New insights in pulmonary cachexia

NEW INSIGHTS IN PULMONARY CACHEXIA:

A MULTIMODAL IMAGING APPROACH

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A MULTIMODAL IMAGING APPROACH

DISSERTATION

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1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Despite well-known health risks, a substantial proportion of people continue to customarily smoke tobacco [1]. Worldwide, over 900 million people smoke, with prevalence rates increasing every year [2]. While there is a gradual downward trend in smoking in the Netherlands, in 2017 still 23.1% of the adults smoked [3]. Of the profuse smoking-related diseases, chronic obstructive pulmonary disease (COPD) is most common and lung cancer is the most deadly [4].

COPD is an airway and lung disease with persistent airflow obstruction, which is the result of small airway remodelling and loss of elastic recoil. The diagnosis is based on the presence and severity of airflow limitation measured by post-bronchodilator spirometry, combined with respiratory symptoms and past exacerbations. Clinically, the disease expresses in a variable combination of thickening and obstruction of the airways (chronic bronchitis) and destruction of the air sacs (emphysema) [5]. Although cigarette smoke is acknowledged as the most important risk factor for COPD, epidemiologic studies show that non-smokers may also develop COPD [6], arguing for other risk factors involved. The list of risk factors for COPD include genetic susceptibility for airway disease, processes occurring during gestation, birth and childhood affecting lung development, high intake of unhealthy or western diet and environmental exposures to particles such as toxic gasses, air pollution and biomass fuel [5,7,8]. In the Netherlands, an estimated 600,000 people suffer from COPD, and it is the sixth leading cause of death [9,10].

Lung cancer is less common than COPD, but accounted for over 10,000 deaths in the Netherlands in 2017 [9]. Based on biological behaviour, lung cancer can be subdivided in two major subtypes, i.e. non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC occurs approximately in 80% of new lung cancer cases [11]. The majority (85%) of lung cancer cases are due to smoking [12], however only 10% of the smokers will develop the disease. Other identified risk factors are occupational exposure to asbestos, nickel and chromium, air pollution and genetic determinants [12,13]. In the Netherlands, an estimated 26,000 people suffer from lung cancer, which tops the list of cancer-related deaths [14,15].

COPD and lung cancer are interrelated. While they share environmental risk factors, epidemiological studies demonstrated that lung cancer is up to five times more likely to occur in smokers with airflow obstruction than those with normal lung function [16]. This risk increases with progressive decline in airflow obstruction [17]. Besides being each other's comorbid disease, the diseases also share extrapulmonary manifestations including unintended weight loss and muscle wasting [18-20].

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Cachexia

Although the lung is the major organ of pathology in both COPD and lung cancer, it is increasingly recognized that systemic manifestation of the disease contribute to symptom burden and health status. As illustrated by the following cases unintended weight loss occurs both in COPD and NSCLC.

> Case 1: COPD
> Patient I, a 58 years old female, has a forced expiratory volume in 1 sec, residual volume and diffusion capacity for carbon monoxide of 28%, 274% and 38% of predicted, respectively. This indicates she suffers from emphysema and severe hyperinflation. Furthermore, she has an arterial oxygen tension of 7.3 kPa, with a carbon dioxide pressure of 4.6 kPa, consistent with arterial hypoxaemia without respiratory insufficiency. Last year she had been admitted to the hospital twice for an acute exacerbation. She is therefore labelled COPD GOLD D. After having smoked 40 pack years she succeeded quitting. During the past years she has lost over 10 kg of body weight and currently she weighs only 36.7 kg resulting in a body mass index of 15.1 kg/m^2. Despite optimal pharmacological treatment, she experiences increased dyspnea upon exertion, which limits her ability to walk to the supermarket. She is therefore homebound and spends most of the day sedentary. Currently, she is referred to the pulmonologist to evaluate whether she might be an appropriate candidate for bronchoscopic lung volume reduction.

> Case 2: NSCLC
> Patient II is a 63 years old female and recently diagnosed with stage III NSCLC for which she started with concomitant chemoradiotherapy 3 weeks ago. Currently, she is hospitalized due to symptoms related to treatment side effects, including neutropenic fever and grade 2 esophagitis. Due to these treatment side effects, the next chemotherapy cycle is postponed. During hospitalization it was noticed that she had lost more than 5% of her body weight since start of treatment. Unlike the patient, her daughter is very distressed about the weight loss. She is worried that malnutrition worsens her mother's condition and affects prognosis.

These cases illustrate two patients, with different pulmonary diseases, who both suffer unintentional weight loss. The subjects differ in weight loss progression, being slower in patient I. Progressive unintentional weight loss is a characteristic of cachexia due to wasting of skeletal muscle and adipose tissue [21]. Cachexia is prevalent in a wide range of illnesses, including COPD and lung cancer. Prevalences ranging from 15 to 40% in COPD and NSCLC depending on definition, disease and disease stage [22-25].

Cachexia associates with a multitude of morbidities affecting clinical outcome. In COPD, patients experience loss of skeletal muscle function, reduced exercise capacity and decreased health status [26,27]. Importantly, cachexia also increases mortality, independent of airflow obstruction [23,28]. Cachectic patients with lung cancer experience diminished quality of life [19], intolerance to anticancer treatment [29] and shorter survival [30,31]. Although the exact contribution of cachexia to mortality is difficult to establish, it is estimated that 20-30% of cancer-related deaths are a direct result of cachexia [32].

Diagnosis of muscle and adipose tissue loss in COPD and NSCLC

The negative impact of cachexia on clinical outcome and healthcare utilization [23,31,33,34], emphasizes the importance of early identification. Weight loss history in a predefined time period or low body mass index are most commonly used in routine clinical practice to assess cachexia [35]. However, low muscle mass is not restricted to those with lean stature [31] and may be masked by adiposity or fluid retention [30,36,37]. Currently, the role of medical imaging is evolving from a primarily diagnostic tool towards a more central role in characterization of patient heterogeneity. In that context there is a growing number of opportunities to combine the imaging for medical purpose with assessment of body composition (reviewed in chapter 2).

Furthermore, computed tomography is increasingly used for single-slice assessment of muscle mass. The third lumbar level is considered as reference for muscle, but chest scans reach generally not further than the first lumbar level. For this reason, in COPD research pectoralis muscle has been proposed as alternative [38,39]. However, to date no studies compared the method with appropriate reference methods or explored alternative locations on chest scans.

Pathophysiology of cachexia

The underlying mechanisms responsible for cachexia in COPD and lung cancer are considered complex and multifactorial, and may differ between the two diseases, between different pulmonary phenotypes and related to disease severity.

The onset of low muscle mass in COPD can arise either from failure to attain a normal skeletal muscle structure and function during early childhood or from an accelerated decline of muscle mass in later life. There is evidence that some COPD patients failed to achieve the normal spirometric plateau because of previous exposures to childhood respiratory infections [40], which might also adversely affect maturation of skeletal muscle. In line with this hypothesis, a constitutionally lower fat-free mass was found among older aged subjects with mild-to-moderate obstructive lung disease compared to smokers without obstructive lung disease. Seven-year follow-up revealed no differences in pattern and progression of age associated changes in fat-free mass [41]. Further, a genetic predisposition for lean stature in COPD patients was found [42] and this lean stature has also been suggested to be a risk factor for development of COPD [43].

There is evidence that a subgroup of COPD patients has a steeper decline of fat-free mass, associated with the number of exacerbations [44,45]. Furthermore, dyspnea upon exertion partially contributes to physical inactivity, which accelerates the downward spiral of deconditioning of the skeletal muscle. However, this does not fully explain the high prevalence and extensiveness of low muscle mass. Additionally, individual differences in the amount of muscle wasting complicate the understanding of the underlying mechanisms.

Great progress has been made in understanding molecular alterations underlying muscle decline. Muscle maintenance is tightly regulated by the balance between muscle cell synthesis and growing on one hand, and muscle breakdown mediated by proteolysis, autophagy and apoptosis on the other hand. In muscle-wasted COPD patients, myogenic signalling and muscle protein turnover is elevated [46]. Recent network analyses also highlight a coordinated regulation of muscle mass and metabolic plasticity [47], being disturbed in COPD [48]. Together, these molecular alterations suggest ongoing muscle repair and remodelling, which might be insufficient for muscle maintenance in COPD.

Muscle wasting commonly occurs in COPD [23] and occurs more frequently in the emphysematous phenotype [18,26] and in advanced disease [23]. During acute disease exacerbations this gradual loss might be accelerated [44,45]. Muscle wasting can occur with and without decrease in body mass index [23], suggesting that different pathophysiological mechanisms of impaired protein turnover versus whole body energy metabolism affect muscles. However, research on whole body energy regulation and fat metabolism has been underexposed.

The classical description of “the pink puffer” by Filley et al. [49] and more recent research using advanced body composition methods, indicate that in particular the emphysematous COPD patient is prone to cachexia. Results from a 3-year follow-up study revealed that patients with more emphysema exhibited an accelerated decline in both lung function and fat-free mass [50]. The emphysema phenotype is hallmarked by a reduction in lung elastic recoil and progressive hyperinflation, resulting in elevated airway resistance and contributing to impaired lung mechanics [51]. Although increased breathing workload in COPD is well documented [52,53], the effect of hyperinflation and emphysema on energy metabolism and muscle mass regulation is unclear.

Activation of brown adipose tissue (BAT) or browning of white adipose tissue has been proposed as putative trigger for hypermetabolism. By dissipating energy stored in triglycerides as heat [54], BAT activation contributes to cold-induced energy expenditure [54,55]. In healthy lean men, energy expenditure increased on average with 17.2% [56]. This implies that BAT thermogenesis may be a significant component of whole body energy expenditure and thereby play a role in regulation of body weight. Indeed, studies employing cold-induced BAT activation

showed that BAT volume and activity are lower in individuals who are obese and BAT activity is inversely correlated with body weight [57-59]. BAT has been primarily researched as a potential target for mitigating obesity as it may facilitate weight loss. Yet few research attempts have been made at the other side of the spectrum, why patients with COPD and lung cancer unintentionally lose weight. Controlled prospective human studies investigating the role of sustained BAT activation in development of cachexia are lacking [60-62].

In cancer cachexia, the tumour is thought to be a potential driver of tissue wasting, partly mediated by tumour and host elicited inflammation. Cachectic NSCLC patients exhibited a prominent systemic inflammation, whereas lower systemic inflammation was apparent in precachectic patients. Furthermore, plasma transfer experiments revealed that plasma of both cachectic and precachectic patients induced inflammatory signalling in skeletal muscle, with increased proteolysis and an impaired anabolic signalling [63]. Cancer therapy aiming to treat the tumour also affects other tissues. Concurrent chemoradiotherapy, which is the standard treatment for patients with unresectable locally advanced NSCLC [64] frequently induces radiation esophagitis and associated dysphagia [65]. It is therefore assumed that weight loss results from radiation esophagitis due to impaired dietary intake. Whether weight loss also occurs before onset of radiation esophagitis and whether this weight loss contributes to prognosis requires further investigation.

In addition, it has been suggested that chemotherapy agents itself also affect cachexia. While some known side effects including nausea, diarrhoea and anorexia may contribute to weight loss, these symptoms do not fully explain muscle wasting. Evidence from experimental rodent cancer models suggest a direct effect of chemotherapeutics on muscle wasting and lipolysis [66, 67]. However, the extent to which muscle mass and adipose tissue mass are affected in a clinical setting is unknown.

Prediction of future muscle loss

There are currently no biomarkers available to identify patients at risk for development of cachexia. In oncology, radiomics is a growing era of interest. Radiomics is a method that quantitatively extracts features including shape, size, intensity and texture that are related to tumour pathophysiology from standard-of-care medical images [68]. In oncologic patients extracted tumour features aid in prognosis prediction [69-72]. Radiomic analysis of muscle features has never been used to predict future muscle loss.

1

AIMS AND OUTLINE OF THIS THESIS

Prevention and timely treatment of cachexia in COPD and NSCLC requires adequate and early identification of patients at risk and detailed understanding of the pathophysiology involved. Comparative clinical research between COPD and NSCLC may shed new light on pulmonary cachexia. Two specific aims were addressed:

- To evaluate opportunities to combine standard-of-care medical images with assessment and prediction of tissue wasting
- To provide insights in putative triggers involved in muscle wasting, including the contribution of impaired breathing mechanics and brown adipose tissue on energy metabolism and the impact of cancer treatment on muscle wasting.

In **Chapter 2**, we review the potential use of diagnostic medical images for characterization of COPD. Furthermore, we describe the potential implications for body composition assessment.

In **Chapter 3**, we investigate whether chest computed tomography scans made in daily clinical practice of respiratory patients can be used to assess cross-sectional and longitudinal body composition.

In **Chapter 4**, we review recent insights on the pathophysiology of cachexia in COPD.

Chapter 5 presents analysis of changes in skeletal muscle mass and adipose tissue in severe emphysematous COPD patients before and after treated with bronchoscopic lung volume reduction.

Chapter 6 reports a proof of concept clinical trial investigating the contribution of impaired lung mechanics on energy balance regulation in severe emphysematous COPD patients treated with bronchoscopic lung volume reduction.

Chapter 7 presents a controlled clinical study on brown adipose tissue activation and its potential contribution to whole body energy expenditure in COPD.

In cancer cachexia, cancer therapies aiming to treat the tumour might also affects other tissues, and thereby contributes to tissue wasting.
Chapter 8 reports the impact of weight loss within 3 weeks after initiation of concurrent administration of chemotherapy and radiotherapy on survival in stage III NSCLC patients.

Chapter 9 presents a longitudinal analysis of computed tomography-derived body composition during chemotherapy in therapy naïve stage IV NSCLC patients.

In **Chapter 10**, we use a radiomics approach to assess whether skeletal muscle radiomic features can predict future muscle loss.

Finally, the results of the studies described in the current dissertation will be discussed and placed in a broader context in **Chapter 11**.

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2

Imaging approaches to understand disease complexity: chronic obstructive pulmonary disease as a clinical model

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ABSTRACT

The clinical manifestations of chronic obstructive pulmonary disease (COPD) reflect an aggregate of multiple pulmonary and extra-pulmonary processes. It is increasingly clear that full assessment of these processes is essential to characterize disease burden and to tailor therapy. Medical imaging has advanced such that it is now possible to obtain in vivo insight in the presence and severity of lung disease associated features. In this review, we have assembled data from multiple disciplines of medical imaging research to review the role of imaging in characterization of COPD. Topics include imaging of the lungs, body composition and extra-pulmonary tissue metabolism. The primary focus is on imaging modalities which are widely available in clinical care settings and which potentially contribute to describing COPD heterogeneity and enhance our insight in underlying pathophysiological processes and their structural and functional effects.

INTRODUCTION

The clinical manifestations of chronic obstructive pulmonary disease (COPD) reflect an aggregate of multiple pulmonary and extra-pulmonary processes. It is increasingly clear that full assessment of these processes is essential to characterize disease burden and to tailor therapy [1,2]. Examples of these COPD associated features include emphysematous destruction and fibrosis of the lung parenchyma, cardiovascular disease, sarcopenia, abdominal adiposity, and osteoporosis [2-6]. Highly effective therapies are available for many of these conditions and yet their prevalence and role in the mortality and morbidity of those with chronic respiratory diseases is often underappreciated [1,2,7].

While no imaging study is critical to the initial diagnosis and management of COPD, many patients with COPD undergo imaging for other reasons such as lung cancer screening, screening for osteoporosis, or acute changes in their clinical status. Although these medical images are often used principally for the indication that prompted their acquisition, they also provide the opportunity to obtain in vivo insight into the presence and severity of these and other conditions [4,8-11]. Exciting new areas of imaging research are focused on singular aspects of these comorbidities, however such specialization often causes us to lose a larger view of efforts in this field. Thus, while we do not advocate the routine use of imaging in COPD without another indication, extraction of morphological information and comorbidity patterns from already available medical images, acquired in routine clinical care, may provide new opportunities to better characterize patients and individualize treatment in the future.

In this review, we have brought together the available information on structural and metabolic imaging with regard to its potential usefulness in improving the understanding of COPD and with particular emphasis on quantitative techniques. More specifically, we focus on the structural information obtained using routine computed tomography (CT) imaging as well as on the information on body composition from both CT imaging and dual-energy X-ray absorptiometry (DXA). Furthermore, we briefly discuss the potential of metabolic molecular imaging using the standard positron emission tomography (PET) radiopharmaceutical fluordeoxyglucose (FDG) in COPD when available for additional scientific purpose. These three imaging modalities allow the noninvasive characterization of many of the underlying pathophysiological processes in COPD and their structural and functional effects. Of note, while magnetic resonance imaging and optical coherence tomography have been and continue to be investigated as potential imaging techniques in COPD, none have yet found a place in routine clinical care and so are beyond the scope of this review. In addition, while ultrasound is increasingly useful in the diagnosis of acute chest diseases such as pneumothorax, and endobronchial ultrasound has been widely adopted for procedures such as lymph node biopsy, neither has been routinely used in COPD and so have also been excluded.

LUNG STRUCTURE

Functional, structural and molecular imaging of the lungs, using a variety of modalities, has revealed new information regarding the complexity of COPD. This discussion will primarily focus on the role of imaging in understanding COPD, but it should be noted that there is extensive work in this area on lung cancer, interstitial lung disease, asthma, pulmonary hypertension, and other chronic lung diseases.

Computed Tomography

Contributions to phenotyping: CT imaging utilizes x-rays to acquire a three dimensional (3D) attenuation image of the body. The images themselves are comprised of voxels, each of which has a density, typically measured in Hounsfield units (HU) that distinguishes water from air, enabling the characterization of various tissue types to that particular area. These voxels are then reconstructed to using various grey values to create the overall CT image. Although CT imaging is not indicated for the routine diagnosis or clinical staging of COPD, due to the overlapping risk factors and clinical manifestations of COPD with other chest diseases such as lung cancer, it is frequently obtained for other reasons in these patients [12,13]. There are a multitude of processes evident in CT imaging of the lungs, which may be grouped by anatomic compartment, namely those that affect the parenchyma, the airways and the vasculature.

With regard to the lung parenchyma, emphysema is the most prominent anatomic and radiologic COPD associated process (figure 1a), and the volume, distribution and subtype of emphysema on CT have yielded insights into the clinical management and physiology of COPD [14,15]. For instance, in the National Emphysema Treatment Trial, 1200 COPD patients with hyperinflation were randomized to either lung volume reduction surgery (LVRS) or optimal medical therapy. While there was no overall survival benefit to LVRS, a non-pre-specified subgroup analysis based on the combination of baseline CT emphysema distribution and exercise testing identified a subset of patients who responded differently to LVRS compared to medical treatment. Subjects with upper lobe or upper zone predominant emphysema and low exercise capacity had significantly lower mortality than subjects randomized to medical treatment [16]. Subsequent work suggests that such clinical differences between those with upper lobe predominant or heterogeneous emphysema and more homogeneous emphysema may reflect not only anatomic differences but physiologic ones as well. For instance, those with homogenous emphysema have been shown to have a greater degree of dynamic hyperinflation during exercise than those with upper lobe predominant disease suggesting potential differences in their lung mechanics and differences in the underlying pathophysiology of their disease [17]. Perhaps more importantly clinically is the role of CT in identifying patients for endobronchial lung volume reduction procedures, which are bronchoscopic alternatives to surgical LVRS including endobronchial valves, coils, glue and other devices. The effectiveness of endobronchial valves in particular may be limited by collateral ventilation in the setting of incomplete interlobular fissures.

Collateral ventilation prevents the valves from fully deflating the target area thus limiting their effectiveness. Small studies have suggested that CT imaging may be sensitive, though not necessarily specific, for the detection of the interlobular fissures, and new automated techniques may improve its performance in this area making it an important tool for the pre-procedure planning of patients undergoing endobronchial lung volume reduction [18-20].

While less immediately clinically applicable, the CT identification of emphysema subtypes provide a non-invasive window into the pathophysiology of the disease, and thus open the door to additional treatment options. Smoking, the most frequent cause of emphysema, is most commonly associated with centrilobular disease, and, to a lesser, but still significant extent, with paraseptal emphysema [21-23]. Additional subtypes include panlobular disease which tends to be rare except in patients with alpha-1-antitrypsin deficiency or who have used certain intravenous drugs [21]. Recent investigations suggest that these subtypes, including both the lobular distribution as well as severity, may have different clinical associations [24,25]. For instance, while nearly all emphysema subtypes are associated with respiratory function, dyspnea, physical capacity and annual number of exacerbations in COPD, paraseptal emphysema may be a marker of more severe clinical manifestations of the disease [25]. Further CT based investigation has shown that there may be genetic and pathophysiologic reasons for such disease heterogeneity. For instance, emphysema matrix-metalloproteinases (MMPs), which are proteolytic enzymes that can break down the extracellular matrix, have been shown to be potentially important in the development of emphysema. While some MMPs, such as MMP-3 and MMP-10 are associated with most emphysema subtypes, others, such as MMP-3 and MMP-10 have been shown to be associated with both paraseptal emphysema and more severe centrilobular disease [26]. In addition, genome wide association studies have suggested that the each emphysema subtypes may have a somewhat unique set of genetic determinants [24]. Taken together, these findings suggest the importance of CT imaging for the understanding of both the clinical manifestations and pathophysiology of the emphysematous component of COPD, especially in terms of identifying specific phenotypes of the disease.

It is increasingly clear, in large part thanks to CT imaging based studies, that tobacco smoke exposure results in not only emphysematous but also interstitial changes in the lung, such as interstitial lung disease. In the extreme, such changes manifest radiologically and clinically as pulmonary fibrosis, but more subtle changes, often termed interstitial lung abnormalities (ILA) are highly prevalent in ever smokers [27-31] (figure 1b). Clinically, smokers with ILA tend to have less COPD, greater respiratory impairment, shorter 6 minute walk distances, and less emphysema as measured by standard densitometry [29,30,32,33]. Furthermore, these abnormalities are associated with a decline in lung function and are strongly predictive of all cause and respiratory specific mortality [31,34]. Finally, ILA have been shown to be associated with a specific single nucleotide polymorphism in the promoter region of the gene

encoding mucin 5B, which has been strongly linked to the presence of more advanced idiopathic pulmonary fibrosis [35,36]. These clinical and genetic associations do not necessarily imply that all ILA will progress to idiopathic pulmonary fibrosis, but rather highlight the potential role of CT in identifying those COPD patients at the greatest risk for adverse events as well as its importance for understanding the complexity of smoking related lung disease. Further work in this area could ultimately lead to the use of novel therapies such as anti-fibrotic agents in those patients with combined emphysema and early stage fibrosis.

The other lung compartment classically affected in COPD is the airways. While usually described as being the location of chronic bronchitis, the distal small airways are likely the site of airflow limitation for COPD more generally [37,38]. These airways are below the resolution of standard clinical CT imaging, however, quantitative CT analysis of more central airways in smokers has revealed that those with smaller airway caliber and thicker airway walls tend to have lower lung function and more frequent exacerbations [39-42]. While these findings are not yet clinically applicable, they may prove especially important in future phenotyping of COPD. For instance, recent work by Lange et al. has shown that some individuals with COPD developed airflow limitation in early adulthood followed by a slow decline in lung function rather than due to a rapid decline from a higher or more normal peak lung function, and it may be that those smokers who have a smaller airway caliber on CT in young adulthood are those more likely to develop COPD [43,44].

The last lung compartment with evident changes on the CT scans of smokers is the intraparenchymal pulmonary vasculature. Pruning of the vasculature and drop out in regions of severe emphysema have long been recognized on angiographic studies, and improved CT scan resolution has made it possible to detect and quantify such changes on CT [45,46]. These changes typically include pruning of the distal vasculature and dilation of the more central vessels [47-49]. While not yet clinically available, 3D analysis of the distal pulmonary vasculature has shown that pruning of the distal vessels is associated with increased respiratory symptoms, reduced exercise capacity, impaired diffusing capacity and multi-component predictors of mortality [47]. Further work is now ongoing to determine how metrics of pulmonary vascular morphology may be further integrated into clinical care to predict outcomes such as patient response to lung volume reduction. Of more immediate clinical importance is the appearance of the central vasculature on CT in patients with COPD. More specifically, Wells et al. has shown that pulmonary artery enlargement as defined by a ratio of the diameter of the pulmonary artery to the diameter of the aorta of greater than 1 is associated with an increased risk for severe respiratory exacerbations in patients with COPD, potentially identifying patients who will benefit the most from strategies to reduce exacerbation risk [49].

Advantages: CT imaging is almost universally available in clinical care settings. It provides high resolution insight into lung structure and there are a multitude of methods published on the standards of image interpretation.

Limitations: The greatest limitation to the broad utilization of CT imaging is ionizing radiation. There is an ongoing debate in the medical community about what is a safe level of such exposure. Because of this, CT manufacturers have invested a tremendous amount of resources to reduce the dose of radiation while maintaining or even improving image quality.

Clinical applications: CT is increasingly becoming a common component to the clinical care of patients with COPD. Despite the reason for image acquisition, the biomedical community is obliged to obtain as much data as possible from these images to improve our understanding of disease and improve patient care.

BODY COMPOSITION

Alterations in body composition frequently occur in COPD, contributing to increased morbidity and mortality. In fact, up to 25% of patients with COPD have a significant loss of skeletal muscle, which has been associated with impaired exercise performance and increased mortality independent of the primary lung impairment [50,51]. However, in overweight and obese subjects, low muscle mass may not be recognized without assessment of body composition [52]. Furthermore, Beijers et al. recently demonstrated in normal weight COPD patients a high prevalence of low muscle mass combined with abdominal obesity. Notably, these patients were characterized by an increased cardiometabolic risk [53]. Clinically available imaging modalities to visualize body composition include dual-energy X-ray absorptiometry (DXA) and CT.

DXA imaging

Contribution to phenotyping: Originally designed for osteoporosis assessment, DXA can also be utilized to measure body composition (figure 1e). The underlying concept for DXA estimation of body composition is based on assumptions regarding the difference in chemical constituents. Briefly, DXA utilizes the variable absorption or attenuation of high and low energy x-ray photons to estimate the fraction of tissue occupied by fat and fat free mass (FM and FFM respectively) [54,55]. While the details of how these measurements are made are beyond the scope of this review, it is important to note that increased tissue thickness is associated with a greater attenuation of low energy photons than high energy photons regardless of the tissue composition, the effect of which is a tendency for the underestimation of FM in obese subjects [56].

Recently, the European Respiratory Society taskforce on nutritional assessment and therapy in COPD has designated DXA as the most appropriate tool for combined screening of osteoporosis,

sarcopenia and adipose tissue redistribution [57]. While two compartment screening methods such as bioelectrical impedance analysis and skinfold thickness can discriminate whole body FM from FFM, DXA is able to analyse tissue depletion and redistribution at regional levels. This is particularly useful in COPD, in which appendicular FFM has been found to be more predictive for exercise performance than whole body FFM [52]. Moreover, DXA analyses have also provided insight into some of the clinical complexity of COPD. For example, despite similar decreases appendicular FFM, whole body and trunk FFM reductions have been found to be more altered in COPD patients with CT based emphysema compared to those without [58,59].

In addition to differences in truncal and whole body distribution, recent advances in DXA imaging have allowed for the differentiation between and the ratio of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) [60-62]. SAT and VAT differ in metabolic and inflammatory characteristics and the corresponding metabolic risk profile [63-65]. Smoking cigarettes is a main cause of both COPD and cardiac disease but other causes may increase the risk. For instance, cardiac disease in COPD associates with VAT [66,67]. DXA measurements of SAT and VAT have thus far only been validated in patients without COPD. Other limitations of DXA include the insensitivity of appendicular FFM for the detection of small loss of muscle fibre CSA, which may limit its role in monitoring subtle changes after intervention [68].

Advantages: DXA is particularly attractive due to low ionizing radiation, which is comparable to one day of normal background radiation [69]. With approximately 10 minutes scan time, DXA is very time-efficient and convenient. It enables non-invasive insight into body composition and its potential metabolic associations.

Limitations: Although the errors are relatively small, one has to keep in mind that the results may be influenced by conditions in which the ratio of extracellular water and intracellular water varies (e.g., severe malnutrition, oedema, diuretics, ageing) [70]. Furthermore, this technique only provides 2D projections of the body, thereby being unable to gain information about muscle groups or quantification of fat depots in the muscle. Regarding DXA-based VAT analysis more validation studies are warranted, as there seems to be a tendency to overestimation of VAT [60,62].

Clinical applicability: DXA is used in clinical routine care for evaluation of osteoporosis in COPD.

CT imaging

Contribution to phenotyping: As discussed above, CT imaging provides a wealth of information regarding lung structure and its implications for lung function in patients with COPD. Extra-pulmonary findings on CT in COPD are of great scientific utility as well. Similar to DXA imaging, CT can also be utilized beyond its “field” to measure body composition in addition to the more

traditional lung measures discussed above. This is done by measuring muscle cross sectional area (CSA) on CT imaging, and while the standard site to measure CSA is the third lumbar vertebra (L3) this is typically outside the field of view of clinically acquired CT imaging in patients with chest diseases [51,53,71,72] (figure 1d).

Therefore, other levels, such as L1, have been evaluated. Although single slice CSA L3 and L1 result in significantly different whole body estimates of muscle mass, the latter may be useful to detect changes during the disease course or after intervention [72]. Other sites that have been evaluated include the pectoralis muscles, where muscle CSA assessed by CT has been correlated with both objective and subjective measures of COPD severity, and mid-thigh muscle CSA which has been found to be strongly related to increased mortality risk [11,73]. Although such measures are not yet routinely clinically available, advances in CT segmentation technology that allows the automated detection of specific radiographic features may soon enable their more routine use.

CT is also able to quantify and evaluate the distribution of fat. For instance, CT-based analysis at the L4-L5 level revealed significantly increased VAT in elderly with obstructive lung disease than non-obstructive subjects despite a comparable SAT and BMI. This adipose tissue distribution also correlated to higher interleukin 6 levels in elderly with obstructive lung disease [74], suggesting that VAT might contribute to increased plasma IL-6, which was also shown to be a strong predictor of all-cause and respiratory mortality.

Finally, patients with chronic diseases such as COPD are known to have ectopic depots of fat beyond the major subcutaneous and visceral fat storage locations, such as intramuscular adipose tissue (IMAT). While, data concerning muscle lipid content in elderly and patients with muscle wasting disease have been initially relied on muscle biopsy studies, improved resolution of CT scanners makes it now possible to non-invasively quantify IMAT [75-77]. CT derived data concerning IMAT have shown fat infiltration in intercostal muscles and mid-thigh muscle in normal to overweight COPD patients. This infiltration in intercostal muscles in particular is correlated with COPD severity and patients with high mid-thigh IMAT tended to have lower physical activity levels [78,79].

Advantages: An advantage of CT is that information about body composition can be collected at the time of routine clinical imaging.

Limitations: Muscle CSA and adipose tissue quantification may be subject to a multitude of factors such as manufacturer, slice thickness and pixel spacing, although their exact influence on body composition quantification is unknown. Furthermore, the radiation exposure of CT limits longitudinal research applications.

Clinical applicability: Although CT imaging is not independently indicated for the diagnosis and management of isolated COPD, images are widely available due to their implementation in excluding other underlying pathology. Measurements of muscle CSA and adipose tissue are not yet routinely performed but new automated techniques may enable their introduction into clinical care in the near future.

TISSUE METABOLISM

Metabolic alterations are not only reflected by body composition but also by underlying changes in tissue metabolism. 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) may improve understanding of biological processes of tissues.

18F-FDG-PET imaging

Contribution to phenotyping: While DXA and CT are informative in tissue mass quantification and distribution, they are limited in monitoring tissue metabolic activity. Standard PET imaging uses a radiolabelled glucose derivate fluordeoxyglucose (FDG) to give a 3D information on glucose metabolism in the body enabling evaluation of local or tissue specific metabolic features [80]. There is a wide range of classical and novel radiopharmaceuticals (tracers) evaluated in (pre)clinical and experimental settings using PET. However, here we focus on FDG-PET because of its universal availability in clinical care settings. Although PET imaging is not routinely used in the workup of COPD assessment, this method allowing quantification of tissue metabolism may be useful in scientific research to contribute to better understanding of different clinical phenotypes as illustrated below.

FDG reflects the metabolic rate of glucose, a process reinforced in inflamed tissue and this distinctive characteristic enables PET to evaluate adipose tissue activity in vivo. One study showed that COPD patients exhibited increased FDG uptake in VAT compared to subjects without COPD. Furthermore, FDG uptake in VAT was found to predict aortic wall FDG uptake, even when adjusted for sex, age, BMI and smoking (figure 1c) [81-83]. It is well known that smoking tobacco is the main risk factor for development of both COPD and vascular inflammatory processes. Nevertheless, these data suggest a possible contributing role of VAT in augmenting atherosclerotic processes in COPD [6]. Further research is however needed including histological confirmation of VAT biological activity.

Several studies confirmed the presence of metabolically active brown adipose tissue (BAT) in the neck and supraclavicular region, the mediastinum as well as along the spine in healthy adults using FDG-PET scanning and biopsy verification [84,85]. BAT activity is inversely correlated to body fat percentage in healthy adults [85]. In addition, in several BAT-associated pathological conditions, such as pheochromocytoma [86] and hyperthyreoidism [87], energy expenditure is increased. Recently, it has been shown from in vitro experiments that lactate stimulate browning

of adipocytes, mediated by intracellular redox modifications [88]. Given the hypermetabolic state observed in COPD in particular in the emphysematous phenotype [89], mitochondrial dysfunction [90,91] and early lactate acidosis during exercise [92], increased BAT activity may be involved in energy homeostasis in COPD. However, no clinical studies are yet available that assessed BAT activity in COPD.

Advantages: Whereas DXA and CT provide information about structures, PET is a unique imaging tool that provides molecular imaging information. Using FDG non-invasive quantitative information of metabolic activity of tissues in vivo is acquired.

Limitations: FDG-PET uses ionizing radiation, is not broadly available and is more expensive than the other modalities. It is important to mention that the radiation burden is lower than conventionally used whole body CT protocols. Additionally, factors influencing the amount of FDG uptake such as overexpression of glucose receptors, might induce false positive or false negative results [80].

Clinical applicability: Not integrated in standard clinical care of COPD patients

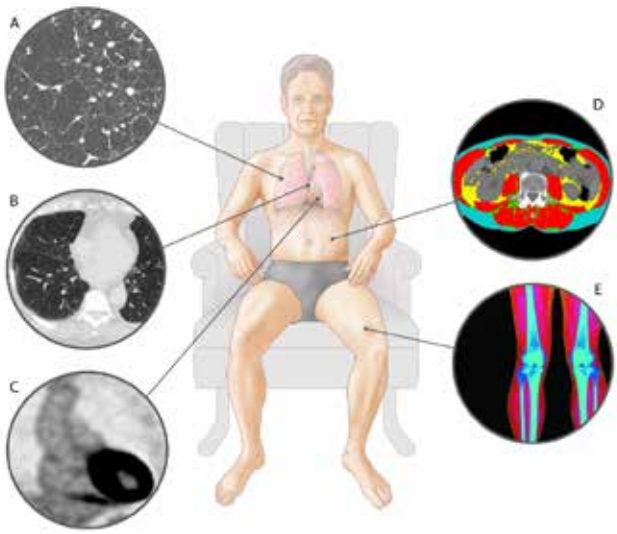

Figure 1. Imaging techniques. (a) Thoracic CT image of patient with emphysema; (b) thoracic CT image of patient with interstitial lung abnormalities; (c) PET image displaying FDG uptake in aortic wall [Image courtesy of Dr. Jan Bucerius]; (d) abdominal CT with different body composition compartments at L3; (e) DXA scan leg with different body composition compartments.

