Università degli Studi di Napoli "Federico II"

Scuola di Medicina e Chirurgia

DOTTORATO DI RICERCA IN SANITÀ PUBBLICA E MEDICINA PREVENTIVA

CICLO XXIX

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NUTRITIONAL STATUS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE: RELATIONSHIP BETWEEN MUSCLE STRENGTH AND BODY COMPOSITION

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ABBREVIATIONS

6MWD = Six-minute walk distance

ASM = Appendicular skeletal muscle mass

ASMI = Appendicular skeletal muscle mass index

ATS = American Thoracic Society

BIA = Bioelectrical impedance analysis

BI index = Bioelectrical impedance index

BIS = Bioelectrical impedance spectroscopy

BMC = Bone mineral content

BMD = Bone mineral density

BMI = Body mass index

BODE index = BMI, obstruction, dyspnoea and exercise

CAT = COPD assessment test

COPD = Chronic obstructive pulmonary disease

CRP = C-reactive protein

CT = Computed tomography

D = Dominant

DLCO = Diffusing capacity of the lung for carbon monoxide

DXA = Dual-energy X-ray absorptiometry

ECW = Extra-cellular water

ERS = European Respiratory Society

ESPEN = European Society of Clinical Nutrition and Metabolism

EWGSOP = European Working Group on Sarcopenia in Older Persons

FEV_1 = Forced expiratory volume in 1 second

FFM = Fat-free mass

FFMI = Fat-free mass index

FM = Fat mass

FM = Fat mass index

FVC = Forced vital capacity

GLM = General linear model

GOLD = Global initiative for chronic obstructive lung disease

HGS = Hand grip strength

HRCT = High resolution computed tomography

IC = Inspiratory capacity

ICW = Intra-cellular water

IQR = Interquartile range

IR = Impedance ratio

ITGV = Intra-thoracic gas volume

LM = Lean mass

MEP = Maximum expiratory pressure

MIP = Maximum inspiratory pressure

MF-BIA = Multi-frequency bioelectrical impedance analysis

mMRC = Modified British medical research council

MNA = Mini nutritional assessment

MUST = Malnutrition universal screening tool

ND = Non-dominant

NMR = Nuclear magnetic resonance

NRS = Nutritional risk screening

PhA = Phase angle

PR = Pulmonary rehabilitation

PRISMA = Transparent reporting of systematic reviews and meta-analysis

RV = Residual volume

SD = Standard deviation

SF-BIA = Single-frequency bioelectrical impedance analysis

SGA = Subjective global assessment

SGRQ = Saint George respiratory questionnaire

TBW = Total body water

TLC = Total lung capacity

VAT = Visceral adipose tissue

VC = Vital capacity

WHO = World health organization

Z = Impedance

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a complex syndrome and an important public health challenge. Although defined as a chronic inflammatory respiratory disease, COPD is heterogeneous, being characterized by a number of systemic consequences and co-morbidities, which contribute to disease severity. Specifically, nutritional disorders (i.e. malnutrition) and nutrition-related conditions (i.e. muscle dysfunction) are highly prevalent extra pulmonary manifestations of COPD, associated with important consequences for risk assessment stratification and management of the disease.

General aim of this thesis was to investigate the occurrence of alterations of body composition and its relationship with muscle strength. More specifically, five studies on COPD patients had been carried out in order to systematically review the use of bioelectrical impedance analysis (BIA) for the assessment of body composition; to evaluate the prevalence of malnutrition and sarcopenia and their relationship with functional parameters; to compare BIA variables between COPD patients and controls and to study the association of muscle strength with body composition estimates and BIA variables. As a final point, this thesis aimed to explore the amount of visceral adipose tissue located in the abdominal region using dual-energy x-ray absorptiometry and to determine its relation with respiratory parameters and other indices of body composition.

In conclusion, this thesis provides a detailed overview of the assessment of nutritional status and body composition in COPD patients, especially in relation with respiratory function and muscle strength, bringing to light the need for prevention strategies and suggesting possible tools for the implementation of personalized approaches for COPD patients.

CHAPTER I

INTRODUCTION

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1. COPD: general introduction

1.1 Background and definition

Chronic obstructive pulmonary disease (COPD) is an important public health challenge that is both preventable and treatable. COPD represents a major cause of morbidity and mortality worldwide. It is currently the fourth leading cause of death in the world. Many people suffer from COPD and die for it or for disease-related complications (1).

According to the current definition, COPD is a common, preventable and treatable disease, characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases (2).

1.2 Burden of the disease

Prevalence

The prevalence of COPD is directly related to the prevalence of tobacco smoking, although in many countries (especially developing countries), outdoor, occupational and indoor air pollution are major COPD risk factors (3). A systematic review and meta-analysis of studies carried out in 28 countries between 1990 and 2004 (4), provides evidence that the prevalence of COPD in adults aged ≥40 years is around 9–10%, being higher in smokers and ex-smokers compared to non-smokers, in those older than 40 years compared to those younger than 40, and in men compared to women.

Mortality

COPD is one of the most important causes of death in the world. According to world health organization (WHO), COPD, which was the sixth leading cause of death in 1990, will become the third by 2020, probably reflecting the increase

in smoking habits, the reduction of mortality from other causes (e.g. infectious diseases), as well as aging of the world population (5). However, data must be interpreted with caution due to the under-recognition and under-diagnosis of COPD, which reduces the accuracy of mortality data (6, 7).

1.3 Risk factors

COPD is a chronic inflammatory process in the lower airways and the lung parenchyma that results from a complex interaction between environment and genes. The most important environmental risk factor for COPD is cigarette smoking (8), but other types of tobacco (e.g. piper, cigar, water pipe) (9, 10) and marijuana (11) are also risk factors for COPD. Passive exposure to cigarette smoke may also contribute to COPD risk (12). Other modifiable documented factors may increase the risk of COPD, i.e. occupational airborne exposure (occupational dust, chemicals and vapors derived from mining, agriculture, and textile, paper, wood, chemical, and food processing), outdoor and indoor pollution, socioeconomic status and early life environmental factors (e.g. smoking mothers, frequent respiratory infections and asthma in childhood).

Besides environmental modifiable risk factors, genes may also play a role in the development of COPD. The genetic risk factor that is best documented is hereditary α1-antitrypsin deficiency (13), a protease inhibitor belonging to the serpin superfamily, which protects tissues from enzymes of inflammatory cells, especially neutrophil elastase. However, homozygous α1-antitrypsin deficiency is very uncommon, being found in fewer than five people per 10000.

1.4 Diagnosis and Assessment

Diagnosis

In presence of symptoms such as dyspnoea, chronic cough, chronic sputum production, recurrent lower respiratory tract infections and/or history of risk

factors, which can increase the probability of the disease, diagnosis of COPD should be considered. Diagnostic criterion is officially based on spirometry, the most reproducible and objective method for the assessment of airflow limitation. It measures the volume of air exhaled from the point of maximal inspiration (forced vital capacity, FVC) and the volume of air exhaled during the first second of this procedure (forced expiratory volume in 1 second, FEV_1). A reduction of the ratio between FEV_1 and FVC (<0,70) confirms the diagnosis of COPD (1).

Disease severity

Specific spirometric cut-points are currently used in order to classify the severity of airflow limitation in COPD (table 1). However, it has been recently reported only a week correlation between spirometrc data, symptoms and impairment of patient's health status (1, 14). Hence, more extended symptomatic assessment is required. The two measures of symptoms most widely used are the Modified British Medical Research Council (mMRC) questionnaire and the COPD Assessment Test (CAT). The former is a simple measurement of breathless, which ranges from 0-4, being related well with health status (15) and mortality risk (16). The latter is an 8-items unidimentional measure of health status impairment in COPD (17), whose score ranges from 0-40 and correlates very well with health status, depression and mortality (18).

In this contest, a new ''ABCD'' assessment tool based on dyspnoea and symptoms (mMRC and CAT), in combination with spirometric data and history of exacerbations, has been proposed in the latest 2017 GOLD document (1).

Assessment of comorbidities

Although the most important symptoms of COPD are breathlessness on exertion and chronic cough, patients with COPD often suffer from other diseases (i.e. hyperglycemia, atherosclerosis, hypertension, dyslipidemia, osteoporosis,

obesity, sarcopenia, renal impairment and anxiety), linked to the same risk factors, i.e. smoking, ageing and inactivity.

Airflow limitation affects gas exchange and cardiac function (19). At the same time, the chronic low-grade inflammation may contribute to weight loss, skeletal muscle wasting and cachexia (20-22) and may cause or worsen comorbidities such as ischaemic heart disease, heart failure, osteoporosis, anaemia, hyperglycemia and metabolic syndrome (23).

Comorbidities were found to be present in the COPD population with frequencies that ranged from 5–54%, with hyperglycemia, atherosclerosis, hypertension, dyslipidemia, and osteoporosis among the five most prevalent comorbidities (24).

Phenotyping

COPD is a syndrome with many phenotypes that influence the clinical progression of the disease. In general terms, a phenotype is an observable characteristic of an organism, which is determined by its genotype and modulated by its environment (25). A clinical COPD phenotype has been defined as "A single or combination of disease attributes that describe differences between individuals with COPD as they relate to clinically meaningful outcomes (e.g. symptoms, exacerbations, response to therapy or death)" (26).

Although a COPD phenotype describes differences between individuals with COPD, clinical phenotyping may be problematical for several reasons. First, some clinical phenotypes may change due to the natural course of the disease or, in response to the therapy. Furthermore, a certain patient can have more than one clinical phenotype. Finally, two prevalent respiratory diseases can coexist (e.g. COPD and obstructive sleep apnoea or asthma) (2).

Only few clinical COPD phenotypes have been validated. They include α_1-antitrypsin deficiency, frequent (two or more per year) exacerbations, chronic bronchitis and emphysema (2). Furthermore, besides the classic description of the clinical phenotypes, several nutritional phenotypes have been recently proposed in COPD by Schols et al., indicating the need for a multidimensional approach (27).

2. Nutritional status in COPD

Although defined as a chronic inflammatory respiratory disease, COPD is heterogeneous, with respect to pulmonary events, systemic consequences (e.g. weight loss and muscle weakness) and co-morbidity (e.g. osteoporosis, diabetes and cardiovascular disease), which contribute to disease severity (28). Nutritional disorders (i.e. malnutrition) and nutrition-related conditions (i.e. sarcopenia) are highly prevalent extra pulmonary manifestations of COPD, associated with important consequences for health risk assessment, stratification and management of the disease.

In this perspective, nutritional assessment is essential and highly recommended in COPD management (2), in order to develop targeted and personalized nutritional interventions.

2.1 Malnutrition

Definition

According to the latest European Society of Clinical Nutrition and Metabolism (ESPEN) guidelines, malnutrition can be defined as "a state resulting from lack of intake or uptake of nutrition that leads to altered body composition and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease" (29).

Malnutrition is a common and serious problem and one of the most relevant systemic manifestation of COPD. Loss of body weight occurs when energy expenditure exceeds energy intake. In COPD, eating is an activity that can increase dyspnoea in patients with severe disease (30). Ageing is also a contributing factor to reduced energy intake in COPD, probably due to its typical symptoms, as loss of taste, poor dentition, dysphagia, poor chewing and swallowing ability; social problems as living or eating alone; and inability to

self-feed (31). Anorexia is, however, not the only determinant of a reduced energy intake in underweight COPD patients (32, 33). In fact, while the normal response to semi-starvation is a reduced metabolic rate and depressed whole-body protein turnover, COPD patients may show increased resting energy expenditure and whole-body protein turnover (34). Furthermore, in addition to an increased cost of ventilation due to abnormal pulmonary mechanics, a higher ATP cost of muscular contraction, due to the muscle fiber shift from type I (oxidative) to type II (glycolytic), may contribute to the elevated daily energy expenditure in some COPD patients (35).

Malnutrition is an important determinant of other the extra-pulmonary effects, including skeletal muscle dysfunction, osteoporosis, cardiovascular disease and poor prognosis (36, 37). In addition, it contributes to a further phenotyping of these patients.

Diagnosis

Several criteria can be used to capture the state of malnutrition, including weight loss, low BMI, anthropometry (mid-arm circumference, calf circumference, triceps skinfolds thickness), body composition (fat-free mass), loss of appetite, reduced food intake and biochemical indicators (albumin, prealbumin, total lymphocyte count) (38-40).

Actually, according to the latest ESPEN consensus statement on malnutrition (41), three variables were chosen to most accurately reflect malnutrition, specifically weight loss, reduced BMI and reduced FFMI. With this perspective, Schols et al. (27) established a nutritional risk diagram for COPD patients, based on prospective assessment of body weight (change) and body composition.

