Skeletal muscle characteristics and physical activity patterns in COPD
To my family
To my family

Örebro Studies in Sport Sciences 10

Gabriella Eliason

Skeletal muscle characteristics and physical activity patterns in COPD
Abstract


Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide. Besides abnormities within the respiratory system COPD is also associated with effects outside the lungs, so called systemic effects. One systemic effect that has been highlighted is skeletal muscle dysfunction, which has also been associated with reduced exercise capacity. Apart from changes in muscle morphology, low levels of physical activity has also been suggested as a plausible mediator of reduced exercise capacity in COPD. The aim of this thesis was to study muscle morphology and physical activity patterns in patients with different degrees of COPD and to examine the associations between muscle morphology, physical activity and exercise capacity in these patients.

Skeletal muscle morphology was found to shift towards a more glycolytic muscle profile in COPD patients and changes in muscle morphology were found to be correlated to disease severity and to exercise capacity. Muscle capillarization was also found to be lower in COPD compared with healthy subjects and to be correlated to disease severity and exercise capacity. When studying signalling pathways involved in muscle capillarization, an overexpression of VHL was observed in patients with mild and moderate COPD compared with healthy subjects. Furthermore, COPD patients were found to be less physically active compared with healthy subjects and the level of physical activity was associated with exercise capacity.

In conclusion, changes in skeletal muscle morphology and low levels of physical activity are present in COPD patients and may partly explain the lower exercise capacity observed in these patients. The more glycolytic muscle profile observed in COPD is suggested to be mediated by hypoxia and low levels of physical activity in this patient group. Furthermore, increased levels of VHL may lead to impaired transduction of the hypoxic signalling pathway, which may contribute to the decreased muscle capillarization observed in COPD.
Abstract


Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide. Besides abnormalities within the respiratory system COPD is also associated with effects outside the lungs, so called systemic effects. One systemic effect that has been highlighted is skeletal muscle dysfunction, which has also been associated with reduced exercise capacity. Apart from changes in muscle morphology, low levels of physical activity has also been suggested as a plausible mediator of reduced exercise capacity in COPD. The aim of this thesis was to study muscle morphology and physical activity patterns in patients with different degrees of COPD and to examine the associations between muscle morphology, physical activity and exercise capacity in these patients.

Skeletal muscle morphology was found to shift towards a more glycolytic muscle profile in COPD patients and changes in muscle morphology were found to be correlated to disease severity and to exercise capacity. Muscle capillarization was also found to be lower in COPD compared with healthy subjects and to be correlated to disease severity and exercise capacity. When studying signalling pathways involved in muscle capillarization, an overexpression of VHL was observed in patients with mild and moderate COPD compared with healthy subjects. Furthermore, COPD patients were found to be less physically active compared with healthy subjects and the level of physical activity was associated with exercise capacity.

In conclusion, changes in skeletal muscle morphology and low levels of physical activity are present in COPD patients and may partly explain the lower exercise capacity observed in these patients. The more glycolytic muscle profile observed in COPD is suggested to be mediated by hypoxia and low levels of physical activity in this patient group. Furthermore, increased levels of VHL may lead to impaired transduction of the hypoxic signalling pathway, which may contribute to the decreased muscle capillarization observed in COPD.

Keywords: COPD, muscle morphology, muscle fibre distribution, muscle capillarization, physical activity, von Hippel-Lindau protein, exercise capacity.

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LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

ATP adenosine triphosphate
BMI body mass index
CAF number of capillaries around a single muscle fibre
CAFA number of capillaries around a fibre in relation to fibre area
cDNA complementary deoxyribonucleic acid
C:F capillary-to-fibre ratio
CFPE-index capillary-to-fibre perimeter exchange index
COPD chronic obstructive pulmonary disease
DXA dual x-ray absorptiometry
FFM fat free mass
FFMI fat free mass index
FEV1,0 forced expiratory volume in one second
FVC forced vital capacity
GOLD Global Initiative for Chronic Obstructive Lung Disease
HIF hypoxia-inducible factor
LC/PF-index length of capillary/perimeter of the fibre
mAb monoclonal antibody
MVPA moderately or vigorously physically active
MyHC myosin heavy chain
N-CAM neural cell adhesion molecule
NO nitric oxide
PaCO2 partial arterial pressure for carbon dioxide
PaO2 partial arterial pressure for oxygen
PCR polymerase chain reaction
pVHL von Hippel-Lindau tumour suppressor protein
RNA ribonucleic acid
SF sharing factor
VEGF vascular endothelial growth factor
VO2max maximal oxygen consumption
INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide [43, 72, 112]. The disease is characterised by irreversible progressive airflow limitation with breathing-related symptoms such as coughing, phlegm, and dyspnea [72, 112]. Besides structural and functional abnormalities within the respiratory system, COPD is also associated with effects outside the lungs, so-called systemic effects. One systemic effect that has been highlighted is skeletal muscle dysfunction. Both morphological and functional changes of the muscle have been described [22, 39, 60, 68]. Skeletal muscle dysfunction has also been suggested to play an important role in reducing exercise capacity in patients with COPD [68]. The mechanisms leading to changes in skeletal muscle are not fully understood; however, systemic inflammation, oxidative stress, use of oral corticosteroids, malnutrition, tissue hypoxia and low levels of physical activity have been suggested as potential mediators [4, 22, 60, 68]. The present thesis elucidates changes in skeletal muscle of COPD patients and highlights physical activity and hypoxia as plausible mechanisms involved in these changes.

Skeletal muscle

Skeletal muscle consists of a number of contractile muscle cells (fibres) that join into a tendon at each end. The muscle is surrounded by a sheath of connective tissue, the epimysium, which is also referred to as the fascia. In the muscle, smaller fascicles are made up of bundles of thousands of muscle fibres surrounded by connective tissue, the perimysium. Each individual muscle fibre is also surrounded by a thin layer of connective tissue, the endomysium, which contains a capillary network and nerve fibres [115] (Figure 1).
Figure 1. Overview of skeletal muscle tissue organization. Illustration from Fundamentals of Anatomy & Physiology by Martini (2008) with permission.

**Muscle fibre types**

In human muscle, three major muscle fibres types have been distinguished. The different fibre types express different myosin heavy chain (MyHC) isoforms (I, IIA, IIX) which give the fibres different contractile properties. Type I fibres are referred to as slow twitch fibres, while type IIX are referred to as fast twitch fibres. The different fibre types also have different metabolic properties. Type I fibres are the most oxidative type of fibres and have a rich mitochondria supply and high concentrations of oxidative enzymes. Fast type IIX fibres are the most glycolytic fibre types, relying almost entirely on anaerobic energy metabolism and containing high concentrations of glycolytic enzymes. Type IIa fibres are considered to have both glycolytic and oxidative properties [115]. In addition to the three main fibre groups, there are also so called hybrid fibres expressing more than one MyHC isoform, usually MyHC I/IIa or MyHC IIa/IIX [7, 91-92]. Classification of muscle fibres can be done according to their morphological or metabolic characteristics. Histochemical staining of myosin adenosine triphosphate (ATPase) made it possible to delineate slow type I and fast type II fibres [87]. More recently, type I, type IIa and type IIb (now a days referred to as type IIX) fibres have been distinguished using this method by pre-incubation at different pH values [15]. Immunohistochemical staining, based on the expression of MyHC isoforms, has made it possible to distinguish both major fibre types and hybrid fibres [92].
Skeletal muscle tissue is extremely heterogeneous in regards to fibre type composition. Moreover, muscle fibres are dynamic structures that are capable of changing phenotype under various conditions such as changes in neuromuscular activity, mechanical load, changes in hormonal profile and aging [91]. Regarding the metabolic properties of skeletal muscle, the oxidative capacity of the muscle has been shown to be affected by environmental changes such as hypoxia. It has been suggested that longterm exposure to hypoxia leads to markedly lowered oxidative capacity of the muscle due to decreased mitochondrial volume and reduced oxidative enzyme activity [54, 73].