CONCLUSION

Over the past century, imaging has become critically important in the routine clinical care of patients and the technologies used have advanced dramatically. The use of new analytic techniques applied to traditional imaging modalities and the development of novel imaging techniques have greatly improved the clinical care and understanding of complex diseases like COPD. These approaches, including CT, DXA, PET or hybrid PET/CT already aid in the non-invasive characterization of COPD phenotypes and severity, and ongoing and future work has the potential to improve our understanding of and care for this disease and the patients it affects even more. We acknowledge that at least in the short term, some of the methods discussed above are limited to the research setting. They may improve phenotyping for clinical observational studies and clinical trials, and may contribute to better risk stratification and personalized medicine.

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3

Cross-sectional and longitudinal assessment of muscle from regular chest computed tomography scans: L1 and pectoralis muscle compared to L3 as reference in non-small cell lung cancer

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ABSTRACT

Background

Computed tomography (CT) is increasingly used in clinical research for single-slice assessment of muscle mass to correlate with clinical outcome and evaluate treatment efficacy. The third lumbar level (L3) is considered as reference for muscle, but chest scans generally do not reach beyond the first lumbar level (L1). This study investigates if pectoralis muscle and L1 are appropriate alternatives for L3.

Methods

CT scans of 115 stage IV non-small cell lung cancer patients were analysed before and during tumour therapy. Skeletal muscle assessed at pectoralis and L1 muscle was compared to L3 at baseline. Furthermore, the prognostic significance of changes in muscle mass determined at different locations was investigated.

Results

Pearson's correlation coefficient between skeletal muscle at L3 and L1 was stronger ($r=0.90$, $P<0.001$) than between L3 and pectoralis muscle ($r=0.71$, $P<0.001$). Cox regression analysis revealed that L3 (HR 0.943, 95% CI: 0.92-0.97, $p<0.001$) and L1 muscle loss (HR 0.954, 95% CI: 0.93-0.98, $p<0.001$) predicted overall survival, whereas pectoralis muscle loss did not.

Conclusion

L1 is a better alternative than pectoralis muscle to substitute L3 for analysis of muscle mass from regular chest CT scans.

INTRODUCTION

It is now widely recognized that loss of skeletal muscle mass adversely impacts clinical outcome and increases healthcare utilization in patients facing cancer or a chronic disease like Chronic Obstructive Pulmonary Disease (COPD) [1-7]. Since low muscle mass is not restricted to those with lean stature [5], there is a growing interest to assess muscle mass with use of techniques that are easily applicable in the clinic or are already being used in regular clinical care for other diagnostic purposes. Whole body skeletal muscle mass can be estimated in clinical settings by measuring fat-free mass using bioimpedance analysis and dual-energy X-ray absorptiometry [8]. Since these modalities are not typically incorporated in routine clinical care, other strategies to evaluate muscle mass have been employed. Computed tomography (CT) is increasingly used as research tool for local quantification of skeletal muscle. These medical images provide the opportunity to obtain information about skeletal muscle quantity and quality. CT-derived skeletal muscle at the third lumbar level (L3) is considered as reference because muscle cross-sectional area (CSA) at L3 is linearly related to whole-body muscle mass assessed by magnetic resonance imaging [9]. As most chest CT scans do not reach beyond the first lumbar level (L1), attempts have been made to assess muscles at other levels. For this reason, in COPD research pectoralis muscle has been proposed as alternative [10-12]. However it is unclear whether this pectoralis muscle reflects a reliable representation of whole body muscle area, as to date no studies compared the method with appropriate reference methods. The first aim of this study is therefore to investigate the association between pectoralis muscle CSA and L3 muscle CSA.

The advantage of L3 and L1 as opposed to pectoralis is that besides skeletal muscle, adipose tissue CSA and distribution can be quantified. Recently, skeletal muscle cut-off values for sarcopenia at L1 have been proposed based on CT analysis in a healthy American population. The authors reported good correlations regarding skeletal muscle CSA at L1 compared to L3. However, pectoralis muscle was not assessed and data comparing adipose tissue between both regions are lacking [13]. The second aim of the study is therefore to compare skeletal muscle and adipose tissue analysis between L1 and L3.

The added value of CT-derived analysis of body composition may not only be to phenotype patients with respiratory disease but even more to detect treatment induced subtle changes in muscle CSA that may adversely affect disease progression or treatment outcome. The third aim of the study is to evaluate the association between changes in skeletal muscle CSA assessed from pectoralis muscle, L1 and L3 after tumour treatment to overall survival.

METHODS

Study subjects

To address the research questions, CT scans derived from a randomized clinical trial involving patients with non-small cell lung cancer (NSCLC) were analysed. This multicentre randomized phase II trial investigated nitroglycerin patches added to paclitaxel-carboplatin-bevacizumab in 223 therapy-naive patients with stage IV non-squamous NSCLC. As part of standard work-up, patients received a 18F-fluorodeoxyglucose positron emission tomography scan combined with CT scan at baseline. The study protocol pre-specified a second 18F-fluorodeoxyglucose positron emission tomography/CT between day 22 and 24 (after second chemotherapy infusion). The study was performed in accordance with the provisions of the Declaration of Helsinki. The study was approved by the medical ethical committee of University Medical Centre Groningen in the Netherlands (METC 2010.241) and registered at clinicaltrial.gov (NCT01171170). All patients provided written informed consent before performing any study-related activities. Adding nitroglycerin to first-line carboplatin-paclitaxel-bevacizumab did not improve progression-free survival and overall survival in this cohort. The detailed methodology of this study has been published previously [14].

Image analysis

CT scans were made at baseline and after the second chemotherapy infusion for response assessment. Cross-sectional measurements of skeletal muscle areas (CSA in cm^2) were made on transverse images. Three images were selected for each patient. The pectoralis muscle slice was selected by scrolling towards the apex of the lungs and identifying the first axial image above the aortic arch. Bilaterally, the area of pectoralis major and minor muscles was measured. For slices at lumbar levels, the first image at the third and first lumbar level with both vertebral transverse processes clearly visible was used in the analysis. The measurements included the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique, and rectus abdominis muscles.

Additionally, adipose tissue compartments including subcutaneous adipose tissue (SAT) CSA and visceral adipose tissue (VAT) CSA were analysed at L3 and L1. Proportional changes in CSA between CT scans were normalized for the time interval between scans. We therefore divided the proportional CSA change by the number of days between scans and multiplied by 100 days to standardise for all patients (percentage change per 100 days).

All analyses were performed with Slice-O-Matic software v5.0 (Tomovision, Montreal, Canada). CSA of these structures were quantified on the basis of pre-established thresholds of Hounsfield units (skeletal muscle-29 to 150, IMAT-190 to-30, SAT-190 to-30, and VAT-150 to-50). Boundaries were corrected manually when necessary.

Statistical analyses

Patients were included if CT scans both at baseline and follow-up were available and contained images of pectoralis, L1 and L3. Descriptive statistics of demographic and clinical variables were obtained. Means and standard deviations (SD) were provided for continuous normally distributed variables, median (range) for continuous not-normally distributed variables and percentages were shown for categorical variables. Comparisons within groups were performed with paired t-test or Wilcoxon signed rank test.

To measure the strength of the linear association of different assay methods (pectoralis, L1 and L3 at baseline and during follow-up) Pearson's correlation coefficient (r) was used. Additionally, a Bland-Altman plot was drawn to investigate the existence of systematic bias and to identify possible outliers. Differences between assay methods are expressed as percentage of the values on the Y-axis. L3 was used as reference method and therefore plotted on the X-axis. If the mean value of the difference between assay methods is different from 0, it indicates a systematic difference. If there is proportional bias, the Bland-Altman plot shows whether or not this bias is constant for all the measures of CSA's.

To evaluate if pectoralis, L1 and L3 measured the same construct (i.e. internal consistency) Cronbach's coefficient alpha was calculated. Cronbach's coefficient alpha is expressed as a number between 0 and 1, and values closer to 1 indicate good reliability among assay methods.

Kaplan meier survival analysis was performed to assess the contribution of skeletal muscle loss measured at different slices to overall survival. Overall survival was defined as the interval from randomization to death from any cause. A mean coefficient of variation between observers of 1.3% for skeletal muscle CSA in a random sample of 15 patients was observed, which is in line with the variation of 0-2% in other studies [5,15]. Therefore, a measurement error of 1.3% was adopted for L1 and L3. Changes of equal or larger than -1.3% were considered as 'loss of tissue', while changes of smaller than -1.3% were considered 'maintenance of tissue'. A variation regarding pectoralis muscle is not described in literature, and therefore the median was chosen as cut-off. The hazard ratio indicates the effect of increased muscle mass on overall survival. In addition, multivariate Cox regression analysis was performed with change in muscle CSA at the level of pectoralis muscle, L1 and L3, gender, age and Charlson comorbidity index as independent variables. The WHO performance score was between 0 and 1 in the majority of the patients and therefore not included in the regression analysis.

All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, version 24.0, IBM, Armonk, NY). Results with two-sided exact p values (<0.05) were considered statistically significant.

RESULTS

Patients and characteristics

In total, 223 patients were enrolled in the randomized controlled trial. One hundred and three patients were excluded due to unavailability of one or both CT scans, three patients were excluded because pectoralis muscle or L3 was not evaluable, one patient was excluded due to lacking OS data and one was excluded for insufficient quality of the scans. After exclusion, CT scans from 115 patients were eligible. Sixty-four patients (56%) were male, with mean age of 61 years and body mass index (mean ± SD) of 25.1±4.2 kg/m^2. Patient characteristics, treatment arm, Charlson comorbidity index and overall survival were not different between patients included (n=115) and patients excluded (n=108) (data not shown).

Skeletal muscle

Mean ± SD skeletal muscle CSA (in cm^2) of pectoralis, L1 and L3 for pre-chemotherapy scans were respectively 36.0 ± 10.1 (figure 1, table 1), 116.1 ± 24.5, and 134.8 ± 28.0, respectively. Baseline r between pectoralis and L3 was 0.71 (p<0.001) (supplemental figure 1a). The associating Bland-Altman plot shows that mean difference between pectoralis muscle and L3 muscle was 116.3 ± 13.6%, which was significantly different from 0 (p<0.001). The plot shows that the bias is relatively constant (supplemental figure 1b). Cronbach's alpha was 0.622.

Correlations, Bland-Altman plots and Cronbach's alpha were also performed for post-chemotherapy scans, showing similar results (data not shown).

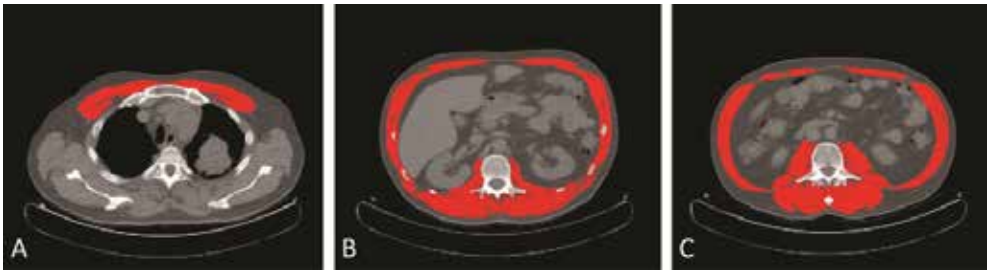

Figure 1. Skeletal muscle area on transverse CT images at (a) pectoralis, (b) first lumbar level and (c) third lumbar level.

Table 1. Computed Tomography measurements of muscle.

	Baseline (cm^2)	Follow-up (cm^2)	Delta absolute (cm^2/100 days)	Delta relative (%/100 days)	P value
Pectoralis muscle	36.0 ± 10.1	33.9 ± 9.8	-4.5 ± 12.9	-4.3 ± 13.3	<0.001
L1 muscle	116.1 ± 24.5	112.8 ± 22.7	-5.7 ± 20.1	-2.3 ± 7.7	<0.001
L3 muscle	134.8 ± 28.0	130.6 ± 27.3	-7.6 ± 18.8	-2.9 ± 6.7	<0.001

Data are expressed as mean ± standard deviation. Abbreviations: L1: first lumbar vertebra; L3: third lumbar vertebra.

Baseline Pearson's r between L1 and L3 was 0.90 (p<0.001) (supplemental figure 1c). The corresponding Bland-Altman plot shows that mean difference between L1 and L3 measuring muscle was 14.9 ± 9.0%, which was significantly different from 0 (p<0.001) (supplemental figure 1d). The plot reveals that there is a bias which is almost constant for all measured CSA's. The muscle CSA of L3 is larger than L1. Cronbach's alpha was 0.859, indicating a high internal consistency of skeletal muscle CSA measurement at L1 opposed to L3.

Adipose tissue

Mean ± SD SAT CSA (in cm^2) of L1 and L3 for pre-chemotherapy scans were respectively 107.0 ± 73.5 and 160.8 ± 85.1 (table 2).

Table 2. Computed Tomography measurements of adipose tissue compartments.

	Baseline (cm^2)	Follow-up (cm^2)	Delta absolute (cm^2/100 days)	Delta relative (%/100 days)	p value
L1 SAT	107.0 ± 73.5	104.5 ± 73.5	-3.6 ± 33.5	-1.4 ± 25.5	0.076
L3 SAT	160.8 ± 85.1	155.3 ± 84.9	-9.6 ± 61.8	-2.1 ± 19.9	0.021
L1 VAT	100.1 ± 65.5	100.1 ± 69.8	-1.3 ± 78.1	4.9 ± 33.2	0.979
L3 VAT	106.9 ± 70.4	104.8 ± 70.5	-5.5 ± 56.9	2.6 ± 29.0	0.386

Data are expressed as mean ± standard deviation. Abbreviations: L1: first lumbar vertebra; L3: third lumbar vertebra; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue.

Baseline r was 0.93 (p<0.001) (supplemental figure 2a). The corresponding Bland-Altman plot shows that mean difference between L1 and L3 measuring SAT was 48.2 ± 26.5%, which was significantly different from 0 (p<0.001). The plot shows that the bias is not constant, as the difference is proportional to the magnitude of L3 muscle (supplemental figure 2b). Cronbach's alpha was 0.959, indicating a high internal consistency of SAT CSA measurement at L1 compared to that at L3.

Mean ± SD VAT CSA (in cm^2) of L1 and L3 for pre-chemotherapy scans were respectively 100.1 ± 65.5 and 106.9 ± 70.4 (table 2). Baseline r was 0.90 (p<0.001) (supplemental figure 2c). The corresponding Bland-Altman plot shows that mean difference between L1 and L3 measuring SAT was 5.4 ± 31.5%, which was significantly different from 0 (p<0.001). The plot shows that the bias is almost constant, with the exception of very low values of L3 VAT. (supplemental figure 2d). Cronbach's alpha was 0.940, indicating a high internal consistency of VAT CSA measurement at L1 opposed to L3. Correlations, Bland-Altman plots and Cronbach's alpha were also performed for post-chemotherapy scans, showing similar results.

Longitudinal changes

Table 1 and 2 display changes in body composition during the course of chemotherapy. Within two cycles of chemotherapy, muscle CSA significantly declined at the level of pectoralis, L1 and

L3. SAT CSA at L3 decreased respectively by-2.1%/100 days ± 19.9 (p=0.021), while no significant changes in SAT CSA at L1 were observed. No changes were observed with respect to VAT.

Survival analysis

Kaplan-Meier survival analysis showed that skeletal muscle loss at L1 (HR 1.6, 95% CI: 1.08-2.36, p=0.020) and skeletal muscle loss at L3 (HR 1.9, 95% CI: 1.27-2.83, p=0.002), but not pectoralis

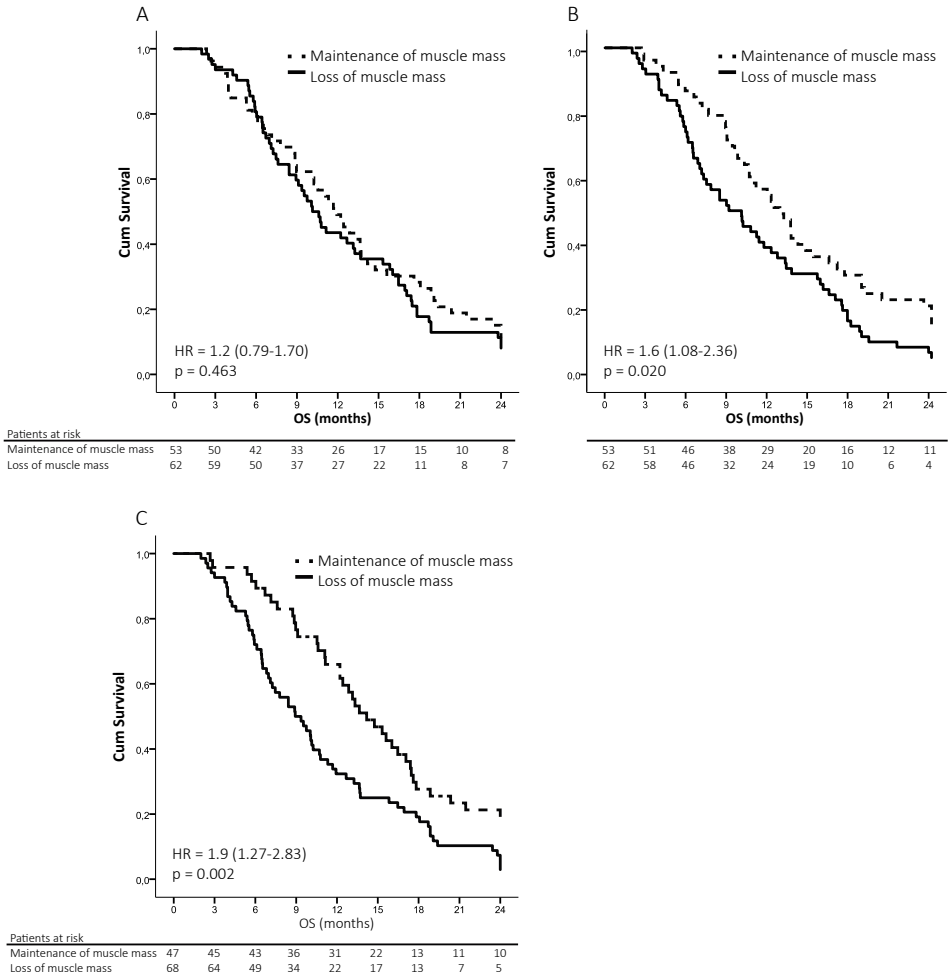

Figure 2. Kaplan Meier overall survival curve for patients with loss of muscle mass compared to patients without loss of muscle mass at the level of (a) pectoralis, (b) L1 and (c) L3.

muscle loss (HR 1.2, 95% CI: 0.79-1.70, p=0.463), were associated with overall survival (figure 2). On multivariate analysis correcting for gender, age and comorbidities, skeletal muscle loss at L3 and L1 levels were associated with overall survival, while pectoralis muscle loss was not (table 3).

Table 3. Multivariate analysis for predictors of overall survival.

	B	HR (95% CI)	P value		B	HR (95% CI)	P value		B	HR (95% CI)	P value
Gender	0.44	1.6 (0.90-2.69)	0.112	Gender	0.48	1.6 (0.94-2.80)	0.085	Gender	0.53	1.7 (0.97-2.98)	0.064
Age	0.01	1.0 (0.98-1.04)	0.577	Age	0.01	1.0 (0.98-1.04)	0.621	Age	0.01	1.0 (0.98-1.04)	0.762
Charlson comorbidity index	0.12	1.1 (0.96-1.33)	0.144	Charlson comorbidity index	0.11	1.1 (0.95-1.31)	0.169	Charlson comorbidity index	0.09	1.1 (0.93-1.29)	0.293
Delta L3 muscle	-0.05	0.9 (0.91-0.98)	0.005	Delta L1 muscle	-0.06	0.9 (0.91-0.98)	0.002	Delta Pectoralis muscle	-0.02	1.0 (0.96-1.01)	0.126

DISCUSSION

The main objective of this study was to investigate whether pectoralis muscle and L1 could be suitable alternatives for L3 to assess muscle CSA. Cross-sectional and longitudinal comparison revealed that L1 is stronger associated to L3 than pectoralis muscle.

To date, several studies have investigated CT-derived muscle to predict clinical outcome. The majority of studies conducted in patients with cancer involve quantification of L3. However, in COPD, a single-muscle approach has been used more frequently. Güerri et al. showed in 20 COPD patients that those with a history of more frequent exacerbations exhibited smaller intercostal muscles, independent of age, gender and body mass index. The authors failed to verify these results among other evaluated muscles including upper limb muscles and psoas muscle [16]. Furthermore, attempts have been made to derive whole body fat-free mass from CT-based pectoralis muscle area by comparing pectoralis muscle with fat-free mass assessed by bioelectrical impedance analysis (BIA), but as BIA is a double indirect measure of fat-free mass [17], this cannot be considered as appropriate reference method such as muscle mass derived by magnetic resonance imaging. In addition, the unadjusted correlation between pectoralis and BIA derived fat-free mass was low (R^2=0.38) [18]. Also in other patient populations, similar attempts have been made to measure one muscle as representative for lumbar muscle CSA, including psoas muscle [19,20]. However, Rutten et al. found that change in psoas muscle area was not representative of total muscle area change [21]. Overall, the idea that an individual muscle as opposed to total lumbar muscle reflects whole body muscle mass or fat-free mass has therefore not yet been appropriately validated.

Our results are in line with a previously published study performed in 90 small-cell lung cancer patients reporting similar baseline correlations between L3 and L1 muscle and between L3 and pectoralis muscle. Longitudinal data and adipose tissue CSA were not assessed [10]. The advantage of lumbar assessment of muscle mass is that also adipose tissue can be assessed. Based on whole body magnetic resonance imaging, single slice adipose tissue quantification at different levels in the abdominal region (L4-5, 5 cm above and below) correlated well with total body adipose tissue [9]. Analysing adipose tissue at the level of L1 might be informative as normal-weight COPD patients exhibited more VAT compared to smoking controls despite similar SAT [22]. Additionally, the presence of excessive VAT contributed to increased plasma interleukin-6 and is associated with increased mortality in persons with obstructive lung disease [23], emphasizing the importance of body composition in chronic disease risk assessment.

The advantage of this study is that it included a well-defined randomized patient cohort. Limitations are that the CT scans were performed in different medical centres, which might have resulted in variation in acquisition and scanning procedures. However, the body composition data derived from pre- and post-chemotherapy scans were made in the same centre, which implies that it will not have a major impact on the outcome of this research. Furthermore, not all CT scans could be retrieved and therefore only a subset of the scans has been analysed. However, the data we presented here are very consistent. We therefore feel confident that this patient sample is representative for the whole study cohort.

In conclusion, the present study shows that L1 but not pectoralis muscle can substitute L3 to determine body composition from regular chest CT scans for cross-sectional and longitudinal purposes.

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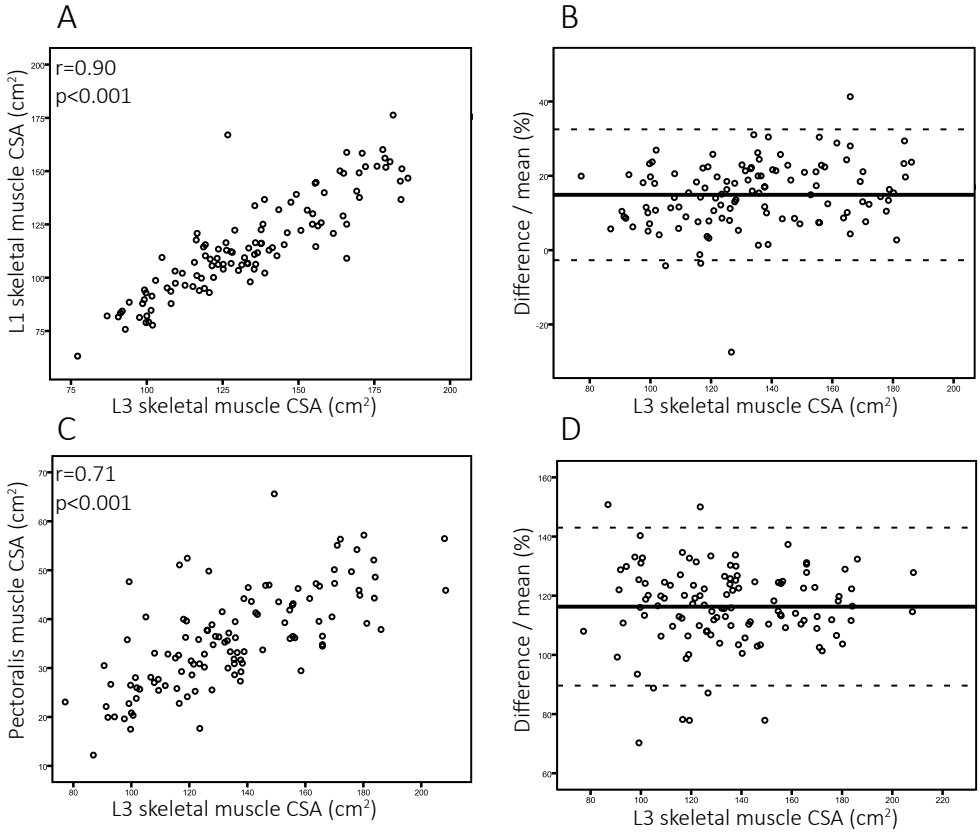
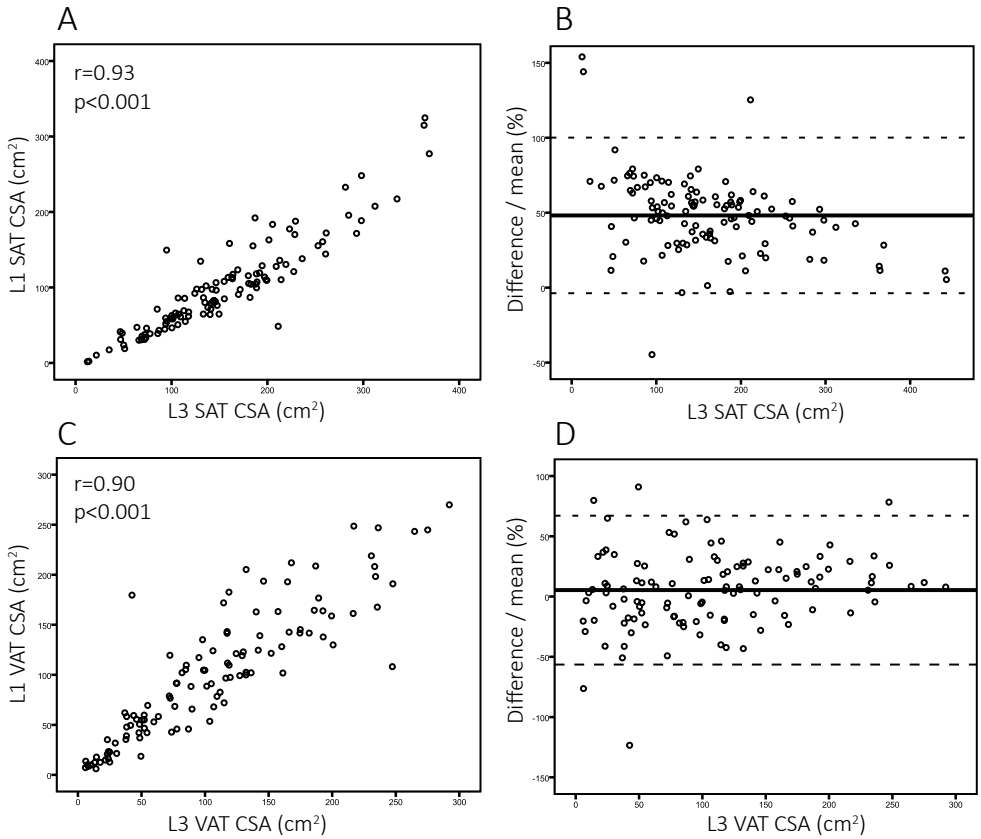
SUPPLEMENTALS



Supplemental figure 1. Scatter plot and Bland-Altman plot of skeletal muscle with data from pre-chemotherapy scans. (a) Intermeasurement correlation of pectoralis and L3, (b) Blant-Altman plot of pectoralis and L3 , (c) intermeasurement correlation of L1 and L3, and (d) Blant-Altman plot of L1 and L3. Abbreviations: L1: first lumbar vertebra; L3: third lumbar vertebra.



Supplemental figure 2. Scatter plot and Bland-Altman plot of adipose tissue with data from pre-chemotherapy scans. (a) Intermeasurement correlation of SAT at L1 and L3, (b) Blant-Altman plot of SAT at L1 and L3, (c) intermeasurement correlation of VAT at L1 and L3, and (d) Blant-Altman plot of VAT at L1 and L3. Abbreviations: L1: first lumbar vertebra; L3: third lumbar vertebra.

4

Cachexia in chronic obstructive pulmonary disease: new insights and therapeutic perspective

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ABSTRACT

Cachexia and muscle wasting are well recognized as common and partly reversible features of chronic obstructive pulmonary disease (COPD) adversely affecting disease progression and prognosis. This argues for integration of weight and muscle maintenance in patient care. In this review, recent insights are presented in the diagnosis of muscle wasting in COPD, the pathophysiology of muscle wasting and putative mechanisms involved in a disturbed energy balance as cachexia driver. We discuss the therapeutic implications of these new insights for optimizing and personalizing management of cachexia in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death worldwide. It has been estimated that COPD related mortality rates will even increase in the coming decades. This increase is not only related to the prevalence of smoking, but also ageing and reduced mortality from other common causes of death play a role [1]. Additionally, COPD is a major contributor to global disease burden, accounting for 43.3 million disability adjusted life years in 2010 [2]. The disease is characterised by persistent airflow obstruction resulting from inflammation and remodelling of the airways, and may include development of emphysema. Furthermore, systemic disease manifestations and acute exacerbations influence disease burden and mortality risk [3]. Extending the classical descriptions of the ''pink puffer'' and ''blue bloater'', recent unbiased statistical approaches [4,5] support the concept that body weight and body composition discriminate pulmonary phenotypes, and are predictors of outcome. Extra-pulmonary degenerative manifestations that may occur in COPD, include osteoporosis and muscle wasting [6]. The prevalence of muscle wasting is relatively high in COPD, 15–40% depending on definition and disease stage [7,8]. Importantly, muscle wasting not only contributes to diminished skeletal muscle function, reduced exercise capacity and decreased health status [9,10], but is also a determinant of mortality in COPD, independent of airflow obstruction [8,11].

Muscle wasting in COPD has been demonstrated by decreases in fat-free mass (FFM) at whole body level, but also specifically at level of the extremities [12]. Whole body and trunk FFM reduction are more pronounced in the emphysematous phenotype, whereas reduced FFM in extremities is not different between the pulmonary phenotypes [13,14]. In addition, muscle wasting is apparent as a decrease in the size of individual muscle fibres, and this muscle fibre atrophy in COPD seems selective for type II fibres in peripheral muscle [15,16], which is in line with other chronic diseases prone to cachexia such as chronic heart failure [17]. Furthermore, a shift in muscle fibre composition from type I (oxidative) to type II (glycolytic), accompanied by a decrease in oxidative capacity culminates in reduced muscle endurance [18]. This not only contributes to reduced exercise capacity [19], but may also affect muscle mass in COPD [20], since type I and II fibres display different responses to anabolic and catabolic signals [21,22].

While unintended weight loss was initially considered to be an indicator of inevitable and terminal progression of the disease process, there is now convincing evidence that it is an independent determinant of survival, arguing for weight maintenance in patient care. There are indications that the pathophysiology of unintended weight loss is different between clinically stable COPD and during acute flare ups of the disease. To date, data in acute exacerbations of COPD is however very limited. Therefore, lung cancer is used as a comparative acute pulmonary cachexia model.

A recent unbiased statistical approach suggests that not all COPD patients, but only the emphysematous phenotype is prone to cachexia [4], although the informative value of available clinical studies is limited by a cross-sectional study design. The last two decades have also yielded insight in the impairments of the processes governing muscle mass and identified putative triggers of muscle wasting in COPD. However, it remains unclear to what extent acute flare ups of COPD may accelerate chronic wasting of muscle mass and whether muscle wasting involves similar mechanisms as in other chronic diseases or in lung cancer cachexia. In this review, we present recent insights in the pathophysiology of muscle wasting in COPD and (putative) mechanisms involved in the pathophysiology of a disturbed energy balance as important driver of cachexia, that may lead to novel targets for clinical management of cachexia in COPD.

RECENT DEVELOPMENTS IN IDENTIFYING MUSCLE WASTING IN COPD

Incorporation of body composition into nutritional assessment has been a major step forward in understanding systemic COPD pathophysiology, since changes in weight and classification of body mass index (BMI) do not account for (hidden) body compositional shifts in fat mass (FM), FFM and bone mineral density. In clinical research bioelectrical impedance analysis is commonly used to identify cachexia. Traditionally, reference values for FFM index in COPD were developed based on age- and gender-specific 10th percentile values [8]. These reference values were defined as abnormally low, based on well-established adverse effects of low FFM index on physical performance and survival in normal to underweight COPD patients [7,11]. However, this might underestimate low muscle mass in the increasing proportion of overweight to obese COPD patients [23]. The recent European Respiratory Society statement on nutritional assessment and therapy in COPD [24] proposed dual energy x-ray absorptiometry as most appropriate method for body composition analysis in COPD, mostly because it combines screening for osteoporosis with assessment of FM and FFM at regional level in addition to whole body level. Consequently, body composition assessed by dual energy x-ray absorptiometry also allows measurement of appendicular skeletal muscle mass, which has been demonstrated to be stronger related to physical functioning than total FFM [23]. Moreover, we recently showed that this particularly applies to overweight and obese COPD patients [23,25].

Whereas low muscle mass is prevalent in ±15% of well-functioning elderly in the general aged population [26], a higher prevalence can be expected in COPD as a reflection of accelerated aging [25]. Indeed, Van de Bool et al. recently identified low appendicular skeletal muscle mass in even 87% of Dutch COPD patients eligible for pulmonary rehabilitation, along with a high persisting prevalence across all BMI categories [23]. The appendicular skeletal muscle mass-wasted phenotype was not only associated with impaired strength, but in men also with decreased endurance capacity. Coexistent abdominal obesity was identified in 78% of muscle wasted patients, which appeared to have a protective effect on physical functioning

[23]. Physiological alterations in terms of less hyperinflation and a larger inspiratory capacity in obese COPD patients contribute to certain advantages during physical activity [27]. In addition, mortality rates in advanced COPD are the lowest among obese subjects [28]. This prognostic advantage typically reflects the 'obesity paradox', because obesity on the other hand is also associated with increased risk of cardiovascular and metabolic diseases.

Although clinically useful estimates can be derived by dual energy x-ray absorptiometry, more precise distinction between muscle mass, visceral and subcutaneous adipose tissue require more advanced imaging technologies. This could be relevant in COPD as Van den Borst et al. and Furutate et al. reported a higher visceral adipose tissue in older age patients with COPD compared to age matched healthy controls, despite comparable subcutaneous adipose tissue and BMI [29,30].

McDonald et al. [31] demonstrated that CT-derived pectoralis muscle area provides a more clinically relevant measure of COPD-related outcomes in comparison to BMI, as lower pectoralis muscle area was associated with more severe expiratory airflow obstruction, lower quality of life, and impaired exercise capacity. Since gender differences have been documented in body composition and its functional implications [23], Diaz et al. explored gender differences in computed tomography (CT)-derived pectoralis muscle area and observed lower pectoralis muscle area in women compared to men [32]. CT scans are often used to exclude other underlying illnesses, and therefore chest CT derived analysis of body composition may be an attractive diagnostic tool to combine screening for pulmonary and systemic pathology. However, it first needs to be properly validated against reference methods of whole body and regional body composition to allow use in clinical practice. Furthermore, due to the radiation exposure, use of CT scans for body composition assessment is only admissible when scans are already performed to screen for pulmonary pathology.