In addition, several nutritional screening tools have been developed for rating the nutritional status of elderly (table 2), such as Malnutrition Universal Screening Tool (MUST), Nutritional Risk Screening (NRS), Mini Nutritional

Assessment (MNA) and Subjective Global Assessment (SGA). Those tools can be used with the purpose of identifying patients at nutritional risk, in order to detect those that will need further nutritional assessment and that will need nutritional therapy.

MUST

The MUST is a nutritional screening tool, validated for use in hospitals (42). It is based on three criteria, BMI, unintentional weight loss over the previous three to six months and significant decrease in food intake associated with acute illness for a period of more than five days (43). The final score of the MUST was obtained by adding the scores for each of the three parameters evaluated, ranking undernutrition risk as low, moderate or high, if the score is 0, 1, or higher than or equal to 2, respectively (43). Different screening and nutritional evaluation tools have been compared, including MUST, MNA, SGA, SGA and NRS in hospital, showing that MUST compared to the other tools reviewed had reasonable to excellent validity, as well as being quick and easy to use and it also demonstrated excellent reproducibility among different users (42).

NRS

The NRS score is the sum of the scores in disease severity, nutritional status, and age, and range between 0 and 7. Disease severity of the patients was scored based on the diagnosis records at admission. The nutritional status of each patient was scored based on the changes in body weight and BMI in the recent three months and food intake in the recent 1 week. Furthermore, 1 score was assigned to the patients who were more than 70 years old. According to the NRS score, the patients are divided in those at nutritional risk (if NRS score≥3) and those not at nutritional risk (NRS score<3). Previous studies supported the association between exercise capacity and nutritional risk according to NRS in severe COPD male patients (44). However, more studies are needed to confirm this relationship in COPD.

MNA

The MNA is one of the most widely used ones. It is an easy, low-cost, fast and non-invasive tool that has been shown to perform well in elderly living with various health conditions (45). The MNA consists of 18 items and evaluates 4 areas of nutritional status—anthropometric, dietary, global and self-rated status. The MNA has a maximum score of 30. A score $\leq$ 16.5 suggests malnourishment; 17–23.5 suggests at risk of malnutrition, and $\geq$ 24 suggests normal (46), suggesting a role of MNA for gaining an insight into the nutritional problems in patients with COPD, providing useful indications for treatment strategies. However, only a few studies have used the MNA to estimate nutritional status of patients with COPD (37, 47-50). According to this nutritional screening tool, 14% of patients with COPD were found to be malnourished and another 55% at risk of malnutrition (49). A positive association of MNA score with lean body mass (49) has been previously proposed, as well as a negative association with dyspnoea in 32 $\geq$ 60-year-old patients with COPD (48). Furthermore, MNA was shown to be an independent predictor of FFMI, being significantly correlated with most other anthropometrics, lung function and exercise capacity indicators (37).

SGA

SGA of nutritional status has been validated as a screening tool to identify patients at risk of malnutrition (51). In Based on SGA, patients can be categorized as well nourished (Category A, SGA-A), mild/moderately undernourished (Category B, SGAB) or severely undernourished (Category C, SGA-C). SGA has been applied successfully as a method of assessing nutritional status in patients with several health-related conditions, i.e. cancer and dialysis, and correlated with objective parameters (anthropometric, biochemical and immunological), measures of morbidity, length of hospital stay, use of medications and quality of life. It also has a high degree of reproducibility (52). However, only few studies has been published on the use

of the SGA in COPD patients (53, 54), showing that SGA scores correlated positively with pulmonary function parameters and negatively with anthropometric parameters.

Nevertheless, an important difference between the risk stratification diagram and these nutritional screening tools is that the latter primary focus on malnutrition and do not take abnormal body composition into account.

2.2 Nutritional phenotypes in COPD

Supporting the concept that body weight and body composition are part of the heterogeneity of the disease, and predictors of outcome independent of lung function impairment (27), a European Respiratory Society Task Force recently identified different nutritional phenotypes of COPD (i.e. cachexia, sarcopenia and sarcopenic obesity) (27).

Cachexia

Although often incorrectly perceived as end-stage malnutrition, the cachectic phenotype has been recently defined by weight loss (>5% in 6 months), reduced BMI and reduced muscle mass and function in combination with an underlying disease that displays biochemical indices of on-going elevated inflammatory activity (as revealed by for example increased serum CRP levels response) (29).

Cachexia occurs frequently in patients with COPD, due to the catabolic inflammatory responses. Besides "generic" cachexia triggers including oxidative stress, inflammation, disuse and malnutrition, also disease specific determinants including hypoxia and glucocorticoids may play a role in particular during advanced disease. Furthermore, a muscle fiber type I to II shift, with a consequent decrease in oxidative capacity has also been observed in COPD (55).

Sarcopenia

Sarcopenia is defined as a syndrome characterized by the progressive and generalized loss of skeletal muscle mass, strength and function (performance) with a consequent risk of adverse outcomes (29). According to European Working Group on Sarcopenia in Older Persons (EWGSOP) (56), the ESPEN Special Interests Groups of Cachexia in Chronic Disease and Nutrition in Geriatrics (57), diagnostic criteria for sarcopenia include loss of muscle mass and strength and/or function. In contrasts with EWGSOP international consensus statements, most studies describing sarcopenia in COPD have focused on only one aspect, predominantly the loss of skeletal muscle mass (58-60), which is clearly considered insufficient. Only one paper examined sarcopenia in COPD using EWGSOP criteria (61), finding a prevalence of sarcopenia of 14.5%, which increased with age and GOLD stages

Sarcopenic obesity

Sarcopenic obesity is defined as obesity in combination with sarcopenia. Mechanisms include inflammation and/or inactivity induced muscle catabolism in obese patients (62). Currently, there are no commonly accepted criteria for sarcopenic obesity beyond those for sarcopenia and obesity separately (29).

3. Body composition in COPD

The assessment of body composition has gained a central role into nutritional assessment of COPD patients. Indeed, several studies have shown the importance of adequate assessment of body composition in COPD patients (63).

While originally considered an indicator of terminal progression of the disease, it is now well known in literature that unintentional weight loss is not an adaptive mechanism to decrease metabolic rate in advanced COPD (64) but an independent predictor of outcome independent of lung function impairment (24).

Several measurements have been considered as potential prognostic indicators. For example, unintentional weight loss and low body mass index (BMI) are associated with significantly worse clinical outcomes (65). Nevertheless, muscle or fat-free mass measurements may be even better predictors of clinical outcomes (66), being associated with impaired physical performance. In addition, a pivotal role of osteoporosis and visceral adiposity in COPD risk and progression has emerged, which places the assessment of body composition as integral part of disease management (27).

With this purpose, the method used is very important, but undoubtedly the criteria for this choice will depend not only on the advantages and disadvantages offered by each one, but also on the patient's limitations, the conditions of the health system and the purpose of the study (67).

Although there is no exact standard diagnostic procedure to evaluate body composition, different methods can be used in clinical practice: anthropometry (weight, height, skinfolds/circumferences measurements), bioelectric impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA) as well as more advanced imaging technologies like computed tomography (CT) and nuclear magnetic resonance (NMR).

3.1 Body mass index (BMI)

Body mass index (BMI: body weight/height^2) is a fairly reliable indicator of body fatness for most people. Hence, BMI is just a variable used to give some indications about body build or shape and about leanness or fatness.

In COPD patients BMI is an important index of malnutrition and disease severity (68-70). Thus, either as a single measure or part of composite score – such as BODE index –, it remains a widely used variable in clinical practice (69, 71-73).

Reduced BMI is seen in COPD patients compared to control subjects (49, 74-77). In the same way, an increased BMI has been described in chronic bronchitis patients compared to both emphysema patients and healthy control subjects (78). Several studies have reported a depletion in nutritional status of COPD patients in terms of BMI <21 kg/m^2 (34, 69, 70, 79-81), or <20 kg/m^2 (54, 74), or <22 kg/m^2 (82).

Low body mass index (<21 kg/m^2) is related to poor exercise tolerance (83), low fat-free mass (FFM) (24) and co-morbid osteoporosis (84). Furthermore, it is incorporated in the prognostic assessment of COPD (71). On the other hand, high BMI ($\geq$30 kg/m^2) is related to increased dyspnoea (85), reduced weight-bearing exercise performance (86) and systemic inflammation in COPD (24). At the same time, reductions in lung volumes have been consistently reported in obese COPD patients (87).

BMI has been shown to be higher in GOLD stages I and II compared with stages III and IV. (50) In fact, low BMI is more prevalent (52%) in the subjects with severe COPD as compared to mild and moderate COPD (26.2%) (88).

From a clinical point of view, in patients with moderate to severe airflow obstruction, a BMI <25 kg/m^2 was consistently associated with increased mortality risk relative to overweight and even obese patients (65, 69, 89). This prognostic advantage of increased BMI in COPD, also referred to as the

''obesity paradox'', could be related to the direct effect of adipose tissue on lung mechanics (e.g. relative reduction in static volumes) in obese COPD patients (27, 90).

Nevertheless, although considered a reliable indicator of body fatness for most people and an important indicator of malnutrition and disease severity in COPD patients (68, 70), weight changes and BMI do not take body compositional shifts, including FFM, appendicular skeletal muscle mass (ASM), fat mass (FM), fat distribution and bone mineral mass into account (27).

In order to quantify the impact of COPD on the different body compartments further techniques are required.

3.2 Fat-free mass (FFM)

The two-compartment model of body composition divides body mass into FM and FFM. FFM can be further divided in an intracellular compartment, which includes muscle mass, bone mineral mass, and other metabolizing tissues, and an extracellular fluid compartment (91). FFM can also be expressed in absolute terms or as percentage of body weight. In COPD patients a height-normalized index, called FFM index (FFMI) [FFM (kg)/height $(m)^2$] is often used.

In order to identify an abnormally low FFMI, several COPD-specific cut-offs were reported in the last decades, based on well-established adverse effects of low FFMI on physical performance and survival (83, 92).

A decrease of FFM was observed in 4 to 35% COPD patients and may occur independently of preserved or even increased BMI (83, 92).

From a clinical perspective, low FFM of FFMI, independently of BMI and fat mass, may have adverse effects on health status (93), increasing the frequency or severity of acute exacerbations of the disease, and is a strong predictor of mortality (27, 94, 95).

3.3 Appendicular skeletal muscle mass (ASM)

Appendicular skeletal muscle mass (ASM), is the sum of the FFM of upper and lower limbs.

Loss of ASM is frequent in COPD, with a prevalence of nearly 30% among all patients, being higher in those with GOLD stage III-IV, compared to GOLD stage I-II (96).

Among the factors that have been implicated as potential triggers of muscle wasting in COPD there are hypoxemia, malnutrition, inflammation and endogenous as well as synthetic glucocorticoids (21, 65, 97, 98).

In addition to ASM depletion, several studies have also shown considerable modifications in the skeletal muscle histochemical characteristics. In general, biopsies of the quadriceps muscle have shown a reduced proportion of type I fibers and an increase in the proportion of type II fibers as compared with normal individuals (99, 100). This change in fiber proportion reduces muscular oxidative capacity increasing fatigability and reducing muscle endurance (101). In fact, COPD patients had been found to show an altered fiber-type distribution, with a shift towards a higher proportion of type II fibers with respect to reported normal values (type I 56.7% and type II 43.3%) (102). The same study also demonstrates that in COPD patients' health status – calculated using Saint George Respiratory Questionnaire (SGRQ) – correlates with peripheral muscle fiber type composition. This data suggest that worse health status is associated with less proportion of type I fibers which are fundamentally oxidative, indicating that pathophysiological changes in peripheral muscles may influence SGRQ total score independent of the degree of airflow limitation.

Although several reference methods (DXA, CT, NMR) are available to evaluate lean tissues (ASM and FFM), BIA is a bedside approach, being widely investigated in clinical research due to the affordability, portability, and ease of use of bioimpedance devices (103-105).

Three predictive equations for the general population available in the literature (106-108) (no disease specific equation has been so far developed yet).

Actually, while BIA has been used to estimate FFM in more than 100 papers on COPD (personal database) by using different predictive equations, to the best of the authors' knowledge, only one paper used BIA to estimate ASM (61).