**Muscle regeneration**

Muscle fibres are postmitotic cells unable to re-enter the cell cycle. In spite of this, the skeletal muscle possesses a remarkable ability to repair and regenerate itself under extraordinary conditions such as extreme physical activity, trauma or injury. The majority of this regeneration is carried out by activation, proliferation and differentiation of satellite cells.

Satellite cells, which were first described by Mauro in 1961 [76], represent undifferentiated myogenic precursor cells that lie between the external lamina and sarcolemma of skeletal muscle fibres (Figure 1). Under normal conditions satellite cells are quiescent, but with appropriate environmental signals they become activated and re-enter the cell cycle to either generate new muscle fibres or provide new myonuclei to the parent fibre [46, 51, 114].

Quiescent satellite cells are present throughout the muscle, but the distribution of satellite cells has been shown to vary between different individuals and between different muscle groups and muscle fibre types [51, 58]. In rodents it has been shown that slow type I fibres have a higher number of satellite cells than fast type II fibres. Thus, muscles containing mainly slow type I fibres tend to contain more satellite cells than muscles containing a large proportion of type II fibres [51]. However, in a study by Kadi et al [57], the satellite cell content in the tibial anterior muscle of healthy untrained humans did not differ between type I and type II muscle fibres. These findings have more recently been confirmed by Verdijk et al [110].

The satellite cell population within the muscle is not static. The proportion of satellite cells has been shown to increase following exercise [58], as a result of low-frequency stimulation [98] and in neuromuscular disorders such as Duchenne muscular dystrophy and neurogenic atrophy [69], while a lower proportion of satellite cells has been reported in elderly men and women compared with in a younger population [57].
The signalling pathways for satellite cell activation are not fully understood, but several mediators of satellite cell activation such as growth factors, nitric oxide (NO), mechanical stimulation and physiological stimuli through exercise have been suggested [114].

Identification of satellite cells has commonly been done using electron microscopy. However, the development of antibodies raised against specific proteins expressed by satellite cells has made immunohistochemical identification of satellite cells possible. The membrane-bound neural cell adhesion molecule (N-CAM) is a cell-surface glycoprotein localised on satellite cells and the N-CAM antibody has been demonstrated to be a successful marker for both quiescent and activated satellite cells in human skeletal muscle [58]. Likewise, it has been shown that antibodies against the transcription factor Pax-7, which is located in the nucleus of the satellite cell, can also be used for identification of satellite cells in sections of human skeletal muscle biopsies [67].

**Muscle capillarization**

The capillary bed in skeletal muscle mainly serves to supply the muscle with oxygen but also to remove different metabolites and heat produced during muscle contraction [55]. The main arteries that supply a muscle with blood lie outside the muscle and it is a secondary artery that enters into the muscle. Inside the muscle, the secondary artery divides into smaller arterioles, which subdivide into metaarterioles. From the metaarterioles, blood moves into the capillaries, which can be identified by their size and cross section. Vessels in the endomysium that have a diameter < 15μm are most commonly capillaries. The capillaries are longitudinally oriented and run between the muscle fibres [17, 29]. The histological structure of the capillary, with the capillary wall being about 1μm thick and consisting of a single layer of endothelial cells, allows a two-way exchange of gases and substances. Capillaries within the muscle are organized as an interconnected network where the entrance to each capillary is guarded by a capillary sphincter. Contraction of the sphincter leads to reduced or altered blood flow in the specific capillary while relaxation of the sphincter results in increased blood flow in the capillary [10]. It is known that highly oxidative muscle fibres have a denser capillary network than glycolytic muscle fibres [55].

**Angiogenesis**

Blood vessel growth is of great importance to match the needs of blood supply to the tissue. Capillary growth through sprouting of capillaries from an already established capillary network is referred to as angiogenesis or
sprouting angiogenesis [41]. This process is carried out by activated endothelial cells that branch out from existing capillaries and assemble into tubes that re-enter the capillary bed [14]. The process of angiogenesis is controlled by both growth promoting and growth inhibitory factors. One of the most central growth factors in the process of initiating skeletal muscle angiogenesis is vascular endothelial growth factor (VEGF), which stimulates proliferation, migration and survival of endothelial cells [85]. Adequate levels of VEGF in skeletal muscle are also essential for maintaining muscle capillarization; it has been shown that lowered levels of VEGF decreases capillary density and capillary-to-fibre ratio [14].

Several factors are involved in the regulation of VEGF. These include growth factors, tumour suppressor factors, exercise and hypoxia [85, 97]. The responses to local hypoxia within the tissue are largely considered to be mediated by the widely-expressed hypoxia-inducible factor (HIF) of which three isoforms (HIF-1α, HIF-2α and HIF-3α) are known to date [47, 77]. Intracellular levels of HIF are tightly regulated by the von Hippel-Lindau tumour suppressor protein (pVHL). pVHL is a component of an E3 ubiquitin ligase that mediates ubiquitylation of the α subunits of the HIF protein, resulting in rapid degradation of the protein by the ubiquitin-proteasome pathway in the presence of oxygen [6, 61, 101]. As a response to local hypoxia within the tissue, intracellular HIF levels are stabilized and increased through decreased degradation. The HIF signal is then transduced to regulate the transcription of a multitude of gene responses including VEGF [101] (Figure 2).

Whether or not systemic hypoxia induces capillary growth in skeletal muscle is controversial. Studies on mice have suggested an induced angiogenesis due to systemic hypoxia [24-25] while increased capillarity as a response to systemic hypoxia in humans has been explained by a parallel reduction in fibre area [53]. Other studies show no change in capillary supply during systemic hypoxia in human skeletal muscle [66, 96] and longterm exposure to hypoxia has even been shown to decrease skeletal muscle capillarization [23, 54]. Taken together, these conflicting results indicate that the degree of hypoxia and the duration of exposure to hypoxic conditions are important factors for angiogenesis in skeletal muscle.
Endurance exercise has been reported to induce capillary growth in skeletal muscle [14, 55, 97]. Furthermore, VEGF and HIF 1-α have been shown to be up-regulated following exercise training [6, 14, 42]. The mechanisms resulting in exercise-induced angiogenesis are not fully understood. However, increased blood flow within the muscle, local hypoxia in the muscle tissue and mechanical stretching of the muscle have been suggested as plausible mediators [55, 97].

**Physical activity**

Physical activity is defined as any bodily movement produced by skeletal muscles resulting in energy expenditure beyond resting energy expenditure [16]. It has been shown that the level of physical activity is an important factor related to a number of health outcomes such as reduced risk of cardiovascular disease, thromboembolic stroke, hypertension, type 2 diabetes mellitus, osteoporosis, obesity, colon cancer, breast cancer, anxiety and depression [84]. In addition to these benefits, physical activity in older adults has been suggested as effective therapy for many chronic diseases such as coronary heart disease, peripheral vascular disease, elevated cholesterol, osteoarthritis, claudication and CODP [84]. Health recommendations state that healthy adults aged 18–65 years should perform 30 minutes...
of at least moderate intensity physical activity most preferably on all weekdays, but at least five days a week, to promote and maintain health [45, 89]. These recommendations have also been shown to be applicable in older adults (>65 years) and in adults aged 50–64 years with clinically significant chronic conditions [84].

Three different dimensions are included when describing a physical activity: frequency, duration and intensity. The frequency refers to how often the activity occurs over a specific time period, the duration refers to how long time the activity is sustained and the intensity refers to how strenuous the activity is. Intensity levels of physical activity can be expressed in terms of absolute or relative intensity. Absolute intensity level is commonly defined as the rate of oxygen required per time unit (l x min⁻¹ or ml⁻¹ x min x kg body weight⁻¹) while relative intensity level often is defined in terms of relative load on physiological markers in relation to maximal capacity (e.g. percentage of maximal oxygen consumption or percentage of maximal heart rate) [78].

Various methods for assessment of physical activity are available. These methods can be broadly divided into two groups: subjective and objective methods. Subjective measurements are also referred to as self-report methods and include questionnaires, activity diaries and interviews, while the most common objective measurements are the double labelled water method, heart rate monitoring and motion sensors such as pedometers and accelerometers [79].