NEW INSIGHTS IN THE PATHOPHYSIOLOGY OF MUSCLE WASTING IN COPD

The loss of muscle mass and cross sectional area in COPD patients as determined by imaging techniques has been confirmed at the cellular level, i.e. a reduction in muscle fibre cross sectional area [16]. As reviewed by Langen et al. [33] and Remels et al. [20] triggers of muscle wasting include hypoxemia, oxidative stress, inflammation, impaired growth factor signalling, oral glucocorticoids, disuse and malnutrition, some of which are influenced by smoking [34]. Wasting of skeletal muscle is due to a net catabolic state, which may result from an imbalance in muscle protein synthesis and breakdown (protein turnover), as well as from an imbalance in myonuclear accretion and loss (myonuclear turnover).

Protein turnover

To get insight in the rate of (muscle) protein turnover in COPD, information on (muscle)

protein synthesis as well as breakdown is required. Both increased and normal rates of whole body protein turnover have been reported in patients with COPD [35,36] but the relative contribution of muscle versus other tissues to protein turnover is unknown. Rutten et al. observed an increase in myofibrillar protein breakdown in cachectic COPD patients compared to non-cachectic patients and controls [35], but no data are available regarding muscle protein synthesis rate, except for a small study showing depressed muscle protein synthesis rates in malnourished patients with emphysema [37]. Numerous studies however have addressed molecular regulation of anabolic and catabolic pathways in the quadriceps muscle of COPD patients, which provides some insight in altered muscle protein turnover in muscle wasted COPD patients.

Proteolytic signalling

Several environmental triggers can lead to catabolic signalling in the skeletal muscle mediated by transcriptional regulators including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and forkhead box O transcription factors (FOXO)s. NF-κB activity is increased in COPD patients compared to controls [38,39] and in cachectic COPD patients compared to non-cachectic COPD patients [38,40]. Furthermore, limb muscle NF-κB activity is increased in patients with lung cancer cachexia [41]. FOXO mRNA and protein expression are increased in patients with COPD [38,39,42-45], seemingly independent of body composition, although it is noticeable that in all studies the patient group showed signs of emphysema. In the COPD patient group, FOXO-1 protein expression was higher in limb muscles than in respiratory muscles, while this difference was not found in controls [46]. Interestingly, the respiratory muscles of COPD patients show an opposite fibre type shift compared to limb muscles, i.e. towards more type I fibres [47,48]. This will have implications for the expression levels of constituents of atrophy signalling pathways [22,49]. Increased catabolic signalling through FOXO and NF-κB can induce gene expression of key factors in both the ubiquitin proteasome system (UPS) [50,51] and the autophagy lysosome pathway [33,52].

Ubiquitin proteasome-mediated degradation

The ubiquitin 26S-proteasome pathway consists of coordinated actions of the ubiquitin conjugating and ligating enzymes, that link ubiquitin chains onto proteins to mark them for degradation by the proteasome [53,54]. These enzymes include tripartite motif containing 63, E3 ubiquitin protein ligase (TRIM63, referred to as MURF1), F-box protein 32 (FBXO32, referred to as ATROGIN1) and neural precursor cell expressed, developmentally down regulated 4, E3 ubiquitin protein (NEDD4).

MURF1 limb- and respiratory muscle mRNA and protein expression appear unaltered in COPD patients compared to controls [38,40,55-57], although one study reported increased MURF1 protein expression in the limb muscles of cachectic COPD patients [39]. In COPD patients,

MURF1 protein expression is relatively increased in limb muscle than in the respiratory muscle, while this difference was not found in controls [46]. Furthermore, cachectic COPD patients show increased limb muscle mRNA expression of MURF1 compared to a control population [44]. ATROGIN1 mRNA and protein expression are increased in limb muscles [38-40,44,56,57], but unaltered in respiratory muscles of patients with COPD [55]. Similarly, ATROGIN1 mRNA expression is increased in the limb muscles of smokers [58]. Furthermore, Doucet et al. found that COPD patients display a higher ATROGIN1 protein expression in limb muscles than in respiratory muscles, while this difference was not found in controls [46]. Additionally, limb muscle NEDD4 protein expression is increased in patients with COPD [57]. Total poly-ubiquitinated protein is increased in limb muscles of COPD patients compared to healthy controls [39,56], and in cachectic COPD patients compared to non-cachectic COPD patients [38].

Taken together, the majority of the literature suggests that wasting in COPD is accompanied by an increase in UPS activation. The increase in catabolic signalling in cachectic COPD patients is site-specific. This may reflect disuse atrophy of the limb muscle with maintained or increased respiratory muscle activity, or it may result from an interaction between inactivity and other triggers of atrophy, such as smoking.

Autophagy-lysosome-mediated degradation

The autophagy-lysosome pathway is a protein degradation pathway, which recently gained interest in the context of COPD-associated muscle dysfunction. Upon activation, autophagosomes form and mature to subsequently fuse with lysosomes. The autophago-lysosomes degrade the cargo and release amino-acids for de novo protein synthesis or other metabolic fates [59].

Signalling through v-akt murine thymoma viral oncogene (AKT) regulates mechanistic target of rapamycin (serine/threonine kinase) complex 1 (MTORC1) activity and downstream of MTORC1, unc-51 like autophagy activating kinase 1 (ULK1) activity, thereby regulating autophagy initiation [60,61]. Inhibitory MTORC1 mediated ULK1 phosphorylation is decreased in limb muscles of COPD patients compared to controls [45], which may implicate an increase in autophagic flux induction. The increase of FOXO mRNA and protein expression in COPD patients may induce the transcription of autophagy related genes. However, it should be taken into account that FOXO transcriptional activity is also regulated by post-translational modifications. Plant et al. found that the mRNA expression of autophagy-related genes beclin-1 and microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A, referred to as LC3) is unaltered in the limb muscles of COPD patients compared to controls [57]. Limb muscle sequestosome 1 (SQSTM1, referred to as P62) mRNA expression, however, is increased in COPD patients [45]. Although the mRNA expression of autophagy related genes and the activation of ULK1 may give some insight in the level of autophagy initiation, it does not directly reflect the level of autophagic flux. In muscle biopsies, the conversion of LC3BI to LC3BII can be used as a measure for autophagic

flux. Furthermore, P62 is used as a marker for autophagic flux since it is broken down by the lysosome. In the limb muscles of COPD patients, the LC3BII/I ratio is increased [39,45], pointing to an increase in the autophagic flux. In contrast, P62 protein expression is increased, pointing to a decrease in autophagic flux [45]. However, it cannot be excluded that the increase in P62 protein expression is due to the increase in P62 transcription. The number of autophagosomes was found to be increased in the limb muscle of COPD patients [39,45], which suggests an increase in autophagic flux. However, it is only possible to speculate on the level of autophagic flux in the limb muscles of patients with COPD based on the currently applied markers, as these incompletely cover autophagic flux, autophagy induction and autophagic-lysosomal degradation. Therefore, autophagic flux markers should be analysed coupled to the activity status of upstream regulators such as MTORC1 and AMP-activated protein kinase (AMPK) in muscle biopsies. Moreover, besides its role in protein breakdown, autophagy also acts as a quality control mechanism for proteins and intracellular components [62,63]. Therefore, an impaired autophagic flux in COPD patients may have consequences for the integrity and function of intracellular components such as the nucleus and mitochondria.

It currently is unknown if the autophagic-lysosome pathway activity is altered during acute exacerbations of COPD, since most studies were conducted in stable COPD patients. However, in lung cancer cachexia LC3BII protein expression and BCL2/ adenovirus E1B 19kDa interacting protein 3 mRNA expression are induced [41], pointing to an increase in autophagy. From this, autophagy induction in skeletal muscle might be anticipated during acute stages of COPD wasting.

Protein synthesis signalling

A major anabolic pathway is the insulin-like growth factor 1 (IGF1)/phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA referred to as PI3K)/AKT pathway. Most studies found an increase in IGF1 mRNA expression in the limb muscle of COPD patients compared to controls [42,43,64], although Crul et al. found a decrease in IGF1 mRNA expression in stable COPD patients [65]. Unfortunately, this study did not provide body composition data. Cachectic COPD patients seem to have a lower limb muscle IGF1 mRNA and protein expression than non-cachectic COPD patients [40]. Furthermore, during an acute exacerbation, muscle IGF1 mRNA expression is lower in COPD patients than in controls, although IGF1 protein expression remains unaltered [65].

Even though IGF1 mRNA expression is increased in limb muscles of COPD patients, AKT activation remains unaltered [39,45,57]. AKT activity is relatively increased in cachectic patients compared to non-cachectic patients and healthy controls [40,44], while the decrease in IGF1 mRNA expression in this group would generally implicate a decrease of IGF1/AKT signalling. Interestingly, an increase in AKT activation is also observed in patients with lung cancer related

cachexia [41], suggesting it may be a common feature pulmonary cachexia. The discrepancy in IGF1 mRNA expression and AKT activation suggests altered regulation at the IGF- or IGF-receptor protein expression level [66].

Signalling through AKT inhibits the upstream inhibitor of MTORC1, thereby inducing MTORC1 activation and subsequent phosphorylation of its downstream targets eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1 also called 4EBP1) and ribosomal protein S6 kinase, 70kDa, polypeptide 1 (PRPS6KB1 also called p70S6K) [67]. The increased AKT activation in the limb muscle of cachectic patients compared to non-cachectic COPD patients is paralleled by an increase in phosphorylation of the downstream targets 4E-BP1 and p70S6K [44]. P70S6K phosphorylation is unaltered in COPD patients compared to controls [44], while ribosomal protein S6 (RPS6 referred to as S6) phosphorylation was even decreased in COPD patients [45]. Together, these studies show an increase in protein synthesis signalling in the limb muscles of cachectic COPD patients compared to non-cachectic COPD patients, but no alteration in the general COPD population. In patients with lung cancer related cachexia, AKT activation is increased without concurrent activation of MTOR or its downstream targets [41]. This may indicate that, although impaired AKT signalling is found in lung cancer cachexia, AKT signalling is largely intact in COPD induced muscle wasting. However, one limitation of these studies concerns the evaluation of the activation status of protein synthesis signalling solely in a basal state. Although the current data may suggest that the protein synthesis pathway is a promising target for the treatment of COPD-induced muscle wasting, the integrity of the anabolic response should be further addressed.

Only limited data is available on anabolic signalling in respiratory muscles of COPD patients, and although the results also point to an increase in anabolic signalling, it remains unclear if this is different between cachectic and non-cachectic COPD patients. Martinez-Llorens et al. found an increase in IGF1 mRNA expression in the intercostal muscles of patients with COPD [68]. Doucet et al. compared the ratio of quadriceps to diaphragm AKT activation in COPD patients with controls and found a lower ratio in COPD [20]. This implicates that the AKT activation is relatively higher in the diaphragm than in the quadriceps. In line with this, the p70S6K phosphorylation is relatively higher in the diaphragm, while 4E-BP1 phosphorylation is higher in the quadriceps [20].

Interestingly, Tannerstedt et al. showed a difference in anabolic response between type-I and type-II muscle fibres, with increased AKT phosphorylation and downstream pathway activation in the type-II fibres [69]. In contrast, in COPD, the respiratory muscle with a shift towards more type-I fibres displayed a larger AKT activation and downstream signalling than the limb muscles with a shift towards more type-II fibres. Therefore, the shift in fibre type does not explain the variation in AKT phosphorylation and downstream signalling between the limb muscle and

respiratory muscle. Other discriminating factors, such as the muscle activity level, may be more closely linked to differences in AKT phosphorylation.

Taken together, anabolic signalling is increased in the skeletal muscle of patients with COPD, with an even larger increase in the diaphragm than the limb muscles. One may speculate that the increased activation of AKT signalling in the respiratory muscles is an attempt to preserve respiratory function by compensating catabolic triggers, although it may also reflect intrinsic alterations in muscle fibre composition.

Myonuclear turnover

Besides the turnover of proteins, the turnover of myonuclei appears essential for muscle regeneration [70-72]. Furthermore, although at a lower rate, myonuclear turnover might be indispensable for the maintenance of skeletal muscle mass. The regulated loss of a nucleus may involve apoptosis. Increased apoptosis, as determined by an increase in terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining and poly (ADP-ribose) polymerase cleavage in the skeletal muscle of cachectic COPD patients has been reported [73]. In the diaphragm of COPD patients with muscle wasting, elevated caspase-3 levels indicated an increase in apoptosis [55]. No difference in apoptosis measured by TUNEL staining or caspase-3 was found in COPD patients with maintained muscle mass compared to controls [74]. A possible alteration in apoptosis in the muscle of COPD patients remains inconclusive due to the lack of studies and the use of different markers. Furthermore, the possible role of apoptotic signalling in the skeletal muscle atrophy remains obscure [75], and multinucleated muscle fibres may utilize other mechanisms for selective myonuclear loss, such as autophagy [76].

To replenish the myonuclear pool, satellite cells are essential [72]. In contrast to muscle wasting in aging [77,78], the number of satellite cells per muscle fibre is unaltered in the limb and respiratory muscles of patients with COPD compared to controls [68,79-81]. Furthermore, no differences in satellite cell number have been reported between muscle wasted and non-muscle wasted COPD patients [80].

Upon activation, satellite cells proliferate, differentiate and fuse with myofibres. Activation and proliferation of satellite cells in the limb muscles does not seem to differ between COPD patients and age matched controls based on number of satellite cells 24h after a resistance-exercise bout [81]. However, molecular markers of satellite cell activation may be more sensitive than satellite cell number to quantify the satellite cell response.

In a basal condition, myogenic factor 5 mRNA expression is unaltered in the limb muscles of patients with COPD compared to controls [57]. Myogenic differentiation 1 (MYOD1) mRNA expression [40] and protein expression appear similar in the limb muscles of COPD patients

and healthy controls [57]. Furthermore, myogenin (MYOG) mRNA expression is unaltered in COPD patients [57]. However, in cachectic COPD patients, MYOD1 [40] and MYOG [38,39] protein expression are reduced compared to controls, while no alteration in MYOG protein expression was found in the limb muscles of cachectic compared to non-cachectic COPD patients [38]. It should be considered that in different populations and disease stages, the course of the satellite cell response might be altered, which may have implications for the timing of the measurements.

A negative regulator of myogenesis is myostatin (MSTN) [82]. Limb muscle MSTN mRNA expression is increased in COPD compared to controls [57,83], while no difference seems to be present between cachectic and non-cachectic COPD patients [40]. The increased MSTN mRNA expression in COPD patients may be partially explained by smoking status, as this increase is also found in smokers [58]. MSTN protein expression is unaltered in the limb muscle of COPD patients, independent of the pulmonary phenotype [38-40], while the circulatory level of MSTN is increased in COPD compared to control subjects [84]. It should be noted that Snijders et al. showed a delayed response in MSTN protein levels upon a single bout of resistance exercise in elderly, while basal levels did not seem to differ [85]. This implies that in absence of a myogenic trigger, intrinsic alterations in satellite cell plasticity responses in muscle of COPD patients may be masked.

Centrally localized myonuclei in myofibres are considered derived from newly fused satellite cells prior to their final location peripherally in the myofibre against the sarcolemma. COPD patients with preserved muscle mass have higher amounts of central nuclei in the limb muscle than muscle wasted COPD patients and controls [80]. This could be interpreted as an attempt to counteract atrophic signalling in COPD patients, which may be essential to preserve muscle mass. However, as there is only indirect evidence that central myonuclei reflect recent regenerative events, central nuclei could also reflect an increase in myonuclear turnover to compensate for increased loss of myonuclei.

To gain further insight in the regulation of myonuclear turnover and possible defects in COPD-induced skeletal muscle wasting, it is essential to incorporate satellite cell activation stimuli and sensitive techniques to monitor myonuclear accretion and turnover in the study design. This will require further development of new techniques, in parallel with novel ex vivo and in vitro approaches to monitor myonuclear accretion and possibly myonuclear loss, and assessment of the role of alterations in myonuclear turnover in muscle atrophy.

Loss of muscle oxidative phenotype

Besides the importance of the muscle quantity for muscle function, also the quality of the muscle should be considered. This is highlighted by the finding that muscle mass-specific

muscle strength and endurance are reduced in patients with COPD [86-88]. A well-established qualitative alteration in the skeletal muscle of COPD patients is the loss of oxidative phenotype (OXPHEN) characterized by a muscle fibre type I to type II shift and a loss of oxidative capacity [20,88,89]. The loss of OXPHEN is associated with increased oxidative stress [88,90], which may render the muscle more susceptible to muscle atrophy [38]. In addition, type II fibres are generally more susceptible to atrophy stimuli including e.g. inflammation [21] and hypoxia [22]. Therefore, the loss of OXPHEN in COPD may accelerate the loss of muscle mass, thereby linking muscle quality to muscle quantity. This is supported by the fact that non-symptomatic smokers already exhibit reduced mitochondrial capacity and a similar fibre type shift [91]. Although less extensively investigated, striking similarities are reported regarding muscle oxidative metabolism in chronic heart failure [92]. As these patients also share other systemic features and lifestyle characteristics (e.g. muscle wasting, low physical activity level) comparative analyses between well phenotyped patients with COPD and chronic heart failure may provide more insight in common and disease specific denominators and mechanisms.

Therapeutic perspective

Since muscle wasting may result from alterations in the protein and myonuclear turnover, targeting key pathways in these processes will be required to combat muscle wasting.

UPS activity is increased in the muscles of cachectic COPD patients, which implicates the atrogenes MURF1 and ATROGIN1 as targets to normalize UPS activity. This is supported by the finding that in a cell culture model and in a mouse model of muscle disuse, MURF1 inhibition and knockout respectively, prevented muscle fibre atrophy [93,94]. Pharmacological inhibitors that target specific ubiquitin-conjugating and deconjugating enzymes are being developed to treat cancer, neurodegenerative disorders and autoimmune diseases [95], but may also be highly relevant for the treatment of COPD-induced muscle wasting. Furthermore, exercise training may attenuate MURF1 expression, as was observed in the skeletal muscle of chronic heart failure patients [50,96]. In contrast to exercise training, one bout of exercise leads to an increase in MURF1 expression, albeit blunted in COPD [97,98], while the increase in proteolytic signalling is reduced by branched-chain amino acid supplementation in a healthy population [97].

Autophagy is disturbed in patients with COPD, although it remains unclear whether there is an increased induction of autophagy or an inhibition of autophagic-lysosomal degradation. Low amino acid availability can activate autophagy by inhibition of MTOR [99]. In line with this, branched-chain amino acid supplementation leads to an inhibition of autophagy by activation of MTOR [100]. Furthermore, overall low energy status, DNA damage and hypoxia can inhibit MTOR through AMPK and hypoxia inducible factor 1, alpha subunit (HIF1-α) [101,102] and thereby induce autophagy. Interestingly, exercise targets these factors, and exercise training

results in elevated levels of basal autophagy [103]. However, exercise leads to an increase in muscle mass and strength in COPD patients [98]. Moreover, autophagy is required for muscle adaptations to training [103,104]. The counterintuitive effect of exercise on autophagy may therefore be more tightly linked to its function in quality control.

A more speculative thought is that autophagy may play a role in selective removal of damaged myonuclei. Besides causing mitochondrial DNA damage, oxidative stress also causes nuclear DNA damage. COPD patients display elevated levels of oxidative stress, which may lead to increased DNA damage and requires increased removal of damaged nuclei. Although exercise induces oxidative stress, which is even accentuated in COPD patients [105], exercise also triggers myogenesis [106]. In COPD, this myogenic response to exercise seems intact, although it is specifically impaired in cachectic COPD [40, 98]. Exercise induced satellite cell activation is mediated by IGF signalling [107]. In contrast, MSTN signalling inhibits satellite cell activation [108]. Since MSTN expression is increased in COPD, pharmacological inhibition of MSTN might be beneficial to prevent COPD-induced muscle wasting. This idea is supported by the finding that in mice with chronic kidney failure, pharmacological inhibition of MSTN blocks muscle atrophy [109], and that pharmacological inhibition of the ActII-receptor, which mediates MSTN signalling, prevents glucocorticoid-induced muscle atrophy [110]. In vitro, glutamine reduced the tumour necrosis factor alpha dependent increase of MSTN [111]. This implicates that availability of amino acids is important for normal satellite cell function in COPD, and that restoration of normal amino acid levels may be required for muscle maintenance. Taken together, the dual function and differential regulation of UPS and autophagy in the maintenance of muscle mass and quality reflects a highly interactive signalling network that is regulated by several upstream pathways. The effect of specific modulation of UPS and autophagy mediators may therefore transcend catabolic signalling and may affect a range of other cellular processes, yielding it difficult to predict long-term side effects. So far, exercise seems to be the only intervention that can target UPS and autophagy leading to improved quantity, as well as an improved quality of the muscle in COPD patients. One prerequisite is that COPD patients, and specifically cachectic COPD patients, have maintained responsiveness to exercise stimuli, which remains to be established. Exercise capacity in COPD may be limited by impaired pulmonary function, leading to incapability to supply a sufficiently strong exercise trigger to the muscles. In this case pharmacological or nutritional activators of AMPK, sirtuin 1 and peroxisome proliferator-activated receptors (PPARs) such as metformin, resveratrol, rosglitazone and poly unsaturated fatty acids could be used as exercise mimetics and may help sensitize the muscle to a following exercise bout. Furthermore, anabolic steroids could be considered in the treatment of COPD induced muscle wasting, although a recent meta-analysis showed that exercise capacity of COPD patients was not improved [112]. It should also be considered that an appropriate nutritional status is necessary for the beneficial effects of exercise, and that exercise (in particular endurance type of exercise) in a malnourished state could even have

detrimental effects by worsening the energy imbalance. Taken together, a multi-modal approach may be required to combat COPD-induced muscle wasting, in which exercise training is central. However, to establish such intervention, further research is crucial to determine whether the response to exercise is intact, or if specific defects occur, in cachectic patients with COPD.

PUTATIVE MECHANISMS INVOLVED IN A DISTURBED ENERGY BALANCE IN COPD

Specific loss of muscle mass in weight stable COPD patients has been observed, which may reflect a tissue specific sensitivity to an overall catabolic state. A net catabolic state may also result from an imbalance in energy expenditure and energy availability.

Increased energy expenditure

It is well established that components of whole body energy expenditure may be increased in patients with COPD [113]. Total daily energy expenditure (TEE) is the sum of resting metabolic rate (RMR), activity induced energy expenditure and diet-induced thermogenesis. Assessment of TEE requires sophisticated methodology including a respiration chamber [114] or doubly labelled water to allow assessment of TEE in free living conditions [115,116]. Data on TEE in COPD are scarce and sometimes contradicting which may be related to the use of different methodology or patient characteristics. Based on the available evidence it seems that in particular the emphysematous phenotype is prone to increased TEE, as high values are more often observed in patients with low carbon monoxide diffusing capacity [117-119]. This is in line with a positive effect of lung volume reduction surgery on body weight in emphysema although surgical intervention might not only decrease energy requirements but also improve dietary intake by alleviating dyspnoea [120]. More research is needed to assess putative differences in whole body energy metabolism and its components among COPD phenotypes under comparable standardized circumstances.

Numerous studies have shown that RMR is raised [121-123]. This is more prevalent in emphysema [124,125], during acute exacerbations [126], and appears inversely correlated with forced expiratory volume in 1 second when comparing different studies [118,119,122,127]. Highest values are found among weight losing patients [122]. This is in contrast with non-pathology-induced malnutrition, where subjects with low BMI have lower RMR due to hypometabolic adjustments [128]. The same results are found for non-small cell lung cancer (NSCLC), where RMR is found to be upregulated in 74% of primary lung cancer patients [129-131]. Hypermetabolism at rest was also found to be more pronounced in weight-losing compared to weight-stable lung cancer patients [132]. Thus increased RMR is a consistent feature of chronic and more acute cachexia and seems to be more pronounced in the emphysematous subtype.

Activity-induced energy expenditure is the most variable component of TEE, and it has been postulated that COPD patients reduce physical activity to compensate for dyspnoea severity

or to anticipate to breathlessness. Indeed lower physical activity levels are seen in COPD [133]. Physical inactivity is associated with advanced disease stage [134-136], exacerbations [137,138] and degree of emphysema [139]. In addition, lung volume reduction surgery in patients with severe emphysema improved exercise performance due to reduced lung hyperinflation, less dyspnoea severity and less energy cost of breathing [140]. However, it did not cause augmentation of physical activity level, implying other factors play a role, including motivation or anxiety [141]. There are several indications that when COPD patients perform physical activities, they require more energy. For examples, Lahaije et al. found a higher daily activity-related oxygen consumption assessed by a face mask measuring ventilatory and metabolic demand in COPD patients compared to healthy controls [142], while Vaes et al. found an increase in FFM adjusted oxygen consumption in COPD patients compared to controls, although total oxygen consumption was not altered [143]. Indirect evidence for altered activity induced energy expenditure is a rise in plasma ammonia in COPD patients during low intensity walking, which is an indicator of muscle ATP depletion and metabolic stress [144]. These collective data may indicate that COPD patients use oxygen less efficient and exhibit an altered energy metabolism during physical activity. This is not surprising in view of the shift in lower limb muscle fibre type composition in COPD towards less oxidative fibres, which appears to be more pronounced in the emphysematous phenotype [18]. The opposite shift in muscle fibre type of the diaphragm relative to the limb muscle [47,48] indicates an adaptation to chronic increase in work of breathing. Together with hyperinflation-induced mechanical inefficiency, this muscle fibre type shift could contribute to enhanced oxygen cost of breathing, illustrated by the effects of lung volume reduction surgery [145] and non-invasive positive-pressure ventilation therapy [146]. For comparison, in lung cancer, physical activity level assessed by accelerometry is also reduced [147,148], but no specific data about activity induced energy expenditure is available.

Diet-induced thermogenesis represents metabolic oxygen cost for processing of ingested nutrients. Green and colleagues [125], reported enhanced diet-induced thermogenesis in emphysematous COPD patients but this was not confirmed by other authors [149,150]. These differences may be due to different test meal composition and portion size. Although oxygen desaturations during meals were noticed in severe COPD patients [152], it is unknown whether this is diet-induced thermogenesis-related or not. Therefore, the thermic effect of dietary intake remains unclear. Taken together, it indicates that energy requirements are increased in COPD and there is certainly no adaptive reduced energy demand.

In addition to the hypermetabolic state, early clinical trials have shown that enhanced systemic inflammation is a contributing factor to elevated RMR, both in COPD [153] and in lung cancer [154], the source of which is yet unclear. Besides pulmonary inflammation [155], also adipose tissue has been suggested to contribute to a higher inflammatory gene expression in adipose tissue, as has been reported in malnourished patients with advanced COPD [156].

Adipose tissue metabolism

In cachexia muscle wasting is accompanied by loss of adipose tissue [14,157]. In fact, in cancer-induced cachexia adipose tissue is often one of the first affected organs, illustrated by decreasing fat cell volume and upregulation of fatty acid metabolism [158]. Regarding COPD, low BMI [11] and fat mass depletion [14] particularly occur in those with advanced disease and in the emphysematous phenotype.

Schols et al. observed low leptin levels in blood of patients with emphysema compared to chronic bronchitis in line with a lower BMI and fat mass [159]. After adjustment for FM and oral corticosteroid use as possible confounders, leptin was associated with systemic inflammation, in particular in the emphysematous patients. More recently, Brusik et al. investigated serum levels and adipose tissue expression of leptin and adiponectin in patients with COPD and reported an association between decreased serum and tissue leptin levels, decreased serum adiponectin and increased RMR adjusted for body weight in underweight patients [160]. In adipose tissue two cell types can be distinguished: white adipose tissue and brown adipose tissue (BAT). BAT is differentiated from white adipose tissue by the presence of cold-induced thermogenesis. Thermogenesis is facilitated by BAT-specific uncoupling proteins that dissipate the proton gradient in mitochondria in order to generate heat [161]. High amounts of mitochondria are responsible for the brown colour of BAT [162]. Additionally, white adipose tissue can be converted in BAT, called browning [163]. BAT activation negatively correlates with BMI, as demonstrated by decreased BAT activation in obese subjects [164] and during aging [165]. Cachexia on the other hand is characterized by fat mass depletion. This raises the question whether there is a role of BAT activity in the hypermetabolic state, as seen in pulmonary cachexia.

No studies are performed to determine BAT activity in COPD patients. With respect to lung cancer, results are conflicting and scarce. Despite negative results of BAT activation reported by some authors [166,167], Shellock and colleagues provided evidence for BAT activation as a cachexia mediator. Autopsy reports of cachectic cancer patients revealed high incidence of BAT in this group compared to age-matched controls [168]. Furthermore, a correlation between BAT activity and neoplastic status has been suggested [169], although the authors also reported high amount of BAT activation in non-malignancy subjects.

There is indirect evidence that BAT activation might be a potential cachexia driver in COPD as well. Hypoxia [170] and hypermetabolism [122] are hallmarks of COPD. In response to hypoxia, cells can produce vascular endothelial growth factor (VEGF) in order to restore oxygen supply [171]. This has been established by Van de Borst et al., who found an upregulation of the VEGF gene in adipose tissue in response to chronic hypoxia in mice. Congruently, adipose tissue showed a brown appearance. This browning of adipose tissue was established by increased

expression of uncoupling protein 1 [172], which proposes a link between hypoxia-induced VEGF activation and browning. Indeed, Sun et al. revealed upregulation of uncoupling protein 1, the main characteristic of BAT, in a VEGF overexpressing mouse model [173]. In addition, recently, increased thermogenesis and energy expenditure were found in mice with VEGF overexpression in BAT and white adipose tissue [174].

Another hypoxia alignment occurs in the form of lactate. In peripheral muscle of COPD patients increased glycolysis metabolism is observed [175], which in turn causes rising lactate levels [176,177]. Lactate is indeed increasingly released by adipocytes in a hypoxic environment [178], which in turn is able to control the expression of uncoupling protein 1. The uncoupling protein 1 regulation is independent of HIF-1α and thereby also promotes browning under normoxic circumstances [179].

Another possible browning factor is beta-adrenergic stimulation mediated by norepinephrine [180, 181]. Emphysematous COPD patients indeed exhibit increased plasma norepinephrine levels [182], indicating a possible activation of the autonomic nervous system. However, Schiffelers et al. showed a blunted beta-adrenoceptor-mediated lipolytic and thermogenic response [183], suggesting desensitization. Additionally, in 10 lean healthy men BAT activity in response to a systemic non-selective beta-agonist was not enhanced [184]. In contrast, blocking the receptors by propranolol decreases BAT activity [185]. Therefore, activation of brown fat through beta-adrenergic stimulation remains disputable.

It can be concluded that there is some indirect evidence pointing towards a role of BAT in pulmonary cachexia, but this area requires more research to identify therapeutic potential.

Compromised dietary intake

In order to compensate for increased energy requirements in COPD, patients should be able to adapt their dietary intake. Systematic analyses of dietary intake in COPD patients are rare. In terms of caloric content, dietary intake was found to be normal compared to healthy controls, but inadequate for measured energy expenditure [118,186-188]. During severe acute exacerbations the gap between energy intake and energy expenditure becomes even wider, which slowly decreases upon recovery [126,189]. To our knowledge, no human studies have systematically investigated the relation between dietary intake and disease severity or putative differences between emphysematous and non-emphysematous patients. Advanced disease stages and acute exacerbations are often characterized by chronic or acute hypoxemia [170]. It is well established that mice under chronic hypoxic circumstances experience weight loss, which is partly due to temporarily decreased dietary intake [172].

Anorexia

It is acknowledged that apparently normal dietary intake in COPD patients may be insufficient to meet elevated energy requirements but reduced food intake may also be caused by anorexia, i.e. loss of appetite [190]. A few underlying causes have been mentioned, including nicotine use [191], physical discomfort, such as dyspnoea and increased breathing effort [192], depression and anxiety [193], seen in COPD [194] as well as in NSCLC [195,196].

Besides pulmonary and psychological symptoms, COPD patients often experience pain. In a Norwegian study, which controlled for age and gender, 45% of the COPD patients reported chronic pain, compared to 34% of the general population [197]. Opioids are commonly used to combat pain in COPD [198]. Side-effects of opioids occur regularly, and opioids are able to cause gastrointestinal motility disorders [199], of which constipation is the most common [200]. People suffering from constipation often present with anorexia [201], probably due to early satiety. Separate from use of pain medication, early satiety and abdominal bloating is highly prevalent in COPD [202].

Chemosensory alterations

Food intake is regulated by taste and smell [203,204], and chemosensory dysfunction could influence dietary intake. Nordén et al. showed that 21 out of 169 stable COPD patients reported taste changes, which contributed to a decreased energy intake [205]. In addition, Dewan et al. compared 20 COPD subjects with long-term oxygen therapy to 20 COPD patients without oxygen therapy and 20 healthy elderly controls. They found reduced smell and taste test scores among COPD patients compared to controls, independent of oxygen supply [206]. Also Wardwell and colleagues found that healthy elderly tended to be able to identify more different tastes correctly than COPD patients, although not statistically significant [207]. Both authors did not report medication use, and therefore the influence of treatment is unknown. Although data are scarce and methodological quality of the studies is limited, these data suggest that COPD or its treatment could modify taste and smell detection.

Food reward system

Fullness is regulated by gastrointestinal hormones, including leptin and ghrelin [208], and their secretion is affected by dietary intake and nutritional status. Clinically stable emphysematous COPD patients exhibit low leptin levels compared to the chronic bronchitis subtype [159]. During acute flare ups these plasma levels rise temporary [189], as seen in NSCLC [209,210]. Likewise, enhanced plasma ghrelin levels are noticed in COPD [211] and NSCLC [212], and are related to cachectic status.

The peripheral hormonal satiety system closely interacts with the central nervous system in order to regulate food intake. Brain imaging studies have revealed reward-specific brain regions

related to food reward [213], and activation of these regions correlate with food rewarding [214]. Different orexigenic and anorexigenic peptides and hormones can stimulate neurons in these specific cerebral regions [208,215]. For instance, leptin inhibits neurons, causing reduced food intake and increased energy expenditure [216]. Ghrelin, considered to be a leptin counterpart, can induce food intake mediated by stimulation of neurons in this area [217].

There is surprisingly no human study available that explored the role of central dysregulation in food reward in patients with COPD. In relation to lung cancer cachexia only one study is performed identifying brain activity in anorectic and non-anorectic patients while receiving pleasant and unpleasant food cues [218]. In contrast to non-anorectic patients, anorectic patients showed no brain activity differences in response to pleasant versus unpleasant pictures. This implies an overall blunted response in the perceptual and motivational system that could also be involved in COPD but requires further investigation.

Therapeutic perspective

The importance of nutritional status is not only emphasized by adverse effects on muscle function and exercise performance, but also by detrimental effects of malnutrition on lung tissue. These effects have mostly been studied in animal models. Following the clinical phenotyping of the pink puffer and the blue bloater in the 1960s, Sahebjami et al. found reinforcement of pre-existing emphysematous processes due to caloric food deprivation in rats [219], which was more pronounced in young rats [220]. These deleterious effects could partly be reversed by refeeding [221]. In contrast, Bishai et al. found no alveolar size changes in calorie restricted mice, although the lungs became stiffer and lung capacity was decreased [222]. Supplementary evidence was provided by emphysema-like changes present in anorexia nervosa patients, which underscores the impact of chronic malnutrition on alveoli [223]. In addition to lung tissue, respiratory muscles also contribute to breathing. Weight loss does not spare the respiratory muscles, since weight loss is related to diminished diaphragm weight [224] and decreased function [225] in experimental models and in humans.