3.4 Bone mineral density (BMD)

Low bone mineral density (BMD) has been recognized as one the most common comorbidities of COPD. A recent meta-analysis of 13 studies in patients with COPD indicated the overall mean prevalence of low bone mineral density of 35.1% (range 9–69%) (109). Proposed mechanisms that likely contribute to increased risk of low BMD in COPD include systemic inflammation, decreased physical activity owing to dyspnoea, low BMI, redunctions in pulmonary function and/or other factors leading to proteolysis, reduced bone formation and increased bone resorption (84, 110-113). Furthermore, pathogenetic links between adipose tissue and bone formation/destruction has recently emerged (114-116). Adiposity, expressed as FM index (FMI), was shown to be positively related to BMD, especially in non-obese patients (116). Both mechanical and endocrine effects of the adipose tissue seem to actively participate to the complex mechanism that regulate bone metabolism (115). Whereas most of the research on the association between fat mass and BMD has focused on total fat mass, it is not clear whether and to what extent abdominal fat, is associated with bone status. Abdominal fat mass was found to be associated with lower BMD (117).

3.5 Body fat and body fat distribution

The overall prevalence of obesity in a large primary care population of patients with COPD in the Netherlands has been found to be 18%, with the

highest prevalence in GOLD stages I and II (16–24%) and the lowest in GOLD stage IV (118).

Generally speaking, in any given subject the effects of obesity on respiratory function depend on the anatomical distribution of the excessive body fat between upper and lower body (119). In healthy obese subjects, abdominal fat mass is recognized as a source for low grade systemic inflammation (defined by plasma CRP concentration) (120) and a determinant for cardiovascular risk (121). However, in spite of the wide interest in muscle mass and muscle wasting, only few studies have focused on body fat and body fat distribution in COPD.

Rutten et al., carefully evaluated fat mass distribution in a large group of patients with moderate to severe COPD, demonstrating that patients with abdominal obesity had higher plasma CRP concentration, diffusion capacity and FEV_1/FVC. No differences in terms of the other lung function parameters were found in the same study, indicating no association between disease severity per se and abdominal FM between the patients with abdominal obesity and those without abdominal obesity.

More specifically, within the abdominal region, visceral adipose tissue (VAT) is now recognized as a key feature of metabolic syndrome metabolic syndrome, diabetes, and cardiovascular disease (122-128). Furthermore, since cardiovascular disease is one of the main comorbidities in COPD, together with type II diabetes, skeletal muscle dysfunction and osteoporosis (24), VAT is receiving increasing attention in clinical assessment of COPD patients.

4. Muscle strength in COPD

Loss of muscle strength (i.e. the ability to develop a maximal effort) or muscle weakness is highly prevalent in COPD. It is one of the most common and best studied systemic manifestations of COPD, being frequently associated with nutritional disorders (i.e. malnutrition) and nutrition-related conditions (i.e. sarcopenia). Muscle weakness can affect both respiratory and limb muscles, probably due to deconditioning, smoking, malnutrition, systemic inflammation, hypoxemia, hypercapnia/acidosis and some drugs such as steroids (129, 130). It carry several important consequences in COPD patients, such as poor quality of life, exercise intolerance, premature mortality and increased health care use (131).

4.1 Respiratory muscles

Respiratory muscles are those involved in ventilation and include inspiratory and expiratory muscles. The former, mainly consisting of the diaphragm, external intercostal and parasternal muscles, generate a more negative pleural and alveolar pressure with their contraction and allow the air to enter into the lungs. The latter are those located in the abdominal wall and the internal intercostals (129). In normal conditions, the relaxation of inspiratory muscles is sufficient to cause expiration, but when an extra expulsive effort is needed, expiratory muscles are recruited. This happens, for example, in the advanced stages of COPD.

Many COPD patients show a reduction in the strength of respiratory muscles (132, 133), with a prevalence of 20–45% in patients with a stable disease (133), and 80–90% in patients with frequent exacerbation (134).

Respiratory muscle weakness can be caused by malnutrition, hypoxemia, steroid myopathy or oxidative stress and requires a specific patient-addressed treatment based on nutritional supplements and muscle training (135).

4.2 Limb muscles

Limb muscles are those involved in ambulation and other important activities of daily life and include muscles of the upper and lower extremities.

Limb muscle weakness's prevalence rate approximates 20% in patients with mild/moderate COPD and 40% in severe disease, with a loss of quadriceps strength of 25% (136), compared to healthy controls. Muscle weakness distribution among muscle groups is not homogeneous. In fact, the strength of the strength of upper limb muscles seems better preserved than that of lower limbs among patients with COPD (130, 137).

Limb muscle strength is a determinant of physical activity and functional capacity in COPD (138). On the other hand, weakness is associated with poor outcomes such as exercise intolerance (139, 140), morbidity, mortality as well as increased health care service use, dyspnoea, and worse quality of life (93, 141).

5. Tables and figures

Table 1. Classification of airflow limitation severity in COPD

GOLD I:	Mild	FEV1/FVC < 0.70 and FEV1 $\geq$ 80% predicted
GOLD II:	Moderate	FEV1/FVC < 0.70 and 50% $\leq$ FEV1 $\leq$ 80% predicted
GOLD III:	Severe	FEV1/FVC < 0.70 and 30% $\leq$ FEV1 $\leq$ 50% predicted
GOLD IV:	Very severe	FEV1/FVC < 0.70 and FEV1 $\leq$ 30% predicted

Abbreviations: GOLD, Global initiative for chronic obstructive lung disease; FEV1, forced expiratory volume in 1 second; FVC; forced vital capacity.

Table 2: Screening tools for rating the nutritional status of elderly patients

Screening tool	Clinical parameters	Score
MUST	BMI Weight loss Presence of acute disease	0, low risk of malnutrition; 1, medium risk of malnutrition; 2, high risk of malnutrition
NRS	Age adjustment (≥70 years) Nutritional score: weight loss, changes in food intake, BMI, general condition Severity of disease score	If score <3, absent, mild or moderate risk of malnutrition; if score≥3, severe risk of malnutrition
MNA	Nutritional status items: anthropometric, dietary, global and self-rated status.	Score between 24-30, normal nutritional status; score between 17–23.5 risk of malnutrition; score ≤ 16.5, malnourishment; score between
SGA	Questionnaire: weight loss, changes in dietary intake, gastrointestinal symptoms, functional capacity Physical examination: muscle, subcutaneous fat, sacral and ankle oedema, ascites Clinician's overall judgement	Stage A, well-nourished; stage B, moderate or suspected malnutrition; stage C, severe malnutrition

Abbreviations: BMI, body mass index; MUST, Malnutrition Universal Screening Tool; NRS, Nutritional Risk Screening; MNA, Mini Nutritional Assessment and SGA, Subjective Global Assessment.

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CHAPTER II

AIM AND OUTLINE OF THE THESIS

SUMMARY: 1. General and specific aims – 2. Outline of the thesis.

1. General and specific aims

Given the important role of nutritional status as an independent determinant of outcome in COPD patients, this thesis has been carried out with the general aim of better understanding the occurrence of alterations of body composition and its relationship with muscle strength.

In this perspective, a number of studies on COPD patients has been carried out with the specific aims of:

- Systematically reviewing the use of bioelectrical impedance analysis (BIA) in the assessment of body composition and nutritional status.

- Evaluating the prevalence of malnutrition and sarcopenia, as defined by the European Society for Clinical Nutrition and Metabolism (ESPEN), and to determine their relationship with functional parameters and raw BIA variables.

- Evaluating body composition using multi-frequency (MF-BIA), in order to compare raw MF-BIA data between COPD patients and controls and to study their relationship with respiratory and functional parameters in COPD patients.

- Studying the association of muscle strength with body composition estimates (i.e. FFM) and raw BIA data (i.e PhA), in order to better understand whether PhA is a predictor of HGS and respiratory muscle strength in COPD patients, independently from anthropometric variables and BIA-derived body composition estimates.

- Assessing visceral adipose tissue using dual-energy x-ray absorptiometry determine its relation with disease severity, BMI, other indices of body composition and static lung volumes.

2. Outline of the thesis

The first study of the thesis is a systematic review, which aimed to investigate the use of BIA in COPD, in order to better understanding how to

interpret BIA data in these patients and how to apply BIA-derived body composition estimates for the diagnosis malnutrition or sarcopenia in COPD.

The second study is cross-sectional study, which aimed to assess the prevalence of malnutrition and sarcopenia, as defined by the European Society for Clinical Nutrition and Metabolism (ESPEN), in COPD and to determine their relationship with functional parameters and raw BIA variables.

The third study is a case-control study. The aim was to compare body composition, using a multi-frequency BIA, between COPD patients and controls and to study the relationship of raw multi-frequency BIA data with respiratory and functional parameters in COPD patients.

The fourth study is a cross-sectional study, which aimed to assess whether PhA, as a raw BIA data, is a predictor of peripheral and respiratory muscle strength in COPD patients, possibly stronger than anthropometric variables and BIA-derived body composition estimates.

The fifth study is a cross-sectional study carried out in order to determine the relation of VAT by dual- energy x-ray absorptiometry (DEXA) in patients with COPD by disease severity, BMI, other indices of body composition and static lung volumes.

CHAPTER III

SYSTEMATIC REVIEW ON THE USE OF BIOELECTRICAL IMPEDANCE ANALYSIS IN COPD

Summary: Abstract − 1. Introduction and aims − 1.1 Single frequency BIA − 1.2 Multifrequency BIA − 2. Methods − 2.1 Inclusion and exclusion criteria − 2.2 Search, screening and selection of included papers − 2.3 Statistical analysis − 3 Results − 3.1 Description of the papers − 3.2 BIA approach − 3.3 Body compartments estimated − 3.4 Raw BIA data − 3.5 Diagnosis of nutritional disorders – 4. Discussion – 5. Figures and tables − 6. References.

Abstract

Bioelectrical impedance analysis (BIA) is as widely used method for evaluating body composition in patients with chronic obstructive pulmonary disease (COPD). However, there is a considerable lack of information on how to apply BIA-derived body composition estimates in order to diagnose malnutrition or sarcopenia in COPD. Furthermore, it is not clear how to interpret raw BIA data in these patients.

The aim of this study was to investigate the use of BIA in COPD, according to the Transparent Reporting of Systematic Reviews and Meta-Analysis (PRISMA) criteria.

A review of original studies published between 1985 to 2016 was undertaken using PubMed and SCOPUS.

Ninety-one original papers were found to be relevant to the research. In the literature there were one COPD-specific BIA equation to predict total body water and four to predict fat-free mass (FFM). These latter was used in 35 papers, while non-specific BIA equations were used in 9 papers and those provided by the manufacturer in 13. Twenty-three papers did not specify which equation was used. Fifty-four studies evaluated both FFM and FFM index (FFMI), 19 only FFM and 7 only FFMI. Appendicular skeletal muscle mass (ASMM) was evaluated with BIA in 5 papers. Only five papers provided data on raw BIA variables (in particular, 3 papers on phase angle, 1 paper on bioelectrical impedance index and 1 paper on multi-frequency impedance ratio). Finally, according to different diagnostic criteria, BIA derived FFM, FFMI or ASMM were used for identifying the occurrence of reduced skeletal muscle mass (6 papers), cachexia (7 papers) or sarcopenia (4 papers).

Most studies did not used COPD-specific BIA equations to predict FFM in COPD patients. Although the increasing interest in the clinical setting, few data are available on raw BIA variables. Available data suggest that BIA may be

useful for diagnosing nutritional phenotypes such as cachexia or sarcopenia in COPD patients.

1. Introduction and aims

Several studies have shown the importance of adequate assessment of body composition in COPD patients. The method used is very important, but undoubtedly the criteria for this choice will depend not only on the advantages and disadvantages offered by each one, but also on the patient's limitations, the conditions of the health system and the purpose of the study (1).

Although there is no exact standard diagnostic procedure to evaluate body composition, different methods can be used in clinical practice: anthropometry (skinfolds/circumferences measurements), bioelectric impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA) as well as more advanced imaging technologies like computed tomography (CT) and nuclear magnetic resonance (NMR).

Bioelectrical impedance analysis (BIA) is a simple, inexpensive, quick and non-invasive technique for evaluating body composition, widely used in patients with COPD. Like most body composition methods, BIA does not directly measure body composition, but provides indirect estimates from the measurement of resistance of body tissues to an electric current passing through the body (2-4).

BIA variables directly measured by the analyzer are impedance (Z) and phase angle (PhA). Resistance and reactance are derived parameters. Z is resistance of the biological compartments to an alternating electric current (5). PhA reflects the relative contributions of fluid (resistance) and cellular membranes (capacitance) of the body (6). In addition, bioelectrical impedance index (BI index), calculated as squared height divided by impedance at 50 kHz, represents an established parameter of TBW(7).