**Exercise capacity**

Exercise is defined as planned, structured and repetitive bodily movement done to improve or maintain components of physical activity and is not synonymous with physical activity [16]. Exercise capacity refers to the ability to perform exercise and is often divided into anaerobic and aerobic capacity. Anaerobic work capacity is dependent on energy-yielding processes that do not require oxygen and can contribute to physical work for a limited period of time. Long-term exercise is mainly dependent on aerobic energy production. Therefore the single most important component in endurance exercise capacity is the ability to provide oxygen for energy supply. The maximum rate at which an individual is able to consume oxygen is referred to as maximal oxygen consumption (VO₂-max) and is an important determinant of exercise capacity. Endurance exercise capacity is often determined by measuring VO₂-max in laboratory settings or by estimating VO₂-max using standardized submaximal tests [78].
COPD

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of morbidity and mortality worldwide [72]. COPD is defined as a preventable and treatable disease characterised by progressive, not fully reversible airflow limitation [2]. The two major components of COPD are chronic bronchitis and emphysema. Chronic bronchitis is clinically defined as chronic coughing for three months in each of two successive years were other causes of coughing have been ruled out. Chronic bronchitis is also associated with inflammation and nonspecific bronchial hyperreactivity causing airway obstruction [72, 112]. Emphysema is characterised by an enlargement of the lower airways and by destruction of their walls without obvious fibrosis, which will cause airflow limitation [103, 112].

Estimating the prevalence of COPD has been shown to be complicated, as it can vary depending on many factors such as diagnostic criteria, age and survey methods. In a systematic review by Halbert et al [43], the prevalence of COPD defined as not fully reversible airflow limitation in adults aged ≥ 40 years was estimated to be 9–10% in European and northern American countries, while the disease prevalence in persons > 45 years in northern Sweden was estimated to be around 14% in a study by Lundbäck et al [65]. In addition, the study by Lundbäck et al reported no gender differences in prevalence of CODP, but showed that prevalence increases rapidly with increased age [65].

The major risk factor for developing COPD is tobacco smoking. Cigarette smokers have a higher prevalence of abnormal lung function and respiratory symptoms as well as a greater rate of annual increase in airflow obstruction than non-smokers [90, 112]. Not all smokers develop COPD suggesting that other factors, such as genetic factors, may modify the individual risk for disease development. The genetic risk factor that is best described is deficiency of α-1-antitrypsin, which generates premature and accelerated development of emphysema and decline in lung function. However, only about 1% of COPD patients have an inherited form of α-1-antitrypsin deficiency [90, 103, 112]. Other known risk factors for development of COPD are air pollution and occupational exposure to dusts and chemicals [90].

Typical symptoms of COPD are coughing, phlegm, and dyspnea. However, there is a weak relationship between symptoms and lung function. Thus, lung function measurements with spirometry are necessary for diagnosing and grading COPD [112]. The presence of a post-bronchodilator forced expiratory volume in one second (FEV₁,₀) value less than 80% of predicted, together with a quotient between FEV₁,₀ and forced vital capacity (FVC) lower than 0.70 confirms the presence of not fully reversible
airflow limitation [2, 72, 90, 112]. Commonly, disease severity in COPD is classified into different stages mainly based on FEV$_{1.0}$ values in relation to predicted FEV$_{1.0}$. The most widely used classification recommendations are the “global strategy for diagnosis, management and prevention of COPD” (GOLD) criteria [2, 90]. However, these criteria have been updated in 2006 and differences in classification may occur in the literature [2]. The GOLD criteria for classification of COPD before and after 2006 are presented in Table 1.

Table 1. Classification of COPD disease severity according to “global strategy for diagnosis, management and prevention of COPD” (GOLD) criteria from 2001 and 2006.

<table>
<thead>
<tr>
<th>Stage</th>
<th>GOLD 2001</th>
<th>GOLD 2006</th>
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<tr>
<td>0: At risk</td>
<td>Normal spirometry</td>
<td>-</td>
</tr>
<tr>
<td>Chronic symptoms</td>
<td>(coughing, sputum production)</td>
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<tr>
<td>I: Mild COPD</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
</tr>
<tr>
<td>FEV$_{1.0}$ ≥ 80% of predicted</td>
<td>FEV$_{1.0}$ ≥ 80% of predicted</td>
<td></td>
</tr>
<tr>
<td>II: Moderate COPD</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
</tr>
<tr>
<td>30% ≤ FEV$_{1.0}$ &lt; 80% of predicted</td>
<td>50% ≤ FEV$_{1.0}$ &lt; 80% of predicted</td>
<td></td>
</tr>
<tr>
<td>III: Severe COPD</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
</tr>
<tr>
<td>FEV$_{1.0}$ &lt; 30% of predicted or the presence of respiratory failure or clinical signs of right heart failure</td>
<td>30% ≤ FEV$_{1.0}$ &lt; 50%</td>
<td></td>
</tr>
<tr>
<td>IV: Very severe COPD</td>
<td>-</td>
<td>FEV$_{1.0}$/FVC &lt; 0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FEV$_{1.0}$ &lt; 30% of predicted or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FEV$_{1.0}$ &lt; 50% of predicted plus chronic respiratory failure</td>
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Besides the pulmonary abnormalities of COPD, the disease is associated with effects outside the lungs, so called systemic effects. Systemic inflammation, nutritional abnormalities and weight loss, cardiovascular effects, effects on the nervous system, osteoskeletal effects and skeletal muscle dysfunction have been reported as potential systemic effects of COPD [4, 22, 40]. The mechanisms mediating these systemic effects are not fully understood; however, systemic inflammation, oxidative stress, use of oral corticosteroids, hypoxia and low levels of physical activity have been suggested as plausible mediators [3, 40].

Skeletal muscle in COPD
Several studies have addressed the fact that skeletal muscle is affected in patients with COPD. Skeletal muscle dysfunction has been reported to be characterised by reduction in muscle strength (defined as the capacity of the muscle to generate force) and muscle endurance (defined as the capacity of muscle to maintain a certain force over time) [60]. Furthermore, several structural changes in peripheral skeletal muscle have been described. The cross sectional area of skeletal muscle fibres has been shown to be lower in COPD patients compared with in healthy subjects and this finding has been associated with muscle atrophy, which is commonly described in this patient group [37, 39, 111]. When studying fibre type distribution in the vastus lateralis muscle, several studies have reported a low proportion of type I fibres in COPD patients compared with in healthy subjects [38, 56, 71, 81, 111] while there are some discrepancies regarding the distribution of type II fibres in this muscle. Maltais et al [71] report a higher proportion of type IIa fibres in COPD patients while Gosker et al [38] as well as Whittom et al [111] report a higher proportion of type IIx fibres but no changes in the proportion of type IIa fibres in COPD patients compared with in healthy subjects. In addition to the reported changes in fibre type distribution, a lower oxidative enzyme activity [38, 70] and lower mitochondrial density [93] have been shown in the vastus lateralis muscle of COPD patients when comparing with healthy subjects. Taken together, changes in fibre type distribution, low levels of oxidative enzyme activities and a low mitochondrial density speak in favour of a reduced oxidative capacity of skeletal muscle in COPD.

Studies on muscle capillarization have reported a lower capillary to muscle fibre ratio in the vastus lateralis muscle of COPD patients compared with healthy subjects [56, 111]. However, when studying capillarization in relation to fibre area, no significant differences were shown between COPD patients and healthy subjects [56, 111]. In a study by Barreiro et al
[11], VEGF protein levels in the vastus lateralis muscle of COPD patients were reported to be lower than those of healthy subjects.

To our knowledge no studies on satellite cell proportion or function have been carried out in a population of COPD patients to investigate the regenerative potential of skeletal muscle. However, it has been suggested that inflammatory mediators, which are increased in COPD due to systemic inflammation, may affect satellite cells and thereby the regenerative capacity of the muscle in COPD [44].