As proof of concept, Efthimiou et al. conducted a randomized controlled trial to investigate the effect of nutritional support on respiratory and peripheral muscle function in malnourished COPD patients. They reported improvement in respiratory muscle strength and hand grip strength, accompanied by less dyspnoea and enhanced distance in 6 minute walk test. Importantly, these effects diminished after quitting the dietary supplementation [226]. The positive effects of dietary support on body weight was verified by Weekes et al., who found weight gain in the intervention group, whereas the control group continued to lose weight. Addition of dietary counselling to dietary support has been shown to maintain weight loss after cessation of intervention [227].

Initially, the focus was primarily on caloric intake to balance energy requirements, but more recent proof of concept experiments also highlighted the importance of optimizing protein intake [228,229]. Low intake of other essential nutrients is identified, including vitamin D and calcium [230], which are also relevant in the context of osteoporosis as clustering comorbid condition.

One should keep in mind that dietary intake does not reflect actual availability of ingested micronutrients. There are indications that intestinal function is impaired in COPD, illustrated by splanchnic hypoperfusion and reduced intestinal permeability [231]. Altered intestinal function translates into reduced splanchnic extraction of amino acids derived from nutritional intake [36,232], but amino acid uptake in the skeletal muscle of clinically stable COPD patients appears increased [233]. Thus the significance for clinical applications remains ambiguous.

Both dietary intake and nutrient availability are controlled by gastrointestinal hormones. By binding to the growth hormone secretagogue receptor, ghrelin can induce secretion of growth hormone [234]. This leads to modulation of the growth hormone/IGF1 axis, which is an important anabolic pathway in human skeletal muscle [235]. Furthermore, ghrelin can induce food intake, mediated by stimulation of specific neurons in the food reward centre [217]. Due to its orexigenic property, ghrelin analogues have been proposed for clinical application in cachexia. One clinical trial with ghrelin analogues have been conducted in COPD patients. They reported improvements of ventilatory efficiency at peak exercise, reflected by increased peak oxygen uptake [236]. However, it did not translate in improved 6-minute walk distance and no data are available about body composition or food intake. Clinical trials in cancer cachexia [237], including lung cancer [238] demonstrate an enhanced lean body mass and quality of life. Hence, ghrelin analogues warrant further investigation in COPD. Besides dietary and pharmacological interventions, cognitive behavioural interventions are relatively underexplored in the management of cachexia in COPD. Although results from different functional neuroimaging studies are inconsistent and sometimes conflicting [239], there might be altered reactivity in the brain reward system in response to perceived food stimuli in people with altered eating patterns, including anorexia nervosa and obesity [240,241]. Therefore, cognitive behavioural therapy may serve as a treatment for patients with an eating disorder like anorexia nervosa [242]. Recently, a randomized controlled trial was conducted in obese subjects, receiving behavioural therapy for 6 months, in order to reduce weight. Analysis of functional magnetic resonance imaging revealed changes in reward system activity in the intervention group versus controls. Further research has to identify whether it is possible to enhance neuroplasticity in the food reward centre in order to increase successfulness for eating disorder treatment [243]. This opens up new insights and therapeutic opportunities for suspected nutritional therapy resistant cachectic COPD patients, if disturbances in the central food reward system are indeed identified.

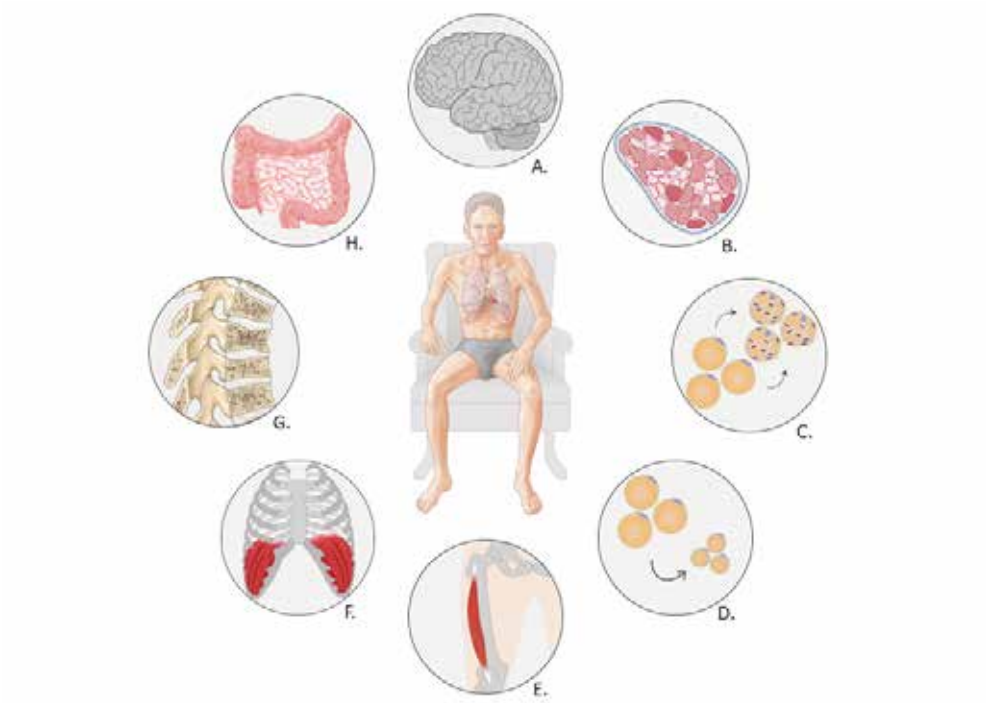

Figure 1. Pulmonary and extra-pulmonary crosstalk in COPD cachexia. (a) Altered brain responses to food stimuli; (b) muscle fibre type shifting and oxidative metabolism; (c) altered adipose tissue metabolism; (d) adipose tissue wasting; (e) limb muscle dysfunction; (f) respiratory muscle dysfunction; (g) osteoporosis; (h) altered gut integrity and reduced splanchnic extraction.

CONCLUSION

It is well established that the prevalence and related disease burden of cachexia is high in COPD and likely to increase in the near future given the high and increasing prevalence of the disease in an ageing population. Nevertheless, cachexia management is still poorly implemented in clinical practice. In 2014 the European Respiratory Society published a statement on nutritional assessment and therapy in COPD including a nutritional risk stratification diagram based on assessment of BMI, weight changes and body composition, that could be useful in patient counselling [24]. In order to increase overall survival and compress morbidity a multi-modal intervention approach is needed, which should target the discussed factors involved in cachexia (figure 1). Such multi-modal intervention approach, encompassing exercise training and improvement of energy balance and nutrient availability is currently feasible as supported by recent statements and meta-analyses, possibly improved in the near future by targeted pharmacological interventions and cognitive behavioural therapy to sensitize patients to anabolic stimuli.

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5

Computed tomography derived muscle remodelling after bronchoscopic lung volume reduction in advanced emphysema

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ABSTRACT

Muscle wasting frequently occurs in severe emphysema. Improving respiratory mechanics by bronchoscopic lung volume reduction using endobronchial valves (EBV) might prevent further loss or even increase muscle mass.

CT-derived skeletal muscle mass gain was observed in 39/49 patients 6 months after EBV. Multiple linear regression showed that gain in muscle (β=2.4 [95%CI 0.2-4.6]; p=0.036) and intramuscular fat (β=3.1 [95%CI 0.2-5.9]; p=0.035) associated with improved 6MWD independent of the change in residual volume.

Skeletal muscle remodelling associates with improved exercise capacity after EBV, independent of hyperinflation reduction.

INTRODUCTION

The emphysematous COPD phenotype is prone to develop cachexia (i.e., unintended weight loss and muscle wasting) [1], adversely affecting morbidity and mortality [1,2]. Patients with severe emphysema have limited treatment options and the efficacy of pulmonary rehabilitation on muscle function and exercise performance is hampered by impaired respiratory mechanics. Recently, lung volume reduction has regained new interest by the arrival of non-invasive bronchoscopic lung volume reduction. Bronchoscopic placement of endobronchial valves (EBV) has been shown to significantly improve symptoms, exercise capacity and quality of life [3].

The impairment of exercise tolerance in severe emphysema results from ventilatory limitation, deconditioning and loss of muscle mass and oxidative metabolism [4,5]. While improved lung function after EBV treatment was accompanied by increased exercise capacity and physical activity [3,6], the effect of EBV on muscle loss is unknown.

We hypothesize that hyperinflation reduction results in restoration of energy balance which increases body weight and maintains or increases muscle mass contributing to improved exercise capacity.

RESULTS

CT scans from 49 enrolled patients were eligible (supplemental figure 1). Baseline characteristics are displayed in supplemental table 1.

Changes in skeletal muscle and adipose tissue are depicted in table 1. After EBV treatment body weight, skeletal muscle, intramuscular fat and subcutaneous fat cross-sectional area significantly increased, while mean skeletal muscle Hounsfield units remained unchanged. Despite a similar improvement in lung function parameters, muscle mass was maintained or increased in the majority of patients (80%), whereas 20% experienced muscle loss.

Table 1. Change in skeletal muscle, adipose tissue and clinical outcome.

	Absolute change	p value
Weight, 95% CI (kg)	1.3 (0.5 to 2.1)	0.001
Skeletal muscle cross-sectional area, median (range) (cm^2)	2.4 (-14.4 to 16.6)	0.002
Hounsfield unit skeletal muscle, 95% CI	-0.1 (-1.3 to 0.6)	0.882
Intramuscular fat cross-sectional area, median (range) (cm^2)	1.3 (-7.0 to 23.8)	0.017
Subcutaneous fat cross-sectional area, median (range) (cm^2)	5.3 (-68.8 to 38.3)	0.014
Residual volume, 95% CI (%)	-17.0 (-20.5 to -13.5)	<0.001

Abbreviations: 6MWD, 6 minute walk distance.

Patients significantly improved in residual volume and their 6MWD after EBV treatment as previously reported for the total cohort [3]. Using multiple linear regression analysis, adjusting for gender and baseline 6MWD, skeletal muscle mass (β=2.4 [95% CI 0.2-4.6]; p=0.036) and intramuscular fat (β=3.1 [95% CI 0.2-5.9]; p=0.035) but not the change in residual volume, associated with 6MWD improvement (table 2).

Table 2. Multivariate analysis for predictors of change in 6MWD, corrected for gender (N=45).

	B	95% CI	p value
Delta skeletal muscle cross-sectional area, cm^2	2.4	0.16 to 4.63	0.036
Delta intramuscular fat cross-sectional area, cm^2	3.1	0.23 to 5.94	0.035
6MWD baseline	-0.3	-0.49 to -0.17	<0.001
Delta residual volume, %	-0.7	-1.93 to 0.48	0.232

Abbreviations: 6MWD, 6 minute walk distance.

DISCUSSION

This is to our knowledge the first study to present changes in skeletal muscle and adipose tissue cross-sectional area and distribution after EBV in patients with advanced emphysema derived from chest CT analysis. Skeletal muscle remodelling associated with increased exercise capacity, independent of the degree of reduced hyperinflation.

Mineo et al. [8] previously investigated body composition in emphysematous patients by dual-energy x-ray absorptiometry, prior to and 1, 3 and 5 year following lung volume reduction surgery. A sustained increase of whole body fat mass and fat-free mass was reported in patients treated with lung volume reduction surgery, while a decline in fat and fat-free mass was observed after 3 and 5 years in patients who received usual care. Dual-energy x-ray absorptiometry analyses however do not allow direct quantification of muscle mass or intramuscular changes.

A remarkable observation in this study was the observed increase in intramuscular fat associated with improved exercise performance independent of muscle cross-sectional area. The observed muscle remodelling may enhance exercise performance by improving muscle strength but also by alleviating fatigue. In line with the athlete's paradox, the observed increase in intramuscular adipose tissue could reflect improved muscle mitochondrial metabolism and insulin sensitivity via an effect of peroxisome proliferator-activated receptor gamma coactivator-1 on intramuscular lipid programming [9]. Supportive for this hypothesis are two studies by Mineo et al. showing that resting metabolic rate after lung volume reduction surgery was associated with a conversion from prevalent lipid to carbohydrate metabolism [8] and demonstrating a reduction in insulin resistance [10], but obviously this requires further in depth investigation.

Although the strength of our study comes from the well-defined randomized patient cohort, there are some limitations. Gender distribution in the study population was unequal with a predominance of females. We adjusted for gender in statistical analyses but the sample size did not allow investigating putative gender differences in the response. We do not expect profound differences as the previous studies by Mineo et al. [8,10] were performed in males. Furthermore, while the standard site to measure muscle tissue as proxy for whole body muscle mass is the third lumbar vertebra, this is typically outside the field of view of clinically acquired CT imaging in patients with COPD. Therefore, in this study we analysed CT scans at the level of the first lumbar vertebra. This hampered analysis of visceral fat mass, because CT scans at this level frequently include the lower lung lobes. Furthermore, a minimal clinically important difference is not yet been determined. We furthermore recognize that CT slice derived intramuscular fat cannot distinguish between intramyocellular and extramyocellular lipids. This requires additional biopsy derived muscle tissue analysis. We did not observe changes in the mean Hounsfield units of the muscle cross-sectional area as proxy for muscle fat deposits, which might be due to the sample size.

In conclusion, bronchoscopic lung volume reduction treatment using one-way endobronchial valves induces muscle remodelling in severe emphysematous patients which associates with improvements in exercise performance.

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SUPPLEMENTALS

Study population and study design

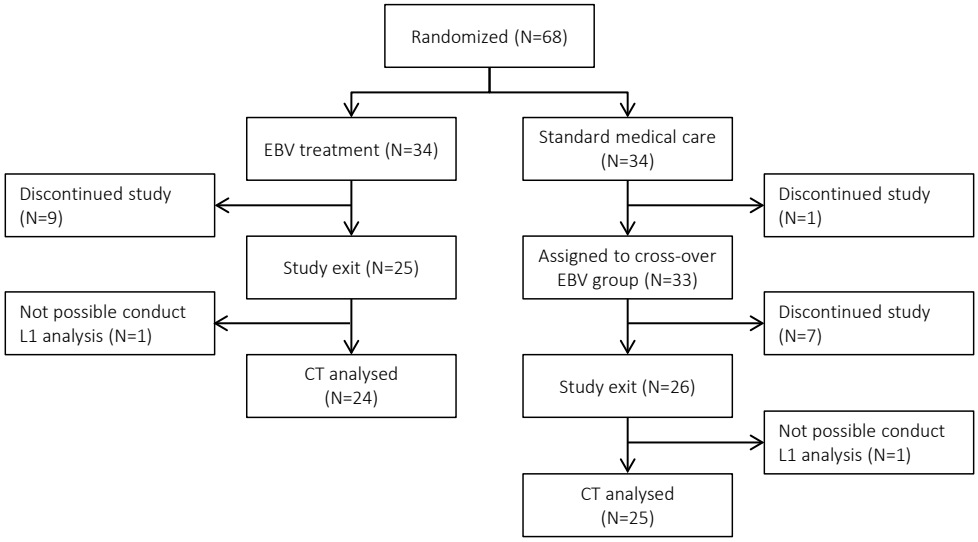
A post-hoc analysis of a randomized controlled crossover trial investigating endobronchial valve (EBV) treatment conducted at the University Medical Centre Groningen in the Netherlands from June 2011 until November 2014 (STELVIO trial NTR2876) was performed. The full and detailed methodology of this study has been published previously [3]. In short, patients with advanced emphysema and a confirmed absence of collateral ventilation by the Chartis measurement were included. Only EBV treated patients who fully completed the study were eligible for analysis in this study. The initial trial randomized patients to active treatment (EBV group), or control (control group), with at 6 months after randomisation crossover to active treatment for the control group. CT images were obtained at baseline and 6 months follow-up after EBV treatment. Spirometry and bodyplethysmography were performed according to the ATS/ERS guidelines [11]. Furthermore, exercise capacity was measured by a 6-minute walk distance (6MWD) test according to the ATS guidelines [12]. Height and body weight were assessed. All tests were performed at baseline and 6 months follow-up after EBV treatment. The study was approved by the ethics committee of the University Medical Centre Groningen.

Image analysis

In this post-hoc analysis of the STELVIO trial skeletal muscle and adipose tissue were analysed on CT scan by assessment of the cross-sectional area at the first lumbar level (L1). Skeletal muscle cross-sectional area, intramuscular fat cross-sectional area and subcutaneous fat cross-sectional area were analysed with Slice-O-Matic software v5.0 (Tomovision, Montreal, Canada). One image was selected for each patient. During anatomical land marking, the first image at L1 with both vertebral transverse processes clearly visible, was used in the analysis. The skeletal muscle measurements included the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique, and rectus abdominis muscles. Cross-sectional area of these structures were quantified by one assessor on the basis of pre-established thresholds of Hounsfield units (skeletal muscle -29 to 150, subcutaneous fat -190 to -30, and intramuscular fat -190 to -30). Boundaries were corrected manually as necessary. Changes in cross-sectional area between CT scans were expressed in squared centimetres. Our group found a mean coefficient of variation between observers of 1.3% for skeletal muscle area [data unpublished], which is in line with a variation of 0-2% in other studies [13-15]. Therefore, changes of larger than -1.3% were considered as 'loss of skeletal muscle', while changes smaller than -1.3% were considered 'maintenance or gain of skeletal muscle'. Additionally, the mean Hounsfield units of the muscle cross-sectional area were assessed, as a measure for muscle fat deposits. Low values reflect increased intramuscular fat.

Statistical analyses

Patients were included if the CT scan contained L1. Descriptive statistics of demographic and clinical variables were obtained. Means (95% confidence interval) were provided for continuous normally distributed variables, median (range) for continuous not-normally distributed variables and percentages were shown for categorical variables. Baseline and 6 months follow up measurements were compared with a paired-samples t test or Wilcoxon signed-rank test. A multiple linear regression model was constructed to assess the contribution of body composition changes to changes of 6MWD (6 months follow-up compared to baseline). The model was constructed including covariates that were found significant in univariate analyses, i.e. change in skeletal muscle cross-sectional area, change in intramuscular fat cross-sectional area, 6MWD at baseline, change in residual volume and gender. Data were tested for multicollinearity and possible influential outliers. All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, Version 24.0, IBM, Armonk, NY). Results with two-sided p values (<0.05) were considered statistically significant.



Supplemental figure 1. Flow chart study population.
Abbreviations: EBV, endobronchial valve; CT, computed tomography.

Supplemental table 1. Patient characteristics (N=49).

Male/Female, N	16 / 33
Age, mean (range),years	59 (42 to 76)
Pack years	37 (31 to 43)
Length, m	1.69 (1.67 to 1.72)
Weight, kg	70.1 (66.5 to 73.7)
BMI, kg/m^2	24.4 (23.4 to 25.5)
FEV1	
Litres	0.8 (0.8 to 0.9)
% of predicted value	30.3 (28.0 to 32.6)
FVC	
Litres	2.6 (2.4 to 2.9)
% of predicted value	78.1 (72.9 to 83.3)
FEV1/FVC	32.6 (30.6 to 34.6)
RV	
Litres	4.5 (4.2 to 4.8)
% of predicted value	215.2 (205.3 to 225.0)
TLC	
Litres	7.5 (7.1 to 7.9)
% of predicted value	130.8 (127.3 to 134.2)
RV/TLC	60.3 (57.7 to 62.8)
6MWD, meter	357.6 (332.8 to 382.3)
Skeletal muscle cross-sectional area, median (range)(cm^2)	88.0 (55.6 to 162.4)

Data are represented as mean (95% confidence interval), unless stated otherwise. Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; 6MWD, 6 minute walk distance.

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6

Effect of bronchoscopic lung volume reduction in advanced emphysema on energy balance regulation

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ABSTRACT

Background

Hypermetabolism and muscle wasting frequently occur in patients with severe emphysema. Improving respiratory mechanics by bronchoscopic lung volume reduction (BLVR) might contribute to muscle maintenance by decreasing energy requirements and alleviating eating-related dyspnoea. The goal was to assess the impact of BLVR on energy balance regulation.

Design

Twenty emphysematous subjects participated in a controlled clinical experiment before and 6 months after BLVR. Energy requirements were assessed: basal metabolic rate (BMR) by ventilated hood, total daily energy expenditure (TDEE) by doubly labelled water, whole body fat-free mass (FFM) by deuterium dilution and physical activity by accelerometry. Oxygen saturation, breathing rate and heart rate were monitored before, during and after a standardized meal via pulse oxymetry and dyspnoea was rated.

Results

Sixteen patients completed follow-up and among those 10 patients exceeded the minimal clinically important difference of residual volume reduction. Residual volume was reduced with median (range) 1285 ml (-2430,-540). Before BLVR, 90% of patients was FFM depleted despite a normal BMI (24.3±4.3kg/m^2). BMR was elevated by 130%. TDEE/BMR was 1.4±0.2 despite a very low median (range) daily step count of 2188 (739, 7110). Following BLVR, the components of energy metabolism did not change significantly after intervention compared to before intervention but BLVR treatment decreased meal-related dyspnoea (4.1 vs 1.7, p=0.019).

Conclusions

Impaired respiratory mechanics in hyperinflated emphysematous patients did not explain hypermetabolism.

INTRODUCTION

Only very recently, a new chronic obstructive pulmonary disease (COPD) phenotype titled 'multi-organ loss of tissue' has been proposed. This phenotype includes those with accelerated emphysema progression and enhanced tissue loss in other extra-pulmonary compartments, including muscle and adipose tissue. Disturbed tissue maintenance is associated with worse clinical outcomes [1], and might be the result of changes in whole body energy expenditure.

Whole body energy expenditure can be distinguished in basal metabolic rate (BMR), diet-induced thermogenesis and physical activity induced energy expenditure. Basal metabolic rate is primarily determined by fat-free mass (FFM) and comprises the largest part of total daily energy expenditure (TDEE) [2]. Diet induced thermogenesis is +/- 10% of TDEE [3] and physical activity induced energy expenditure largely depends on physical activity level [4]. Whole body energy expenditure can only be measured over a prolonged period in daily life using doubly labelled water [5]. This stable isotope methodology is very expensive and requires analytical technology that is available in a limited number of centres worldwide.

In COPD, an increased BMR relative to predicted values has repeatedly been demonstrated [6], which is more aggravated in weight losing patients [6] and in those with emphysema [7]. Although hypermetabolic at rest, COPD patients do not exhibit increased diet-induced thermogenesis [8]. Besides the proposed triggers for hypermetabolism including activation of brown adipose tissue, inflammation and increased whole body protein turnover, impaired lung mechanics might also result in hypermetabolism [9]. Emphysema is hallmarked by a reduction in lung elastic recoil and progressive hyperinflation, resulting in elevated airway resistance and contributing to impaired lung mechanics [10]. This results in an increased workload of breathing (ml oxygen cost per litre ventilation) [11]. The increased breathing workload has shown to be more pronounced in patients with low body weight and correlated with the degree of hyperinflation [12].

Pharmacological interventions may alleviate dyspnoea, reduce exercise limitation and improve quality of life in COPD by decreasing airway resistance and reduce hyperinflation. However, response is limited in patients with predominant emphysema [13]. In selected severe emphysematous patients bronchoscopic lung volume reduction (BLVR) is an additional treatment option that results in marked benefits in terms of pulmonary function, dyspnoea, exercise capacity and also physical activity [14, 15]. Furthermore, in a recent post-hoc analysis of the STELVIO trial [15] we illustrated a significant increase in body weight, skeletal muscle and fat tissue, suggesting a positive effect on energy balance regulation [16].

BLVR is an unique model to test the influence of lung mechanics on energy balance regulation, as it diminishes thoracic hyperinflation, reduces breathing frequency, and reduces mechanical

constraints on lung volume expansion, thereby improving ventilatory mechanics [17]. Efficacy of this treatment highly depends on advanced patient selection to identify responders to the treatment, and thereby creating a homogeneous study population.

We hypothesize that a decline in breathing workload following BLVR would decrease energy expenditure, which might positively influence components and determinants of energy balance. Secondly, BLVR may also improve dietary intake by alleviating eating associated dyspnoea and meal-related oxygen desaturation [18].

METHODS

Participants

Twenty patients with advanced emphysema, an identified target lobe with confirmed absence of collateral ventilation by the Chartis measurement who underwent BLVR treatment using one-way endobronchial valves were included in this study.

Patients were recruited from Maastricht University Medical Centre (MUMC+) and University Medical Centre Groningen (UMCG) in the Netherlands from September 2016 until April 2017. The Ethics Committee of Maastricht University Medical Centre approved the study protocol, and all participants provided written informed consent. Procedures were conducted according to the principles of the Declaration of Helsinki. The trial was registered at ClinicalTrial.gov (NCT02500004).

Study design

The study design is shown in figure 1.

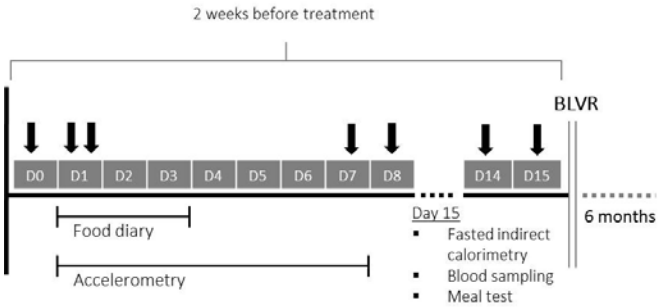


Figure 1. Study design in days. Black arrows indicate urine sampling.

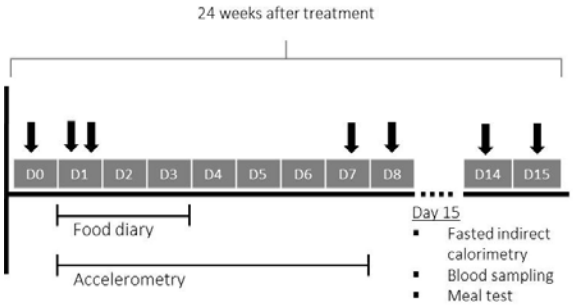


Figure 1 Continues.

Prior to BLVR treatment, patients underwent a 2-week assessment period.
At day 1, patients were visited at home and received a dose of doubly labelled water. They were also instructed to collect urine samples for assessment of TDEE and to wear an accelerometer for registration of physical activity. Furthermore, patients were asked to record their dietary intake in order to assess if they were in a state of stable energy balance. On day 15, fasted state urine and blood samples were collected, weight, height and BMR were assessed and a meal test was performed (vide infra). This 2-week assessment period was repeated 6 months after BLVR treatment.

Body composition
Body height was determined to the nearest 0.5 cm while subjects were standing barefoot. Weight was assessed with a beam scale to the nearest 0.1 kg while subjects were standing barefoot and in light clothing. FFM was calculated from total body water assessment using the deuterium dilution technique assuming a hydration fraction of FFM of 73%.

Resting metabolic rate
BMR was measured by indirect calorimetry using a ventilated hood (EZCAL; Maastricht Instruments, Maastricht, the Netherlands and COSMED QUARK; TulipMed B.V., the Netherlands). Patients received their maintenance inhalation according to their normal habits. The time interval between medication use and start of indirect calorimetry was documented. During the second 2-week assessment period 6 months following BLVR treatment, the same time interval between medication use and start of indirect calorimetry was employed. Patients were in a fasting state for at least 10 hours and had a period of 30 minutes bed rest prior to the measurement during which subjects were lying on bed in supine position. After stabilization, BMR was recorded during a period of 30 minutes. BMR was calculated from oxygen consumption (VO_2) and carbon dioxide (VCO_2) production using the abbreviated Weir formula [19]. BMR was also predicted using the equation from Slinde et al., which was especially designed for COPD patients [20].

Total daily energy expenditure

TDEE was determined by the doubly labelled water technique over two 2-week periods (before and after BLVR treatment) according to the Maastricht protocol [21]. In the evening, prior to dosing, a urine sample was collected for determination of background isotope enrichment. Each patient received a weighted oral dose of water labelled with deuterium and oxygen-18. The given dose was calculated based on the subjects' total body water, which was estimated based on body mass index, age and gender. Subjects received a dose of 2.5 g/L total body water containing 250 ppm deuterium and 2200 ppm oxygen-18. After overnight equilibration, a second urine sample was collected from the second morning voiding. Additional urine samples were collected in the evening of days 1, 7 and 14 and in the morning of days 8 and 15. TDEE was calculated by the linear regression from the difference between elimination constants of deuterium and oxygen-18.

Physical activity

Actigraph GTX3 accelerometers (Actigraph, Pensacola, FL, USA) were used to assess the physical activity level. This activity monitor has been validated against activity related energy expenditure measured by doubly labelled water in patients with different stages of COPD [22]. The tri-axial accelerometers were attached to the lower back with an elastic belt and worn for 7 consecutive days. Subjects were instructed to wear the accelerometer during the time they were not asleep, except when showering or bathing. Only days with ≥8 hours of wear time were accepted as valid days.

Energy expenditure for activities was calculated by (0.9*TDEE)-BMR, assuming a diet-induced thermogenesis of 10% of TDEE.

Dietary intake

Food intake was recorded by a food diary for 2 week days and 1 weekend day to estimate baseline energy balance.

Meal test

On the measurement day at the hospital, subjects received a standardized breakfast with wheat bread, butter, eggs, and milk. This meal contained a total of 502 kcal derived from protein (24%), carbohydrate (28%) and fat (48%). Oxygen saturation, breathing rate and heart rate were monitored before, during and after the breakfast via pulse oximetry. Before and immediately after the meal, dyspnoea was rated using the Borg dyspnoea scale.

Systemic Inflammatory status

High sensitive C-reactive protein (hsCRP) was assessed from frozen stored plasma collected from a venepuncture after overnight fasting.

Statistics

Descriptive statistics of demographic and clinical variables were obtained. Means (±SD) were provided for continuous normally distributed variables, medians (interquartile range) for continuous not-normally distributed variables and percentages were shown for categorical variables. Baseline and 6 months follow up measurements were compared with a paired-samples t test or Wilcoxon signed-rank test. All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, Version 24.0, IBM, Armonk, NY). Results with two-sided p values (<0.05) were considered statistically significant.

RESULTS

Patient characteristics

Twenty patients (7 men, 13 women) with severe emphysema (N=10 MUMC+ and N=10 UMCG) were enrolled in this study and 16 patients completed the follow-up. Reasons for drop out were patients' decision due to deterioration in health (N=2), patients' decision due to lack of efficacy of BLVR treatment (N=1) and diagnosis of bladder cancer (N=1). In four of the 16 patients who completed follow-up, endobronchial valves were removed due to granulation tissue around endobronchial valves (N=2), torsio bronchus (N=1) and recurrent pneumothorax (N=1) (figure 2).

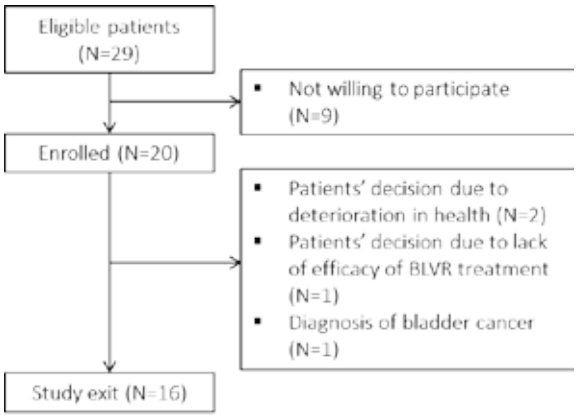


Figure 2. Study flow chart.

Baseline characteristics are depicted in table 1. The study population represented a COPD population with normal BMI (24.1±4.4 kg/m^2) and low FFM (FFMI males 15.1 kg/m^2 [14.7-16.2], females 13.5 kg/m^2 [12.1-18.1]). The prevalence of depletion of FFM, defined as FFMI≤17 kg/m^2 for males or ≤15 kg/m^2 for females [23], was 90%.

Table 1. Baseline characteristics (N=20).

General	
Male/Female, N	7/13
Age, years	63±7
Pack years	42±25

Lung function	
FEV1 (% predicted)	23.3±6.6
FVC (% predicted)	76.2±15.9
FEV1/FVC	28.5±6.3
RV (% predicted)	238.3±38.2
TLC (% predicted)	136.6±17.9
RV/TLC	64.7±9.4

Body composition	
Weight (kg)	70.3±16.3
BMI (kg/m^2)	24.1±4.4
FFM, kg	38.2 (32.1-57.4)
FFMI, kg/m^2	
Male	15.1 (14.7-16.2)
Female	13.5 (12.1-18.1)

Data are represented as mean ± SD or median (minimum-maximum).
Definition of abbreviations: FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index.

6

Baseline assessment

At baseline, the mean BMR was 1537±259 kcal/day, which corresponded to 130% of predicted, indicating pronounced hypermetabolism. The average TDEE over two weeks was 2133±294kcal/day. The average daily TDEE of week 1 was not statistically significantly different from the average daily TDEE of week 2. Energy expenditure for activities was median (range) 275 kcal/day (138-827) (11% of TDEE).

Among those who completed follow-up, from all subjects but two (due to an accelerometer device defect), 6.5±1.1 valid accelerometry days were available with a mean of 13±1 hours of wear time per day. Median (range) steps per day was 2188 (739-7110). Patients spent a significant part of the day in sedentary state (79.7 % of the wear time [56.5-89.6]) (table 2).

Table 2. Clinical variables and components of energy balance at baseline and 6 months after BLVR treatment (N=10).

	Baseline	After BLVR	p value
Lung function and symptom burden			
FEV1, % of predicted value	27.5±6.9	34.9±8.3	0.003
FVC, % of predicted value	74.4±15.0	95.1±17.1	<0.001
FEV1/FVC	30.5±7.2	29.5±5.6	0.591
RV, % of predicted value	236.2±37.6	181.3±27.5	<0.001
TLC, % of predicted value	135.0±20.0	125.1±14.7	0.007
RV/TLC	65.8±6.2	51.3±6.1	<0.001
COPD Assessment Test, points	18.6±3.4	14.3±5.8	0.022
6MWD, meter	378±98	427±84	0.030

Table 2 Continues.