1.1 Single frequency BIA

Single frequency BIA (SF-BIA) devices measure impedance variables (i.e., Z, and PhA) at a single frequency, typically 50 kHz.

SF-BIA is useful to estimate a body compartment (i.e. total body water, TBW and FFM) in patients with COPD, using several disease-specific predictive equations (8-11), which include BIA variables. However, general predictive equations and manufacturer's equations have also been frequently used. The choice of a prediction equation (from the abundant literature on the topic) that ideally matches the patient that he or she wishes to assess, is one of several limitations to the application of SF-BIA in the clinical setting (12).

Alternatively, as suggested by a growing number of authors (12, 13), a potentially useful approach to the use of SF-BIA might be to use the raw data generated, in particular, PhA.

1.2 Multifrequency BIA

Multi-frequency BIA (MF-BIA) devices measure Z at several frequencies, usually in the range between 1-1000 kHz (13).

Since at low frequency the current cannot pass through the cell membrane due to its capacitance, low frequency currents are conducted only through extra-cellular water (ECW), whereas high frequency currents penetrate cell membranes and are thus used to estimate total body water (TBW) (14). As a consequence, high to low frequency impedance ratio (i.e. Z at 250 kHz / Z at 5 kHz) is a raw MF-BIA variable which provides information on water distribution between intra and extra-cellular compartments and therefore on body cell mass and muscle quality (15-17).

Although BIA is largely used both in research and in clinical practice, there is considerable lack of information on how to apply BIA-derived body

composition estimates in order to diagnose malnutrition or sarcopenia in COPD. Furthermore, it is not clear how to interpret raw BIA data in these patients.

To our knowledge, no recent systematic review of the international scientific literature has been published yet. In this perspective, we aimed to systematically investigate the use of BIA in COPD, according to the Transparent Reporting of Systematic Reviews and Meta-Analysis (PRISMA) criteria.

2 Methods

A systematic literature search was performed to identify published studies, which used BIA for assessing body composition in patients with COPD, using specified inclusion and exclusion criteria.

2.1 Inclusion and exclusion criteria

Type of studies

Cross-sectional, case-control, longitudinal and intervention studies were included. If appropriate, reviews, case reports, editorials and comments were considered only for the discussion.

Types of participants

Studies assessing body composition using BIA in COPD patients of any age or nationality were considered. Studies on healthy controls were also included when compared to COPD. Studies involving samples of patients suffering from any respiratory disease different from COPD were excluded.

Other criteria

Studies included were limited to those published in the English language. We limited the search to articles regarding humans. Studies published in abstract or thesis format only were excluded.

2.2 Search, screening and selection of included papers

A comprehensive and systematic search of the literature was conducted on PubMed and Scopus by two independent reviewers, for relevant papers published from 1985 to August 2016, using the following keywords: "BIA or "biompedance" or "bioelectrical impedance analysis" and "chronic obstructive

pulmonary disease" or "COPD". Additional publications were identified from references provided in original papers.

Two reviewers screened the title and abstract of each study independently against the inclusion/exclusion criteria.

Two reviewers then screened full texts of remaining articles against the review criteria. Where disagreements were acknowledged, a third reviewer made a final decision.

2.*3 Statistical analysis*

Data from each included study were extracted and checked by two reviewers into standardised tables. Data extracted included information regarding sample characteristics, type of BIA device, raw variables measured, body compartments estimated, diagnosis of nutritional disorders, relation of body composition with respiratory function or other outcome. Statistical analysis was performed using SPSS version 20.0.

3 Results

3.1 Description of the papers

After checking for duplicates, 141 potential articles were initially included. Of those, 39 papers were excluded because they were not of interest or published in different languages from English. From the resulting 102 articles, 4 were excluded because they were reviews, 3 because conference papers, 1 because case report, 1 because book chapter and 1 because pilot study. Furthermore, one study was excluded after full-text review because of a not clear use of BIA for assessing body composition. None of the papers had a study design that could infer causality. Finally, 91 original papers met the review criteria (figure 1). An overview of the studies included in this review is reported in table 1.

Study design

Sixty-nine papers had a cross-sectional design, of which 22 had also a control group. Seventeen papers had a longitudinal design, 7 retrospective and 10 prospective. Of these latter, 2 were also case control studies. Furthermore, a total of 5 intervention studies was included.

Country of the study

The majority of the studies presented data from Europe (23 Dutch, 6 Sweden, 6 Norwegian, 5 Italian, 4 German, 3 French, 3 Polish, 2 Slovakian, 1 Czech, 1 Danish, 1 Greek, 1 Romanian, 1 Spanish and 1 Swiss). Moreover, there were 8 papers on Brazilian data, 8 on data from United Kingdom, 4 Chinese papers, 4 from Japanese, 1 from Taiwan, 1 from Russia, 1 from Taiwan, 1 from USA. Furthermore, 3 multicentric studies were also analysed

3.2 BIA approach

Fifty-five studies used a SF-BIA device, twenty-eight a MF-BIA device and four studies a BIS device. Four studies did not specified which approach they used.

3.3 Body compartments estimated

As reported in table 2, one COPD-specific BIA equation to predict total body water was found in the literature and four COPD-specific BIA equation to predict fat-free mass (FFM). These latter were used in 35 papers, while non-specific BIA equations were used in 9 papers and those provided by the manufacturer in 13. Twenty-three papers did not specify which equation was used (figure 2).

Fifty-four studies reported data on both FFM and FFM index (FFMI), 19 only on FFM and 7 only on FFMI. Appendicular skeletal muscle mass (ASM) was evaluated with BIA in 5 papers, four of which also as ASMI (figure 3).

3.4 Raw BIA data

Finally, only five papers provided data on raw BIA variables. In particular, 1 paper on BI index (18), 3 papers on phase angle (19-21) and 1 paper on multi-frequency impedance ratio (22).

3.5 Diagnosis of nutritional disorders

According to different diagnostic criteria, BIA derived FFM, FFMI, ASM or ASMI were used for identifying the occurrence nutritional disorders, i.e. cachexia (7 papers) or nutrition-related conditions, i.e. sarcopenia (4 papers).

4 Discussion

The present review has been carried out with the aim of systematically analyse the use of BIA for assessing body composition in COPD. With this purpose, 91 original studies has been selected.

There are different ways that BIA has been applied in COPD. i.e. using data generated by SF-BIA or MF-BIA or BIS devices. SF-BIA is the most widely used approach in literature, generally in order to obtain estimates of FFM or TBW. Compared with SF-BIA, MF-BIA was used in a smaller number of studies on COPD patients and, generally, in order to estimate within TBW, the volume occupied by intra-cellular water (ICW) and extra-cellular water (ECW) through predictive equations (18, 23).

The present review has found that the 80% of the included studies, used BIA in order to estimate FFM (n=73) and, FFMI (67%; n=61), while a smaller percentage of papers, to estimate FM (47%; n=43) and FMI (29%; n=26).

In the clinical setting, for estimating FFM, three disease specific BIA predictive equations were developed using DXA as reference method in studies that exclusively (10, 11) or predominantly(9) involved COPD patients. Schols et al. (8) also proposed a disease-specific predictive formula for TBW.

This review has pointed out that, although the presence of several COPD-specific BIA equations for predicting FFM, a number of studies did not use any of them, choosing equations provided for the general population instead. Furthermore, the great majority of studies found in literature did not specified which predictive equation was used. Manufacturer's equations have also been frequently applied.

ASM, i.e. the sum of FFM of upper and lower limbs, has also been evaluated in COPD, although much less frequently compared to FFM. In fact, while BIA has been used to estimate FFM (as such or indexed to height, i.e. FFMI) in

almost all the papers included in this review, only 5 papers used BIA to estimate ASM by using several predictive equations for general population. In fact, differently from FFM, no disease specific equation has been so far developed yet. As a matter of fact, evaluating ASM (and ASMI) is receiving increasing interest in clinical setting, since this variable is one of the diagnostic criteria for sarcopenia in older people (24).

As described above, BIA is a widely used method for estimating body compartments in COPD. Alternatively, directly measured (i.e. raw) BIA variables are receiving increasing interest in the clinical management of COPD, providing direct information on cellular mass and integrity, without the assumptions inherent in estimating body compartments (21). However, only 5 studies provided data on raw BIA variables, specifically focusing on PhA.

Finally, body composition estimates derived from BIA (i.e. FFM and ASM) has been applied in order to diagnose nutritional phenotypes, such as cachexia or sarcopenia, in a small number of studies. Nevertheless, the choice of criteria used is not homogeneous among papers. The European Society for Clinical Nutrition and Metabolism (ESPEN) and the European working Group on Sarcopenia in Older People (EWGSOP) have recently provided valuable criteria for identifying cachexia and sarcopenia, respectively (24, 25). However, diagnostic criteria varied among COPD studies, being still debated.

Concluding, available data in literature suggest that BIA has been widely used in order to assess body composition in COPD. However, very few data are available on raw BIA variables.

Furthermore, although standardized and reproducible criteria should be applied, the results of the present study highlights that BIA may be useful for diagnosing nutritional phenotypes such as cachexia or sarcopenia in COPD patients.

5. Figures and Tables

Figure 1. Flow chart of papers screened for this review
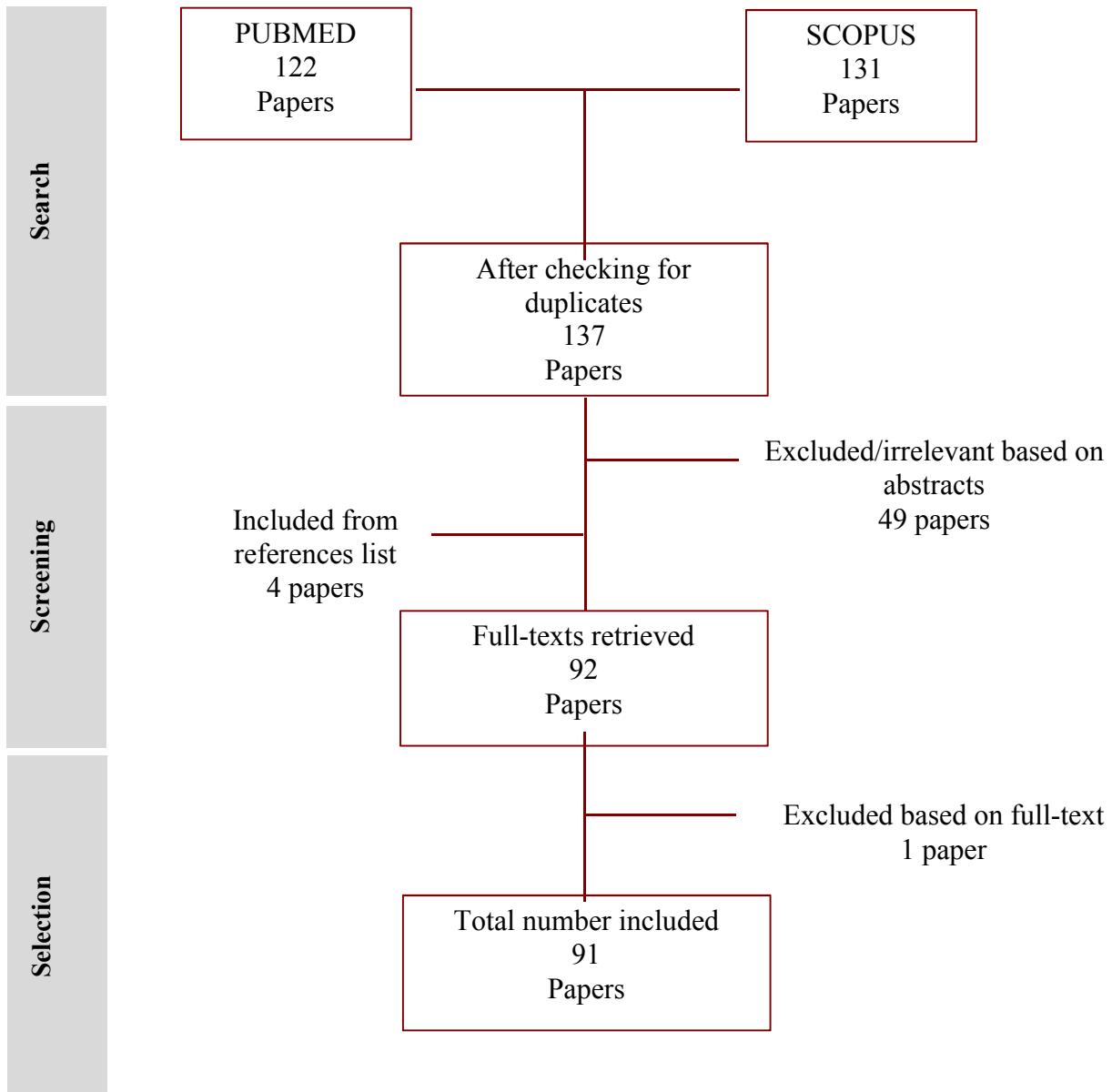
Table 1. Overview of the 91 studies included in this review

