven, is het gehalte IMP, dat ook verkregen wordt uit de deaminase reactie, verhoogd bij stabiele COPD patiënten.

In plasma bleek het totale gehalte aan vrije aminozuren verlaagd te zijn. Dit werd vooral veroorzaakt door verlaagde gehaltes aan alanine, GLN en GLU. De LBP concentratie was verhoogd bij stabiele COPD patiënten en was gerelateerd aan het energie verbruik in rust. Bovendien bleken LBP concentraties negatief gerelateerd te zijn aan het totaal gehalte aan vrije aminozuren in plasma. Dit verband bleek voornamelijk bepaald te worden door de verlaagde GLN en alanine concentraties in plasma. De LBP concentraties in plasma bleken niet gerelateerd te zijn aan de aminozuur concentraties in de spier. Het is op dit moment niet geheel duidelijk hoe de gevonden verbanden verklaard kunnen worden. In dieronderzoeken naar acute ontstekingsprocessen zijn aanwijzingen gevonden dat aminozuren vanuit de spier gebruikt worden in de lever voor de aanmaak van acute fase eiwitten en voor gluconeogenese. In **hoofdstuk 5** werd verondersteld dat het gevonden verlaagde totale gehalte aan vrije aminozuren in plasma en het verband met de LBP concentraties verklaard zouden kunnen worden uit een vergrote behoefte aan aminozuren voor aanmaak van acute fase eiwitten, terwijl de afgifte van aminozuren vanuit de spier tekort schiet.

Bij patiënten met stabiel COPD zijn aanwijzingen gevonden dat er sprake kan zijn van systemische ontsteking. Rode bloedcellen kunnen gezien worden als circulerende dragers van antioxidanten. In rode bloedcellen kan de concentratie van het antioxidant glutathion (GSH) toenemen na blootstelling aan ontstekingsmediatoren of aan oxidatieve stress. In **hoofdstuk 6** werd de relatie tussen de acute fase respons (gemeten als de plasma concentratie van C-reactive protein (CRP)) en de concentratie van GSH in de rode bloedcellen onderzocht bij patiënten met stabiel COPD. Bij meer dan de helft van de patiënten met COPD werden aantoonbare CRP concentraties in plasma gevonden. Bij patiënten waarbij aantoonbare plasma CRP gehaltes gevonden werden, bleek de concentratie van GSH in de rode bloedcel gerelateerd te zijn aan het CRP gehalte in het plasma. Er werden geen verbanden gevonden tussen het GSH gehalte in de rode bloedcel en de longfunctie, het gebruik van medicatie of roken. Het gevonden verband tussen het GSH gehalte in de rode bloedcel en de CRP concentratie in plasma werd geïnterpreteerd als een adaptieve respons van de rode bloedcel op oxidatieve stress veroorzaakt door ontsteking.

Hoofdstuk 7 behelst de algemene discussie van dit proefschrift.

Dankwoord

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Curriculum vitae

Ellen Maria Pouw werd geboren op 19 juni 1960 te Rotterdam. In 1978 behaalde zij het eindexamen Gymnasium ß aan de Gemeentelijke Scholengemeenschap in Emmen. Omdat ze aanvankelijk niet werd ingeloot voor de studie Geneeskunde, begon ze met de studie Psychologie en behaalde haar propedeuse in 1979. In 1979 kon ze toch met de studie Geneeskunde aan de Rijksuniversiteit Groningen beginnen. De co-schappen werden doorlopen in ziekenhuis Ziekenzorg in Enschede. Tijdens haar studie deed ze op de afdeling Longziekten van het Academisch Ziekenhuis Groningen onderzoek naar beademing bij patiënten met een status astmaticus (begeleider Prof. Dr. G.H. Koëter). In 1987 behaalde zij haar artsexamen (cum laude). Vervolgens werkte ze een halfjaar als AGNIO op de afdeling Thoraxchirurgie van het Academisch Ziekenhuis Groningen. In het Academisch Medisch Centrum te Amsterdam werkte ze tot 1989 als onderzoekscoördinator van het SGO-CARA project. Vervolgens werkte ze enkele maanden als AGNIO op de afdeling Longziekten van het Academisch Ziekenhuis Maastricht en keerde in september 1989 weer terug in het Academisch Medisch Centrum in Amsterdam om daar te starten met de Interne vooropleiding (opleider Prof. Dr. J. Vreeken). In 1991 startte ze met de opleiding tot longarts in het Academisch Ziekenhuis Maastricht (opleider Prof. Dr. E.F.M. Wouters). In 1993 verkreeg zij een stipendium van ASTRA BV ten behoeve van het verrichten van onderzoek naar het spiermetabolisme bij patiënten met COPD en werd begonnen met het onderzoek dat leidde tot dit proefschrift. Bovendien werkte ze tijdens haar opleiding mee aan een internationaal onderzoek naar primaire pulmonale hypertensie. In 1995 voltooide ze haar opleiding tot longarts en werkte tot 1997 als staflid op de afdeling Longziekten van het Academisch Ziekenhuis Maastricht. Vanaf 1997 werkt ze als staflid op de afdeling Longziekten van het Erasmus Medisch Centrum te Rotterdam, en heeft chronische beademing als aandachtsgebied.