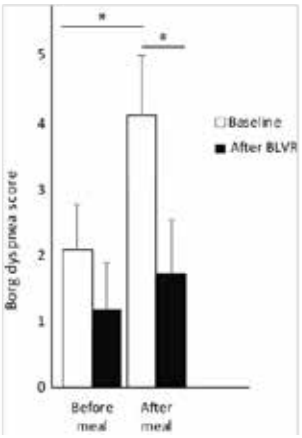
	Baseline	After BLVR	p value
FFMI, kg/m^2			
Male	15.5 (14.7-16.2)	16.6 (15.5-18.6)	0.068
Female	13.2 (12.4-17.6)	13.6 (12.1-17.3)	0.600
Body composition			
Weight, kg	71.4±17.4	73.0±18.7	0.096
BMI, kg/m^2	24.3±4.3	24.8±5.0	0.127
FFM, kg	40.4 (32.2-57.4)	41.1 (29.4-60.2)	0.074
Energy expenditure			
VCO_2 (ml/min)	179.5±26.1	180.5±28.6	0.854
VO_2 (ml/min)	224.7±40.0	224.0±3.1	0.903
RQ	0.81±0.07	0.81±0.05	0.916
BMR measured (kcal/day)	1537±259	1549±231	0.778
BMR predicted (kcal/day)	1213±155	1245±189	0.103
BMR measured /BMR predicted ratio	1.3±0.2	1.2±0.1	0.655
TDEE (kcal/24h)	2133±294	2192±480	0.576
TDEE/BMR ratio	1.4±0.2	1.4±0.3	0.934
Energy expenditure for activities (kcal/day)	275 (138-827)	397 (18-1262)	0.694
Energy expenditure for activities/TDEE ratio	0.2±0.1	0.2±0.1	0.995
Physical activity level			
Mean steps/day	2188 (739-7110)	2429 (990-6983)	0.161
Time spent in sedentary PA, % of wear time	79.7 (56.5-89.6)	79.4 (52.7-84.2)	0.123
Time spent in lifestyle PA, % of wear time	17.6 (10.1-34.2)	18.6 (14.1-37.9)	0.123
Time spent in MVPA, % of wear time	0.0 (0.0-1.1)	0.2 (0.0-1.4)	0.028
Inflammation			
hsCRP, mg/L	3.0±2.7	2.5±1.8	0.463

Data are represented as mean ± SD or median (minimum-maximum).
Definition of abbreviations: FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; 6MWD, 6-minute walk distance; MVPA, moderate-to-vigorous physical activity; PA, physical activity; BMR, basal metabolic rate; TDEE, total daily energy expenditure; hsCRP, high sensitive C-reactive protein. COPD Assessment Test, missing N=2

Systemic inflammation measured by hsCRP was 3.0±2.7 mg/L. BMR or TDEE was not associated with hsCRP or residual volume (RV) (% of predicted) [data not shown].

Reported dietary intake comprised 2065±507 kcal/24h, which equalled measured TDEE. Patients experienced more dyspnoea after eating (4.1±1.8 after meal vs 2.1±2.1 before meal, p=0.013). No significant change was shown in oxygen saturation, respiration rate and heart beat rate during the course of the meal (figure 3).

A



B

		Baseline	After BLVR	p value
Borg dyspnoea score	Before meal	2.1 ± 2.1	1.3 ± 2.1	0.359
	After meal	4.1 ± 1.8	1.7 ± 2.4	0.019
Oxygen saturation (%)	Before meal	93.2 ± 2.4	92.2 ± 3.1	0.193
	During meal	92.5 ± 3.6	91.9 ± 3.3	0.272
	After meal	92.0 ± 3.1	92.4 ± 3.7	0.406
Respiration rate (respirations/min)	Before meal	16.1 ± 3.7	16.5 ± 2.6	0.647
	During meal	16.7 ± 3.7	16.6 ± 3.4	0.850
	After meal	16.0 ± 4.5	17.1 ± 3.2	0.465
Heart rate (beats/min)	Before meal	74.6 ± 8.7	70.1 ± 10.2	0.155
	During meal	82.2 ± 8.4	77.4 ± 10.4	0.167
	After meal	83.1 ± 9.8	77.3 ± 11.3	0.134

Figure 3. (a) Borg dyspnoea score, (b) Borg dyspnoea score, oxygen saturation, respiration rate and heart rate before, during and after completion of a standardized meal, before and after BLVR treatment (N=10). Data are presented as mean values (± standard deviation).

Response after BLVR

Not all patients benefited from the BLVR treatment, in terms of hyperinflation reduction. We therefore took a closer look to the 10 patients who responded beyond the MCID for RV reduction of >430 ml [24]. At 6 months follow-up, patients significantly improved in RV and forced expiratory volume in 1 second, with respectively 1285 ml (-2430, -540) and 190 ml (10-390).

BMR did not significantly change over time (1537±259 kcal/day vs 1549±231 kcal/day, p=0.778) and patients remained hypermetabolic (BMR was 130% of predicted). No changes in TDEE were observed (2133±294 kcal/day vs 2192±480 kcal/day, p=0.576), in accordance to an unaltered physical activity expressed by mean number of daily steps. Although 6MWD increased significantly, the mean step count and activity-induced energy expenditure, did not change over time. hsCRP also remained unchanged (table 2).

A significant effect of BLVR treatment on meal-related dyspnoea was observed. Compared to baseline meal-related dyspnoea after the meal was significantly lower after BLVR treatment (1.7±2.4 vs 4.1±1.8, p=0.019). No changes were found in oxygen saturation, respiration rate or heart rate during the meal (figure 3).

DISCUSSION

This is the first study presenting a comprehensive analysis of energy balance in a homogeneous group of patients with severe emphysema and investigating the effect of BLVR. In contrast to our hypothesis, a median reduction of hyperinflation with 25% did not decrease BMR or TDEE adjusted for physical activity level. Eating-related dyspnoea however was diminished.

In line with the 'multi-organ loss of tissue' phenotype [1], we observed a very high prevalence of FFM depletion indicative for disturbed muscle maintenance. Nearly all patients were FFM depleted, but this was disproportionate to the FM as the majority of patients fell within a normal BMI range. Before BLVR, BMR was very high, up to 130% of predicted and energy expenditure for physical activities was very low (11%). This implies that in this patient group and at this stage of the disease, fat mass regulation is primarily determined by the balance between energy intake and whole body energy requirements and less or not yet by fat catabolism (i.e. increased lipolysis or brown adipose tissue activation). The normal BMI in this population hides FFM depletion, emphasizing the importance of body composition assessment for estimation of metabolic risk as proposed by the European Respiratory Society task force Nutritional Assessment and Therapy in COPD [25].

No studies to date have investigated the effect of lung volume reduction on TDEE, but a few studies previously reported the effect of lung volume reduction surgery on BMR. Mineo et al. showed a reduction of BMR with 5% [26], while Takayama et al. observed no change in BMR [27]. The degree of hyperinflation reduction was comparable to our cohort. Nevertheless, one needs to consider that, although our patients improved importantly after intervention, they still remain severely hyperinflated with a mean residual volume of 181% of predicted.

A contributor to BMR is whole body protein turnover, which explained approximately 20% of the between subject variation of BMR, in healthy young individuals [28]. Also in COPD, increased rates of whole body protein turnover have been reported [29, 30], which associated with BMR [31]. Increased muscle turnover signalling was accompanied with elevated myogenic signalling [32], which was most prominent in patients with FFM depletion. Therefore, persistence of high BMR after BLVR might be the result of energy cost of protein anabolism, supported by increased muscle mass observed previously in chest CT scans [16].

In the absence of catabolic drivers, fat mass is primarily regulated by the balance between energy intake and energy metabolism. In line with others [33, 34], our patients experienced an eating induced increase in dyspnoea. Vermeeren et al. reported the effects of different meals on dyspnoea sensation and found a significantly greater increase in dyspnoea after ingestion of a fat-rich meal than after a carbohydrate-rich meal [33]. Here, we show for the first time that dyspnoea after the same, standardized meal was significantly less following

BLVR. In line with two other studies, these effects could not be explained by changes in meal related oxygen saturation [18,33].

Systemic inflammation has been proposed as putative trigger for hypermetabolism in particular during acute exacerbations [35, 36]. Indeed elevated CRP levels have previously been associated with higher BMR in clinically stable COPD [37, 38]. In this study, CRP levels were slightly elevated but did not change after BLVR.

The strength of this prospective well-controlled clinical proof of concept study comes from the well-defined patient cohort and from the use of gold standard methods to assess body composition, BMR and TDEE. We recognize that the study power was based on detection of changes in energy metabolism in relation to changes in lung function but not for changes in body composition. The technique of pulse oximetry has the advantage of providing a continuous and non-invasive measurement of oxygen saturation. However, this technique is limited by a poorer accuracy of 1-3% when compared to arterial blood sampling [39].

To conclude, the present work showed that impaired respiratory mechanics in hyperinflated emphysematous patients did not explain hypermetabolism.

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7

Brown adipose tissue activation in relation to hypermetabolism in emphysematous chronic obstructive pulmonary disease patients

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Submitted.

ABSTRACT

Introduction

Brown adipose tissue (BAT) has been primarily researched as a potential target for mitigating obesity. However, the physiological significance of BAT in relation to cachexia remains unknown. The objective of this study was to investigate the putative contribution of BAT on different components of energy metabolism in patients with emphysema.

Methods

Twenty emphysematous patients (mean ± SD age 62 ± 6, median [range] BMI 22.4 [15.1-32.5] kg/m^2, 85% low fat-free mass index [FFMI]) were studied. Basal metabolic rate (BMR) was assessed by ventilated hood, total daily energy expenditure (TDEE) by doubly labelled water and physical activity by tri-axial accelerometry. BMR was adjusted for fat-free mass (FFM) as assessed by deuterium dilution. Analysis of BAT and white adipose tissue was conducted in a subset of ten patients and six age, gender and BMI matched healthy controls. BAT glucose uptake was assessed by means of cold-stimulated integrated [18F]FDG positron-emission tomography and magnetic resonance imaging. White adipose tissue was collected from subcutaneous abdominal biopsies to analyse metabolic and inflammatory gene expression levels. Lung function was assessed by spirometry and bodyplethysmography and systemic inflammation by high sensitivity C-reactive protein.

Results

Mean TDEE was 2209 ± 394 kcal/day and mean BMR was 1449 ± 214 kcal/day corresponding to 120% of predicted. Fat-free mass adjusted BMR did not correlate with lung function or systemic inflammation.

Upon cooling, energy expenditure increased, resulting in a non-shivering thermogenesis of (median [range] 20.1% [3.3-41.3] in patients and controls. Mean BAT glucose uptake was comparable between COPD and controls (1.5 [0.1-6.2] versus 1.1 [0.7-3.9]). In addition, no correlation was found between BMR adjusted for fat-free mass and BAT activity and between cold-induced non-shivering energy expenditure and BAT activity. Gene expression levels of the brown adipocyte or beige markers were also comparable between the groups.

Conclusions

Although COPD patients were hypermetabolic at rest, no correlation was found between energy expenditure and BAT activity. Furthermore, both BAT activity and gene expression levels of the brown adipocyte or beige markers were comparable between COPD and controls.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an airway and lung disease with persistent airflow obstruction, which is the result of small airway remodelling and loss of elastic recoil [1]. Disturbances in whole body and cellular energy metabolism are common in COPD and contribute to weight loss and muscle wasting in particular in the emphysematous phenotype [2-4].

Total daily energy expenditure (TDEE) consists of basal metabolic rate (BMR), diet induced thermogenesis and activity induced energy expenditure (AEE). AEE has been shown to be elevated due to decreased mechanical efficiency [5, 6] but may also be (relatively) decreased in patients with very low physical activity levels [3]. Diet induced thermogenesis was shown to be normal in previous research [7, 8]. Increased BMR relative to predictive values has been consistently reported in COPD [2] and found more aggravated among weight losing patients [2, 3] and among those with emphysema [9, 10]. Hypermetabolic patients do not necessarily exhibit an elevated TDEE [11]. Systemic inflammation has been proposed as cause of elevated BMR in cachexia induced by cancer [12] or COPD [13]. Another contributing factor to hypermetabolism in COPD might be the workload of breathing [14] due to lung hyperinflation. Nevertheless, we recently showed that decreasing hyperinflation with 25% by endoscopic lung volume reduction in patients with emphysema did not decrease BMR (own results, accepted for publication).

Activation of brown adipose tissue (BAT) or browning of white adipose tissue (WAT) has been proposed as putative trigger for hypermetabolism in cachexia but to date no evidence from human research is available [15, 16]. BAT tissue is characterized by abundance of mitochondria and by heat production via the process of proton leakage over the mitochondrial membrane mediated by uncoupling protein 1 [17]. Compared to BAT-negative healthy young lean subjects, those with BAT had a higher cold-induced energy expenditure, with an average difference of 370 kcal/day [18]. In healthy young lean men, cold induction increased energy expenditure by on average 17% [19]. This increase in energy expenditure upon cold in the absence of shivering (i.e. non-shivering thermogenesis) implies that BAT thermogenesis may be a significant component of BMR and thereby plays a role in regulation of body weight as suggested by an inverse association between cold-induced BAT activity and body mass index in adults [20-22].

BAT has been primarily researched as a potential target for mitigating obesity [23]. Yet few research attempts have been made to explore the role of BAT in relation to cachexia as observed in patients with COPD and no clinical studies are available. There is some indirect evidence pointing towards a possible role for BAT as driver of hypermetabolism in the emphysematous COPD subtype. Patients with advanced emphysema have high plasma levels of norepinephrine at rest [24], which is a potential browning factor [25]. Furthermore, whole body glucose

production [26] and skeletal muscle glycolysis [27, 28] is increased in COPD patients, which result in increased plasma lactate levels, even at rest [29]. Lactate in turn is able to control expression of uncoupling protein 1 (UCP1), which also promotes browning. We therefore hypothesized that BAT or browning of WAT contributes to energy expenditure in emphysematous COPD patients. Since limited studies are available regarding TDEE and the different components in mixed COPD phenotypes, we first measured energy expenditure in emphysematous COPD patients and related its components to airflow obstruction and hyperinflation. We then conducted a controlled clinical experiment in a subgroup to assess presence of BAT by cold induced BAT activity and BAT and beige gene expression markers in WAT.

METHODS

Participants

Whole body energy metabolism and its components were assessed in twenty Caucasian COPD patients with emphysema. The contribution of BAT and WAT was studied in a subgroup of ten patients and a control group, matched for ageing, male gender and adiposity, since these factors are known to negatively correlate with BAT activity [22]. Furthermore, BAT volumes may differ between ethnicities [30]. In addition, since cigarette smoke is acknowledged as the most important risk factor for COPD and control subjects may not be diagnosed with COPD yet, non-smoking controls were included.

Patients were recruited from Maastricht University Medical Centre (MUMC+) and the healthy controls were recruited via advertisements in local newspapers. The Ethics Committee of MUMC+ approved the study protocol, and all participants provided written informed consent. Procedures were conducted according to the principles of the Declaration of Helsinki. The study was registered at ClinicalTrial.gov (NCT02500004).

Exclusion criteria for the COPD as well as the healthy controls were diabetes mellitus, severe clotting disorder, active malignancy, claustrophobia, long-term oxygen therapy, history of radiotherapeutic radiation in the neck and/or upper chest, cervical or thoracic sympathectomy and the presence of MRI contra-indications, such as pacemaker, cochlear implant, vascular clips etc. In addition, subjects using medication that influences the sympathetic nerve system, including ß-blockers, α-blockers, central antihypertensive drugs, certain anti-depression drugs (MAO inhibitors, tricyclic anti-depressives), reserpine, cocaine, calcium channel blockers, and certain tranquillizers (fenothiazines) were also not eligible. Furthermore, patients with pancreatic cancer were included, with similar exclusion criteria as mentioned above.

The original intention was to compare the COPD patients not only to healthy controls, but also to matched lung cancer and pancreatic cancer patients. However, the BAT protocol was too strenuous, regarding eligibility criteria as well as methodology used, for most of the cachectic

cancer patients. We were able to measure only two pancreatic cancer patients and no lung cancer patients before study closure.

Study design

The overall study design is shown in figure 1. All participants completed the 2-week study period focussing on whole body energy metabolism. A subset underwent the protocol focussing on the contribution of BAT and subcutaneous adipose tissue (SAT) to energy metabolism. This protocol included an individualized cooling protocol [31], [18F]FDG positron-emission tomography (PET) and magnetic resonance imaging (MRI) scanning in order to measure BAT activity and a subcutaneous fat biopsy obtained after an overnight fast.

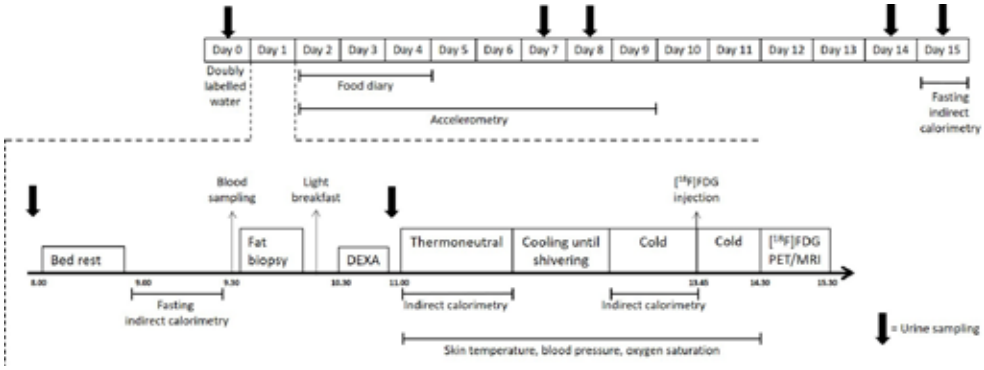

Figure 1. Schematic illustration of the study protocol.

Basal metabolic rate

BMR was measured by indirect calorimetry using a ventilated hood (EZCAL; Maastricht Instruments, Maastricht, the Netherlands). Patients were in a fasting state for at least ten hours and were requested to abstain from heavy exercise, inhalation medication, and smoking for 24 hours. Prior to the measurement the subjects had a period of 30 minutes bed rest during which they were lying on bed in supine position. After stabilization, BMR was recorded during a period of 30 minutes. BMR was calculated from oxygen consumption (VO_2) and carbon dioxide (VCO_2) production using the abbreviated Weir formula [32]. BMR was also predicted using the equation from Slinde et al. [4].

Doubly labelled water

TDEE was determined by the doubly labelled water technique over 2 weeks according to the Maastricht protocol [33]. In the evening, prior to dosing, an urine sample was collected for determination of background isotope enrichment. Each patient received a weighted oral dose of water labelled with ~ 7% deuterium and 10% oxygen-18. The given dose was calculated based on the subjects' total body water, which was estimated based on body mass index, age and gender. Subjects received a dose of 2.4 g per litre total body water resulting in an initial

excess enrichment of approximately 150 ppm for deuterium and 200 ppm for oxygen-18. The basic principle of DLW is that after equilibration with total body water, deuterium and oxygen-18 are eliminated from the body at a different rate. Deuterium is eliminated from the body as water whereas oxygen-18 is eliminated as water and carbon dioxide. The difference in elimination rates is hence a measure of CO_2-production. After ingestion of doubly labelled water, urine samples were collected at standardized time points (in the evening of days 1, 7, 14 and in the morning of days 8 and 15). CO_2-production was calculated by the linear regression from the difference between elimination constants of deuterium and oxygen-18. TDEE was calculated from CO_2-production assuming an RQ of 0.85, corresponding to the RQ of an average Western diet.
Energy expenditure for activities was calculated by (0.9*TDEE)-BMR, assuming a diet-induced thermogenesis of 10% of TDEE.

Body composition
Body height was determined to the nearest 0.5 cm while subjects were standing barefoot. Weight was assessed with a beam scale to the nearest 0.1 kg while subjects were standing barefoot and in light clothing. FFM was calculated from total body water assessment using the deuterium dilution technique assuming a hydration fraction of FFM of 73%.

Physical activity
Actigraph GTX3 accelerometers (Actigraph, Pensacola, FL, USA) were used to assess physical activity. This activity monitor has been validated against activity related energy expenditure measured by doubly labelled water in patients with different stages of COPD [34]. The tri-axial accelerometers were attached to the lower back with an elastic belt and worn for seven consecutive days. Subjects were instructed to wear the accelerometer during the time they were not asleep, except when showering or bathing. Only days with ≥8 hours of wear time were accepted as valid days.

Dietary intake
Energy intake was recorded by a food diary for two week days and one weekend day to judge if subjects were in a stable energy balance.

Individualized cooling and PET/MRI scanning
For individualized cooling, subjects were wrapped in a water-perfused suit. During the cooling procedure, skin temperature, blood pressure, heart rate and oxygen saturation were monitored. Skin temperatures were measured continually at 14 positions by means of iButtons (type DS1291H, Dallas Maxim Semiconductor Corp., USA) [35] and on three additional positions (supraclavicular, underarm and fingertip). Blood pressure and heart rate were measured via an automated blood pressure monitor (Omron Healthcare Inc, Field Court Lake Forest, USA)

and oxygen saturation was measured via pulse oximetry (Nellcor Puritan Bennett NPB-40, Pleasanton, CA, USA).

The first 45 minutes, subjects were kept at thermoneutral conditions with water temperature of the water-perfused suit at 34°C. Following this thermoneutral condition, a gradual step-wise decrease in temperature followed (water temperature was lowered with 4°C every 15 minutes) until shivering occurred. After the onset of shivering, subjects were warmed up for 5 minutes after which the temperature was set slightly above shivering level for 30 minutes. At thermoneutral condition and during cold exposure, indirect calorimetry was performed to measure energy expenditure. Subsequently, 150 MBq of 18F-FDG was injected intravenously while mild cold stimulation remained for 45 minutes after injection. At 60 minutes a hybrid PET/ magnetic resonance imaging (MRI) scan (Siemens Healthcare GmbH, Erlangen, Germany, software version VB20P) was acquired.

PET/MRI analysis

PET/MRI scans were analysed using PMOD software (version 3.7, PMOD Technologies, Zurich, Switzerland). For assessment of BAT activity, volumes of interest (VOIs) were drawn which encompassed supraclavicular adipose tissue. A sphere with radius 6.2 mm was deposited on the location where the average value was maximal in the selected VOI. Additionally, VOIs were placed in the liver, several skeletal muscle groups (biceps and triceps brachii, deltoid, erector spinae), subcutaneous adipose tissue and visceral adipose tissue. For each VOI and sphere, the measured activity concentration was corrected for radioactive decay, total administered activity and body weight, resulting in the mean standardized uptake value, SUVmean.

Blood analysis

Venous blood was obtained after an overnight fast for analysis of C-reactive protein (CRP), glucose, insulin, thyroid stimulating hormone, free fatty acids, cholesterol, triglycerides, high- and low-density lipoprotein.

Fat biopsy

A subcutaneous fat biopsy was obtained para-umbilically during fasting conditions through needle biopsy. This biopsy was snap-frozen in liquid nitrogen and stored at-80°C until analysing gene expression by qPCR.

Statistics

Descriptive statistics of demographic and clinical variables were obtained. Means (± SD) are provided for continuous normally distributed variables, median (range) for continuous not-normally distributed variables and percentages are shown for categorical variables. Between group differences were tested with an independent t-test, Mann-Whitney U test or Chi square

test. All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, Version 24.0, IBM, Armonk, NY). Results with two-sided p values (<0.05) were considered statistically significant.

RESULTS

Baseline characteristics of participants

Ten male and ten female COPD patients with emphysema were enrolled. Baseline characteristics are depicted in table 1.

Table 1. Baseline characteristics of COPD patients (N=20) and healthy controls (N=6).

	COPD	Controls
General		
Male/Female, N	10/10	4/2
Age, years	62 ± 6	60 ± 6
Pack years	36 ± 17	4 ± 8
Smoking status, N		
Never	0	4
Current	4	0
Former	16	2
Body composition		
Weight, kg	67 ± 15	70.8 ± 3.8
BMI, kg/m^2	22.4 (15.1-32.5)	23.1 (21.2-30.8)
FFM, kg	44.7 (31.7-57.4)	50.5 (44.6-63.8)
FFMI, kg/m^2		
Male	15.7 (14.2-18.2)	16.9 (15.3-19.7)
Female	13.5 (12.7-17.6)	18.0 (16.2-19.8)
Lung function		
FEV1, % predicted	36.0 ± 15.4	not applicable
FVC, % predicted	82.2 ± 18.3	not applicable
FEV1/FVC	39.2 ± 15.4	not applicable
RV, % predicted	209.7 ± 61.2	not applicable
TLC, % predicted	128.7 ± 23.7	not applicable
RV/TLC	1.6 ± 0.3	not applicable
DLCO, % predicted	38.0 ± 9.4	not applicable

Data are represented as mean ± SD or median [range].
Definition of abbreviations: BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; DLCO, diffusion capacity of lung for carbon monoxide.

The COPD population was characterized by moderate to severe airflow obstruction and low diffusion capacity (FEV1: 36 ± 15% of predicted and DLCO: 38 ± 9% predicted). Median BMI (22.4 [15.1-32.5]) was within the normal range but median (range). FFM index was below normal (FFMI males 15.7 kg/m^2 [14.2-18.2], females 13.5 kg/m^2 [12.7-17.6]) and 85% was depleted according to current definition (FFMI ≤17 kg/m^2 for males or ≤15 kg/m^2 for females [36]).

A subset of ten COPD patients (five males and five females) and six controls (BMI 23.1 [21.2-30.8] kg/m^2, FFMI males 16.9 kg/m^2 [15.33-19.7] and females 18.0 kg/m^2 [16.20-19.8]) underwent individualized cooling and PET/MRI scanning in order to measure BAT activity. PET/MRI imaging was impossible in one COPD patient, because she experienced a panic attack immediately before initiation of the scan. Compared to controls, COPD patients exhibited statistically higher insulin (27.2 [12.0-86.6] versus 13.0 [12.0-19.0] pmol/L), while fasting glucose was comparable (5.8 ± 1.0 versus 4.9 ± 0.5 mmol/L). Groups were comparable regarding thyroid function and lipid profile. CRP levels on average were low but tended to be higher in the COPD patients (2.5 [2.0-21.0] versus 0.0 [0.0-3.0] mg/L) (supplemental table 1).

Participans are hypermetabolic at rest

Mean TDEE was 2209 ± 394 kcal/day in the COPD patients and not significantly different from dietary intake (2235 ± 600 kcal/day). AEE amounted to 538 ± 258 kcal/day (23% of TDEE) in COPD and 812 ± 255 (32% of TDEE) in healhy controls, indicating a low physical activity level in the COPD group. Indeed, median (range) steps per day were 3097 [468-8792] and 8519 [6517-13518] for respectively COPD patients and healthy controls. Patients compared to controls spend a larger part of the day in sedentary state (70.2% of the wear time [56.5-90.6] vs 51.3% of wear tiime [41.7-66.8]) (figure 2). TDEE was highly correlated to activity-induced energy expenditure (r=0.80, P<0.001).

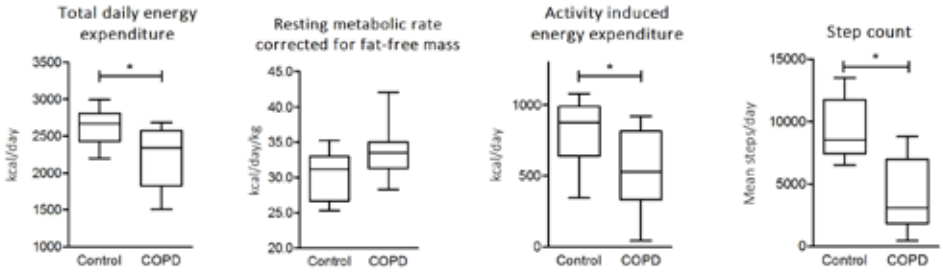

Figure 2. Components of energy balance of healthy controls (N=6) and COPD patients (N=20).

In COPD, the mean BMR of 1449 ± 214 kcal/day corresponded to 120% of predicted. 17 patients were characterized as hypermetabolic based on measured BMR >110% of predicted. No significant correlations could be found between adjusted BMR and FEV1 % predicted (r=-0.22, P=0.366) and between adjusted BMR and RV % predicted (r=0.04, P=0.888). Furthermore, adjusted BMR was not correlated to CRP (r=-0.18, p=0.621).

Hypermetabolism is not associated with BAT activation

Upon cooling in both COPD patients and controls, mean skin temperature decreased from 31.9 ± 2.3 to 27.6 ± 3.3 °C and supraclavicular skin temperature decreased from 35.3 ± 0.9 to 30.4 ± 5.2 °C. The cooling protocol increased energy expenditure (4.5 [3.1-5.0] versus 5.1 [4.1-7.0] kJ/min), resulting in an increment of average non-shivering thermogenesis of 20.1% (3.3-41.3). Blood pressure and heart rate increased upon cold exposure, while oxygen saturation remained unchanged. This increment in blood pressure and heart rate was not different between groups, indicating a comparable cold stress reaction.

Mean SUV of the region of BAT was comparable between COPD and controls (1.5 g/mL [0.1-6.2] versus 1.1 g/mL [0.7-3.9]). Furthermore, cold-induced [18F]FDG uptake in skeletal muscle, subcutaneous adipose tissue and visceral adipose tissue were neither different (figure 3). In addition, no correlations were found between FFM adjusted BMR and BAT activity (r=-0.06, p=0.881) and between non-shivering thermogenesis and SUV uptake in skeletal muscles (r=0.011, p=0.970). Additionally, no significant relation was found between non-shivering thermogenesis and BAT activity (r=-0.044, p=0.877).

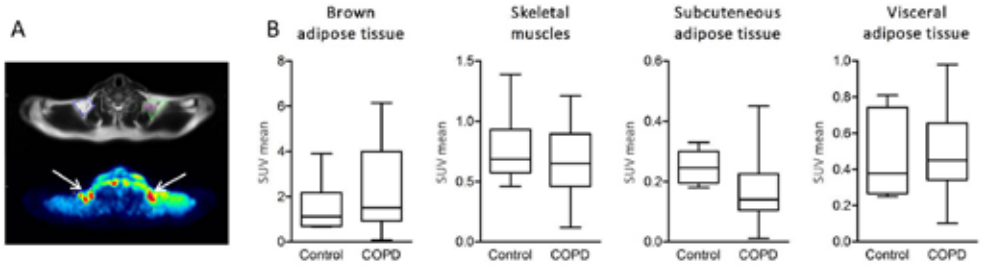

Figure 3. (a) area of brown adipose tissue on MRI and PET. (b) mean uptake of [18F]FDG in brown adipose tissue and other tissues during cold exposure. SUV, standardized uptake value.

Gene expression levels of BAT or beige markers in white fat are not upregulated

In subcutaneous adipose tissue UCP1 mRNA, which is considered as an important BAT and beige marker [37], was undetectable in most subjects. Furthermore, we did not detect differences in mRNA expression of the brown adipocyte marker Cidea or beige markers TMEM26 and CD137 between COPD patients and controls (figure 4).

Hypoxia marker GLUT-1 mRNA expression level was higher in COPD compared to controls and HIF1 alpha tended to be more expressed in COPD than in control subjects. Gene expression levels of markers of inflammation, macrophages, glycolysis, lipolysis and adipokines were not differentially expressed (supplemental table 1).

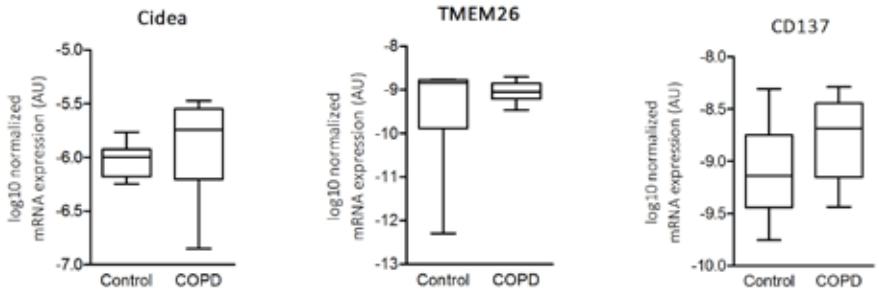

Figure 4. Adipose tissue gene expression levels of BAT and beige markers. AU, Arbitrary Units.

Role of BAT in cachectic cancer patients: a case report

A 61-year old male (BMI 23.9 kg/m^2) and a 70-year old female (BMI 23.8 kg/m^2) diagnosed with pancreatic cancer were both enrolled in one arm of the current study that was closed due to recruitment difficulties (see methods section for explanation). The male underwent a pancreaticoduodenectomy, and because of a relapse he started with chemotherapy which comprised oxaliplatin, folinezuur, irinotecan and fluorouracil. The female was diagnosed with pancreatic cancer with pulmonary and lymph node metastasis, and she was treated with gemcitabine. Both were cachectic at time of enrollement with a weight loss of more than 5% of body weight in the previous 6 months and both were hypermetabolic at rest (mean BMR was 120% of predicted). Despite a cold induced increased RMR from 4.5 to 5.3 kJ/min, no BAT activity was found. In line with the results from COPD patients and controls, no upregulation of BAT and beige markers were found in subcutaneous fat.

DISCUSSION

Activation of BAT or browning of WAT has been proposed as putative trigger for hypermetabolism and cachexia in wasting disorders including COPD. This is the first study that investigated the contribution of BAT on hypermetabolism in patients with emphysema in comparison to appropriately matched healthy controls. In contrast to our hypothesis, emphysematous patients did not exhibit enhanced BAT activity or altered gene expression of BAT markers in WAT compared to age-, gender- and BMI matched healthy non-smoking controls.

Consistent with previous publications [2, 9], we found that BMR was elevated among those with COPD. Although, upon cooling energy expenditure increased with an average non-shivering thermogenesis of 20.1 [3.3-41.3] %, no relation between BMR and BAT activity was found. This is in line with some [23, 38, 39], but not all [40, 41] previous studies using healthy young subjects. Absence of a relation between BMR and BAT activity and between non-shivering thermogenesis and BAT activity could be due to the low overall BAT activity in the current study population (also certainly related in part to the age). The SUV value of BAT was 1.5 [0.1-6.2], which was considerably lower than reported by others [18, 42, 43] who found SUV values in

the range of 2-18 among 20-30 year old healthy study subjects. The relatively low metabolic rate of BAT depots may not be entirely unexpected, as BAT is inversely correlated with age, and BAT is barely detected in healthy subjects above 60 years old [44]. Nevertheless, one autopsy study [45] and one retrospective imaging study [46] reported the presence of BAT activity among elderly with cancer, suggesting that independent of age, disease might be a trigger for BAT activity. However, in those studies [45, 46] no data are presented considering the stage of cancer. Furthermore, regarding the available imaging study, the results might be biased by the retrospective design, which did not allow to control environmental temperatures. In male patients, 2.8% of scans demonstrated BAT activity compared with 7.2% of scans in female patients [46].

Systemic inflammation has been proposed as another trigger for hypermetabolism and also as BAT activator in cancer cachexia [47, 48]. In the studied patients with clinically stable COPD, systemic inflammation assessed by CRP was low and not associated with BMR. Additionally, no altered gene expression of inflammation or macrophage markers was observed compared to healthy controls. In this study also two pancreatic cancer patients were enrolled. In line with the results in COPD patients, no BAT activity was found.

Interestingly, despite that no BAT activity was detected, the non-shivering thermogenesis was relatively high in the whole study cohort. Non-shivering thermogenesis reported in BAT negative healthy young subjects was on average 4% compared to 30% in BAT positive subjects [18]. Others reported average non-shivering thermogenesis of 12% in BAT positive subjects [41, 49, 50], which is remarkably lower compared to our results. No correlation between non-shivering thermogenesis and SUV of skeletal muscles was found, arguing that the elevated energy expenditure upon cold could not be attributed to involuntary muscle contractions. Furthermore, some studies indicate a role of insulin resistance that point towards either impaired glucose uptake by BAT [50] or reduced BAT activity [51]. However, the current cohort was not insulin resistant. Another explanation for the relatively high non-shivering thermogenesis might be that there is an effect of diet-induced energy expenditure. Three hours before cold-induced energy expenditure was assessed, participants had a light breakfast. In general, after three hours most of the thermic effect of a meal dissolved [52]. Although individual differences might exist, this cannot fully explain the high non-shivering thermogenesis.

This study is not without limitations. The major limitation in this study was the smaller number of enrolled subjects than intended for multiple group comparison (10 subjects per subgroup, in total 50 subjects). Accrual of COPD patients was hampered by the exclusion of subjects using medication that influences the sympathetic nerve system, including ß-blockers and calcium channel blockers. Partly due to shared risk factors such as smoking, cardiovascular diseases commonly coincide COPD [53]. Therefore, ß-blockers and calcium channel blockers are widely

used by COPD patients. Another factor contributing to low accrual was the use of MRI instead of computed tomography, since a significant part of the COPD patients who were approached to participate suffered from claustrophobia. This might not be totally unexpected because the patients already suffered from dyspnoea, which might be reinforced by MRI scanning. In addition, it appeared impossible to include lung cancer patients and only two pancreatic cancer patients were enrolled since the extensive study methodologies turned out to be too severe in this critically ill population. [54-56].