It is also important to highlight the fact that changes in skeletal muscle of COPD patients are not homogenous between various muscle groups. The most pronounced reductions in oxidative properties have been reported in the lower limb muscles [13]. When studying the diaphragm and other inspiratory muscles of COPD patients a higher proportion of type I fibres, increased mitochondrial density and increased oxidative capacity have been reported [20, 63, 105]. These changes are thought to be caused by overload of the ventilatory muscles due to increased work of breathing brought on by airflow obstruction and hyperinflation in COPD [20].

What causes the observed changes in skeletal muscle of COPD patients is poorly understood. Muscle morphology and function have been suggested to be influenced by several factors such as systemic inflammation, oxidative stress, nutritional abnormalities, use of corticosteroids, hypercapnia, hypoxia and low levels of physical activity [13, 36, 40, 44].

**Physical activity in COPD**

Physical activity is an important clinical parameter related to lung function decline, hospitalisation and mortality in COPD [32, 34]. Likewise, patients with higher levels of physical activity have been shown to report a better health-related quality of life compared with more inactive patients [83]. Furthermore, low levels of physical activity have been associated with extrapulmonary effects such as skeletal muscle dysfunction and systemic inflammation in COPD patients [108, 113].

The most widely used research methods for measuring physical activity in COPD are subjective measurements such as questionnaires and activity diaries. The advantages with these types of measurement are the low costs and easy applications while the disadvantages are low accuracy and large individual variability [94]. Objective measurements have been recommended for more accurate and detailed measurements [12, 94] and motion sensors such as accelerometers have been shown to provide objective measurements of duration, intensity and frequency of physical activity in daily life in COPD patients [94].
In a previous study, addressing objectively measured free-living physical activity in COPD patients, it was reported that COPD patients in GOLD stage II–IV are significantly less physically active compared with control subjects who were considered at risk for developing COPD (GOLD stage 0) [109]. Furthermore, Pitta et al [95] have found that COPD patients spend less time walking and standing but more time sitting and lying compared with healthy subjects.

**Exercise capacity in COPD**

Exercise intolerance has been suggested as one of the most common symptoms in COPD [5]. It has also been shown that exercise capacity is correlated to mortality and reduced exercise capacity has been associated with reduced quality of life in this patient group [68, 86]. Lung function impairment shows only a weak relation to exercise capacity and it has been suggested that other factors play an important role in reducing exercise capacity in COPD [104]. It has been shown that skeletal muscle function and fat free mass are associated with exercise capacity in COPD [5, 9, 30, 100], raising speculations that muscle weakness and wasting may play important roles in exercise capacity in these patients. These speculations are strengthened by studies that have shown improved muscle function and increased exercise tolerance as a response to exercise training in COPD patients [5, 104]. It has also been suggested that de-conditioning due to inactivity is an important determinant to low exercise capacity [104] and increased levels of physical activity have been shown to have positive effects on exercise tolerance in COPD patients [33].
AIM OF THE THESIS

The overall aim of this thesis was to study muscular characteristics of the tibial anterior muscle and physical activity patterns in patients with different degrees of COPD. Furthermore, this thesis aimed to study the relationships between muscle characteristics and exercise capacity as well as between physical activity and exercise capacity in patients with different degrees of COPD.

The specific aims of this thesis were to investigate:

- fibre type distribution, fibre area, satellite cell content and muscle capillarization of the tibial anterior muscle in patients with different degrees of COPD (studies I, II and III).
- exercise capacity in patients with different degrees of COPD (studies I, III and IV).
- the relationship between muscle characteristics and exercise capacity in different degrees of COPD (studies I and III).
- the expression of VHL, VEGF and HIF in the tibial anterior muscle of patients with different degrees of COPD (study II).
- physical activity patterns and their relationship to exercise capacity in patients with different degrees of COPD (study IV).
MATERIALS

Subjects
Patients included in study I, II and III were recruited from the Department of Respiratory medicine at Örebro University Hospital. Twenty-three patients (13 women and 10 men) who were selected in a stable condition and were not suffering from any respiratory tract infections or exacerbations of their disease four weeks prior to the sampling date participated. Exclusion criteria were malignancy, cardiac failure and severe endocrine, hepatic or renal disorder. Twelve age-matched, healthy, non-smoking subjects (6 women and 6 men) were recruited as a control group. Test subject characteristics are given in Table 2.

In study IV, patients were recruited from primary health care centres in central Sweden through the “Diagnosdatabasregistret”. Fifty patients (33 women and 17 men) who were selected in a stable condition and were not suffering from any respiratory tract infections or exacerbations of their disease four weeks prior to the sampling date participated in the study. Exclusion criteria were malignancy, cardiac failure and severe endocrine, hepatic or renal disorder. Nineteen age-matched, healthy, non-smoking subjects (13 women and 6 men) were recruited as a control group. Of the recruited subjects, six patients and two healthy subjects were excluded from the statistical analysis due to incomplete data sampling. Test subject characteristics are given in Table 3.

All patients and age-matched healthy subjects underwent spirometry to determine forced expiratory volume in one second (FEV$_{1.0}$) with the highest value from at least three technically acceptable assessments being used. In studies I–III, both patients and controls underwent spirometry with reversibility tests, while in study IV the patients underwent post-bronchodilatory spirometry and the healthy subjects underwent spirometry without administration of bronchodilators. FEV$_{1.0}$ was then used to divide patients into subgroup based on the “Global Initiative for Chronic Obstructive Lung Disease (GOLD)” criteria [2, 90].

In study I, patients were divided into two subgroups according to GOLD criteria from 2006 [2]. Group one (n=12) was considered to have mild or moderate COPD (FEV$_{1.0}$ ≥ 50% of predicted) and group two (n=11) was considered to have severe or very severe COPD (FEV$_{1.0}$ < 50% of predicted).

In study II and III, patients were divided into three subgroups according to GOLD criteria from 2001 [90]. Group one (n=8) was considered to have mild COPD (FEV$_{1.0}$ > 80% of predicted), group two (n=9) was considered
to have moderate COPD (FEV$_{1,0}$ 30-80% of predicted) and group three (n=6) was considered to have severe COPD (FEV$_{1,0}$ < 30 of predicted). The healthy subjects had no airway obstruction (mean FEV$_{1,0}$ 115% of predicted).

In study IV, patients were divided into three subgroups according to GOLD criteria from 2006 [2]. Group one (n=11) was considered to have mild COPD (FEV$_{1,0}$ ≥ 80% of predicted), group two (n=21) was considered to have moderate COPD (80% > FEV$_{1,0}$ ≥ 50% of predicted) and group three (n=12) was considered to have severe COPD (50% > FEV$_{1,0}$ ≥ 30% of predicted). The healthy subjects had no airway obstruction (mean FEV$_{1,0}$ 103 % of predicted).

For all test subjects, height and weight were measured and body mass index (BMI) was calculated as weight (kg)/(height (m))$^2$ (Table 2 and Table 3). All test subjects included in studies I–III were sampled for arterial blood gases from the radial artery at rest. The samples were analysed for partial arterial pressure for oxygen (PaO$_2$) and carbon dioxide (PaCO$_2$) using the Radiometer ABL 500 blood gas analyser (Table 2).

All studies were approved by the regional ethical review board in Uppsala, Sweden (dnr 2004:M-355).