FIRST AUTHOR (reference)	YEAR	BODY COMPOSITION ESTIMATED BY BIA	RAW BIA VARIABLES	PREDICTIVE BIA EQUATIONS
ABBATECOLA (20)	2014	FFM, FM and FFM/FM	PhA	Piccoli et al., 1998 (26)
AL-SHAIR (27)	2009	FFMI	-	Not specified
BAARENDS (18)	1998	ECW and TBW	BI index	Not specified
BROEKHUIZEN (28)	2005	FFM, FM and FFMI	-	Steiner et al., 2002 (10)
BROEKHUIZEN (29)	2006	FFM, FM and FFMI	-	Steiner et al., 2002 (10)
BROEKHUIZEN (30)	2005	FFM, FM and FFMI	-	Schols et al., 1991 for COPD (8) Lukaski et al., 1985 for controls (31)
BUDWEISER (32)	2008	FFM, FM, FFMI and FMI	-	Kyle et al., 2001 (33)
CAMILLO (34)	2008	FFM, FM, FFMI and FMI	-	Kyle et al., 1998 (9)
CREUTZBERG (35)	1998	FFM, FFMI and FM	-	Schols et al., 1991 for COPD (8) Lukaski et al., 1986 for controls (36)
CREUTZBERG (37)	2003	FFM and FFMI	-	Schols et al., 1991 (8)
DE BLASIO (22)	2016	FFM, FM, FFMI and FMI	Impedance ratio	Rutten et al., 2010 (11)
DI MARCO (38)	2013	FFMI and FMI	-	Not specified
DORE' (39)	1997	FFM, FM, and ACM	-	Manufacturer
EAGAN (40)	2010	FFMI and FMI	-	Not specified

EAGAN (41)	2012	FFMI and FMI	-	Schols et al., 1990 (42)
EISNER (43)	2007	FFM and FM	-	Sternfeld et al., 2002 (44)
EMTNER (45)	2014	FFM and FFMI	-	Not specified
FAISY (46)	2000	TBW, ECW, ICW, FFM, FM and ACM	-	Boulier et al., 1990 for FFM (47) Jenin et al, 1975 for ECW (48)
FRANSSEN (49)	2004	FFM, FFMI and FM	-	Schols et al., 1991 (8)
FRANSSEN (50)	2005	FFM, FFMI, FM and FMI	-	Schols et al., 1991 (8)
GIRON (51)	2009	FFM and FM	-	Manufacturer
GODOY (52)	2000	FFM and FM	-	Schols et al., 1991 (8)
GOLOGANU (53)	2014	FFM, FFMI, FM, VAT, ASM and ASMI	-	Schols et al., 1991 (8)
GOSKER (54)	2003	FFM and FFMI	-	Schols et al., 1991 for COPD (8) Lukaski et al., 1985 for controls (31)
GOSKER(55)	2002	FFM and FFMI	-	Schols et al., 1991 (8)
GROENBERG (56)	2005	FFM and FFMI	-	Manufacturer
GURGUN (57)	2012	FFMI and FMI	-	Not specified
HALLIN (58)	2011	FFM, FM, FFMI and TBW	-	Not specified
HITZL (59)	2010	FFM, FM, FFMI and FMI	-	Kyle et al., 2001 (33)
HOPKINSON (60)	2007	FFM	-	Steiner et al., 2002 (10)

HOPKINSON (61)	2008	FFM and FFMI	-	Steiner et al., 2002 (10)
HRONEK (62)	2013	FFM, FM, FFMI and FMI	-	Manufacturer plus Steinter et al. 2002 (10)
HSU (63)	2014	FFM, FFMI and FM	-	Not specified
ISCHAKI (64)	2007	FFMI	-	Not specified
JONES (65)	2015	ASM, ASMI	-	Janssen et al., 2000 (66)
JOPPA (67)	2016	FFM, FM, FFMI and FMI	-	Steiner et al., 2002 (10)
KAYMAZ (68)	2015	FFM and FFMI	-	Not Specified
KILDUFF (69)	2003	FFM	-	Kyle et al., 1998 (9)
KOBAYASHI (70)	2000	FFM and FM	-	Not specified
KUROSAKI (71)	2009	FFMI and FMI	-	Not specified
KYLE (72)	2005	FFM, FFMI, FM and FMI	-	Kyle et al., 2001 (33)
LEI (73)	2014	FFM and FM	-	Manufacturer
LENARTOVA (74)	2016	VAT	-	Not specified
LERARIO (1)	2006	FFM, FM and FFMI	-	Schols et al., 1991 (8)
LIU (75)	2009	FFM and FM	-	Manufacturer
LUO (76)	2005	FFM, FM	-	Manufacturer
LUO (77)	2016	FFMI	-	Not specified
MADDOCKS (21)	2015	FFM, FFMI	PhA	Not Specified
MAMOTO (78)	2003	TBW, ICW, ECW, upper extremity water, trunk water, lower extremity water and FM	-	Not specified

MARINARI (79)	2013	FFMI and FMI	-	Not specified
MEKAL (80)	2015	FFM, FM, FFMI and TBW	-	Not Specified
MILLER (81)	2009	FFM, FM, FFMI, FMI and TBW	-	Schols et al., 1991 (8) plus Lukaski et al., 1985 (31)
MONTEIRO (82)	2012	FFM, FM and FFMI	-	Kyle et al., 1998 (9)
MOSTERT (83)	2000	FFM and FFMI	-	Schols et al., 1991 (8)
MULLER (19)	2006	FFM, FM, FFMI, FMI, BCM, ECM and ECM/BCM	PhA	Not specified
NORDEN (84)	2015	FFM and FFMI	-	Manufacturer
NORDENSON (85)	2010	FFM and FFMI	-	Manufacturer
PERSSON (86)	2015	FFMI and FMI	-	Not Specified
RAMIRES (87)	2012	FFM, FM, ASM and ASMI	-	Segal et al., 1988 for FFM (88) Janssen et al., 2000 for ASM (66)
RUBINSZTAJN (89)	2014	FFM, FM and FFMI	-	Not specified
RUBINSZTAJN (90)	2015	FFM, FM, FFMI ASM, ASMI and TBW	-	Not Specified
RUTTEN (11)	2010	FFM and FFMI	-	Steiner et al., 2002 (10)
RUTTEN (91)	2011	FFM, FM, FFMI and FMI	-	Rutten et al., 2010 for COPD (11) Dey et al., 2003 for controls (92)
SABINO (93)	2010	FFMI and FMI	-	Kyle et al., 1998 (9)

SANCHEZ (94)	2011	FFM and FFMI	-	Kyle et al., 1998 (9)
SAURE (95)	2012	FFMI and FMI	-	Not specified
SCHOLS (42)	1990	FFM and Rx	-	Schols et al., 1990 (42)
SCHOLS (8)	1991	FFM and TBW	-	Schols et al., 1991 (8)
SCHOLS (96)	2005	FFMI FMI and ASMI	-	Schols et al., 1991 for FFM (8) Janssen et al., 2000 for ASM (66)
SCHOLS (97)	1991	FFM	-	Schols et al., 1991 (8)
SCHOLS (98)	1991	FFM	-	Schols et al., 1990 (42)
SCHOLS (99)	1993	FFM and ASM	-	Schols et al., 1990 (42)
SCHOLS (100)	1996	FFM, FFMI and FM	-	Schols et al., 1991 (8)
SERRES (101)	1998	FFM	-	Schols et al., 1991 (8)
SEYMOUR (102)	2009	FFM and FFMI	-	Steiner et al., 2002 (10)
SILVEIRA (103)	2014	FFM and FFMI	-	Schols et al., 1991 (8)
SLINDE (104)	2005	FFM and FFMI	-	Manufacturer
STEINER (10)	2002	FFM and FFMI	-	Steiner et al., 2002 (10)
STEUTEN (105)	2006	FFMI	-	Not specified
TEOPOMPI (106)	2013	FFM and FFMI	-	Segal et al., 1988 (88)

TKACOVA (107)	2010	FFMI and FMI	-	Not specified
VAKHLAMOV (108)	2015	FM and VAT	-	Not Specified
VERMEEREN (109)	2006	FFM, FM, FFMI and FMI	-	Steiner et al., 2002 (10)
VERMEEREN (110)	1997	FFM, ICW and ECW	-	Not specified
VESTBO (111)	2006	FFM, FM and FFMI	-	Segal et al., 1988 (88)
VILARO' (112)	2010	FFM, FM and TBW	-	Not specified
VRIEZE (113)	2007	FFM and FFMI	-	Not specified
WAATEVIK (114)	2012	FFM, FM, FFMI and FMI	-	Not specified
WAATEVIK (115)	2016	FFM, FM, FFMI and FMI	-	Not specified
WATZ (116)	2008	FFM and FFMI	-	Not specified
YOSHIKAWA (23)	2012	ICW, ECW and ECW/ICW	-	Not specified

Abbreviations: FFM, fat-free mass; FM, fat mass; FFMI, FFM index; FMI, FM index, BI index, bioelectrical impedance index; ECW, extra-cellular water; ICW, intra-cellular water; TBW, total body water; ACM, active cell mass; VAT, visceral adipose tissue; ASM, appendicular skeletal muscle mass; ASMI, ASM index, PhA, phase angle; BCM, body cell mass.

Table 2. Details of four disease-specific equations with BIA variables to calculate body composition in patients with COPD

Author	Population	Validation method	FFM predictive equation
Schols et al., *Am J Clin Nutr*, 1991	24 M, 65±10 years 8 F, 57±11 years	TBW	M and F: $3.32 + 0.44 \times Ht^2/R + 0.13 \times Wt)/0.73$
Kyle et al., *Eur Respir J,* 1998	60 M, 66.8±8.2 years 15 F, 60.8±11.,1 years	DXA	M and F: $6.06 + 0.83 \times Ht + 0.207 \times Wt - 0.024 \times R\ (+ 4.036\ \text{if M})$
Steiner et al., *Eur Respir J,* 2002	53 M, 67.7±8.4 years 32 F, 65.6±8.7 years	DXA	M: $8.383 + 0.465 \times Ht^2/R + 0.213 \times Wt$ F: $7.610 + 0.474 \times Ht^2/R + 0.184 \times Wt$
Rutten et al., *Resp Med,* 2010	641 M, 65.4±9.4 years 446 F, 60.1±9.3 years	DXA	M and F: $-11.81 + 0.245 \times Wt + 0.298 \times Ht^2/R + 0.148 \times Ht\ (+ 5.284\ \text{if M})$

Abbreviations: M, males; F, females; Ht, height; Wt, weight; BMI, body mass index; R, resistance