Nevertheless, the consistency of our findings in the hypermetabolic COPD patients and confirmed in the two cancer cases do not support a role for BAT activation as trigger of disease induced hypermetabolism and cachexia.

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SUPPLEMENTALS

Supplemental table 1. Blood analysis and qPRC analysis of subcutaneous fat biopsies of COPD patients (N=10) and healthy controls (N=6).

	COPD	Controls
Blood analysis		
C-reactive protein, mg/L	2.5 (2.0 to 21.0)	0.0 (0.0 to 3.0)
Fasting glucose, mmol/L	5.8 ± 1.0	4.9 ± 0.5
Insulin, pmol/L	27.2 (12.0 to 86.6)	13.0 (12.0 to 19.0)
Thyroid stimulating hormone, mU/L	1.8 (1.0 to 7.2)	1.8 (1.5 to 2.8)
Free T4, pmol/L	14.6 (1.7 to 20.5)	13.6 (12.3 to 18.1)
Free fatty acids, mmol/L	0.7 (0.4 to 1.8)	0.6 (0.3 to 0.9)
Cholesterol, mmol/L	4.8 ± 0.7	4.5 ± 0.7
High-density lipoprotein, mmol/L	1.6 ± 0.3	1.7 ± 0.2
Low-density lipoprotein, mmol/L	2.8 ± 0.8	2.4 ± 0.7
Triglycerides, mmol/L	1.0 ± 0.3	0.9 ± 0.3
qPRC subcutaneous fat biopsies		
BAT and beige markers, AU		
Cidea	-6.14 (-6.85 to -5.47)	-6.00 (-6.25 to -5.77)
TMEM26	-8.99 (-9.47 to -8.82)	-8.84 (-12.29 to -8.76)
CD137	-8.60 (-9.44 to -8.40)	-9.14 (-9.75 to -8.31)
Hypoxia markers, AU		
GLUT1	-7.63 (-7.86 to -7.45)	-7.51 (-7.86 to -7.45)
HIF1a	-7.13 (-7.25 to -7.01)	-7.19 (-7.37 to -7.12)
VEGF-a	-7.62 (-7.66 to -7.53)	-7.61 (-7.89 to -7.15)
Inflammation and macrophages markers, AU		
IL-8		
MCP-1	-7.79 (-8.61 to -7.25)	-8.54 (-9.59 to -7.48)
CD68	-7.35 (-7.75 to -6.98)	-7.62 (-7.75 to -7.22)
CD163	-6.75 (-7.26 to -6.54)	-6.87 (-7.09 to -6.29)
CD206	-6.74 (-7.26 to -6.48)	-7.00 (-7.25 to -6.39)
CD11b	-7.11 (-7.59 to -6.81)	-7.42 (-7.65 to -6.88)
	-7.98 (-8.80 to -7.81)	-8.34 (-8.80 to -7.59)
Glycolysis and lipolysis markers, AU		
HKII	-6.22 (-6.41 to -6.03)	-6.26 (-6.51 to -6.11)
CGI58	-6.38 (-6.52 to -6.18)	-6.32 (-6.50 to -6.16)
ATGL	-5.90 (-6.12 to -5.81)	-5.95 (-6.22 to -5.67)
HSL	-6.13 (-6.20 to -5.85)	-6.10 (-6.24 to -5.91)
Perilipin1	-6.05 (-6.12 to -5.76)	-6.04 (-6.21 to -5.77)

Supplemental table 1 Continues.

	COPD	Controls
qPRC subcutaneous fat biopsies		
Adipokine markers, AU		
PAI-1	-8.25 (-8.45 to -7.91)	-8.31 (-8.59 to -7.89)
Adrenomedullin	-7.29 (-7.52 to -6.88)	-7.43 (-7.80 to -7.13)
Visfatin	-6.41 (-6.87 to -6.15)	-6.71 (-6.86 to -6.06)
Osteoprotegrin	-7.92 (-8.03 to -7.54)	-8.05 (-8.14 to -7.89)
Chemerin	-5.84 (-5.99 to -5.67)	-5.89 (-6.14 to -5.70)
Adiponectin	-5.57 (-5.80 to -5.33)	-5.49 (-5.81 to -5.28)
Leptin	-5.82 (-6.14 to -5.55)	-6.16 (-6.26 to -5.70)
Irisin	-8.48 (-8.54 to -8.18)	-8.30 (-8.70 to -8.17)

qPCR data are visualised by log 10 normalized mRNA expression in arbitrary units.
AU, arbitrary units.
Data are represented as mean ± SD or median (range).

8

Early weight loss during chemoradiotherapy has detrimental impact on outcome in non-small cell lung cancer

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ABSTRACT

The aim of this study was to assess the effect of early weight loss before the onset of radiation esophagitis on overall survival (OS) in non-small cell lung cancer (NSCLC) patients treated with concurrent chemoradiotherapy.

Characteristics (e.g., patient weight, radiation esophagitis score, gender, World Health Organisation performance status, chemotherapy dose, nodal status, and gross tumour volume) of 151 patients who received concurrent chemoradiotherapy (in 2006-2013) were retrospectively correlated with OS. Early weight loss was defined as weight loss of more than 5% between the start and third week of radiotherapy in patients whose weight was stable before treatment initiation.

In 17% of the patients early weight loss was observed. Median OS (95% confidence interval [CI]) was significantly shorter in the early weight loss group (OS = 13.0 months, 95% CI: 2.0-24.0) versus in the non-early weight loss group (OS = 23.0 months, 95% CI: 14.7-31.3) (hazard ratio [HR] = 1.8, 95% CI: 1.12-2.96, p = 0.017). On multivariate analysis gender (HR = 2.1, 95% CI 1.33-3.29, p = 0.001), World Health Organization performance status (HR = 1.9, 95% CI 1.20-2.97, p = 0.006), nodal status (HR = 2.9, 95% CI 1.38-6.01, p = 0.005) and early weight loss (HR = 1.9, 95% CI 1.10-3.19, p = 0.022) were associated with OS.

Early weight loss in patients with non-small cell lung cancer was found to be associated with worse prognosis. These data warrant further investigation into the efficacy of tailored intervention to prevent early weight loss.

INTRODUCTION

Attention towards management of involuntary weight loss in patients with non-small cell lung cancer (NSCLC) is increasing [1]. Although unintended weight loss was initially considered to be an indicator of terminal progression of the disease, there is now convincing evidence that it is an independent determinant of survival [2]. Besides their implications for survival, weight loss and muscle wasting have a detrimental impact on response to chemotherapy and quality of life [3]. Not only tumour-related factors but also treatment-related factors can contribute to weight loss and muscle wasting [4].

Because of improved survival rates in comparison with sequential chemoradiotherapy, concurrent chemoradiotherapy (CCRT) has become the standard treatment for patients with unresectable locally advanced NSCLC [5]. However, compared with sequential chemoradiotherapy, CCRT has more serious side effects including oesophageal toxicity [6]. Grade 3 to 4 dysphagia due to acute radiation esophagitis, which impairs spontaneous dietary intake resulting in weight loss, will develop in approximately 20% of patients [3, 7]. Symptoms of radiation esophagitis gradually start at the end of week 4 of CCRT and commonly dissipate 1 to 3 weeks after completion of therapy [7]. Nevertheless, our group has previously shown that weight loss may already occur in the first 3 weeks of CCRT, before onset of clinically relevant radiation esophagitis and irrespective of decreased dietary intake [8]. Furthermore, this early weight loss was accompanied by a rapid decline of muscle strength in the lower limbs. Taken together, the presence of weight loss and muscle weakness is indicative of early metabolic alterations that may warrant tailored nutritional or multimodal intervention.

The objective of the current study was to evaluate the impact of early weight loss (>5% between the start and third week of radiotherapy [RT]) on overall survival (OS) in patients with stable weight before initiation of treatment.

METHODS

Patient selection

Medical records of all consecutive patients with advanced stage NSCLC (stage IIIA, IIIB and oligometastatic stage IV) who were undergoing a radical course of RT in a single institute but receiving concurrent chemotherapy in one of two neighbouring Dutch teaching hospitals between January 2006 and December 2013 were retrieved and retrospectively analysed. For staging purposes all patients received a diagnostic 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan combined with a computed tomography (CT) scan and magnetic resonance imaging or CT of the brain. Patients were excluded from this analysis if they had a neuroendocrine carcinoma, another malignancy within 5 years of diagnosis, pre-existing swallowing difficulties, weight loss of more than 5% preceding lung cancer treatment, weight loss before diagnosis, unknown and/or no weight recorded at the start of RT or week 3 of RT, or

an uncompleted course of CCRT. This retrospective study was approved by the medical ethical committee of the Maastricht University Medical Centre+ and the Institutional Review Board of the Department of Radiation Oncology (MAASTRO clinic, the Netherlands).

Treatment

Patients were treated with CCRT. Chemotherapy was administered according to national and international guidelines (chemotherapy treatment scheme displayed in table 1). Generally, patients received one or more cycles of induction therapy followed by CCRT. The concurrent chemotherapy regimen in most cases consisted of platinum-based chemotherapy combined with etoposide. Other regimens used included cisplatin-vinorelbine, cisplatin-vinorelbine-cetuximab [9] and carboplatin-vinorelbine.

Two different RT schedules were used. The first consisted of individualized hyperfractionated accelerated external beam RT [10]. The schedule consisted of a maximum total dose of 69 Gy, first delivered in fractions of 1.5 Gy twice-daily up to 45 Gy, followed by an individualized dose of 8 to 24 Gy delivered in once-daily 2.0 Gy fractions, limited by the dose to surrounding organs at risk (e.g., mean lung dose and maximum dose to the spinal cord). The second was the delivery of RT within the PET-boost trial, in which the radiation dose to the entire tumour volume or on FDG-avid subvolumes is escalated until normal tissue dose constraints have been reached (see van Elmpt et al. [11] for details). RT planning was performed on the XiO system (Elekta Instrument AB, Stockholm, Sweden) until July 2012, using a convolution–superposition algorithm with inhomogeneity corrections and according to International Commission on Radiation Units & Measurements 50 guidelines. As of July 2012, radiation treatment was planned using RapidArc (Eclipse version 11.0, Varian Medical Systems, Palo Alto, CA), with a type B dose calculation algorithm (AcurosXB-10.0 [Varian Medical Systems]).

Data collection

All medical records were retrospectively reviewed. Data collected included patient demographics (age and gender); World Health Organization performance status (WHO PS); medical history; Charlson comorbidity score; clinical tumour stage and clinical nodal stage (cN); tumour, node, and metastasis (TNM) disease stage (according to TNM6 until 2009 or TNM7 from 2010 onward); weight loss before initiation of CCRT; premorbid weight, height and body mass index; weight weekly assessed from start of CCRT until 4 weeks after completion of CCRT; chemotherapy used (type and dose reductions); hospitalization and episodes of febrile neutropenia.

From the treatment planning system, the gross tumour volume (GTV) encompassing the primary tumour and the metastastic hilar/mediastinal lymph nodes (confirmed by histopathology after endobronchial/endoesophageal ultrasonography, enlarged or presence of malignant features on CT scan, and/or FDG-PET positivity) was retrieved. Additionally, RT parameters

were extracted, including total RT dose and mean oesophagus dose.

Patients were monitored weekly by a dietician. During and after completion of treatment, patients were followed up weekly to evaluate weight and onset/resolution of acute radiation esophagitis. Radiation esophagitis was evaluated according to the Common Toxicity Criteria version 3.0 grading scale. Local follow-up policy to evaluate tumour response included a CT scan 3 months after completion of RT, followed by three monthly chest X-ray and annual CT scan.

The primary objective of this study was to assess the impact of early weight loss on OS. Early weight loss was defined as weight loss of more than 5% between the start and third week of RT. A cut-off limit of 5% was chosen according to the international consensus of cancer cachexia [12]. The secondary objective included the determination of the contribution of gender, age, WHO PS, tumour- and nodal status, histological diagnosis, and early weight loss to OS. In addition, the relation between aforementioned parameters and progression-free survival (PFS) was evaluated.

Statistical analysis

All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, Version 22.0, IBM, Armonk, NY). Descriptive statistics of demographic and clinical variables were obtained. Means (± SD) were provided for continuous variables and percentages were shown for categorical variables. Continuous variables were compared using Student's t-test for independent samples, categorical variables were compared using chi-square test, and non-parametric variables were tested using Mann-Whitney U test. OS was calculated from day of pathologically proven diagnosis of NSCLC until death. PFS was defined as day of pathologically proven diagnosis of NSCLC until first documented disease progression assessed on a CT scan or death. For survival analysis, patients lost to follow-up were censored at the time of their last visit or observation. The probabilities of both OS and PFS were calculated using the Kaplan-Meier method. Survival curves of patients with and without early weight loss were tested for significance using the log-rank test. To assess the contribution of early weight loss to OS, multivariate Cox regression analysis was performed with several clinical parameters, including gender, age, WHO PS, tumour and nodal status, histological diagnosis, and early weight loss as independent variables. Proportional hazard assumption was tested using visual inspection of log-minus-log survival plots. Results with two-sided exact p values (<0.05) were considered statistically significant.

RESULTS

Patient and treatment characteristics

A total of 287 patients with NSCLC were identified during the study period. One hundred thirty-six patients were excluded because of histological diagnosis (5), another malignancy within

5 years of the diagnosis of NSCLC (15), pre-existing swallowing difficulties (11), weight loss of more than 5% before treatment (58), missing weight records before treatment (9), start of RT (9), or week 3 of RT (15), and no completion of CCRT (14). As a result, 151 patients were eligible for analysis (figure 1).

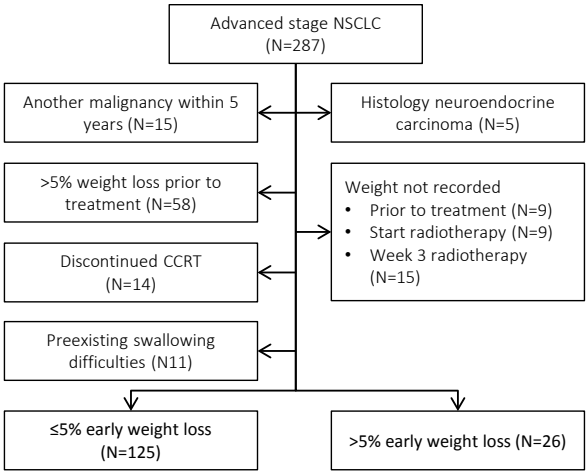

Figure 1. Flow chart study population.

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Baseline patient and treatment characteristics are described in table 1. The median age was 63 years and 42% of the participants were female. Seventy-five patients with stage IIIA disease, 70 patients with stage IIIB disease, and six patients with oligometastatic stage IV disease were included. Twenty-six patients (17%) experienced weight loss of more than 5% during the first three weeks of RT. Mean (± SD) weight loss in the non-early weight loss group and early weight loss group were -0.5 ± 2.3 kg and -5.4 ± 1.6 kg, respectively. The course of mean weight loss during therapy is displayed in supplemental data.

Table 1. Comparison of chemotherapy regimen between patients without early weight loss and with early weight loss.

	≤5% early weight loss (N=125)	>5% early weight loss (N=26)
Number induction chemotherapy cycles 0 / 1 / 2 / 3	11.2 / 72.0 / 11.2 / 2.4	3.8 / 76.9 / 7.7 / 11.5
Platinum-based induction chemotherapy Cisplatin / carboplatin	52.8 / 33.6	61.5 / 34.6
Type induction chemotherapy Etoposide / gemcitabine / paclitaxel / vinorelbine	35.2 / 40.8 / 1.6 / 0.8	42.3 / 42.3 / 7.7 / 3.8
No. of concurrent chemotherapy cycles 2 / 3	86.4 / 12.0	92.3 / 3.8
Platinum-based concurrent chemotherapy Cisplatin / carboplatin	81.6 / 16.8	84.6 / 11.5

Table 1. Continued.

Type concurrent chemotherapy Etoposide / vinorelbine / vinorelbine-cetuximab	68.0 / 24.0 / 6.4	61.5 / 26.9 / 3.8

Data are presented as %.

Patients stratified by early weight loss were comparable with regard to age at diagnosis, WHO PS, and number and severity of comorbidities. There was a trend towards higher nodal staging and GTV in the patients with early weight loss; however this was not statistically significant and the GTV had a large and overlapping range. Furthermore, treatment regimens were comparable with respect to RT (table 2).

Table 2. Comparison of clinical and treatment characteristics between patients without early weight loss and with early weight loss.

	≤5% early weight loss (N=125)	>5% early weight loss (N=26)	P value
Age, years	63 ± 9.5	61 ± 9.9	0.276
Male/female, %	56.0/44.0	69.2/30.8	0.276
Weight change, kg	-0.5 ± 2.3	-5.4 ± 1.6	0.000
Weight change prior to treatment initiation, kg	-0.58 ± 1.2	-0.92 ± 1.5	0.287
Weight change during induction chemotherapy, kg	-0.27 ± 2.6	0.76 ± 3.1	0.077
WHO performance status 0 / 1, 2, %	37.6 / 62.4	50.0 / 50.0	0.274
Charlson comorbidity index	5 ± 1.5	4 ± 1.8	0.208
Disease stage, % IIIA / IIIB / IV	52.8 / 43.2 / 4.0	34.6 / 61.5 / 3.9	0.213
Clinical tumour stage, % Tx, T1, T2 T3, T4	 50.8 49.2	 34.6 65.4	 0.195
Clinical nodal status, % N0, N1 N2, N3	 17.5 82.5	 3.8 96.2	 0.129
Histology, % Squamous Adenocarcinoma Large cell carcinoma NSCLC NOS	 24.6 42.1 12.5 20.8	 42.3 23.1 15.4 19.2	 0.092
Total dose radiotherapy (Gy)*	65.5 (60.1- 69.0)	69.0 (65.0- 69.0)	0.132
Gross tumour volume*	79.9 (51.2- 158.2)	181.7 (43.5- 280.8)	0.248
Mean oesophagus dose (Gy)*	20.0 (14.0- 26.3)	18.2 (16.5- 29.2)	0.477

Data are presented as mean ± SD or %, unless indicated otherwise. *Median (interquartile range).
Abbreviations: NSCLC NOS, non-small cell lung cancer not otherwise specified.

Adverse events

No statistically significant differences were found between the groups with respect to adverse events (table 3). Percentage of patients receiving chemotherapy dose reductions was not statistical significantly different between the early weight loss and non-early weight loss group. Additionally, no significant differences were found for hospitalization rates, neutropenic fever episodes, and incidence of esophagitis.

Table 3. Comparison of adverse events between patients without early weight loss and with early weight loss.

	≤5% early weight loss (N=125)	>5% early weight loss (N=26)	P value
Chemotherapy dose reduction	11.2	19.2	0.339
Hospitalization	55.2	73.1	0.122
Neutropenic fever	18.4	34.6	0.112
Esophagitis week 3 radiotherapy grade ≥2	32.5	32.0	1.000

Data are presented as %.

Survival

Median follow-up was 40.0 months (range 28.4 to 51.7), and at the time of analysis 86 patients (56.6%) had died. Median OS (95% confidence interval [CI]) was significantly shorter in the early weight loss group compared with the non-early weight loss group (OS = 13.0 months, 95% CI: 2.0-24.0 versus OS = 23.0 months, 95% CI: 14.7-31.3) (hazard ratio [HR] = 1.8, 95% CI: 1.12-2.96, p = 0.017; [figure 2a]). Although not statistically significant, a trend was observed regarding the influence of early weight loss on PFS. The early weight loss group has a shorter PFS compared with the non-early weight loss group, with a median PFS of 8.0 months (95% CI: 5.5-10.5) versus 15.0 months (95% CI: 9.5-20.5) (HR = 1.5, 95% CI: 0.95-2.51, p = 0.08 [figure 2b]).

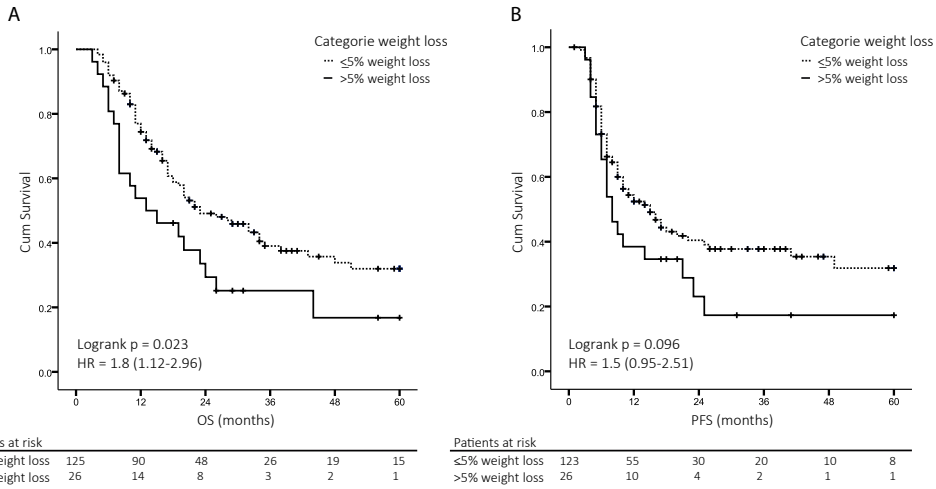

Figure 2. Survival analyses. (a) Kaplan Meier OS curve; (b) Kaplan Meier PFS curve of patients experiencing >5% early weight loss (solid line) compared to patients with ≤5% early weight loss (dotted line).

On multivariate analysis gender (HR = 2.1, 95% CI: 1.33-3.29, p = 0.001), WHO PS (HR = 1.9, 95% CI: 1.20-2.97; p = 0.006), nodal status (HR = 2.9, 95% CI: 1.38-6.01, p = 0.005) and early weight loss (HR = 1.9, 95% CI: 1.10-3.19, p = 0.022) were associated with OS (table 4). Gender (HR = 2.4, 95% CI: 1.48-3.79, p < 0.001), WHO PS (HR = 1.9, 95% CI: 1.18-2.98, p = 0.008), nodal status (HR = 2.4, 95% CI: 1.16-5.14, p = 0.019), histology (HR = 2.4, 95% CI: 1.33-4.17, p = 0.003) and early weight loss (HR = 1.8, 95% CI: 1.01-3.05, p = 0.046) were associated with PFS (data not shown).

Table 4. Multivariate analyses for predictors of overall survival.

Variable	HR (95% CI)	P value
Gender female vs male	2.092 (1.329-3.293)	0.001
Age at diagnosis <65 vs ≥65 years	1.353 (0.889-2.061)	0.159
WHO performance status 0 vs 1-2	1.888 (1.201-2.967)	0.006
Clinical tumour status cTx, cT1, cT2 vs cT3, cT4	1.304 (0.839-2.027)	0.238
Clinical nodal status cN0, cN1 vs cN2, cN3	2.883 (1.382-6.015)	0.005
Histology non-squamous vs squamous	1.635 (0.950-2.814)	0.076
Weight stable vs early weight loss	1.868 (1.095-3.187)	0.022

DISCUSSION

Despite weekly advice from a dietician, in our retrospective analysis 17% of patients who were weight stable before curative intent CCRT experienced early weight loss and this adversely affected overall survival (HR = 1.9, 95% CI: 1.10-3.19, p = 0.022). Furthermore, early weight loss could not be attributed to a higher incidence of radiation esophagitis, restricted chemotherapy dose, or differences in GTVs. Therefore, early weight loss may provide predictive information additional to known prognostic factors, such as TNM staging, WHO PS and comorbidities. Attention towards early weight loss despite stable nutritional intake is therefore mandatory.

Weight loss is a frequent problem in lung cancer, as up to 54% of patients with NSCLC report weight loss before diagnosis, including weight loss of 10% or more in up to 30% of patients with lung cancer [2, 13]. Previously, patients with stage IIIB NSCLC with and without weight loss during treatment were compared, and it was reported that the latter group had a statistically significantly superior OS and PFS [14]. However, in that study weight loss was defined as a reduction body mass index of more than 0.5 kg/m^2 at the end of CCRT. Furthermore, among weight-losing patients, the need for reduction of chemotherapy dose or abandonment of chemotherapy as well as the incidence of grade 3 or 4 adverse events were significantly higher. At present, there are no published data on the effects of weight loss occurring before the onset of radiation esophagitis on OS and PFS. Therefore, it is unclear whether the weight loss by itself or the adverse events and/or restricted chemotherapy/RT dose was cause of the worse prognosis. It is of note that weight loss is associated with delivery of fewer chemotherapy

cycles and increased treatment delay [15]. It is therefore unknown from the previous literature whether weight loss is a causal factor of decreased survival or merely a symptom that occurs during disease progression. This is the first study showing the impact of early weight loss on outcome in previously weight stable patients, independent of treatment characteristics, treatment side effects and GTV.

Our observations regarding early weight loss are in line with body weight changes during CCRT in other malignancies. Weight loss has been observed from initiation of CCRT until several weeks thereafter in patients treated for head and neck cancer [16]. Recently however, Ghadjar et al. [17] found no association between weight loss during treatment and survival outcomes in head and neck cancer patients. Nonetheless, weight loss in this tumour type is often attributed to tumour- and treatment-related swallowing difficulties. Notably, however, the authors only recorded weight at week 1 and week 5 of RT and no dysphagia data were presented. Furthermore, a trend towards more advanced disease and higher WHO PS in the weight-losing group may obscure its impact on survival.

Although it did not reach statistical significance in univariate analysis, we observed a trend concerning the impact of early weight loss on PFS. This is in contrast with Topkan et al. [14] reporting a clear statistically significant difference in median PFS of 4.6 months in patients with stage IIIB NSCLC. However we recognize that (both in the study of Topkan et al. and in the current study), follow-up was not standardised and the time-line of follow-up was – owing to the retrospective analysis of the data - not uniformly defined. This limits us to draw hard conclusions about the impact of early weight loss on PFS.

From RT studies it is known that a large GTV, representing the tumour load, is associated with worse survival rates [18,19]. In our study, GTVs in the early weight loss group were larger even though not statistically significant, but no effect of the GTV on OS or PFS was found. Additionally, cN2-cN3 involvement tended to be somewhat higher among the weight loss group, again not statistically significant. This may indicate that in part the lower survival rates seen in the early weight loss group may be attributed to more advanced disease. Nevertheless, early weight loss adjusted for disease stage was found to have a statistically significant detrimental impact on OS in multivariate analysis.

Although the strength of our study is the well-defined patient cohort, there are also some limitations. The day of pathological proven diagnosis of NSCLC was used as starting point of the current study. However, we recognize that the time-line of diagnosis until start of the treatment may be different between subjects. Weight measurement was not standardized, with respect to the use of different scales and different time points of measurement during the day. We tried to overcome these differences by define early weight loss as weight loss of

more than 5%, which is considered to be beyond the range explained by the aforementioned factors. Nevertheless, patients were closely monitored by a dietician, who was able to start nutritional intervention, and therefore attention was paid to weight change. The nature of weight loss is unclear because there are no data available about body composition changes and putative catabolic stimuli like systemic inflammation. It is well established that enhanced levels of inflammatory mediators associate with weight loss [4, 20].

In conclusion, the present study provides evidence for the negative impact of early weight loss on outcome in patients with NSCLC who are receiving curative intent CCRT. Lower survival rates were demonstrated for patients experiencing weight loss of more than 5% during the first 3 weeks of treatment. Given the anabolic potential of dietary protein, we suggest aggressive supplementation of proteins initiated from start of CCRT, in combination with a tailored exercise regime to combat loss of muscle mass and strength. The feasibility and effectiveness of early intervention to prevent or postpone CCRT-induced weight loss needs to be explored in a prospective setting.

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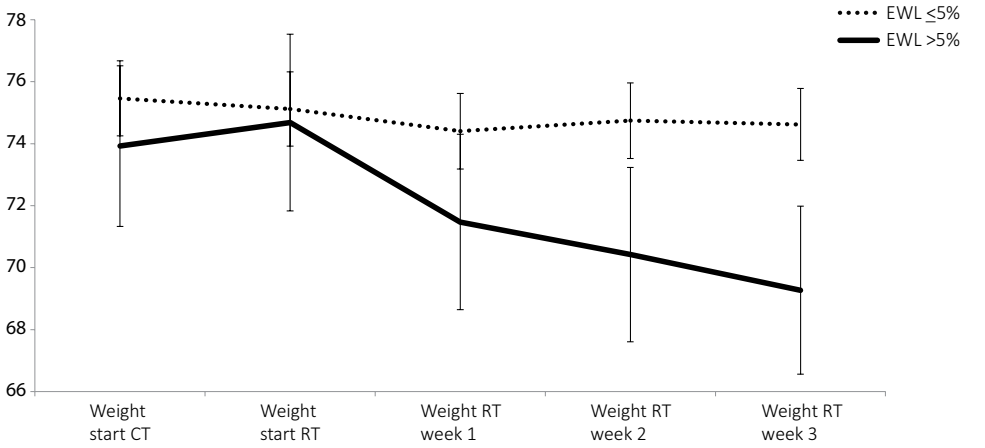
SUPPLEMENTALS



Supplemental figure 1. The course of mean (± standard error of the mean) weight loss during therapy of patients experiencing >5% early weight loss (solid line) compared to patients with ≤5% early weight loss (dotted line). Abbreviations: CT, chemotherapy; RT, radiotherapy.

9

The prognostic value of early onset CT derived loss of muscle and adipose tissue during chemotherapy in metastatic non-small cell lung cancer

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ABSTRACT

Objectives

To evaluate the relationship between early changes in muscle and adipose tissue during chemotherapy and overall survival in stage IV non-small cell lung cancer (NSCLC).

Methods

In this post-hoc analysis of the first line NVALT12 trial (NCT01171170) in stage IV NSCLC, skeletal muscle, radiation attenuation, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were assessed at the third lumbar level on computed tomography scans obtained before initiation of chemotherapy and shortly after administration of the second cycle. The contribution of changes in different body compartments to overall survival was assessed.

Results

Computed tomography scans of 111 patients were included. Analysis of body composition changes between the baseline and the follow-up scan, revealed that overall skeletal muscle cross sectional area (CSA), radiation attenuation and SAT CSA decreased respectively by -1.2 ± 2.9 cm^2/m^2 ($p<0.001$), -0.7 ± 3.3 HU ($p = 0.026$) and -1.9 ± 8.7 cm^2/m^2 ($p = 0.026$), while no significant changes in VAT tissue were observed. Longitudinally, median overall survival was significantly shorter among patients losing skeletal muscle compared to patients with preserved skeletal muscle (9.4 versus 14.2 months; hazard ratio [HR] = 1.9, 95% confidence interval [CI]: 1.23-2.79, p=0.003). Multivariate analyses showed that proportional loss of muscle mass was associated with poor overall survival (HR = 0.949, 95% CI: 0.915-0.985, p=0.006) independent from important clinical prognostic factors including gender, age, World Health Organisation performance status and Charlson comorbidity index.

Conclusion

Early loss of skeletal muscle during first line chemotherapy is a poor prognostic factor in stage IV NSCLC patients. Future studies have to reveal whether early supportive intervention guided by initial computed tomography muscle response to chemotherapy can influence the wasting process and related mortality risk.

INTRODUCTION

Despite recent developments in cancer diagnostics and treatment modalities, mortality rates maintain high in non-small cell lung cancer (NSCLC) [1]. One of the factors contributing to high mortality rates is progressive unintentional weight loss of body weight and muscle mass (i.e. cachexia) [2].

Computed tomography (CT) has emerged as a promising tool in assessment of cancer cachexia. CT scans are routinely acquired in cancer patients for disease diagnosis, staging and treatment follow-up and thereby readily available from medical records to extract body composition data. Skeletal muscle- and adipose tissue depletion, both characteristics of cachexia [3], are clinically important as predictors of cancer outcomes [4-6]. Cachexia is frequently observed in lung cancer [7] and low skeletal muscle mass at presentation has been linked to functional deterioration, chemotherapy intolerance, and poor survival rates [8-10].

Muscle tissue is plastic, undergoing constant remodelling in response to anabolic and catabolic signals related to aging [11], exercise [12], malnutrition [13] inflammation [14] and drug interventions [15]. Evidence from experimental research suggests that chemotherapy might contribute to muscle wasting, via activation of nuclear factor kappa B and upregulation of myostatin [16]. Thereby, even weight stable patients with normal muscle mass before therapy initiation might lose muscle mass during the course of treatment [17].

To date, a few longitudinal studies addressed changes in muscle mass during anti-tumour therapy in NSCLC. In 35 stage IV NSCLC patients treated with palliative chemotherapy, muscle mass loss was observed in half of the study population over the duration of chemotherapy (approximately 9 weeks). However, the authors found no significant survival effect in univariate analysis [18]. Another small study reported muscle depletion in 30 stage III and IV NSCLC patients, which was accompanied by physical functional decline. The prognostic effect of muscle depletion was not examined [19].

Next to skeletal muscle depletion, cachexia might also effect other tissues, including adipose tissue. Although adipose tissue mass has been emerging as a prognostic factor, results to date are confusing. Patients with low baseline subcutaneous adipose tissue in gastrointestinal- and lung cancer [20] or low visceral adipose tissue in renal cell carcinoma exhibited lower survival rates [21]. In contrast, high adipose infiltration in the skeletal muscle as a measure of muscle quality, reflected on CT analysis by low skeletal muscle radiodensity, was associated with shorter overall survival and shorter disease free survival in patients with various other cancer types [5,22-25].

The prognostic value of early onset changes in muscle- and adipose tissue compartments in therapy naive NSCLC patients during chemotherapy is lacking. Particularly, in this patient population known for poor survival rates, timely information on body compartment changes could be of clinical relevance for patient guidance and treatment decision making. Therefore, the primary goal of our study is to evaluate early body composition changes in relation to survival in therapy naive metastatic NSCLC patients undergoing chemotherapy.

METHODS

Study population and study design

This study was a post-hoc analysis of a multicentre randomized phase II trial investigating the effect on survival of nitroglycerin patches added to paclitaxel-carboplatin-bevacizumab in 223 therapy naive patients with stage IV non-squamous NSCLC (NCT01171170). The full and detailed methodology and results of this study have been published previously [26].

In short, patients were randomized between paclitaxel-carboplatin-bevacizumab with (experimental arm) and without nitroglycerin patches (control arm). Adding nitroglycerin to first-line carboplatin-paclitaxel-bevacizumab did not improve progression-free survival and overall survival (OS) in patients with stage IV non-squamous NSCLC. Exploratory endpoint of the study was to assess whether nitroglycerin was related with an early decrease in 18F-fluorodeoxyglucose (18F-FDG) uptake. According to the protocol, a second 18F-FDG positron emission tomography (PET) scan combined with CT scan was assessed for patients with a baseline PET/CT after the second cycle of chemotherapy [27], with median follow-up time of 23 (17-50) days between follow-up scan and initiation of therapy. Specific patient characteristics (age, gender, World Health Organisation performance status [WHO PS], smoking status, histology, body mass index [BMI] and Charlson comorbidity index) were selected from patient records [28]. Patients were selected from this study because the population is a well-defined randomized cohort in which all patients have received a standardized chemotherapeutic regime. Next to this, all patients were assessed by a physician with follow-up scans at pre-determined time points.