Table 2. Characteristics of test subjects included in studies I–III.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n=12, 6 women and 6 men)</th>
<th>COPD patients (n=23, 13 women and 10 men)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 ± 7</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 10</td>
<td>169 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 ± 13</td>
<td>76 ± 18</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.7 ± 3.7</td>
<td>26.3 ± 5.0</td>
</tr>
<tr>
<td>FEV$_{1,0}$ (% of predicted)</td>
<td>113 ± 12</td>
<td>56 ± 25*</td>
</tr>
<tr>
<td>PaO$_2$ (kPa)</td>
<td>11.1 ± 1.3</td>
<td>9.7 ± 1.3*</td>
</tr>
<tr>
<td>PaCO$_2$ (kPa)</td>
<td>5.2 ± 0.3</td>
<td>5.1 ± 0.6</td>
</tr>
</tbody>
</table>

* = significantly lower compared with healthy subjects, p<0.001
** = significantly lower compared with healthy subjects, p=0.005
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy subjects (n=17, 11 women and 6 men)</th>
<th>COPD patients (n=44, 28 women and 16 men)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 5</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 8</td>
<td>169 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 10</td>
<td>78 ± 19</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 ± 1.8</td>
<td>27.0 ± 5.5</td>
</tr>
<tr>
<td>FEV1.0 (% of predicted)</td>
<td>103 ± 18</td>
<td>65 ± 20*</td>
</tr>
</tbody>
</table>

* = significantly lower compared with healthy subjects, p<0.001
METHODS

Muscle biopsy sampling and analysis
Muscle biopsies were taken from the bulk of the tibial anterior muscle, which is an important postural muscle active daily for long periods and involved in balance control and foot stability during walking [64]. The biopsies were obtained under local anaesthesia (Xylocaine® 2%) using the semi-open biopsy technique described by Henriksson [48] (Figure 3). The biopsies were frozen in isopentane cooled to its freezing point in liquid nitrogen, and stored at -80°C until analyses were performed. Biopsies were then studied further using immunohistochemistry and polymerase chain reaction (PCR) techniques. Biopsies analysed using immunohistochemistry were prepared by cutting serial transverse section, 5μm thick, at -22°C using a microtome (Leica CM1850, Leica Microsystems, Germany) and mounted on glass slides. Biopsies used for PCR analyses were prepared by isolating total RNA using the RNeasy Fibrous Tissue Mini Kit (Qiagen, USA) according to the manufacturers’ instructions. The integrity and quantity of the isolated RNA was evaluated with the Agilent 2100 bioanalyzer using the RNA 6000 Nano Assay Kit (Agilent Technologies) and all isolated RNA was stored at -80°C until use.

Figure 3. Muscle biopsy sampling from the tibial anterior muscle using the semi-open biopsy technique.

Fibre type distribution
For determination of fibre types immunohistochemistry was used. Primary monoclonal antibodies (mAb) against myosin heavy chains (MyHC) that were used were mAb A4.951 and mAb N2.261 (Developmental Studies Hybridoma Bank, University of Iowa). Using mAb A4.951, type I fibres are strongly stained while type IIa, IIx and type IIx fibres are unstained.
Using mAb N2.261, type IIa fibres are strongly stained, type IIx fibres are unstained while type I and type IIxa fibres are slightly stained. Type I–IIa fibres are stained with both antibodies [59] (Figure 4). Analyses were performed using a light microscope (Nikon Eclipse E400) connected to a computerised image system (SPOT Insight: Diagnostic Instrument, Sterling Heights, Michigan). Fibre type distribution was analysed on the whole cross section and a mean of 401 fibres was counted for each subject. The fibres were designated as type I, type IIa, type IIx, type I–IIa or type IIxa.

![Figure 4. Muscle biopsy from the tibial anterior muscle stained using mAb N2.261 (A) and mAb A4.951 (B) for determination of fibre type.](image)

**Fibre area and perimeter**

For each test subject four to ten randomly selected areas on the cross section were selected and photographed at a magnitude of x20. Fibre area and fibre perimeter for type I and type IIa fibres were then determined for a mean of 49 type I and 31 type IIa fibres. Due to the low number of type IIx, type IIxa and type I–IIa fibres, areas and perimeters for these fibres types were not included in the statistical analyses. Obliquity in fibre sectioning was assessed using the form factor that represents: \((4\pi \times \text{fibre area})/\left(\text{fibre perimeter}\right)^2\). There were no differences in form factor between the different study groups (p=0.31).
Satellite cells
For visualization of satellite cells, mAb CD56 (Novocastra Laboratories), which is similar to N-CAM, was used [57] (Figure 5). Following staining, the sections were visualized using a light microscope (Nikon Eclipse E400) and analyses were performed at a magnitude of x40–x60. In each cross section, the number of satellite cells and number of muscle fibres was counted on the whole muscle biopsy. The ratio between number of satellite cells and number of muscle fibres was then calculated.

![Figure 5](image)

**Figure 5.** Muscle biopsy from the tibial anterior muscle immunohistochemically stained using mAb CD56 for visualization of satellite cells.

Capillary network
The identification of capillaries was done using the mAb CD31 (Dako, Glostrup, Denmark; MO823), an antibody that recognizes PECAM-1, a transmembranous glycoprotein that is strongly expressed in vascular endothelial cells. Sections were also counterstained with eosin [17] (Figure 6). Analyses were performed using a light microscope (Nikon Eclipse E400) connected to a computerised image system (SPOT Insight: Diagnostic Instrument, Sterling Heights, Michigan). Four to ten randomly selected cross-sectional areas corresponding to a mean of 80 fibres were photographed at a magnitude of x20 and used for determination of the capillary network.
Capillary parameters

Muscle capillary parameters were in the present thesis calculated as previously described by Charifi et al [17]. The capillary supply in skeletal muscle can be measured by counting the number of capillaries around each muscle fibre (CAF) or by assessing CAF in relation to fibre area (CAFA). CAFA is a parameter based on the diffusion distance between the capillary and the centre of the fibre. Capillary parameters determining the diffusion distance may not detect actual disturbances in muscle capillarization. It has previously been suggested that the muscle fibre-to-capillary interface is an important factor involved in oxygen supply to the muscle [17-18, 35, 49-50, 52] and may thereby be used as a more sensitive marker for changes in the capillary bed compared with CAF and CAFA. Muscle fibre-to-capillary interface can be assessed using the capillary to fibre perimeter exchange index (CFPE-index) or the index of tortuosity (LC/PF-index). CFPE-index represents the quotient between the capillary-to-fibre ratio (C:F_i) and the fibre perimeter, where C:F_i is calculated by counting the number of capillaries around the fibre in question followed by determination of the sharing factor (SF) for each capillary and thereafter taking the sum of the fractional contributions of all capillary contacts around the fibre [49]. LC/PF-index is calculated as the ratio between the length of capillary in contact with the muscle fibre membrane and the fibre perimeter and represents the percentage of muscle fibre perimeter in contact with the capillary wall. CAF and CAFA were assessed in study II while CFPE-index and LC/PF-index were assessed in study III.
**VHL-identification**

In study II, identification of VHL at protein level was done immunohistochemically using the von Hippel-Lindau protein mAb (GTX111899, GeneTex, Inc, Texas, USA). The number of VHL-immunoreactive cells was quantitatively scored by counting the number of VHL-positive cells from six randomly selected cross sectional areas photographed at a magnitude of x40. Only cells containing visible nuclei were included.

TaqMan PCR was used for gene expression analyses. Reverse transcription of RNA was performed for synthesis of complementary DNA (cDNA) and the final cDNA product was stored at -20°C until use. The VHL (Hs00184451_m1) gene designed and premixed by Applied Biosystems (Foster City, California, USA) was then used for expression analysis.

**HIF and VEGF identification**

In study II, gene expression of HIF and VEGF was studied using TaqMan PCR. Reverse transcription of RNA was performed for synthesis of cDNA and the final cDNA product was stored at -20°C until use. Genes included for expression analysis were HIF-1α (Hs00936368_m1), HIF-3α (Hs00541709_m1), VEGF (Hs00900054_m1), VEGFB/VEGFC (Hs 00). All genes were designed and premixed by Applied Biosystems (Foster City, California, USA).

**Exercise capacity**

To determine exercise capacity, the six-minute walk test was used. This test has been described as a submaximal test that can be performed by patients who do not tolerate maximal exercise testing [80]. Furthermore, the test is widely used in clinical practice and is generally recommended as a functional assessment for patients suffering from COPD [1, 28]. The test was performed on a 25m (studies I and III) or a 20m (study IV) court where the test subject walked back and forth as fast as possible for six minutes and the distance walked after six minutes was measured. The test subjects did not use any bronchodilators or oxygen supplementation before or during the test.