Figure 2. Number of papers that estimated fat free mass with several BIA predictive equation available in the literature.
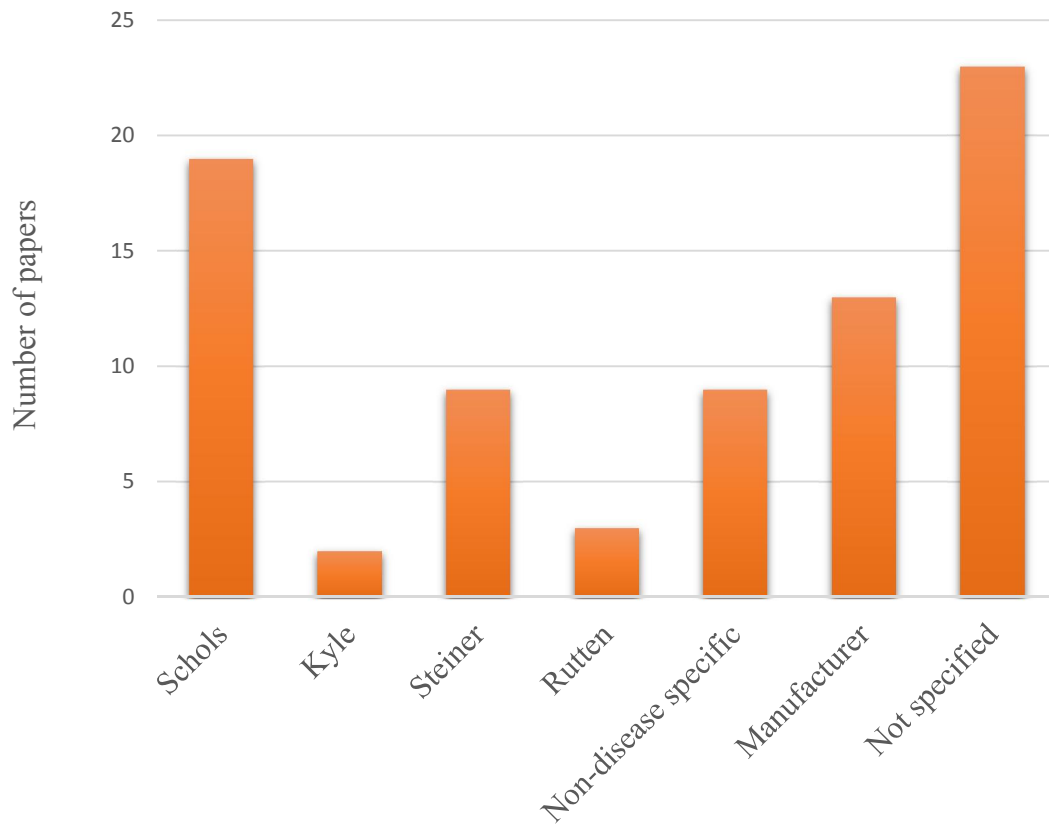
Figure 3. Estimates of body composition derived from BIA
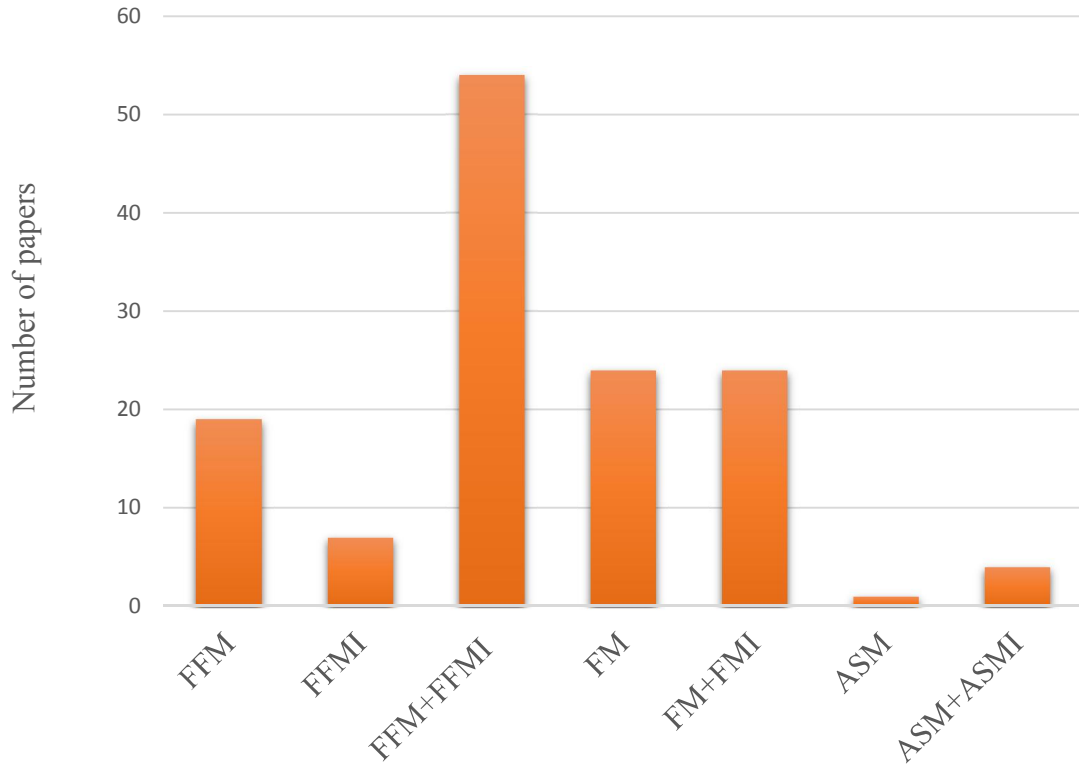
Abbreviations: FFM, fat-free mass; FFMI, FFM index; FM, fat mass; FMI, FM index; ASM, appendicular skeletal muscle mass; ASMI, ASMM index.

Table 3. List of studies, which used BIA for assessing cachexia

Author	BIA equation used	Number of subjects	Criteria to define cachexia	Prevalence of cachexia
Schols et al., 2005(117)	Schols et al.(8)	412 COPD (317 M and 95 F)	BMI <21 kg/m² plus FFMI<16 kg/m² in M or <15 kg/m² in F (118)	28.5% (29.0% in M and 27.0% in F)
Broekhuizen et al., 2005(119)	Schols et al.(8)	99 COPD (69 M and 30 F) 20 controls (15 M and 5 F)	FFMI<16 kg/m² in M or <15 kg/m² in F	35.4% in COPD
Eagan et al., 2010(120)	Not specified	409 COPD (249 M and 160 F)	FFMI<17 kg/m² in M or <14 kg/m² in F (121)	29.1% (31.0% in M vs 27.5% in F)
Eagan et al., 2012(41)	Not specified	408 COPD (249 M and 159 F)	FFMI<17 kg/m² in M or <14 kg/m² in F (121)	28.7% (31.0% in M and 27.5 in F)
Gologanu et al., 2014 (53)	Not specified	36 COPD (33 M and 3 F)	BMI ≤ 21 kg/m² and FFMI ≤ 16 kg/m² in M and ≤14 in F.	8.3%
Persson et al., 2015 (86)	Not specified	526 COPD	FFMI<17 kg/m² in M or <14 kg/m² in F (121)	31.7% vs 26.8% in patients with Vitamin D deficiency vs those without and 29.7%, 27.1% and 29,4% in those with VDBP <200 µg/mL, between 200 and 299 µg/mL and > 300 µg/mL, respectively.
Waatevik et al., 2015 (115)	Not specified	370 COPD (223 M and 147 F)	FFMI<17 kg/m² in M or <14 kg/m² in F (121)	27.0% (25.5% in patients with desaturation during 6MWT vs 74.5% in those without desaturation)

Abbreviations: BMI, body mass index; FFMI, fat-free mass index; VDBP, vitamin D binding protein; 6MWT, 6-minute walk distance.

Table 4. List of studies, which used BIA for assessing sarcopenia

Author	BIA equation used	Number of subjects	Criteria to define sarcopenia	Prevalence of sarcopenia
Ramires et al., 2012(87)	Janssen et al.(66)	20 COPD 20 controls	$5.76 < ASMI < 6.75$ or $ASMI \leq 5.75$ kg/m^2 in F; $8.51 < ASMI < 10.75$ or $ASMI \leq 8.50$ kg/m^2 in M; to denote moderate or high physical disability risk, respectively (122)	Not assessed
Gologanu et al., 2014 (53)	Not specified	36 COPD (33 M and 3 F)	BMI > 21 kg/m^2 and FFMI ≤ 16 kg/m^2 in M and ≤ 15 kg/m^2 in F.	8.3%
Jones et al., 2015 (65)	Not specified	622 COPD (354 M and 268 F)	EGWSOP (24)	14.5% (16.1% in M vs 12.3% in F)
Joppa et al., 2016 (67)	Steiner et al.(10)	2000 COPD (1314 M and 686 F) 548 controls (272 M and 276 F)	FFMI $< 10^{th}$ percentile of age, sex, and BMI-specific reference values (123)	24.0% in COPD 15.0% in controls

Abbreviations: ASMI, appendicular skeletal muscle mass index; BMI, body mass index; FFMI, fat-free mass index; EWGSOP, European working group on sarcopenia in older people.

Table 5. List of studies, which used raw BIA variables

Author	Number of subjects	BIA approach (SF, MF or BIS)	BIA variable	Mean values
Baarends et al., 1998 (18)	40 COPD (32 M and 8F)	BIS	BI index	36.2 cm^2/ohm in M and 28.9 cm^2/ohm in F
Muller et al., 2006 (19)	164 COPD (100 M and 64 F) 120 controls (56 M and 64 F)	MF	PhA	4.4 and 4.5 degrees in M and F with COPD; 5.8 and 5.5 degrees in M and F controls
Abbatecola et al., 2013 (20)	132 COPD (86 M and 46 F)	SF	PhA	5.5 and 4.7 degrees in M and F
Maddocks et al., 2015 (21)	502 COPD (295 M and 207 F)	MF	PhA	4.7 degrees (4.9 degrees in M and 4.3 degrees in F)
De Blasio et al., 2016 (22)	212 COPD (144 M and 68 F) 115 controls (53 M and 62 F)	MF	IR	124.1 and 122.3 in M and F with COPD; 126.8 and 126.0 in M and F controls

Abbreviations: BIS, bioelectrical spectroscopy; MF, multi-frequency; SF, single frequency; BI index, bioelectrical impedance index; PhA phase angle; IR, impedance ratio

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CHAPTER IV

EVALUATION OF THE PREVALENCE OF MALNUTRITION AND SARCOPENIA IN PATIENTS WITH COPD

Submitted for publication

SUMMARY: Abstract – 1. Introduction and aims – 2. Methods − 2.1 Subjects − 2.2 Lung function − 2.3 Body composition − 2.4 Diagnosis of malnutrition − 2.5 Diagnosis of sarcopenia − 2.6 Statystical Analysis − 3. Results − 3.1 Prevalence of malnutrition and sarcopenia − 3.2 Characteristics associated with malnutrition − 3.3 Characteristics associated with sarcopenia − 3.4 Characteristics of malnourished patients, with and without sarcopenia − 3.5 The high IR and low PhA group of patients − 4. Discussion − 5. Tables − 6. References

Abstract

Malnutrition and sarcopenia are two major consequences of chronic obstructive pulmonary disease (COPD). However, no previous study focused on the prevalence of overlapping malnutrition and sarcopenia in these patients.

The aim of this study was to determine the prevalence of malnutrition and sarcopenia, as defined by the European Society for Clinical Nutrition and Metabolism (ESPEN), in COPD and to determine their relationship with functional parameters and raw BIA variables.

Two-hundred and sixty COPD patients (183 males and 77 females) underwent respiratory, anthropometric, bioelectrical impedance analysis (BIA), handgrip strength (HGS), six-minute walk distance (6MWD) and biochemical measurements. Malnutrition was diagnosed based on low body mass index (BMI <18.5 kg/m^2) or between 18.5 - 22.0 kg/m^2, plus low fat-free mass index (FFMI<17 kg/m^2 for males and <15 kg/m2 for females). Malnourished patients with CRP levels>5 mg/dL were defined as cachectic. Sarcopenia was diagnosed as low appendicular muscle mass plus low HGS or 6MWD (or both, in case of severe sarcopenia).

The overall prevalence of malnutrition and sarcopenia was 20.4% and 25.4% respectively, being significantly higher across global initiative for chronic obstructive lung disease (GOLD) stages. Nor malnutrition or sarcopenia differed by age. The prevalence of sarcopenia was significantly higher in patients with malnutrition and, among malnourished patients, in those with inflammation (cachexia). Among malnourished patients, a significant reduction in BMI, forced expiratory volume in 1 second, inspiratory capacity/total lung capacity, FFM, HGS emerged in those with sarcopenia, compared to non sarcopenic patients.

In conclusion, we identified a 20.4% prevalence of malnutrition and a 25.4% prevalence of sarcopenia in patients with COPD. A clear discrepancy

between the two conditions emerged, since 42.4% of sarcopenic patients do not fulfil the ESPEN criteria of malnutrition. An association of the presence of malnutrition and sarcopenia with GOLD stages also emerged, with a prevalence of the two conditions (separately or combined) that increased with disease severity.

1. Introduction and aims

COPD is a heterogeneous disease not only in terms of pulmonary characteristics but also with respect to both systemic consequences such as weight loss and muscle weakness, and co-morbidity (e.g. osteoporosis, diabetes and cardiovascular disease) (1). Malnutrition and nutrition-related phenotypes (for example, sarcopenia) are highly prevalent extra-pulmonary manifestations, which are associated with important consequences for health risk assessment, stratification and management of the disease (2). For this reason, early diagnosis of both malnutrition and sarcopenia are highly recommended.