Image analysis

In this post-hoc analysis body composition was analysed on low dose CT scan by assessment of the cross-sectional area (CSA) at the third lumbar level by two assessors. CSA of skeletal muscle, subcutaneous adipose tissue and visceral adipose tissue were analysed with Slice-O-Matic software v5.0 (Tomovision, Montreal, Canada). One image in each scan was selected. During anatomical land marking, the first image at the third lumbar level with both vertebral transverse processes clearly visible was used for analysis. CSA of these structures were quantified based on pre-established thresholds of Hounsfield units (skeletal muscle -29 to 150, subcutaneous adipose tissue -190 to -30, and visceral adipose tissue -150 to -50). Boundaries were corrected manually when necessary.

Baseline CSA was normalised for height in meters squared and reported in cm^2/m^2. Changes in CSA between CT scans were expressed as a percentage. We found a mean coefficient of variation between observers of 1.3% for skeletal muscle area, subcutaneous- and visceral adipose tissue in a random sample of 15 patients, which is in line with a variation of 0-2% in other studies [7,29-31]. Therefore, a measurement error of 1.3% was adopted. Changes of equal or larger than -1.3% were considered as 'loss of tissue', while changes of smaller than -1.3% were considered 'maintenance of tissue'. Furthermore, skeletal muscle radiation attenuation was assessed as the average Hounsfield units of the total skeletal muscle area within the range -29 to 150 (i.e. excluding intramuscular adipose tissue).

Study endpoints and statistical analyses

Patients were included if CT scans both at baseline and follow-up were available, were assessed within 3 weeks after the second chemotherapy and contained images of the third lumbar level. Descriptive statistics of demographic and clinical variables were obtained. Means (±SD) were provided for continuous normally distributed variables, median (range) for continuous not-normally distributed variables and percentages were shown for categorical variables. Comparisons within groups were performed with paired t-test and between groups with an independent t-test.

The primary endpoint of this study was OS. OS was defined as the interval from randomization to death from any cause. The probabilities of OS were calculated using the Kaplan-Meier method. Survival curves of patients with and without skeletal muscle loss were tested for significance using the log-rank test. To assess the contribution of different body compartments to OS, multivariate Cox regression analysis was performed with body composition changes, gender, age, Charlson comorbidity index, and BMI as independent variables. Variables were tested for interactions. Proportional hazard assumption was tested using visual inspection of log-minus-log survival plots. All analyses were performed using SPSS statistical software (SPSS Statistics for Windows, Version 24.0, IBM, Armonk, NY). Results with two-sided exact p values (<0.05) were considered statistically significant.

RESULTS

Patients and characteristics

CT scans from 111 of the 223 enrolled patients were eligible (figure 1). Baseline patient characteristics are shown in table 1.

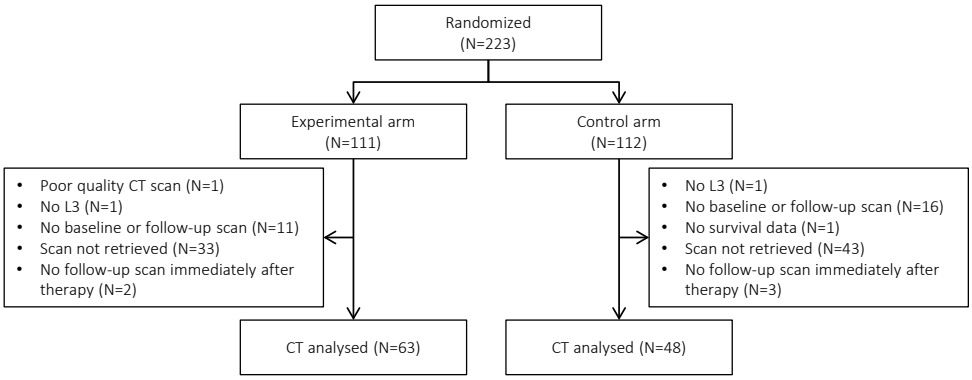

Figure 1. Flow chart study population.

Table 1. Patient characteristics (N=111).

Experimental arm/control arm, N	63 / 48
Male/female, N	60 / 51
Age, mean (range)	61 (39- 79)
WHO performance status, N (%) 0 / 1 / 2	 50 (45.0) / 46 (41.1) / 3 (2.7)
Comorbidity, N (%) COPD Cardiovascular disease Diabetes mellitus Gastrointestinal disease >1 comorbidity	 16 (14.4) 41 (36.9) 12 (10.8) 15 (13.5) 50 (45%)
Smoker, N (%) Current / ex / never	 48 (43.2) / 49 (44.1) / 14 (12.6)
Histology, N (%) Adenocarcinoma Large cell Other	 96 (86.5) 6 (5.4) 7 (6.4)
Body weight, kg	74.1 ± 14.7
BMI, kg/m^2	25.0 ± 4.3

WHO performance status missing N=12, histology missing N=2.

Table 2 shows baseline measurements and body composition changes parameters of the whole group. In total 65 (58.5%) lost muscle mass during the course of chemotherapy. Analysis of body composition changes between baseline and follow-up scan revealed that muscle CSA, radiation attenuation and subcutaneous adipose tissue CSA decreased respectively by -1.2 ± 2.9 cm^2/m^2 ($p<0.001$), -0.7 ± 3.3 HU ($p = 0.026$) and -1.9 ± 8.7 cm^2/m^2 ($p = 0.026$), while no significant changes in visceral adipose tissue were observed.

Table 2. Body composition at baseline and change during therapy.

	Baseline	Change, absolute	Change, percentage	p value
Skeletal muscle CSA, cm^2/m^2	44.4 ±6.9	-1.2 ± 2.9	-2.7 ± 6.6	<0.001
Radiation attenuation (housefield units)	31.5 ± 7.2	-0.7 ± 3.3	-1.9 ± 12.2	0.026
Subcutaneous adipose tissue CSA, cm^2/m^2	54.5 ± 31.8	-1.9 ± 8.7	-2.1 ± 20.2	0.026
Visceral adipose tissue CSA, cm^2/m^2	34.3 ± 22.5	0.6 ± 8.0	2.1 ± 29.3	0.438

Data are represented as mean ± SD. Abbreviations: CSA, cross-sectional area.

Survival

Median OS (95% confidence interval [CI]) was similar between subjects with normal versus low skeletal muscle index at baseline (12.2 months, 95% CI: 9.8-14.7 versus 10.3 months, 95% CI: 7.8-12.7, hazard ratio [HR] 1.1, 95% CI: 0.8-1.7, p = 0.496 [data not shown]).

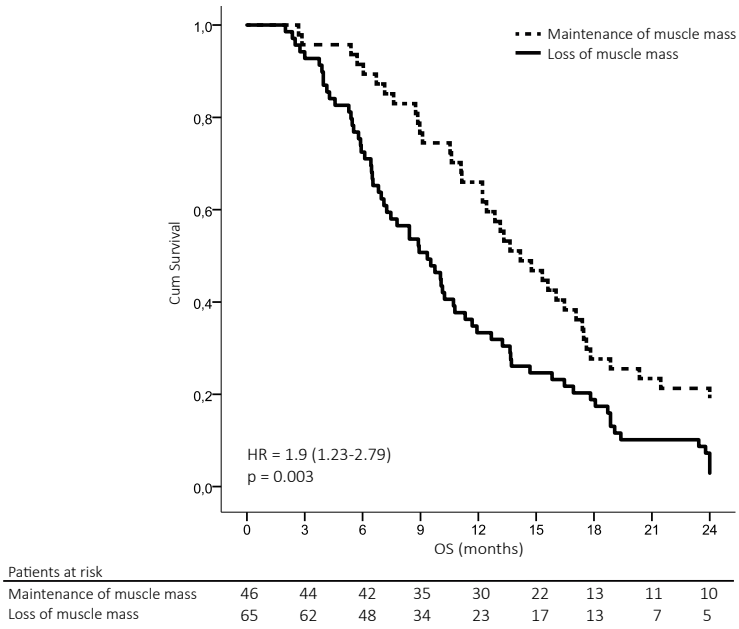

Figure 2. Kaplan Meier overall survival curve for patients with loss of muscle mass compared to patients without loss of muscle mass.

Longitudinally, median OS was significantly shorter among patients losing skeletal muscle mass compared to patients with preserved skeletal muscle mass (9.4 months, 95% CI: 7.1-11.6 versus 14.2 months, 95% CI: 11.3-17.1, HR 1.9, 95% CI: 1.23-2.79, p = 0.003) (figure 2).
Multivariate analyses showed that proportional loss of muscle mass (HR 0.949, 95% CI: 0.915-0.985, p = 0.006) was associated with poor OS independent from important clinical prognostic factors including gender, age, WHO PS and Charlson comorbidity index (table 3).

Table 3. Multivariate analysis for predictors of overall survival.

Variable	HR (95% CI)	p value
Delta muscle mass (%)	0.949 (0.915-0.985)	0.006
Age (years)	1.008 (0.978-1.039)	0.593
Gender	1.593 (0.915-2.772)	0.100
WHO performance status	1.084 (0.847-1.387)	0.520
Charlson comorbidity index	1.132 (0.960-1.335)	0.139

Adipose tissue compartments

On multivariate analysis, changes in skeletal muscle mass were associated with OS (table 3). We therefore stratified patients in two groups; 'maintenance of skeletal muscle mass' and 'loss of skeletal muscle mass'. At baseline, there were no differences between groups regarding weight and body composition. Furthermore, groups did not differ in tumour progression (data not shown). Muscle loss was accompanied by a generalized loss of tissue as both subcutaneous fat and visceral fat decreased. Furthermore, radiation attenuation decreased, which could reflect gain in intramuscular fat (table 4) [32].

DISCUSSION

In the current study, we identified body composition changes after two cycles of chemotherapy treatment initiation in stage IV NSCLC patients. Skeletal muscle CSA, muscle radiation attenuation and to lesser extent subcutaneous adipose tissue decreased throughout treatment indicating a rapid loss in muscle mass and quality. Sixty-nine percent of the patients lost muscle mass and exhibited worse survival rates compared to those with preserved muscle mass.

Next to the loss of muscle mass, a rapid decline in skeletal muscle radiation attenuation was observed during the course of treatment. Reduced radiation attenuation is believed to reflect fat infiltration (myosteatosis) in the muscle [33]. Myosteatosis in the muscle has indeed been observed in muscle biopsies from cancer patients and was more aggravated in those with weight loss [34]. The phenomenon of myosteatosis is shown in several patient groups including both obese patients [35] and patients with low BMI. This suggests that high muscle fat infiltration is not merely a result of disturbed energy balance but could be driven by disturbances in muscle oxidative metabolism.

Table 4. Characterization of the overall wasting pattern in groups stratified by muscle response.

	Maintenance of muscle mass (n=46)			Loss of muscle mass (n=65)				
	Baseline	Change, %	Within group p value	Baseline	Change, %	Within group p value	Between group baseline p value	Between group change p value
Skeletal muscle, cm^2/m^2	43.8 ± 6.9	2.9 ± 4.0	<0.001	44.9 ± 7.0	-6.6 ± 5.1	<0.001	0.392	<0.001
Radiation attenuation (HU)	30.6 ± 7.6	-0.3 ± 11.9	0.600	32.1 ± 6.9	-3.0 ± 12.4	0.015	0.243	0.233
Subcutaneous adipose tissue, cm^2/m^2	58.3 ± 35.2	7.9 ± 18.5	0.043	51.9 ± 29.1	-9.3 ± 18.3	<0.001	0.291	<0.001
Visceral adipose tissue, cm^2/m^2	36.4 ± 24.6	11.0 ± 23.4	0.004	32.8 ± 21.1	-4.1 ± 31.5	0.029	0.445	0.006
Weight, kg	74.7 ± 15.7	0.7 ± 2.4	0.068	73.7 ± 14.1	-1.7 ± 3.2	<0.001	0.811	<0.001

Data are represented as mean ± SD. Abbreviations: HU, hounsefield units.

A recent study, comparing changes in skeletal muscle during treatment with chemotherapy or targeted therapy (EGFR or ALK tyrosine kinase inhibitor) showed significant differences in the loss of skeletal muscle in advanced NSCLC patient [36]. Skeletal muscle was decreased in both groups, but the loss of muscle mass was significantly lower in the targeted therapy group compared to chemotherapy.

In this study, all patients were treated with the same chemotherapeutic regimen (carboplatinum, paclitaxel, bevacizumab). Therefore, it is interesting to speculate about the influence of anti-cancer drugs and toxicity on skeletal muscle changes in advanced NSCLC. From experimental research it is known that chemotherapy can directly affect regulation of muscle maintenance, possibly mediated by nuclear factor kappa B activation [37]. Cisplatin is known to induce nuclear factor kappa B, which triggers muscle wasting. In addition, also the tumour itself is able to increase nuclear factor kappa B. Thereby, muscle wasting might be both tumour- and chemotherapy induced via common pathways mediated by nuclear factor kappa B. In this study, nineteen (17%) of the patients experienced an adverse event graded with a CTCAE of ≥ 3. There was no significant difference in toxicity between the group “muscle loss” and “muscle maintenance”. The underlying mechanism why some patients experience chemotherapy-induced muscle wasting, while others maintain muscle mass is unknown.

The added value of the current study is that also in patients receiving standardized chemotherapy, clinically relevant inter-individual responses in body composition were observed (46 patients maintained muscle mass and had a stable body weight) which may partly be reversible by early supportive interventions. Importantly in this context and in contrast to some previous studies [4,6,9,10,25], low muscle mass and attenuation at baseline were not prognostic for overall survival.

The main strength of our study comes from the well-defined randomized patient cohort, receiving a standardized chemotherapeutic regime [26]. However, this study is not without limitations. All patients were treated with the same chemotherapeutic agents however in 63 of 111 patient nitroglycerin patches were added to the therapy. No difference in OS between the two groups was found [26]. The follow-up CT scans were executed according to the protocol on predefined times, CT scans were performed in different medical centres, whereby minor variation in scanning procedure between hospitals and quality of the images cannot be ruled out. Additionally, data on pre-treatment weight loss were unavailable.

To conclude, this study shows prognostic value of body composition changes after two cycles of chemotherapy treatment in stage IV NSCLC patients. Therefore, CT derived assessment of body composition may provide an additional tool for the treating physician to judge disease severity and related prognosis. Future studies have to reveal whether early supportive intervention, for example with intensive nutritional support, guided by initial CT muscle response to chemotherapy can influence the systemic consequences of NSCLC (treatment) and related elevated mortality risk.

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10

Can radiomics help to predict skeletal muscle response to chemotherapy in stage IV non-small cell lung cancer?

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ABSTRACT

Background

Muscle depletion negatively impacts treatment efficacy and survival rates in cancer. Prevention and timely treatment of muscle loss requires prediction of patients at risk. We aimed to investigate the potential of skeletal muscle radiomic features to predict future muscle loss.

Methods

A total of 116 patients with stage IV non-small cell lung cancer included in a randomised controlled trial (NCT01171170) studying the effect of nitroglycerin added to paclitaxel-carboplatin-bevacizumab were enrolled. In this post hoc analysis, muscle cross-sectional area and radiomic features were extracted from computed tomography images obtained before initiation of chemotherapy and shortly after administration of the second cycle. For internal cross-validation, the cohort was randomly split in a training set and validation set 100 times. We used least absolute shrinkage and selection operator method to select features that were most significantly associated with muscle loss and an area under the curve (AUC) for model performance.

Results

Sixty-nine patients (59%) exhibited loss of skeletal muscle. One hundred ninety-three features were used to construct a prediction model for muscle loss. The average AUC was 0.49 (95% confidence interval: 0.36-0.62). Differences in intensity and texture radiomic features over time were seen between patients with and without muscle loss.

Conclusions

The present study shows that skeletal muscle radiomics did not predict future muscle loss during chemotherapy in non-small cell lung cancer. Differences in radiomic features over time might reflect myosteatosis. Future imaging analysis combined with muscle tissue analysis in patients and in experimental models is needed to unravel the biological processes linked to the radiomic features.

INTRODUCTION

Cachexia is a frequently observed phenomenon of skeletal muscle and adipose tissue depletion among patients with non-small cell lung cancer (NSCLC) [1,2]. The progressive loss of muscle has a devastating impact on the quality of life [3] and survival rates in patients with NSCLC [2,4-6]. Although both muscle and fat become depleted, there is evidence that body fat is lost more rapidly than muscle [7,8]. Cancer may therefore shift lipid metabolism to a catabolic state, which in turn may affect skeletal muscle. In cancer, depletion of subcutaneous fat is driven by increased lipolysis [9,10]. Experimental research has shown that lipolysis generates fatty acids which are able to transport into myocytes and stimulate protein degradation [11]. Indeed, skeletal muscle of patients with cancer contained more intramyocellular fat than age- and gender-matched controls [12]. However, there are currently no (non-invasive) biomarkers available to predict muscle loss.

Radiomics is a method that quantitatively extracts features including shape, size, intensity and texture that are related to pathophysiology from standard-of-care medical images [13-17]. Until now, radiomics has mainly been applied to extract tumour features in oncologic patients to visualise tumour heterogeneity [17] and to assess prognosis [18-20]. Besides quantitative evaluation of Hounsfield units, radiomics also explores patterns. Given the quantitative and qualitative differences in skeletal muscles of cachectic patients, radiomics might be helpful to predict future muscle loss. Therefore, the primary goal of this exploratory study is to investigate whether baseline skeletal muscle radiomic features are different between patients who develop muscle loss and those who maintain their muscle mass after chemotherapy. We furthermore investigated longitudinally if muscle loss is associated with changes in muscle radiomic features.

METHODS

Patient cohort

Computed tomography (CT) scans derived from the multicentre randomised phase II trial (NVALT12 trial, NCT01171170) were investigated. In this trial the effect of nitroglycerin added to paclitaxel-carboplatin-bevacizumab on progression-free survival in chemotherapy naive stage IV non-squamous NSCLC was investigated. The methodology and results of this trial have been published previously [21].

Image analysis

CT scans made at baseline and after the second cycle of chemotherapy, as part of a secondary end point, were used [22]. To evaluate whether or not patients lost skeletal muscle, cross-sectional measurements of skeletal muscle areas were made on transverse images at the third lumbar level using Slice-O-Matic software version 5.0 (Tomovision, Montreal, Canada). One slice at the third lumbar level in each scan was selected for each patient. During anatomical land marking, the first image at the third lumbar level with both vertebral transverse processes

clearly visible was used for analysis. Skeletal muscle cross-sectional area was quantified on the basis of pre-established thresholds of Hounsfield units (-29 to 150). Boundaries were corrected manually when necessary. It is of note, that this delineation excludes intramuscular fat. Changes in muscle cross-sectional areas between CT scans were expressed as a percentage. A measurement error of 1.3% was adopted, based on previous reported literature [2,4]. Changes greater than or equal to -1.3% were considered as 'loss of skeletal muscle', while changes less than -1.3% were considered 'maintenance of skeletal muscle'. In addition, the mean Hounsfield units of the muscle CSA were assessed, as a measure for muscle fat deposits. Low values reflect increased muscle fat.

Then, to evaluate radiomic features, skeletal muscle cross-sectional area was delineated at the third lumbar level using the same thresholds of Hounsfield units as described previously. Now, it was extended one slice in the cranial direction and one slice in the caudal direction using Mirada software (Mirada Medical, Oxford, UK), to be able to calculate three-dimensional image features. Segmentation for radiomics was a semi-automatic process which was manually adjusted if needed. Image features were calculated on both baseline and follow-up scans, using an adapted version of Computational Environment for Radiotherapy Research extended with in-house developed radiomic image analysis software (Matlab 2014a; The Mathworks, Natick, MA). Before the extraction of features, a grey-level discretisation using a bin width of 25 Hounsfield units was applied. To minimise the possible effect of the variation in image parameters, all scans were resampled to a voxel size of $1 \times 1 \times 3\ mm^3$ using a cubic interpolation as recommended in the study by Larue et al. [23]. Although collection of the CT scans was predefined in the clinical trial, the analysis of muscle mass and radiomics was not part of the pre-registered outcomes of the original trial. Because of this well-defined randomised patient cohort, with CT scans executed according to study protocol-predefined time points, this might be an appropriate data set to explore our hypothesis.

Radiomic feature selection and statistics

Intensity and texture features were analysed. Shape and size features were excluded because the volumes of interest has been segmented manually, which might influence the outcome of these feature categories. In addition, a three-dimensional wavelet transformation was applied to the CT scan to create filtered, next to the unfiltered intensity and texture features. Features without a range (i.e. features with an exact similar value in all patients), which were not able to discriminate patients, were excluded.

Spearman's correlation coefficient (ρ) was used to assess the correlation between all texture and intensity radiomic features. Of each feature pair with $\rho>0.85$, the feature that was strongest correlated to all other features was excluded. This process was repeated until no feature pair with all $\rho>0.85$ was remaining.

To calculate to which extent the variation among the radiomic features on baseline scans is explained by muscle loss, a logistic Least Absolute Shrinkage and Selection Operator (LASSO) regression model adopting a 100-fold Monte Carlo cross-validation in Matlab 2017b was applied (The Mathworks, Natick, MA).

The cohort was randomly split into a training set (approximately two-third) and validation set (approximately one-third). Patients were randomised such that the ratio between patients with and without skeletal muscle loss was similar in each group. Features in the training and validation sets were standardised by subtracting the respective mean feature value in the training set and dividing by the feature standard deviation in the training set. The logistic LASSO model was used to reduce the number of features and estimate regression coefficients for the remaining features. The model-intrinsic parameter λ was estimated using an internal five-fold cross-validation on the training set. The out-of-sample area under the curve (AUC) of the Receiver operating characteristic curve (ROC) was computed on the validation set to assess the prognostic power. This process was repeated 100 times, each time with a different randomisation of the patients into training and validation sets, and the average AUC over the hundred different models was calculated. This analysis was carried out for the radiomic texture and intensity features of the baseline and for the absolute difference in feature values between the baseline scan and the follow-up scan (delta features). For all radiomic features that were selected at least once in the LASSO feature selection procedure the difference in feature value between patients with and without muscle loss was compared by plotting a heatmap. For the heatmap all radiomic features were normalised to have values between 0 and 1. A hierarchical clustering was applied on the same heatmap to identify clusters of patients with different radiomic texture and intensity values.

RESULTS

Patients and characteristics

In total, 223 patients were enrolled in the randomised controlled trial. One hundred three patients were excluded because of unavailability of one or both CT scans, two patients were excluded because L3 was not evaluable, one patient was excluded because of lacking overall survival (OS) data and one was excluded for insufficient quality of the scans. After exclusion, CT scans from 116 patients were eligible. The mean age was 61 years, and 64 patients (55%) were male; survival rates of the included group were comparable with the whole study group of 223 patients.

Muscle maintenance and muscle loss

Delineations of skeletal muscle cross-sectional area made using Slice-O-Matic for cross-sectional area and using Mirada for extraction of radiomic features are shown in figure 1. Analysis of skeletal muscle cross-sectional area at baseline and during follow-up revealed that in the whole

cohort, skeletal muscle decreased with mean (± standard deviation) 2.9 ± 6.7%. Of those, 69 patients (59%) exhibited loss of skeletal muscle.

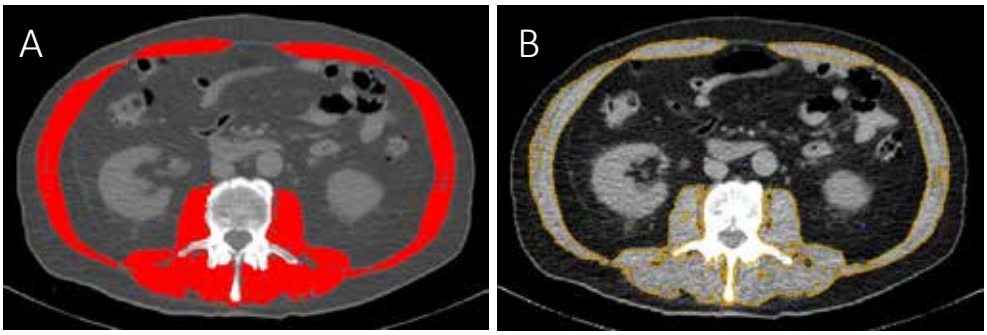

Figure 1. Skeletal muscle area on transverse computed tomography images at the third lumbar level, using (a) Slice-O-Matic for evaluation of cross-sectional area and (b) Mirada software for extraction of radiomic features.

Radiomic features

A total of 1298 radiomic features were extracted. For analysis of baseline radiomic features, those who had no range (n=9) were excluded. After removing the redundant features using the spearman correlation method, 193 radiomic features, 11 unfiltered and 182 filtered, could be used for the analysis.

Figure 2 shows a heatmap in which radiomic features that are selected at least once in the LASSO models are plotted against the patients ranked in descending order of the decrease in muscle mass. The upper half of the graph shows the patients with muscle loss, and the lower half of the graph the patients with a stable muscle mass. No difference in radiomic features is seen between those with maintenance of muscle and those with muscle loss. The average AUC over 100 repetitions for radiomic features of the baseline scan, with muscle loss as outcome, is 0.49 (95% CI: 0.36-0.62). In addition, no differences were observed in muscle mass changes between patients treated with and without nitroglycerin. A list with the selected radiomic features in alphabetic order can be found in the supplementals.

We also analysed the delta features (differences in feature values between the baseline scan and the follow-up scan) with muscle loss as outcome. The average AUC was 0.68 (95% CI: 0.51-0.84). In this model the grey-level co-occurrence matrix (GLCM) was most often selected (data not shown).

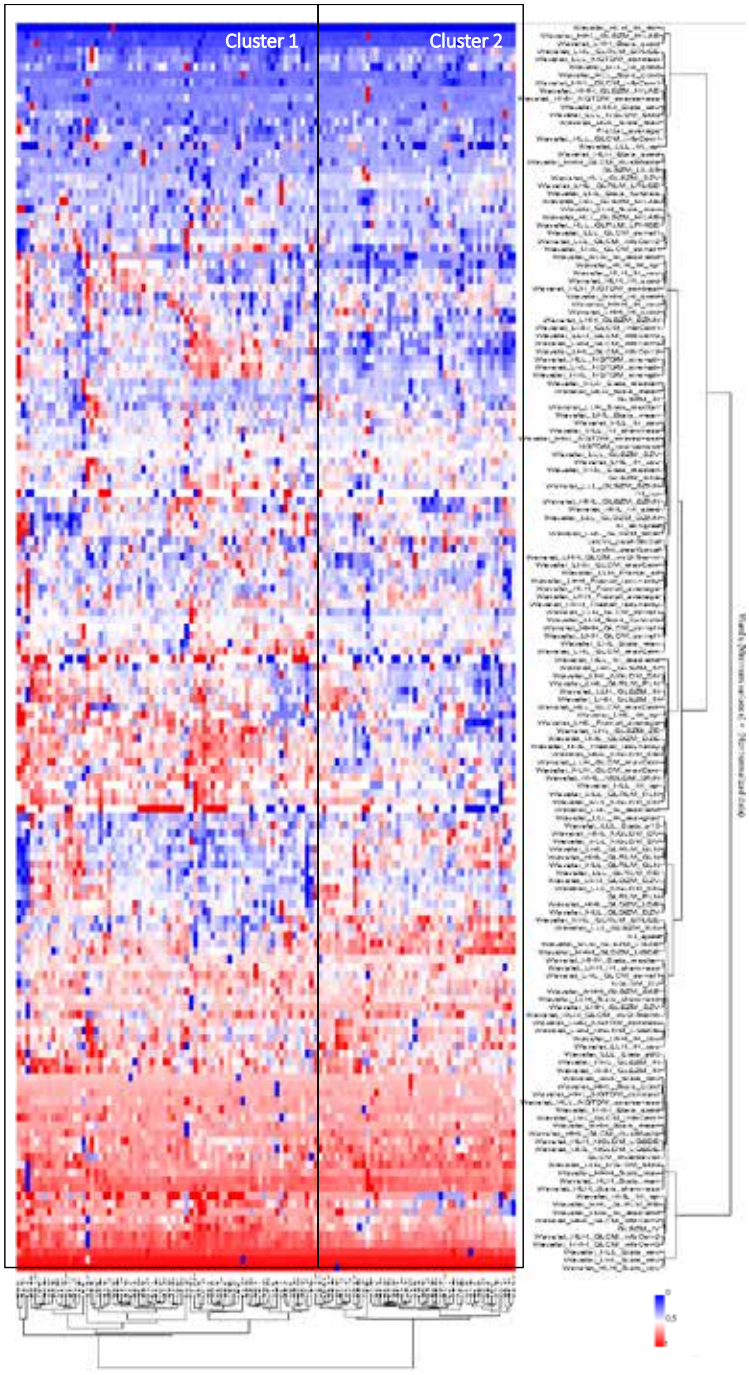

Figure 2. Heatmap of the normalised selected delta radiomics features in alphabetic order (list of features is reported in supplementals). Patients are ranked according to the descending difference in muscle mass; upper half are the patients with muscle depletion, and lower half patients with stable muscle mass.

DISCUSSION

To the best of our knowledge, this is the first exploratory study evaluating the potential of skeletal muscle radiomics to predict skeletal muscle loss. Some cross-sectional studies show that baseline low CT-derived muscle mass is an important prognostic factor for OS [4,24], which contradicts to other studies [25,26]. The lack of properly validated and population-specific cut-off values for CT-derived low muscle mass may explain this discrepancy. We therefore were interested to know if radiomic features of baseline skeletal muscle are prognostic for longitudinal muscle loss. In this study, baseline radiomic features had no discriminatory value with regard to longitudinal skeletal muscle changes. However, longitudinal differences in radiomic features were seen between those who lost muscle and those who maintained muscle mass.

The most distinct delta radiomic feature was a feature in the GLCM category. To calculate the GLCM, each pixel in an image is assigned a numerical value depending on the combination of grey-level intensity values in two neighbouring pixels. Second-order statistics calculate mathematical algorithms to derive textural homogeneity, contrast, variance etc. [27]. GLCM features depend on the grey-level pattern, which might implicate that patients with muscle loss develop more muscle fat deposits, leading to a more heterogeneous grey-level intensity pattern (as low grey levels reflect increased intramuscular fat). Indeed analysis of muscle biopsies demonstrated that compared with controls, patients with cancer exhibited increased numbers of lipid droplets in skeletal muscle. Moreover, the amount of lipid droplets increased with progression of weight loss [28]. Muscle fat depots assessed by muscle radiation attenuation on CT are associated with poor survival in cancer [4]. A phantom study showed concordance between radiation attenuation and muscle lipid content [29], therefore, reduced muscle radiation attenuation is believed to reflect fat infiltration. However, it is unclear if this radiation attenuation indicates intramyocellular or extramyocellular lipids. Therefore, the aetiology and prognostic significance of muscle lipids in cachexia progression is object for further research.

While the strength of our study comes from the well-defined randomised patient cohort, with CT scans executed according to study protocol-predefined time points, there are some limitations. Pre-treatment changes in muscle mass are unknown. Patients currently identified as having ‘muscle maintenance’ could have exhibited muscle loss before the first CT scan, which may influence radiomic features. The NVALT12 trial is a multicentre study. Consequently, the CT scans included in this analysis are performed in different hospitals on different CT scanners. Although all scans were low-dose CT scans, there might be differences in scan acquisition and the use of contrast. A phantom study showed that slice thickness variability influenced radiomic features, however this variability can be reduced by resampling to a standardised voxel size [23]. While it is known that different exposures do not influence the radiomic features values [23], the impact of use of contrast is unknown.

In conclusion, the present study shows that baseline skeletal muscle radiomics did not predict future muscle loss in patients with metastatic NSCLC. Nevertheless, longitudinal differences in intensity and texture muscle radiomic features are detected, which slightly differ between patients with muscle loss and patients with preserved muscle. Future research in experimental models and human radiomics combined with muscle tissue analysis is required to unravel the biological processes linked to the radiomic features.

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SUPPLEMENTALS

Fractal_average, GLCM_inverseVar, GLRLM_RLN, GLSZM_IN, GLSZM_IV, GLSZM_LILAE, GLSZM_SAE, IH_iqr, IH_mingrad, IH_qcod, LocInt_peakGlobal, LocInt_peakLocal, NGLDM_DV, NGTDM_coarseness, Wavelet_HHH_Fractal_lacunarity, Wavelet_HHH_GLCM_clusShade, Wavelet_HHH_GLCM_correl1, Wavelet_HHH_GLCM_infoCorr2, Wavelet_HHH_GLDZM_LISDE, Wavelet_HHH_GLSZM_HILAE, Wavelet_HHH_GLSZM_IN, Wavelet_HHH_GLSZM_SAE, Wavelet_HHH_IH_cov, Wavelet_HHH_IH_qcod, Wavelet_HHH_NGTDM_coarseness, Wavelet_HHH_Stats_cov, Wavelet_HHH_Stats_max, Wavelet_HHH_Stats_mean, Wavelet_HHH_Stats_median, Wavelet_HHH_Stats_qcod, Wavelet_HHL_Fractal_lacunarity, Wavelet_HHL_GLCM_clusShade, Wavelet_HHL_GLCM_correl1, Wavelet_HHL_GLCM_infoCorr1, Wavelet_HHL_GLCM_infoCorr2, Wavelet_HHL_GLDZM_DZE, Wavelet_HHL_GLDZM_LDE, Wavelet_HHL_GLRLM_GLN, Wavelet_HHL_GLRLM_RE, Wavelet_HHL_GLRLM_SRLGE, Wavelet_HHL_GLSZM_HILAE, Wavelet_HHL_GLSZM_IN, Wavelet_HHL_GLSZM_SZNN, Wavelet_HHL_IH_iqr, Wavelet_HHL_IH_qcod, Wavelet_HHL_NGLDM_DE, Wavelet_HHL_NGLDM_DNN, Wavelet_HHL_NGLDM_DV, Wavelet_HHL_NGLDM_LGSDE, Wavelet_HHL_NGTDM_coarseness, Wavelet_HHL_NGTDM_contrast, Wavelet_HHL_NGTDM_strength, Wavelet_HHL_Stats_cov, Wavelet_HHL_Stats_max, Wavelet_HHL_Stats_median, Wavelet_HHL_Stats_qcod, Wavelet_HLH_Fractal_average, Wavelet_HLH_GLCM_infoCorr2, Wavelet_HLH_GLCM_invDiffnorm, Wavelet_HLH_GLCM_maxCorr, Wavelet_HLH_GLSZM_LISAE, Wavelet_HLH_IH_cov, Wavelet_HLH_IH_iqr, Wavelet_HLH_IH_mediand, Wavelet_HLH_IH_min, Wavelet_HLH_IH_qcod, Wavelet_HLH_NGLDM_LGSDE, Wavelet_HLH_NGTDM_contrast, Wavelet_HLH_Stats_cov, Wavelet_HLH_Stats_max, Wavelet_HLH_Stats_mean, Wavelet_HLH_Stats_median, Wavelet_HLH_Stats_qcod, Wavelet_HLH_Stats_skewness, Wavelet_HLL_GLCM_infoCorr1, Wavelet_HLL_GLCM_maxCorr, Wavelet_HLL_GLDZM_DZV, Wavelet_HLL_GLRLM_GLN, Wavelet_HLL_GLRLM_LRHGE, Wavelet_HLL_GLRLM_RLN, Wavelet_HLL_GLSZM_HILAE, Wavelet_HLL_GLSZM_SZV, Wavelet_HLL_IH_cov, Wavelet_HLL_IH_iqr, Wavelet_HLL_IH_mediand, Wavelet_HLL_IH_qcod, Wavelet_HLL_IH_skewness, Wavelet_HLL_NGLDM_DN, Wavelet_HLL_NGLDM_DV, Wavelet_HLL_NGTDM_coarseness, Wavelet_HLL_NGTDM_strength, Wavelet_HLL_Stats_cov, Wavelet_HLL_Stats_qcod, Wavelet_LHH_Fractal_average, Wavelet_LHH_Fractal_lacunarity, Wavelet_LHH_GLCM_correl1, Wavelet_LHH_GLCM_infoCorr1, Wavelet_LHH_GLCM_infoCorr2, Wavelet_LHH_GLCM_invDiffnorm, Wavelet_LHH_GLCM_maxCorr, Wavelet_LHH_GLDZM_DZV, Wavelet_LHH_GLSZM_IN, Wavelet_LHH_GLSZM_SZNN, Wavelet_LHH_GLSZM_SZV, Wavelet_LHH_IH_cov, Wavelet_LHH_IH_mediand, Wavelet_LHH_IH_qcod, Wavelet_LHH_IH_skewness, Wavelet_LHH_NGLDM_LGSDE, Wavelet_LHH_NGTDM_contrast, Wavelet_LHH_Stats_qcod, Wavelet_LHL_Fractal_average, Wavelet_LHL_GLCM_correl1, Wavelet_LHL_GLCM_infoCorr1, Wavelet_LHL_GLCM_infoCorr2, Wavelet_LHL_GLCM_maxCorr, Wavelet_LHL_GLDZM_SDE, Wavelet_LHL_GLRLM_GLN, Wavelet_LHL_GLRLM_LRLGE, Wavelet_LHL_GLRLM_RLN, Wavelet_LHL_GLRLM_SRLGE, Wavelet_LHL_GLSZM_HILAE, Wavelet_LHL_GLSZM_IN, Wavelet_LHL_GLSZM_ZE, Wavelet_LHL_IH_cov, Wavelet_LHL_IH_iqr, Wavelet_LHL_IH_mediand,

Wavelet_LHL_NGLDM_DN, Wavelet_LHL_NGTDM_strength, Wavelet_LHL_Stats_cov, Wavelet_LHL_Stats_kurtosis, Wavelet_LHL_Stats_max, Wavelet_LHL_Stats_mean, Wavelet_LLH_Fractal_sd, Wavelet_LLH_GLCM_correl1, Wavelet_LLH_GLCM_infoCorr2, Wavelet_LLH_GLCM_maxCorr, Wavelet_LLH_GLSZM_IN, Wavelet_LLH_IH_cov, Wavelet_LLH_NGLDM_SM2, Wavelet_LLH_Stats_kurtosis, Wavelet_LLH_Stats_max, Wavelet_LLH_Stats_median, Wavelet_LLH_Stats_skewness, Wavelet_LLL_GLCM_correl1, Wavelet_LLL_GLCM_infoCorr2, Wavelet_LLL_GLDZM_DZNN, Wavelet_LLL_GLRLM_RE, Wavelet_LLL_GLSZM_INN, Wavelet_LLL_GLSZM_SZNN, Wavelet_LLL_GLSZM_SZV, Wavelet_LLL_IH_iqr, Wavelet_LLL_IH_maxgrad, Wavelet_LLL_NGLDM_DN, Wavelet_LLL_NGLDM_SM2, Wavelet_LLL_NGTDM_contrast, Wavelet_LLL_Stats_p10, and Wavelet_LLL_Stats_p90.