**Body composition**

In study I, fat free mass (FFM) and fat free mass index (FFMI) were determined using dual x-ray absorptiometry (DXA) where a double photon beam generated by an x-ray source is used to distinguish between different body tissues. A whole body scan was conducted in a Lunar DPX-L scanner (Lunar cooperation, Madison, WI, USA) with the subjects in a supine posi-
tion. Lunar software (DPX-L v 4.7E) was used to calculate body composition.

In study IV, body composition was established by measuring skinfold thickness with a Harpenden® skinfold calliper at four sites; the triceps, the biceps, the subscapula and the suprailiaca. Body fat content was estimated from the sum of the skinfolds using the body density equation by Durnin and Womersley [26] and the Siri equation for body fat [102].

Physical activity
To assess physical activity in study IV, the uniaxial accelerometer ActiGraph, model GT1 M (Manufacturing Technology IC, Fort Walton Beach, Fl, USA) was used (Figure 7a). The accelerometer consists of a piezoelectric sensor that is sensitive to vertical accelerations and samples voltage signals in proportion to detected accelerations (range 0.05–2.0 g with a frequency rate of 0.25–2.5 Hz) with a sample rate of 10 measures per second. The signal first becomes filtered to eliminate artefacts such as vibrations and then becomes converted into a digital set of numbers, so called “counts”. Finally, all counts are summarised over a user-specified time frame (epoch). The accelerometer measures both the magnitude and frequency of body movement, which makes quantification of the intensity and duration of physical activity possible [19].

The test subjects wore the accelerometer on an elastic belt around the waist (Figure 7b) all waking hours except during water activities, for seven days and data were sampled in 60s epochs. Data were reduced using the ActiGraph analysis software MAHUffe (available from http://www.mrc-epid.cam.ac.uk/Research/PA/Downloads.html). Continuous periods of zero values exceeding 20 min were regarded as “accelerometer not worn” and were not included in the calculation of total registered time. 500 minutes of registration per day were required for a day to be considered as valid and at least three valid days of which at least one was a Saturday or Sunday were required for the registration to be included in the statistical analysis.

Based on previously reported validation studies, activity count cut-off points applied to assign accelerometer outcomes to physical activity categories were as followed:

Time spent sedentary was defined as where counts/min were < 100 [75], light activity was defined as activity resulting in 100–2019 counts/min [74], moderate activity was defined as activity resulting in 2020–4944 counts/min [107] and vigorous activity was defined as activity resulting in more than 4944 counts/min [106].
Figure 7. The uniaxial accelerometer ActiGraph, model GT1 M (a) used for registration of physical activity and a test subject wearing the accelerometer on an elastic belt around the waist (b).

Statistical analyses

Statistic analyses were performed using Statistix ®8 (Analytic Software, Tallahassee, Florida, USA) (studies I–III) and PASW Statistics 18.0 (former SPSS) (study IV). In studies I–III p < 0.05 was considered significant while in study IV p ≤ 0.05 was considered significant. In studies I and III, all data were presented as mean ± standard deviation. Comparison between groups was done using Kruskal-Wallis one way ANOVA followed by the Kruskal-Wallis all pairwise comparison post-hoc test when significance was found and relationship between variables was studied using Spearman’s rank correlation test. In study II, capillary variables were presented as mean ± standard deviation. Comparison between groups was done using Kruskal-Wallis one way ANOVA followed by the Kruskal-Wallis all pairwise comparison post-hoc test when significance was found. To estimate differences between groups regarding gene expression of VHL, HIF and VEGF and quantitative analysis of VHL immunohistochemistry regression analysis was applied. In study IV, variables were checked for normality using Shapiro-Wiik’s test for normality. All values except time spent MVPA showed a satisfactory pattern and after logarithm transformation time spent MVPA also fitted within the normal distribution and parametrical tests were applied. Data were presented as mean ± standard deviation and comparison between groups was done using analysis of variance (ANOVA) followed by Tukey’s post-hoc test when significance was found. To examine associations between variables, linear regression analysis was applied.
RESULTS

Study I
Physical performance and muscular characteristics in different stages of COPD

Aim
The aim was to study exercise capacity as well as muscle fibre type distribution, fibre area and satellite cell content in the tibial anterior muscle of patients with different degrees of COPD and to investigate the existence of a relationship between exercise capacity and muscle morphology.

Results
Significant differences in exercise capacity expressed as distance walked in six minutes were observed between the study groups (p < 0.001). Patients with mild and moderate COPD on average walked a 21% shorter distance compared with the healthy subjects and patients with severe and very severe COPD on average walked a 41% shorter distance compared with the healthy subjects. Furthermore, a significant correlation between exercise capacity and degree of airflow obstruction expressed as percent of predicted FEV$_{1.0}$ was shown (r=0.89, p < 0.001).

Patients with severe and very severe COPD had a significantly lower proportion of type I fibres (p=0.01) and a significantly higher proportion of type IIa fibres (p=0.01) compared with the healthy subjects, while the proportion of type IIx, type IIxa and type I–IIa fibres did not differ between the study groups.

The fibre area of type IIa fibres was significantly lower in the patients with severe and very severe COPD compared with the healthy subjects (p=0.007), while no differences in fibre area of type I fibres were observed between the study groups. A correlation between degree of airflow obstruction and area of type IIa fibres was also observed (r=0.55, p < 0.001). Furthermore, the area of type IIa fibres was found to significantly correlate to exercise capacity (r=0.63, p < 0.001).

Body composition expressed as fat free mass index (FFMI) was not found to differ between the study groups. However, FFMI was significantly correlated to the fibre area of both type I fibres (r=0.48, p=0.005) and type IIa fibres (r=0.71, p < 0.001).

The number of satellite cells per muscle fibre did not differ between the study groups, indicating an intact regenerative capacity of the muscle.
Study II

Overexpression of von Hippel-Lindau protein in skeletal muscles of patients with chronic obstructive pulmonary disease

Aim

The aim was to investigate the hypothesis that impaired coupling of the hypoxic-angiogenic signalling cascades mediates decreased skeletal muscle capillarization in patients with COPD.

Results

Significant differences in the number of capillaries around a single muscle fibre (CAF) for both type I (p=0.006) and type IIa fibres (p=0.002) were observed between the study groups with patients with moderate and severe COPD having a significantly lower number of capillaries/fibre compared with the healthy subjects. However, when CAF was adjusted for fibre area (CAFA) the significant differences did not remain.

A significant overexpression of VEGF-A, VEGF-B and HIF 3-α was observed in the patients with moderate COPD compared with the healthy subjects, while a trend towards overexpression of HIF 1-α, HIF 3-α, VEGF-A, VEGF-B and VEGF-C was observed in mild COPD compared with the healthy subjects. Likewise, a trend towards overexpression of HIF 1-α and VEGF-C was observed in moderate COPD compared with the healthy subjects. No significant differences in gene expression of HIF or VEGF were observed between the patients with severe COPD and the healthy subjects (Table 4).

The mRNA levels of VHL were significantly higher in the patients with mild COPD compared with the healthy subjects (p=0.008). In the patients with moderate COPD, a trend towards overexpression of mRNA VHL was observed (p=0.077), while mRNA levels of VHL in severe COPD were similar to the levels in the healthy subjects (Table 4). On a protein level, the number of cells displaying pVHL immunoreactivity was significantly higher in the patients with mild (p=0.001) and moderate COPD (p=0.001) compared with the healthy subjects, while the number of cells displaying pVHL immunoreactivity in severe COPD was lower, although not significantly, than in the healthy subjects.
Table 4. mRNA expression of VEGF, HIF and VHL in COPD patients compared with healthy subjects analysed using regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>Mild COPD vs healthy subjects</th>
<th>Moderate COPD vs healthy subjects</th>
<th>Severe COPD vs healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>2.8 (-15.9 to 21.5)</td>
<td>0.764</td>
<td>17.9* (0.5 to 35.2)</td>
</tr>
<tr>
<td>VEGF-B</td>
<td>7.4 (-16.4 to 31.2)</td>
<td>0.533</td>
<td>22.7* (0.7 to 44.7)</td>
</tr>
<tr>
<td>VEGF-C</td>
<td>1.5 (-4.0 to 7.0)</td>
<td>0.573</td>
<td>3.8 (-4.0 to 7.0)</td>
</tr>
<tr>
<td>HIF 1-α</td>
<td>5.0 (-3.6 to 13.6)</td>
<td>0.245</td>
<td>7.0 (-0.9 to 15.0)</td>
</tr>
<tr>
<td>HIF 3-α</td>
<td>14.7 (-13.9 to 43.4)</td>
<td>0.303</td>
<td>29.9* (3.4 to 56.5)</td>
</tr>
<tr>
<td>VHL</td>
<td>11.9* (3.3 to 20.6)</td>
<td>0.008</td>
<td>7.1 (-0.8 to 15.1)</td>
</tr>
</tbody>
</table>

*= significant difference compared with healthy subjects (p<0.05)
Study III
Alterations in the muscle-to-capillary interface in patients with different degrees of chronic obstructive pulmonary disease.