Malnutrition is more common in advanced GOLD stages (3) and in acute exacerbations compared to stable COPD (4). Indeed, wide difference in the prevalence of malnutrition in COPD patients, as found in the literature, may also be expected due to the use of different diagnostic criteria. Overall, the definition of standardized approaches for identifying malnourished patients is a key issue in clinical nutrition. In the last few years, international criteria for the diagnosis of malnutrition were defined by the American Society of Parenteral and Enteral Nutrition (ASPEN) together with the Academy of Nutrition and Dietetics (5) and, more recently, by the European Society of Clinical Nutrition and Metabolism (ESPEN) (6). According to these latter, malnutrition is diagnosed based on low body mass index (BMI) or combined weight loss plus low BMI or low fat-free mass index (FFMI). In addition, the ESPEN consensus statement (2016) recognized a disease-related malnutrition with inflammation (or cachexia) and a disease-related malnutrition without inflammation (no cachexia), depending on the presence of biochemical indices of either ongoing or recurrent inflammation. ESPEN criteria have been recently applied in several kind of patients, such as acutely ill middle-aged and diabetic hospitalized older patients, geriatric outpatients, geriatric post-acute care settings, healthy old and healthy young individuals (7, 8), finding out a prevalence of malnutrition from 0-14% in the diverse populations, but never in in COPD.

Sarcopenia, which is another systemic manifestation of COPD (9), is a syndrome characterized by the progressive and generalized loss of skeletal muscle mass, strength and performance with an increased risk of adverse outcomes (10-12). Widely accepted criteria for sarcopenia have been developed by the European Working Group of Sarcopenia in Older People (EWGSOP), recently endorsed by the ESPEN (6), and include an algorithm based on loss of appendicular muscle mass (ASM) plus reduced strength and/or performance (10, 13). However, in contrast with international consensus statements for sarcopenia, most studies on COPD have focused just on one aspect of sarcopenia (14-22), the loss of muscle mass, and only one paper on reduced muscle mass, strength and performance (23).

Overall, only few studies focused on the overlapping presence of malnutrition and sarcopenia (8), suggesting a relation between these two conditions. Nevertheless, no study previously has been carried out in COPD.

Thus, the aim of the present study was to determine the prevalence of malnutrition and sarcopenia, as defined by ESPEN, in COPD and to determine their relationship with functional parameters and raw BIA variables.

2. Methods

2.1 Subjects

A cross-sectional study in consecutive hospitalized COPD patients was carried out in the Pulmonary Rehabilitation Section of Clinic Center S.p.A (Naples, Italy). This unit focusses on rehabilitation and recovery during a defined period of time, usually about 6 weeks before a scheduled home discharge.

The study population consisted of 260 consecutive COPD patients, which met the following inclusion criteria: age >50 years and a baseline post-bronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) < 0.7. Exclusion criteria were related to diagnosis of known

respiratory disorders other than COPD, known history of significant inflammatory disease other than COPD and a COPD exacerbation within 4 weeks of enrolment. The Ethics Committee of the "Federico II" University of Naples approved the research protocol and all patients gave informed consent to participate in the study.

2.2 Lung function

All COPD patients performed a baseline post-bronchodilator spirometry and body plethysmography (QBOX® COSMED) according to American Thoracic Society (ATS)/European Respiratory Society (ERS) standardization (24). FEV_1 and FVC were assessed in accordance with the latest GOLD guidelines (25).

Patients were classified into four stages according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. If $FEV_1 \geq 80\%$ of the predicted, patients were classified as GOLD stage I (mild), if $50\% \geq FEV_1 > 80\%$ of the predicted, as GOLD stage II (moderate), $30\% \geq FEV_1 > 50\%$ of the predicted as GOLD stage III (severe), and if $<30\%$ of the predicted as GOLD stage IV (very severe). Inspiratory capacity (IC) and total lung capacity (TLC), were assessed. IC/TLC ratio (26) was calculated by dividing IC for TLC, and was used as a marker for hyperinflation, the abnormal increase in the volume of air remaining in the lungs at the end of spontaneous expiration (27).

2.3 Body composition

Body weight and body height were measured to the nearest 0.1 kg and 0.5 cm respectively, using a mechanical column scale (SECA 711+220; Hamburg - Germany), and BMI was calculated as body weight/height.[2] Body composition was assessed by performing a multifrequency BIA in standardized conditions (i.e. ambient temperature between 23 and 25°C, fast of > 3 h, empty bladder, supine position for at least 10 minutes before starting the measurement), using a Human Im-Touch analyzer (© DS Medica S.r.l., Milan, Italy). In addition,

after cleaning skin surface, patients were asked to lay with legs and arms slightly abducted at 30° so there was no contact between the extremities and trunk.

A standard tetra-polar technique was used, with measuring electrodes placed on the anterior surface of the wrist and ankle, and injecting electrodes placed on the dorsal surface of the hand and the foot, respectively. Z and phase angle were obtained at five frequency kHz for both dominant (D) and non-dominant (ND) sides with an imperceptible electrical current of 800 mA. In addition, bioelectrical impedance index (BI index) was calculated as squared height divided by impedance at 50 kHz, representing an established parameter of total body water (TBW) (28). The impedance ratio between high (250 kHz) to low (5 kHz) and phase angle at 50 kHz, expressed as a degree, provide information on hydration status, cellular mass and quality (29, 30). FFM and FFM index (FFMI kg/m^2 = FFM/height^2) were estimated from Z at 50 kHz using a disease specific BIA equation (31). Appendicular skeletal muscle mass (ASM) was estimated using a BIA equation for general population (32).

2.4 Diagnosis of malnutrition

Malnutrition was diagnosed according to the main outcome variables indicated by the latest ESPEN consensus (6, 13). Patients were classified as malnourished when they had BMI<18.5 kg/m^2 or BMI between 18.5 and 22 kg/m^2, combined with low FFMI (<17 kg/m^2 for men and <15 kg/m^2 for females). In absence of elevated serum CRP concentrations (CRP<5 mg/dL), patients were defined as affected by *malnutrition without inflammation*. On the contrary, the simultaneous presence of elevated serum CRP concentrations (CRP ≥5 mg/dL), combined with malnutrition, was used for diagnosis of *cachexia* (or *malnutrition with inflammation*) (13).

2.5 Diagnosis of sarcopenia

Sarcopenia was diagnosed in accordance with EWGSOP criteria (10). Patients with low muscle mass without impact on muscle strength or physical performance were classified as *pre-sarcopenic*. Patients were classified as *moderately sarcopenic*, when affected by low muscle mass, plus low muscle strength or low physical performance. *Severe sarcopenia* was identified when all three criteria of the definition were met (low muscle mass, low muscle strength and low physical performance).

Low muscle mass was assessed by BIA as described above.

Low muscle strength was assessed by handgrip strength, a surrogate measurement of overall muscle strength (33), measured at baseline with a digital hand-held dynamometer (Dynex, MD systems Inc. Ohio USA) and expressed in kilograms. Patients performed a maximum voluntary isometric contraction of finger flexor muscles. Three measurements were taken for both body sides (dominant and non-dominant). Mean value of the two body sides was indicated as whole body HGS (or HGS). The maximum values were considered for statistical analysis (34).

Physical performance was assessed with 6-minute walk distance (6MWD) test in patients who were able to walk at baseline (35). Participants were instructed to stand with both feet touching the starting line and, after a verbal command, to begin walking with usual aids (canes or walkers) and at their usual pace.

For the purpose of this analysis, low walking speed was defined as walking slower than 250 m, as previously indicated by Celli et al (36).

2.6 Statistical Analysis

Data are presented as mean (SD) or percentage (%). The prevalence of malnutrition and sarcopenia, with 100% of cells greater than 5, was determined

and compared according to gender, age and GOLD staging, using a non-parametric test (Chi Square test for categorical variables, i.e. gender and GOLD; Mann-Whitney U Test for continue variables, i.e. age). P values less than 0.05 were considered as statistically significant. SPSS 20.0 (IBM Corporation, SPSS, INC., Chicago, IL, USA) was used for data analyses.

3. Results

Two hundred and sixty patients (183 males and 77 females) with stable COPD were studied.

3.1 Prevalence of malnutrition and sarcopenia

The overall prevalence of malnutrition was 20.4% (53 patients). Prevalence did not differ either between genders (p=0.281) or age (p=0.71), but significantly increased among GOLD stages (11.2%, 18.0% and 32.5% in GOLD I+II, III and IV, respectively). There were 20 malnourished patients with inflammation (12.7% of total group) and 33 malnourished without inflammation (7.7%).

Pre-sarcopenia was diagnosed in 4 out of 260 patients (1.5 %), who were grouped together with non-sarcopenic patients for this study. The overall prevalence of sarcopenia was 25.4% (66 patients). Of these, 22 patients had a moderate sarcopenia and 44 had a severe sarcopenia. Low muscle mass was combined with low muscle strength (low HGS) in 54 patients or with low physical performance (low 6MWD) in 56 patients. Prevalence of sarcopenia was significantly higher in males compared to females (32.8% and 7.8%, respectively; p<0.001), and significantly different among GOLD stages (7.5%, 22.0% and 47.5% in GOLD I+II, III and IV, respectively; p<0.001), but did not differed by age (p=0.61).

3.2 Characteristics associated with malnutrition

General and body composition characteristics of 260 patients stratified for grades of malnutrition and sarcopenia are shown in tables 2 and 3, respectively.

Malnourished patients with and without inflammation did not differ compared to each other, but exhibited lower values of weight, BMI, FFM, FM and ASM (p<0.05), compared to non-malnourished (table 1).

Lower values of FEV_1 (% pred) were found in malnourished patients with inflammation, compared to non malnourished patients (p=0.003). However, no significant difference was reported in malnourished patients without inflammation compared to the other two groups of patients, with different grades of malnutrition. A significant reduction of IC/TLC was also observed both in malnourished patients with (p=0.001) and without (p=0.01) inflammation, compared to non-malnourished (table 1).

HGS was significantly lower in malnourished patients with inflammation, compared to non malnourished (p=0.001), although no differences emerged between malnourished patients with and without inflammation (table 2).

With respect to raw BIA data, high to low frequency impedance ratios (specifically, 250/5 kHz IR and 100/5 kHz IR) were significantly higher in malnourished patients with inflammation compared to non-malnourished patients (p=0.005) and, within the group of malnourished patients, significantly higher in those with inflammation, compared to those without (p=0.05). Similarly, PhA was significantly lower in malnourished patients with inflammation, compared to non-malnourished (p=0.001). Within the group of malnourished patients, PhA tended to be lower in those with inflammation compared to those without (p=102) (table 1).

3.3 Characteristics associated with sarcopenia

With respect to grades of sarcopenia, both patients with moderate or severe sarcopenia exhibited lower values of height, weight, BMI, FM and ASM, compared to non-sarcopenic patients.. Differently, FFM was found to be significantly reduced only in severe sarcopenic patient, compared to non-sarcopenic.

A significant reduction of FEV_1 and IC/TLC was also observed both in patients with moderate (p<0.001) and severe (p<0.01) sarcopenia, compared to non-malnourished (table 2).

With respect to muscle strength, lower values of HGS emerged in patients with moderate (p<0.001) and severe (p<0.001) sarcopenia, compared to non-sarcopenic patients (table 2).

Similarly to what emerged across grades of malnutrition, differences in raw BIA data emerged also across grades of sarcopenia: higher values of 250/5 kHz IR, 100/5 kHz IR and 50/5 kHz IR emerged in patients with severe sarcopenia, both compared to those with moderate sarcopenia and to non-sarcopenic patients (table 2). Likewise, a significant reduction in PhA values emerged only between sever sarcopeia and non-sarcopenic patients.

3.4 Characteristics of malnourished patients, with and without sarcopenia

The cross tabulation data of malnutrition and sarcopenia are shown in table 3. The prevalence of sarcopenia was significantly higher in patients with malnutrition: 71.7% vs 13.5% (p<0.001). Among malnourished patients, the prevalence of sarcopenia was still higher in those with inflammation (cachectic), compared to those without (non-cachectic) (80.0% vs 63.6%; p<0.001)

Table 4 shows general and body composition characteristics of 53 malnourished COPD patients, divided in those with and without the simultaneous presence of sarcopenia. With respect to disease severity, 35 out of

38 malnourished patients with sarcopenia had a GOLD stage III/IV. Compared to those without sarcopenia, malnourished patients with sarcopenia exhibited a significant reduction in FEV_1 (28.7±12.1 vs 50.5±24.1, p<0.001), CI/CPT (0.15±0.05 vs 0.20±0.07, p=0.004), FFM (37.6±4.1 vs 41.2±3.6, p=0.004) and FFMI (data not shown). A similar tendency to be reduced emerged also for raw BIA variables. Not any reduction emerged with respect to FM. Indeed, when indexed to height, FMI was even higher in malnourished patients with sarcopenia compared to non-sarcopenic patients (data not shown).