11

Summary, general discussion, and future perspectives

SUMMARY AND GENERAL DISCUSSION

Cachexia is a frequently observed phenomenon in chronic obstructive pulmonary disease (COPD) and lung cancer and encompasses loss of skeletal muscle and adipose tissue. Tissue loss and redistribution is associated with worse clinical outcomes and interferes with treatment efficacy. Prevention and timely treatment of cachexia requires adequate and early identification of patients at risk and detailed understanding of the pathophysiology involved. In this thesis, standard-of-care medical images for respiratory disease evaluation were used to characterize muscle and adipose tissue. In addition, skeletal muscle radiomic features derived from these images were evaluated for predicting future cachexia development in lung cancer. We furthermore investigated the putative contribution of brown adipose tissue and breathing mechanics to elevated energy requirements in COPD. In this final chapter, these findings are positioned in a broader scientific and clinical perspective considering the latest developments in the management of COPD and lung cancer.

(EARLY) IDENTIFICATION OF PATIENTS AT RISK

It is now widely recognized that loss of skeletal muscle mass adversely impacts clinical outcome and increases healthcare utilization in patients facing cancer or a chronic disease like COPD [1-4]. Low muscle mass is not restricted to those with lean stature and even obese patients can have substantial ongoing muscle depletion [5,6]. Furthermore, stable weight may mask changes in body composition, such as a shift from muscle towards fat mass. There is a growing interest to assess muscle mass with use of techniques that are easily applicable in the clinic or are already being used in regular clinical care for other diagnostic purposes. Thereby, the role of medical imaging is evolving from a diagnostic tool of lung pathology towards an instrument for more extensive disease phenotyping (chapter 2).

Cross-sectional measurement of skeletal muscle

Muscle mass constitutes the major part of fat-free mass, which can be measured in clinical practice using bioimpedance analysis and dual-energy x-ray absorptiometry (DEXA) [7,8]. Since, these modalities are not typically incorporated in routine clinical care, other strategies have been employed. Currently, computed tomography (CT) scans are increasingly utilized in clinical research to measure body composition [9]. This technique allows assessment of individual muscles separately and discriminates different tissue types. Also, assessment of the degree of fat infiltration into the muscle is possible, which is suggested to be an indirect measure of muscle quality [10].

CT derived evaluation of body composition is done by measuring muscle cross sectional area at the level of the third lumbar vertebra [10,11]. Thoracic scans in non-small cell lung cancer (NSCLC) patients usually extend to the third lumbar vertebra level, considering scan protocols include the adrenals to detect the presence of metastasis. However, this is not the case for

other lung disease-related clinical purposes as most reach not further than the first lumbar vertebra. Therefore, in COPD a single-muscle approach using the pectoralis muscle has been proposed as alternative, based on associations between low pectoralis muscle with markers of disease severity, symptoms and exercise capacity [12-14]. However, pectoralis muscle was suggested as marker of whole body muscle mass based on moderate associations with clinical outcome measures. Moreover, pectoralis muscle has never been compared with other measurements including a gold standard such as whole body potassium or the gold standard for CT (third lumbar level). In line, we showed a poor correlation between skeletal muscle at the third lumbar level and the pectoralis muscle (chapter 3). Therefore, one might speculate that there is no true association between the two and that causality does not exist. However, the correlation between first and third lumbar level was better in cross-sectional and longitudinal comparison and would therefore, rather than the pectoralis muscle, be a better alternative for analysis of muscle mass from regular chest CT scans (chapter 3). In COPD and aging research, DEXA derived whole body fat-free mass or appendicular lean mass are commonly used to assess muscle mass [15-17]. Correlations reported between DEXA lean mass and CT muscle cross sectional area were moderate [18,19]. One study reported CT derived muscle mass and multiplied the measured area with 1.04 [20], which is the assumed constant density of fat-free skeletal muscle [21]. However, higher degrees of intermuscular fat will decrease density and thereby impacting the accuracy. Therefore, current single slice CT scans cannot substitute DEXA for assessment of cross-sectional whole body muscle mass or vice versa. Furthermore, the current skeletal muscle cut-offs that define cachexia or sarcopenia are based on DEXA and not yet standardized and tailored towards CT measurements [22]. Thereby, one cannot diagnose cachexia or sarcopenia based on a single time point CT scan.

Early changes of skeletal muscle

Longitudinally, there is evidence that repeating DEXA increases measurement errors. DEXA reliability studies have demonstrated good repeatability (less than 2% coefficient of variation) [23]. However, the percentage of change in lean tissue required to be accurately detected by DEXA has been reported in the range of 2.7–6.6% for individual extremities [23]. In this study serial scans were performed within 5 minutes and after repositioning of the subject, indicating that the measured differences can be attributed to machine errors. Changes of muscle mass or changes in hydration status might magnify these measurement errors. Additionally, few longitudinal comparison studies have reported moderate to poor agreement between DEXA and CT derived changes [24,25].

We showed that longitudinally, CT is able to detect subtle changes in body composition which correlated with clinical outcome (chapter 3, chapter 5, and chapter 9). From a clinical practice point of view, future studies to reveal if these changes are also detected by other clinical assessment methods including DEXA would be worth an effort.

Prediction of future muscle loss

Baseline low muscle mass does not necessarily result in longitudinal loss and till now, there are no biomarkers or techniques available to predict muscle loss. In oncology, tumour features are used to visualize tumour heterogeneity and to aid in prognosis [26,27]. This is carried out with the use of radiomics. The concept underlying the process of radiomics is that standard-of-care medical images contain qualitative and quantitative information regarding tumour shape, size, intensity and texture that are related to the underlying pathophysiology of a tissue [28,29]. In this thesis the use of skeletal muscle radiomics was explored for its potential to predict future cachexia development in lung cancer. Skeletal muscle radiomic features had no discriminatory value with regard to longitudinal loss of skeletal muscle. However, we did observe longitudinal differences in radiomic features between those who lost muscle and those who maintained muscle mass. Future imaging analysis combined with muscle tissue analysis may unravel the biological processes linked to the radiomic features (chapter 10). However, radiomic features are impacted by constant developments in radiology regarding scanner acquisition protocols, exposure control, dose modulation and reconstruction algorithms and parameters. Up to now, despite encouraging results these challenges prevented the use of radiomics in routine practice to aid in clinical decision making. Within this context it is therefore crucial to focus on stable parameters across different scanners and to validate these parameters in a multicentric context [30-33].

ADIPOSE TISSUE

In cachexia, skeletal muscle loss is often accompanied by depletion of adipose tissue, driven by increased lipolysis [34,35]. Lipolysis generates free fatty acids which are able to transport into myocytes and through a process of oxidation stimulate protein degradation [36,37]. Indeed, skeletal muscle of cancer patients contains more intramyocellular fat compared to age- and gender matched controls [38]. Furthermore, a rise in the number of lipid droplets has been demonstrated in relation to increasing weight loss and loss of adipose tissue in cancer [39]. Inhibition of lipolysis in a lung cancer mouse model not only prevented depletion of fat but also prevented loss of muscle mass [40]. Together these observations point towards a possible muscle-fat crosstalk in cachexia.

While lipolysis represents the loss of white adipose tissue, brown adipose tissue (BAT) or browning of white adipose tissue uses stored energy for thermogenesis, which is an energy costly process [41]. Thereby, the association between BAT and elevated energy expenditure in cachexia has been suspected for a long time [42]. It was shown that activation of BAT contributed to development of cachexia in mice with colorectal cancer. Furthermore, BAT activation was not present in non-cachectic mice with colorectal cancer [43]. Human research of BAT in relation to cachexia is scarce and conflicting. Autopsy reports of cachectic cancer patients revealed higher incidence of BAT compared to age-matched controls [42]. Unfortunately,

no data were presented regarding the stage of cancer. In contrast to the autopsy reports, a retrospective imaging study reported a similar prevalence of BAT between subjects with and without cancer [44]. However, these results might be biased by the retrospective design, which did not allow to control environmental temperatures as BAT is known to be activated upon cold in healthy young subjects [45,46]. As direct evidence linking BAT activity, energy metabolism and cachexia was not available, this thesis investigated the contribution of BAT on different components of energy metabolism in muscle depleted patients. In COPD, muscle wasting [47,48] and hypermetabolism [49] have particularly been demonstrated among those with emphysema. Therefore, this thesis studied the contributing role of BAT to energy metabolism in emphysematous COPD patients. In contrast to our hypothesis, hypermetabolic COPD patients with the emphysematous phenotype did not exhibit enhanced BAT activity or altered gene expression of BAT or beige markers in white adipose tissue compared to age-, gender- and body mass index matched healthy non-smoking controls (chapter 7). The original intention was to compare the COPD patients not only to healthy controls, but also to matched cachectic lung cancer and cachectic pancreatic cancer patients. However, the BAT protocol was too strenuous, regarding eligibility criteria as well as methodology, for most of the cachectic cancer patients. The current study protocol used cold to activate BAT. Study subjects were wrapped in a water-perfused suit for several hours while temperature decreased slightly from 36 to on average 20 degrees. Furthermore, medication influencing the sympathetic nerve system and thereby interfering with BAT activity, including ß-blockers and calcium channel blockers was prohibited. Since ß-blockers and calcium channel were widely used among our study population, many potential particiants were excluded. This resulted in the inclusion of only two pancreatic cancer patients before study closure. Both pancreatic patients lost > 5% body weight in the previous six months and were both hypermetabolic at rest. Surprisingly no BAT activity was found among those two cachectic cancer patients. Although based on only two cancer patients, these results indicate that BAT does not play a role in the pathophysiology of cancer associated cachexia.

SUPPORTIVE INTERVENTIONS IN COPD

In addition to the loss of lung tissue resulting in emphysema, a subgroup shows a concordant enhanced loss of muscle tissue [50]. Muscle depletion observed in emphysema is associated with hypermetabolism (chapter 4, chapter 6). It has been proposed that the working load of breathing increases energy metabolism. We indeed showed that patients with severe emphysema and hyperinflation were hypermetabolic at rest (130% of predicted) (chapter 6). Innovative endoscopic interventions including endobronchial valves to reduce hyperinflation are a growing new therapeutic field [51]. After hyperinflation reduction CT analysis showed that patients gained skeletal muscle and fat mass (chapter 5) implying an overall positive energy balance. Nevertheless, no impact of hyperinflation reduction by endobronchial valves on the different components of energy metabolism was observed after six months and patients remained hypermetabolic at rest (chapter 6).

Improving body composition abnormalities and aiming to provide the best possible baseline condition before treatment with EBV, might result in greater clinical benefits from EBV. To improve body composition, improve exercise capacity and positively influencing the clinical course of the disease in patients with COPD, pulmonary rehabilitation is currently accepted as evidence-based intervention strategy [52]. Mean improvements following pulmonary rehabilitation are comparable after stratification for baseline airflow limitation [53] and static lung hyperinflation [54]. However, data on pulmonary rehabilitation outcomes in patients with severe emphysema are scarce.

The National Emphysema Treatment Trial performed in the United States evaluating the effect of lung volume reduction surgery in patients with severe emphysema included a mandatory preoperative pulmonary rehabilitation program. The rationale was to facilitate early postoperative mobilization and to provide optimized preoperative exercise capacity [55]. Although the study was not specifically designed to evaluate the effect of pulmonary rehabilitation, significant improvements in exercise capacity, dyspnoea and health related quality of life were observed [56]. This provides evidence that pulmonary rehabilitation is feasible among patients with severe emphysema and that it alleviates symptoms and improves functional capacity [57]. One study evaluated whether preoperative improvements due to pulmonary rehabilitation can augment the benefits from lung volume reduction surgery or reduce postoperative complication rates. A cohort of 22 COPD patients who received 3 weeks of pulmonary rehabilitation prior to lung volume reduction surgery significantly improved in 6 minute walk distance. However, no additional postoperative gain in 6 minute walk distance was observed [58]. Nevertheless, follow-up time postoperative was only 5 weeks and data regarding complication rates and quality of life were lacking. There are currently no data available whether pre- and postoperative pulmonary rehabilitation can augment the clinical outcome of patients receiving EBV.

SUPPORTIVE INTERVENTIONS IN NSCLC

Despite increased understanding of the pathophysiology, there are still no evidence-based guidelines on the management of cancer cachexia. Given the multifaceted pathophysiology, it is thought that it requires a multimodal treatment approach encompassing nutrition as well as physical exercise, to preserve muscle mass, strength and function [59,60]. The optimal time to initiate such treatment might be early in the disease trajectory. A significant part of non-resectable stage III NSCLC patients who were weight stable before concurrent chemoradiation experienced weight loss during the first three weeks after therapy initiation (chapter 8). Furthermore, nearly two-third of stage IV NSCLC patients lose skeletal muscle after only two cycles of first line chemotherapy (chapter 9). Together these results argue for implementing cachexia-directed therapy in the anti-tumour treatment.

The average caloric deficit in patient with advanced-stage NSCLC has been estimated to be approximately 200 kcal per day. Moreover, the mean protein intake of these patients was 0.9 g/kg/day [61,62], which was significantly lower than the recommended daily protein intake of 1.0-1.5 g/kg/day [63]. Although dietary counselling in patients with various tumour types including NSCLC has found to improve body weight [64-66], systematic reviews and meta-analyses of randomised controlled trials found limited evidence of benefit of dietary counselling alone on clinical outcomes in lung cancer in terms of quality of life and mortality [67-69].

Regarding physical activity, low activity levels have been reported among NSCLC patients [70] with an average daily step count of approximately 4500 steps [71,72], and it is estimated that only 20% of the patients followed the advice regarding daily exercise [73]. As a potent anabolic stimulus, exercise might antagonize muscle atrophy in cachexia. Although proven feasible [74] and despite positive effects following exercise intervention in lung cancer patients at an earlier stage of disease [75-77], studies evaluating the effects of exercise in advanced lung cancer to combat cachexia are scarce. Only two exercise intervention studies have included advanced lung cancer patients. A feasibility single-arm study reported a trend towards increased functional capacity and muscle strength following a 8-week aerobic and resistance training in advanced lung cancer [78]. Others found significant improvement in functional capacity and muscle strength following a similar exercise intervention [74]. Despite that no significant changes in quality of life were found [74,78], an improvement in lung cancer symptoms was reported [78]. The evidence suggests benefit of combined resistance and aerobic muscle training; however randomized clinical trials in patients with advanced cancer are warranted to establish such programmes to counteract cachexia [79,80].

In stage III and stage IV NSCLC patients evidence of a multimodal cachexia treatment is very scarce. One study examined the effects of treatment with an appetite stimulant medroxyprogesterone and an anti-inflammatory drug celecoxib with food supplementation, recommendation of regular exercise and psychological assistance in 15 stage III and IV cachectic NSCLC patients. After 6 weeks, caloric intake increased with 22% and 13 patients either had stable or gained weight [73]. A feasibility study evaluated the effects of a combined intervention with polyunsaturated fatty acid nutritional supplements, exercise and anti-inflammatory drug celecoxib in advanced NSCLC and pancreatic cancer patients. Most of the included patients exhibited stage IV NSCLC with good performance score and who lost on average 5% of body weight prior to the intervention. Although the study was not specifically designed to evaluate the effect of the intervention, weight maintenance compared to standard care was reported [81].

Currently, results of the phase III multimodal-exercise, nutrition and anti-inflammatory medication for cachexia trial (NCT02330926) are awaited to fully assess the effect of the

multimodal intervention [82]. This trial aims to prevent the development of cachexia and/or to attenuate cachexia progression in patients with stage III-IV NSCLC, stage III-IV pancreatic cancer or cholangiocarcinoma, who commence first or second line anticancer treatment. The intervention consists of a 6-week program with nutritional supplements and advice, home-based exercise program, anti-inflammatory drug ibuprofen and standard care. The primary endpoint is weight loss and secondary endpoints are change in muscle mass and change in physical activity.

Two studies integrated exercise intervention in anticancer therapy in patients with advanced lung cancer [74,78]. Although the authors concluded that the intervention during anticancer therapy was feasible, exercise adherence was low since on average 42% of the participants completed all exercise sessions. Patients were unable to complete mainly due to clinical deterioration, hospitalization or loss of motivation.

Up to now unimodal strategies aiming to counteract cachexia have failed to demonstrate consistent clinical benefits and results of multimodal interventions are awaited. There is a need for intervention studies recruiting patients early during their disease trajectory which are feasible for this vulnerable population and which target both metabolism, nutritional intake and muscle anabolism.

FUTURE PERSPECTIVES

When looking at the future, after a period without major innovations in the field of cachexia prevention and treatment, ongoing research at different domains have the potential to change the landscape.

Energy balance: from an intake perspective

A net catabolic state is the result from an imbalance in energy expenditure and energy intake. In order to compensate for increased energy requirements patients should be able to adapt their dietary intake. Dietary intake is controlled by the central nervous system, which integrates peripheral signals of satiety and adiposity. Brain imaging studies have revealed specific brain regions that are related to food reward-value [83,84]. Functional neuroimaging studies reported altered reactivity in the brain reward system in people with altered eating patterns, including anorexia nervosa and obesity [85-87].

In relation to cancer only one study has been performed identifying brain activity in anorectic and non-anorectic lung cancer patients. In contrast to non-anorectic patients, anorectic patients showed no brain activity differences in response to pleasant versus unpleasant food cues, implying an overall blunted response in the perceptual and motivational system. Unfortunately, the relation between blunted food reward and cachexia was not evaluated and requires further investigation [88].

Cachexia and the efficacy of anticancer treatment

There is some evidence from experimental research suggesting that cancer therapy aiming to achieve tumour control might stimulate catabolic processes in the muscle via activation of nuclear factor kappa B and upregulation of myostatin, and thereby contribute to onset or progression of cachexia [89]. Also the other way around, cachexia impacts the efficacy of anticancer treatment. For example, low muscle mass in NSCLC patients is associated with haematological toxicity [90], which is thought to be the result of a high absolute dose of chemotherapy with a reduced volume of distribution. Currently, chemotherapy dosing utilizes body surface area [91], which does not account for the distribution of muscle and adipose tissue. Some studies have tried to explore the use of body composition to guide chemotherapy dosing in patients with advanced cancer [92,93]. CT derived lean body mass might provide a basis for better dosing chemotherapy individually. However, prospective clinical trials are warrant.

Currently, the landscape of (locally) advanced NSCLC treatment is evolving rapidly. In the absence of treatable oncogenic alterations, platinum-based chemotherapy remains the cornerstone of treatment [94]. In patients with advanced NSCLC, immune checkpoint inhibitors, which unleash a patients' own T lymphocytes to kill tumours, have demonstrated to improve outcomes in stage IV patients [95-100]. More recently, immunotherapy has shown to be also promising in stage III patients following concurrent chemoradiotherapy [101], and trials regarding (neo-) adjuvant immunotherapy are ongoing (NCT03425643, NCT02998528).

Although premature, it has recently been suggested that the exposure-response relationship for immunotherapy is confounded by cachexia. A post-hoc analysis of immunotherapy treated NSCLC and melanoma patients reported that rapid clearance of pembrolizumab was strongly linked to decreased overall survival, and that those with rapid clearance exhibited more weight loss compared to those with normal-to-slow clearance [102]. Additionally, nivolumab clearance was greater in patients with lower albumin levels (mild hepatic impairment did not affect clearance) [103]. T cells rely on glucose for cytolytic activity and availability of adequate nutrients is crucial. Experimental research showed that high glycolysis within the tumour cells cause depletion of extracellular glucose which restricts T cells in its direct microenvironment [104,105]. This metabolic competition between tumour and immune cells may lead to T cell hyporesponsiveness, emphasizing the close relationship between cellular nutritional status and T lymphocyte function. Whole body catabolism such as seen in cachexia may also influence the T-cell microenvironment, however studies are lacking. Together these studies argue for a possible role for cachexia in efficacy of immunotherapy.

To conclude, the underlying mechanisms of cachexia are still poorly defined hampering effective medical interventions including targeted drug therapies. With the results of this thesis we were able to add relevant insights into the pathophysiology, but there is still potential for improvement. New molecular and clinical studies are running which hopefully prove valuable in the management of cachexia in COPD and lung cancer.

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Samenvatting

SAMENVATTING

Cachexie is een frequent voorkomend fenomeen in chronische obstructieve longziekten (COPD) en niet-kleincellig longkanker (NSCLC) en omvat het verlies van spier- en vetweefsel. Cachexie beïnvloed het klinisch beloop van de ziekte, heeft een negatieve invloed op de kwaliteit van leven en verhoogt het sterfterisico. Preventie en tijdige behandeling van cachexie vereisen adequate en vroege identificatie van risicopatiënten en vragen een gedetailleerd inzicht in de betrokken pathofysiologie. Dit proefschrift richt zich op het diagnosticeren van cachexie met behulp van beeldvorming dat wordt gebruikt in de huidige reguliere zorg en evalueert de invloed van ademhalingsmechanica en bruin vet weefselactiviteit op het energiemetabolisme in cachexie bij longziekten.

Momenteel evolueert de rol van medische beeldvorming van een diagnostisch hulpmiddel naar een uitgebreidere fenotypering van ziekten. **Hoofdstuk 2** bespreekt de mogelijkheid van nieuwe analysetechnieken die kunnen worden toegepast op traditionele beeldvormingsmodaliteiten om het begrip van de complexiteit van longziekten te verbeteren. Het extraheren van morfologische informatie en patronen van comorbiditeiten uit reeds beschikbare medische beelden die zijn gemaakt in routine klinische zorg, kan helpen om de ziektelast in kaart te brengen en om therapie te personaliseren.

Om skeletspierweefsel op computer tomografie scans te kwantificeren in de context van analyse van lichaamssamenstelling, wordt meestal het derde lumbale wervel niveau gebruikt. Echter, borstscans reiken doorgaans niet verder dan het eerste lumbale wervel niveau. **Hoofdstuk 3** laat zien dat het eerste lumbale wervel niveau kan worden gebruikt als alternatief voor het derde lumbale wervel niveau voor analyse van spiermassa op reguliere borstscans.

De huidige kennis over de pathofysiologie van cachexie bij COPD wordt in **hoofdstuk 4** besproken. Hoewel vaststaat dat verschillende componenten van het energieverbruik van het lichaam kunnen zijn verhoogd bij patiënten met COPD, is er weinig bekend over de invloed van ademhalingsmechanica en bruin vet op hypermetabolisme bij longziekten gerelateerde cachexie.

Patiënten met ernstig emfyseem hebben vaak hyperinflatie. In **hoofdstuk 5** wordt aangetoond dat succesvolle behandeling van deze patiënten met bronchoscopische longvolumereductie resulteert in toename en remodellering van skeletspieren. Bovendien is de toename van skeletspier en intramusculair vet geassocieerd met verbeterde inspanningscapaciteit na bronchoscopische longvolumereductie, onafhankelijk van hyperinflatiereductie.

Bronchoscopische longvolumereductie werd ook gebruikt om de invloed van ademhalingsmechanica op de energiebehoefte bij emfyseem te bestuderen, aangezien longvolumereductie thoracale hyperinflatie vermindert, ademhalingsfrequentie vermindert en mechanische beperkingen op longvolume expansie vermindert, waardoor de ademmechanica verbetert. **Hoofdstuk 6** laat zien dat 90% van de patiënten met hyperinflatie die in aanmerking kwamen voor bronchoscopische longvolumereductie, een lage vetvrije massa hadden, ondanks een normaal BMI. Bovendien waren de patiënten hypermetabool (130% van voorspeld) en was het energieverbruik voor fysieke activiteit erg laag wegens de zeer lage fysieke activiteit. Een mediane hyperinflatie reductie van 25% (residuale volume daalde van 236% van voorspeld naar 193% van voorspeld) verbeterde hypermetabolisme niet bij patiënten met ernstig emfyseem.

Er werd gedacht aan activatie van bruin vetweefsel (BAT) of verbruining van wit vetweefsel, als mogelijke trigger voor hypermetabolisme. Terwijl wit vetweefsel het vermogen heeft om energie op te slaan, bezit BAT de eigenschap om, middels oxidatie van vetzuren en glucose, energie vrij te zetten in de vorm van warmte. **Hoofdstuk 7** presenteert de eerste prospectieve humane studie die de rol van BAT activatie in de ontwikkeling van cachexie in COPD onderzoekt. Vergeleken met leeftijd, geslacht en BMI gematchte niet-rokende controles, vertoonden COPD patiënten geen toegenomen BAT of veranderde genexpressie van BAT-markers in wit vetweefsel. Deze studie pleit tegen een rol van activering van BAT of verbruining van wit vetweefsel als een trigger voor hypermetabolisme in COPD. In deze studie was ook beoogd om het effect van BAT op kanker gerelateerde cachexie te bestuderen, maar het was onmogelijk om voldoende proefpersonen te includeren omdat de invasieve studiemethoden niet haalbaar bleken in deze kwetsbare populatie.

In kanker-gerelateerde cachexie hebben experimentele studies gesuggereerd dat kankertherapie die gericht is op de behandeling van de tumor ook andere weefsels kan aantasten en daardoor kan bijdragen aan verlies van die weefsels. Gelijktijdige chemoradiatie, de standaardbehandeling voor irresectabel lokaal uitgebreid NSCLC, veroorzaakt vaak bestralingsoesofagitis en bijbehorende dysfagie. Er wordt daarom aangenomen dat gewichtsverlies het gevolg is van bestralingsoesofagitis resulterend in verminderde voedselinname. Desalniettemin ondervond één op de zes patiënten met een stabiel gewicht voor aanvang van de gelijktijdige chemoradiatie, gewichtsverlies voorafgaand aan het begin van bestralingsesophagitis (**hoofdstuk 8**). Dit betekent dat er andere mechanismen zijn die gewichtsverlies veroorzaken. Bovendien werden lagere overlevingspercentages aangetoond voor patiënten met vroeg gewichtsverlies.

Daarnaast kan chemotherapie zelf ook de lichaamssamenstelling beïnvloeden. **Hoofdstuk 9** laat inderdaad zien dat 69% van de patiënten met stadium IV NSCLC spiermassa verloren na twee cycli van eerstelijns chemotherapie. In vergelijking met patiënten met behouden spiermassa, vertoonden patiënten die spiermassa verloren, slechtere overlevingskansen. Een meer gedetailleerde kijk op de veranderingen in spierweefsels onthulde dat naast het verlies van spiermassa een afname van skeletspier radiatie attenuatie werd waargenomen, waarvan wordt aangenomen dat het vetinfiltratie in de spier weerspiegelt. Waarom sommige patiënten gevoeliger zijn voor spierafbraak dan anderen, vereist nader onderzoek.

In **hoofdstuk 10** werd het potentieel van radiomics beeldeigenschappen van skeletspieren onderzocht om toekomstig spierverlies te voorspellen. Radiomics is een methode om kwantitatieve kenmerken zoals vorm, grootte, intensiteit en textuur te extraheren uit medische beelden. Tot nu toe is radiomics vooral toegepast om tumorkenmerken te extraheren bij oncologische patiënten voor visualisatie van tumorheterogeniteit en het inschatten van de prognose. Radiomics beeldeigenschappen van skeletspieren waren niet voorspellend voor skeletspierverlies. We hebben echter longitudinale verschillen in radiomics beeldeigenschappen waargenomen tussen degenen die spiermassa hebben verloren en degenen die spiermassa hebben behouden. Toekomstige beeldvormingsanalyse gecombineerd met spierweefselanalyse kan de biologische processen ontrafelen die verband houden met de radiomics beeldeigenschappen.

Hoofdstuk 11 positioneert de bevindingen van het huidige proefschrift in een breder wetenschappelijk en klinisch perspectief, rekening houdend met de nieuwste ontwikkelingen op het gebied van COPD en longkanker management.

Valorisation

This chapter describes the valorisation of the research illustrated in this thesis, which is essentially the creation of value from knowledge [1,2]. This chapter therefore summarizes and reflects on how the findings of this thesis can be utilized outside the scientific field. First we will describe the main public relevance. Next, the main findings and their impact for health care and the future plans will be discussed.

According to the World Health Organization, chronic obstructive pulmonary disease (COPD) and lung cancer are both in the top 10 world's biggest killers. Globally, it is estimated that in 2015 3.17 and 1.69 million deaths were caused by COPD and lung cancer, respectively [3,4]. With ageing and rising smoking prevalence in developing nations, the prevalence of COPD and lung cancer is expected to further increase in the coming years [5,6]. Although COPD and lung cancer are primarily lung diseases, both often coexist with cachexia [7,8], which encompasses depletion of skeletal muscle and/or adipose tissue. Cachexia is associated with a range of adverse clinical outcomes including reduced exercise capacity [9], decreased health status [10] and diminished quality of life [11], it interferes with treatment efficacy [12] and impacts mortality rates [13].

Prevention and timely treatment of cachexia in patients with COPD and lung cancer requires adequate and early identification of patients at risk and detailed understanding of the pathophysiology involved. Overall, the research described in this doctoral thesis focussed on new insights in order to disentangle disrupted energy metabolism in cachexia. Cachexia is a complex process being a consequence of changes in the control of metabolism. This thesis focussed on putative triggers of increased energy metabolism in COPD and in lung cancer, amongst others with use of standard-of-care medical images.

In COPD, the prevailing believe is that increased energy metabolism is caused by high workload of breathing. An intervention to enhance breathing mechanics by reducing hyperinflation is an unique model to test this hypothesis. While this intervention to enhance breathing mechanics has resulted in improved pulmonary function and improved physical activity level, no systemic effects on the energy balance were observed.

Cachexia has long been regarded as a muscle wasting disorder, but more recently adipose tissue has started to gain attention as possible target for cachexia. In the overall disease trajectory in COPD there were no leads for a role of increased adipose tissue metabolism in cachexia. However, its role in a phase when cachexia develops progressively and patients exhibited metabolic derangements is unknown. In order to measure increased adipose tissue metabolism, extensive cold exposure followed by scanning is currently the only reliable protocol in a clinical setting. Nevertheless, this study protocol turned out to be too strenuous for the fragile cachectic population. Consequently, development of more appropriate study methodologies

to investigate the role of brown adipose tissue for this vulnerable population is subject for future research.

In the context of cachexia in lung cancer, extensive analysis of baseline standard-of-care medical images found no potential to predict future cachexia development. However longitudinally, this thesis showed a clear added value for the use of clinically acquires computed tomography scans for evaluation of body composition. Muscle wasting is clinically relevant and body composition data have the potential to improve risk stratification and personalize lifestyle interventions. It is therefore important to link longitudinal body composition analysis to evaluation of regular care images in order to gain more insight in cachexia development at an early stage. Currently, quantification of body composition occurs manually, which is a time consuming process. Automated methods will facilitate integration into regular clinical care. Thereby, imaging based tumour response evaluation during anti-tumour therapy can be combined with body composition analysis and can be captured in radiology reports. This should be combined with recording of body weight during each hospital visit of the patient in order to closely monitor and to assess risk stratification.

Optimal therapeutic intervention in cachexia depends on both proper insight into the precise mechanisms and timely identification of patients at risk. This research provided new scientific insights in the aetiology and showed a clear added value for the use of clinically acquires computed tomography scans for evaluation of body composition. Hopefully this will prove valuable for future research and contribute to optimization of current treatments and contribute to new treatment options for cachexia.

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List of publications

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Chris Burtin*, Jacques Bezuidenhout*, **Karin J.C. Sanders***, Anne-Marie C. Dingemans, Annemie M.W.J. Schols, Stephanie Peeters, Martijn A. Spruit, Dirk De Ruysscher. Handgrip weakness and low fat-free mass are prognostic factors for overall survival in patients with non-small cell lung cancer treated with curative-intent radiotherapy. J Cachexia Sarcopenia Muscle. 2020 Apr;11(2):424-431. *authors contributed equally.

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Curriculum Vitae

Karin Sanders was born on February 2nd 1987, in Eindhoven, the Netherlands. After the completion of both an economics and sociology track and a health track in secondary school, she studied Molecular Life Sciences at the Maastricht University (Maastricht, the Netherlands). She received her Bachelor's degree in 2010, and subsequently enrolled in the 4-year Physician-Clinical Investigator Research Master at the Maastricht University. In 2014 she graduated and started her Ph.D. project under the supervision of prof. dr. Schols, prof. dr. Dingemans and prof. dr. Mottaghy at the department of Respiratory Medicine at Maastricht University Medical Centre+ (Maastricht, the Netherlands). In 2019 she started with her residency training of pulmonology at the Maastricht University Medical Centre+ (Maastricht, the Netherlands) under the supervision of prof. dr. Wesseling and dr. Hochstenbag.