Aim
The aim was to investigate the muscle fibre-to-capillary interface in patients with different degrees of COPD and its correlation to the degree of airflow obstruction and exercise capacity.

Results
The C:F for type I fibres was significantly lower in the patients with moderate and severe COPD compared with the healthy subjects (p=0.007) and the C:F for type IIa fibres was significantly lower in the patients with severe COPD compared with the healthy subjects (p=0.002), indicating that each capillary is shared by more fibres in these patient groups. Furthermore, the CFPE-index for type I fibres was significantly lower in the patients with moderate and severe COPD compared with the healthy subjects (p=0.002).

No significant differences in LC/PF were observed between the study groups.

The degree of airflow obstruction expressed as percent of predicted FEV$_{1.0}$ was found to correlate positively to CFPE-index for both type I fibres (r=0.61, p < 0.001) and type IIa fibres (r=0.37, p=0.04). Furthermore, a positive correlation was observed between exercise capacity expressed as distance walked in six minutes and CFPE-index for both type I fibres (r=0.67, p < 0.001) and type IIa fibres (r=0.40, p=0.02), indicating a parallel reduction in exercise capacity and muscle capillarization.
Study IV
Physical activity patterns in patients with different degrees of chronic obstructive pulmonary disease.

Aim
The aim was to assess exercise capacity, body composition and physical activity in daily life in patients with different degrees of COPD and to use physical activity data to estimate how much time patients spend at least moderately physically active. Furthermore, the study aimed to examine the associations between exercise capacity, pulmonary function and physical activity.

Results
Exercise capacity expressed as distance walked in six minutes was found to differ significantly between the study groups (p < 0.001) with all patient groups walking a significantly shorter distance than the healthy subjects.

No differences in fat free mass were observed between the different study groups.

Regarding physical activity, time spent moderately or vigorously physically active (MVPA), as well as mean physical activity level, were found to differ between the study groups with patients having moderate and severe COPD spending on average less time MVPA (p=0.02) and having on average lower mean physical activity level (p=0.01) than the healthy subjects. Time spent sedentary did not differ between the study groups. The recommendation of 30 minutes of at least moderate physical activity/day was reached by 47% of the healthy subjects, 27% of the subjects with mild COPD, 10% of the subjects with moderate COPD and 17% of the subjects with severe COPD.

When studying only the patients, a significant association between exercise capacity and percent of predicted FEV\textsubscript{1.0} was observed (β=0.363, R\textsuperscript{2} adjusted=0.110, p=0.02), while when studying only the healthy subjects no association between exercise capacity and percent of predicted FEV\textsubscript{1.0} was shown.

In the patients, significant associations between exercise capacity and time spent MVPA (β=0.335, R\textsuperscript{2} adjusted=0.090, p=0.03) as well as between exercise capacity and mean physical activity level (β=0.336, R\textsuperscript{2} adjusted=0.091, p=0.03) were observed, while in the healthy subjects a significant association between exercise capacity and mean physical activity level (β=0.487, R\textsuperscript{2} adjusted=0.187, p=0.05) but not between exercise capacity and time spent MVPA was shown. No associations between exercise capacity and time spent sedentary were observed in the patients or in the
healthy subjects. The significant association between exercise capacity and time spent MVPA in the patients was found to remain after controlling for percent of predicted FEV$_{1.0}$. 
DISCUSSION

Muscle fibre type and fibre area

Previous studies have suggested a more glycolytic muscle profile and a fibre type shift with a high number of type II fibres and low number of type I fibres in the vastus lateralis of COPD patients [38, 56, 70-71, 81, 93, 111]. This is in line with the findings in the present thesis, where a lower number of oxidative type I fibres and a higher number of more glycolytic type IIa fibres were found in the tibial anterior muscle of patients with COPD compared with healthy subjects. The changes in fibre type composition within the tibial anterior muscle appear to be more pronounced in severe and very severe COPD than in mild and moderate CODP. A plausible explanation to these findings is that changes in muscle characteristics towards a more glycolytic muscle profile is an adaptive response to hypoxia. As patients with severe and very severe COPD were found to have a significantly lower PaO$_2$ compared with healthy subjects, these patients most probably suffer from a chronic state of hypoxia. Likewise, a reduced cross-sectional fibre area has been associated with chronic hypoxia [53-54, 73] and in study I the area of the type IIa fibres was found to be lower in the patients with severe and very severe CODP compared with the healthy subjects. The cross sectional area of the muscle fibre has also been associated with muscle volume and reduced fibre area has been suggested to be a marker for muscle atrophy in COPD [37, 39, 111]. The finding of a reduced fibre area of type IIa fibres together with the significant correlation shown between fibre area and FFMI indicates the presence of muscle atrophy in the studied patient group. However, FFMI was not found to differ significantly between the study groups, probably due to the small number of patients recruited to each group, and therefore muscle atrophy could not be confirmed in the patients in the present thesis.

The shift towards a more glycolytic muscle profile and a low muscle mass, reflected by reduction in muscle fibre area, may partly account for the low exercise capacity expressed as distance walked in six minutes shown in the patient groups. However, as the morphological changes appear to occur mainly in the later stages of disease while exercise capacity begins to be reduced in mild and moderate CODP, other mechanisms such as low levels of physical activity and lung function impairment most likely also contribute to the decline in exercise capacity.
Muscle regenerative capacity

The majority of skeletal muscle regeneration is carried out by activation, proliferation and differentiation of satellite cells. As no differences in the number of satellite cells/muscle fibre in the tibial anterior muscle were found between COPD patients and healthy subjects, the regenerative capacity of this muscle appears to be intact in COPD patients. Increasing muscle mass and thereby fat free mass is of importance for COPD patients suffering from muscle wasting as low fat free mass has been identified as an independent predictor of mortality and has been suggested to have an adverse effect on muscle function, exercise capacity and health status in this patient group [9, 27, 82, 88, 99]. Satellite cells have been suggested to be activated in response to stimuli such as physical activity [114] and it can be speculated that increasing the level of physical activity may result in increased muscle regeneration and thereby increased muscle mass in COPD patients. However, as the biopsies were not taken following any form of stimulation the ability of the satellite cells to re-enter the cell cycle and contribute to muscle regeneration is not clarified in the present thesis and needs further investigation.

Muscle capillarization

The finding of a lower number of capillaries/muscle fibre (CAF) in the tibial anterior muscle of COPD patients compared with healthy subjects indicates a decreased muscle capillarization in COPD and is in line with previous studies examining muscle capillarization in the vastus lateralis muscle of COPD patients [56, 111]. However, as the ratio between the number of capillaries around a fibre and the area of the muscle fibre (CAFA) did not differ between the study groups, the lower number of capillaries/muscle fibre may be explained by the observed reduction in fibre area and not reflect an actual change in muscle capillarization. This is also in line with a study by Whittom et al [111], where a parallel reduction in the number of capillaries around a muscle fibre and the fibre area was observed. CAF and CAFA are variables based on the diffusion distance between the capillary and the centre of the muscle fibre and may not detect actual disturbances in muscle capillarization. It has been suggested that the muscle fibre-to-capillary interface, which has been shown to be a factor involved in oxygen supply to the muscle [17-18, 35, 49-50, 52], may be a more sensitive marker for changes in the capillary bed compared with CAF and CAFA. To assess muscle fibre-to-capillary interface in the present thesis, the capillary-to-fibre perimeter exchange index (CFPE-index) and the index of tortousity (LC/PF-index) were used. Patients with moderate and
severe COPD were found to have a lower CFPE-index compared with healthy subjects indicating that each capillary is shared by more muscle fibres, which may impair oxygen delivery to the muscle fibre. Taken together, the findings in the present thesis speak in favour of a disturbed muscle capillarization in the tibial anterior muscle of patients with moderate and severe COPD.

As a positive correlation was observed between CFPE-index and exercise capacity, impaired muscle capillarization and subsequently impaired oxygen delivery to the muscle appears to have a contributing role in the development of reduced exercise capacity in COPD. The changes in muscle capillarization are more pronounced in type I fibres and as these fibres are the most oxygen dependent and also the main fibre type involved in endurance exercise, it is likely that disturbed capillarization within these fibres will affect endurance exercise capacity negatively.

The mechanisms mediating decreased muscle capillarization in COPD have not previously been clarified. However, it is known that physiological stimuli, such as exercise, as well as environmental stimuli, such as hypoxia, affect muscle capillarization [14, 23, 54-55, 97]. It has previously been suggested that chronic hypoxia has negative effects on muscle capillarization [23, 54] and one plausible explanation to the lower capillarization observed in COPD may be the presence of systemic hypoxia. The underlying mechanisms by which hypoxia impairs muscle capillarization have not yet been fully understood. Contrarily, local hypoxia within the muscle has been suggested to enhance muscle capillarization by increasing VEGF expression due to increased levels of intracellular HIF through decreased degradation [101]. However, the increased levels of VHL found in mild and moderate COPD may affect the transduction of the hypoxic signalling pathway negatively and may therefore contribute to decreased capillarization in COPD. The correlation between CFPE-index and disease severity expressed as percent of predicted FEV₁,0 indicates a gradual decline in tissue capillarization with increased disease severity. It can be speculated that overexpression of VHL does not lead to complete blockage of the HIF signalling pathway but, more likely, a partial blockage leading to expression of VEGF at levels that are inadequate to maintain normal capillarization over a long period of time. This could explain the gradual decrease in muscle capillarization.

It has been suggested that physical activity enhances muscle capillarization through increased blood flow within the muscle, mechanical stretching of the muscle and local hypoxia in the muscle tissue [55, 97]. Thus, another plausible explanation to the decreased muscle capillarization in
COPD may be the low levels of physical activity shown in these patients in study IV.

**Physical activity**

In the present thesis, COPD patients with moderate and severe COPD were found to be significantly less physically active compared with healthy subjects. Patients were found to have a lower mean physical activity level and to spend less time at least moderately physically active. Furthermore, the observed association between degree of airflow obstruction, expressed as percent of predicted FEV$_{1.0}$, and physical activity indicates that increased disease severity can be associated with decreased levels of physical activity. These findings are in line with previous studies, suggesting that COPD patients are less active compared with age-matched healthy subjects [95, 109].

It has previously been discussed that decreased exercise capacity in COPD patients may partly be caused by inactivity [4]. Interestingly, no significant differences in time spent sedentary were observed between the study groups in the present thesis. However, it has to be clarified that this result applies to time spent sedentary during registered time. It cannot be ruled out that patients wear the accelerometer shorter periods/day and spend more time sedentary when not wearing the accelerometer. Yet, as physical activity was positively associated with exercise capacity in both patients and healthy subjects while no associations between time spent sedentary and exercise capacity were observed in any of the study groups, it appears as if physical activity plays a more important role than time spent sedentary in the low exercise capacity observed in COPD patients.

As previously mentioned, low exercise capacity was found to correlate to changes in skeletal muscle in COPD patients and taken together with the observed association between exercise capacity and time spent physically active, it can be suggested that physical activity partly contributes to skeletal muscle changes observed in COPD. However, as it appears that changes in muscle morphology occur mainly in the later stages of disease, i.e. in patients with low PaO$_2$, it is most likely that a combination of hypoxia and low levels of physical activity strongly contributes to the more glycolytic muscle profile shown in these patients. Likewise, physical activity has been shown to have positive effects on skeletal muscle. Endurance training is known to promote a more aerobic muscle profile with increased number of type I fibres, increased mitochondrial density, increased oxidative enzyme activity and increased muscle capillarization [8, 14, 18, 21, 31, 55, 92]. As changes in muscle fibre type composition and capillarization are most pro-
nounced in the later stages of disease, it is important to promote physical activity in the early stages of disease to inhibit these changes.

Health recommendations for healthy adults aged 18–65 years, stating that 30 minutes of at least moderate physical activity/day is preferable for promoting and maintain health [45, 89], have been suggested to be applicable also for older adults (<65 years) and adults aged 50–64 years with clinically significant chronic conditions [84]. As it is shown in the present thesis that the intensity of physical activity affects exercise capacity, it can be suggested that these health recommendations can be applied to COPD patients. However, it appears as if few COPD patients reach the recommended levels, which highlights the need of focusing on moderate and high intensity physical activity on a regular basis in rehabilitation of COPD patients.

The low levels of physical activity recorded in study IV may also be a result of seasonal variations in physical activity. It has previously been discussed that seasonal variations may affect physical activity patterns [62] and as the present study was carried out during the winter season it is possible that test subjects were less active than they would have been during the summer season. However, data sampling was carried out during the same season for all subjects. Therefore, seasonal variations cannot explain differences in physical activity patterns shown between the different study groups.
CONCLUSIONS

From the work of this thesis it was concluded that:

- Exercise capacity expressed as distance walked in six minutes is lower in COPD patients compared with healthy subjects and is associated with disease severity.
- Skeletal muscle morphology shifts towards a more glycolytic muscle profile in COPD and changes in muscle morphology are correlated to disease severity.
- The number of satellite cells/muscle fibre does not differ between COPD patients and healthy subjects.
- Skeletal muscle capillarization is affected in COPD and the number of capillaries/muscle fibre, as well as the CFPE-index is lower in COPD patients compared with healthy subjects. CFPE-index is also correlated to disease severity.
- VHL is overexpressed in the tibial anterior muscle of patients with mild and moderate COPD compared with healthy subjects.
- Changes in skeletal muscle morphology are associated with decreased exercise capacity in COPD.
- Patients with moderate and severe COPD are less physically active compared with healthy subjects.
- Physical activity of at least moderate intensity is positively associated with exercise capacity while time spent sedentary is not associated with exercise capacity in COPD patients.
FUTURE PERSPECTIVES

In the present thesis it is shown that morphological changes within the tibial anterior muscle are most pronounced in the later stages of COPD, i.e. in patients with low PaO$_2$ and low physical activity levels. Systemic hypoxia and inadequate levels of physical activity are therefore suggested as plausible mediators of changes in skeletal muscle morphology in patients with COPD. However, the underlying mechanisms by which hypoxia influences skeletal muscle in COPD are not fully understood and need further investigation. Increased levels of pVHL are in study II suggested to impair hypoxic signalling pathways and thereby reduce muscle capillarization. Whether the overexpression of VHL is a consequence of systemic hypoxia or has other causes is not clarified in the present thesis and needs to be addressed further.

In the present thesis it is speculated that increasing the amount of physical activity of at least moderate intensity may generate a more aerobic muscle profile and may also increase exercise capacity in patients with COPD. Therefore, investigating the effects of specific exercise protocols on skeletal muscle morphology and exercise capacity in COPD would be of great interest.

As the satellite cell population was not found to differ between COPD patients and healthy subjects it is suggested that the regenerative capacity of the muscle is intact in COPD patients. However, the ability of the satellite cells to be activated and re-enter the cell cycle has not been addressed in this thesis and needs further investigation.
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