3.5 The high IR and low PhA group of patients

Fifty-one patients (19.6%) had an IR above the 75° percentile (>0.834) and a PhA below the 25° percentile (<4.3 degrees) and were defined as those with worst muscle quality. Within this group of patients, the prevalence of malnutrition was 27.5% (14 out of 51 patients), and slightly differed from the rest of the COPD patients (18.7%, 39 out of 209 patients; p=0.07). The prevalence moderate/severe sarcopenia was 35.3% in the group of patients with highest IR and lowest PhA (18 out of 51 patients), and tended to be different compared to the rest of the COPD patients (23.0%, 48 out of 209 patients; p=0.07).

Differences between the two groups of patients, stratified according to IR and PhA values, accentuate when considering only GOLD stages III and IV patients (table 5).

4. Discussion

The present study provides data on overall prevalence of malnutrition as defined by ESPEN (13) and sarcopenia according to the EWGSOP criteria (10) in COPD patients. Furthermore, this is the first study that focused concurrently on the presence of both conditions, highlighting their effects on clinical characteristics of these patients.

Malnutrition is a common and serious problem in COPD. It's prevalence has been reported to vary between 10 and 60% for the entire COPD group, divided into 10-45% of outpatients and 30-60% of COPD inpatients (37), possibly due to varying definitions and criteria of malnutrition used (38).

According to the ESPEN (6, 13), malnutrition can be diagnosed based on low BMI, low FFMI and loss of body weight. In addition, the latest 2016 ESPEN consensus statement (13) proposed two different concept of disease-related malnutrition, i.e. with inflammation (also indicated as cachexia) and without inflammation, based on the simultaneous presence of an underlying disease and/or biochemical indices of either ongoing or recurrent inflammation (13).

No previous paper focused on the assessment of malnutrition as defined by the latest ESPEN consensus. The present study identified a 20.4% prevalence of malnutrition, demonstrating an association between the presence of malnutrition and GOLD stages. In fact, 83% of malnourished patients suffer from severe or very severe disease (GOLD stage III or IV) and, considering only malnourished patients with inflammation, the percentage of those with the most advanced disease stage increases to 95%.

Beyond some obvious differences between non-malnourished vs malnourished patients, the two groups of malnourished patients (with and without inflammation) do not differ from each other in terms of a number of nutritional variables, such as BMI, FFM and ASM estimates, suggesting that the greatest differences were between malnourished vs non malnourished patients.

However, beyond body composition estimates, the use of raw variables generated by a BIA device, such as PhA or IR, could have utility independently of body composition estimates, as markers of extracellular/intracellular distribution of water, body cell mass and muscle quality (39-45). In this perspective, we aimed to compare raw BIA data among patients with different

grades of malnutrition in order to find out whether these variables could contribute to further phenotype these patients.

No studies have previously examined the relationship between diagnosis of malnutrition and raw BIA data in COPD. This study shows a clear worsening of raw BIA data in malnourished patients with inflammation, compared to non-malnourished and, within the group of malnourished patients, in those with inflammation. This is probably due to the effect of the chronic low-grade inflammation on muscle quality (46-48).

Sarcopenia is another recognized extra-pulmonary manifestation of COPD, being related to contributing factors, including physical inactivity, malnutrition and chronic disease.

Several definitions of sarcopenia have been previously proposed, some of those only consider low muscle mass, as suggested by the recent European Respiratory Society (ERS) statement for nutritional assessment and therapy (2). However, the current international consensus developed by the EWGSOP (and endorsed by ESPEN) is that the sarcopenia syndrome includes the presence of both low muscle mass and function (i.e. strength and performance) (6, 10, 13).

Only few papers have examined sarcopenia in COPD using EWGSOP criteria. Jones et al. (49) have recently found out a prevalence of 14.5% of sarcopenia among stable COPD patients, which increased with age and disease severity (49).

According to the present study, the overall prevalence of sarcopenia is 25.0%, being higher across GOLD stages. Indeed, 90% of sarcopenic patients are in GOLD stages III or IV, with a percentage that remain stable for those with moderate or severe sarcopenia, separately.

With respect to nutritional variables, BMI and estimates of FFM and ASM are reduced in severe sarcopenic patients. However, of these three variables,

only BMI has been found to be reduced also in moderate sarcopenia, compared to non-sarcopenic patients.

Similarly to what described in malnourished patients, the present study showed some differences between grades of sarcopenia. These differences were particularly marked with respect of HGS and raw BIA variables (IR and PhA).

Finally, the present study pointed out some discrepancies between prevalence of malnutrition and sarcopenia. In fact, 42.4% of sarcopenic patients do not fulfil the ESPEN criteria of malnutrition, while 28.3% of malnourished patients do not fulfil the EWGSOP criteria of sarcopenia.

We acknowledge argument around the use of BIA for the assessment of muscle mass, as compared with reference methods, such as dual-energy X-ray absorptiometry (DEXA) or MRI. Nevertheless, BIA offers a simple, portable and non invasive method for the assessment of muscle mass that was considered acceptable by the EWGSOP.

Defining nutritional disorders and nutrition-related conditions in COPD should be of primary importance in future studies. According to the preliminary results of the present study, there is an urgent need of using standardized and reproducible criteria for diagnosing malnutrition and sarcopenia in COPD. As a matter of fact, the use of ESPEN criteria provide an valuable approach for differencing cachectic and non-cachectic patients. In fact, withing the group of malnourished patients, several differences emerge between patients with and without inflammation, especially with respect to IR and PhA. In particular, the most evident BIA alterations emerge in malnourished patients with inflammation. Further research should also analyse whether malnutrition and/or sarcopenia are predictive factors for clinical outcomes in these patients.

5. Tables

Table 1: General characteristics, body composition, BIA variables and HGS in COPD patients across stages of malnutrition.

	No malnutrition (N. 207)	Malnutrition without inflammation (N. 33)	Malnutrition with inflammation (N.20)
Age (years)	69.9±7.9	69.9±7.9	69.1±8.8
Males/females (number)	142/65	23/10	18/2
Patients GOLD III-IV (number)	136 (65.7%)	25 (75.8%)	19 (95.0%)
Height (cm)	160.5±9.2	160.3±7.4	163.1±9.0
Weight (kg	72.1±14.1[ab]	48.9±6.6[a]	47.2±7.6[b]
BMI (kg/m^2)	28.0±4.8[ab]	18.9±1.7[a]	17.8±2.2[b]
FEV_1 (% pred.)	43.8±18.2[b]	38.1±21.7	29.6±12.3[b]
IC/TLC	0.23±0.09[ab]	0.18±0.07[a]	0.15±0.05[b]
HGS (kg)	25.1±5.6[b]	23.4±6.3	20.3±7.2[b]
Low HGS (number)	126 (60.9 %)	24 (72.7 %)	14 (70.0 %)
FFM (kg)	48.2±5.7[ab]	39.2±4.2[a]	37.6±4.3[b]
Low FFMI (number)	13 (6.3 %)	33 (100%)	20 (100%)
FM (kg)	24.1±9.2[ab]	9.8±4.8[a]	7.4±5.3[b]
ASM (kg)	22.7±3.0[ab]	18.4±2.7[a]	16.9±2.4[b]
Low ASMI (number)	29 (14.0 %)	23 (69.7%)	18 (90.0%)
250/5 kHz impedance ratio	0.814±0.027[b]	0.817±0.026[c]	0.838±0.031[bc]
100/5 kHz impedance ratio	0.869±0.022[b]	0.876±0.024[c]	0.892±0.025[bc]
50/5 kHz impedance ratio	0.909±0.018[b]	0.915±0.018	0.926±0.022[b]
Phase angle (degrees)	4.88±0.80[b]	4.63±0.94	4.15±0.88[b]

Note: Data are presented as mean values ± standard deviation or count data and percentage. FFM, ASM, impedance ratios (250/5, 100/5 and 50/5 IR), phase angle, and HGS data adjusted for gender and age. [a] or [b] or [c] = $p<0.05$ between the two groups

Abbreviations: GOLD, global initiative for chronic obstructive lung disease; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; IC/TLC, inspiratory capacity/ total lung capacity; HGS, handgrip strength; FFM, fat-free mass; FFMI, FFM index; ASM, appendicular skeletal muscle mass; ASMI, ASM index.

Table 2: General characteristics, body composition, BIA variables and HGS in COPD patients across stages of sarcopenia

	No sarcopenia (N. 194)	Moderate sarcopenia (N. 22)	Severe sarcopenia (N.44)
Age (years)	69.6±7.9	68.4±8.8	71.3±7.5
Males/females (number)	123/71	19/3	41/3
Patients GOLD III-IV (number)	120 (61.9 %)	20 (90.9%)	40 (90.9%)
Height (cm)	159.5±9.0[ab]	165.9±7.7[a]	163.3±8.4[b]
Weight (kg	70.9±15.5[ab]	59.1±11.5[a]	55.2±13.7[b]
BMI (kg/m^2)	27.8±5.3[ab]	21.4±3.3[a]	20.7±4.2[b]
FEV$_1$ (% pred.)	45.7±18.4[ab]	32.3±12.7[a]	30.4±16.0[b]
IC/TLC	0.023±0.09[ab]	0.018±0.08[a]	0.016±0.06[b]
HGS (kg)	25.7±5.5[b]	24.9±4.4[c]	19.1±5.5[bc]
Low HGS (number)	110 (56.7 %)	10 (45.5 %)	44 (100 %)
FFM (kg)	48.0±6.0[b]	42.4±4.9	40.0±5.9[b]
Low FFMI (number)	19 (9.8 %)	15 (68.2 %)	32 (72.7 %)
FM (kg)	23.6±9.9[ab]	14.9±7.7[a]	12.5±8.5[b]
ASM (kg)	22.9±3.0[ab]	18.8±2.2[a]	17.7±2.6[b]
Low ASMI (number)	4 (2.1 %)	22 (100 %)	44 (100 %)
250/5 impedance ratio	0.813±0.027[b]	0.813±0.028[c]	0.831±0.028[bc]
100/5 impedance ratio	0.869±0.022[b]	0.871±0.023[c]	0.888±0.023[bc]
50/5 impedance ratio	0.908±0.018[b]	0.911±0.019[c]	0.923±0.018[bc]
Phase angle (degrees)	4.91±0.80[b]	4.76±0.94	4.29±0.84[b]

Note: Data are presented as mean values ± standard deviation or count data and percentage. FFM, ASM, impedance ratios (250/5, 100/5 and 50/5 IR), phase angle, and HGS data adjusted for gender and age. [a] or [b] or [c] = p<0.05 between the two groups

Abbreviations: GOLD, global initiative for chronic obstructive lung disease; BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; IC/TLC, inspiratory capacity/ total lung capacity; HGS, handgrip strength; FFM, fat-free mass; FFMI, FFM index; ASM, appendicular skeletal muscle mass; ASMI, ASM index.

Table 3. Cross tabulation data of patients with and without malnutrition and/or sarcopenia

	No Malnutrition	Malnutrition without inflammation (non-cachectic)	Malnutrition with inflammation (cachectic)	Total
No sarcopenia	179	12	3	194
Moderate/severe sarcopenia	28	21	17	66
Total	207	33	20	260

Table 4: General characteristics, body composition, BIA variables and HGS in malnourished COPD patients with and without sarcopenia

	Malnourished without sarcopenia (N. 15)	Malnourished with sarcopenia (N. 38)	P values
Age (years)	68.9±8.2	69.9±8.2	0.682
Males/females (number)	5/10	36/2	**<0.001**
Patients GOLD III-IV (number)	9 (60.0 %)	35 (92.1 %)	**0.005**
Height (cm)	157.7±8.5	162.8±7.4	**0.036**
Weight (kg	48.8±5.5	48.1±7.5	0.764
BMI (kg/m^2)	19.5±1.5	18.1±2.0	**0.023**
FEV_1 (% pred.)	50.5±24.4	28.7±12.1	**<0.001**
IC/TLC	0.20±0.07	0.15±0.05	**0.004**
HGS (kg)	26.9±6.3	20.4±6.0	**0.001**
Low HGS (number)	6 (40.0 %)	32 (84.2 %)	**0.001**
FFM (kg)	41.2±3.6	37.6±4.1	**0.004**
Low FFMI (number)	15 (100 %)	38 (100 %)	**<0.001**
FM (kg)	8.9±2.9	9.5±4.1	0.589
ASM (kg)	20.3±2.1	16.9±2.2	**<0.001**
Low ASMI (number)	3 (20.0 %)	38 (100 %)	**<0.001**
250/5 impedance ratio	0.816±0.030	0.828±0.028	0.180
100/5 impedance ratio	0.871±0.024	0.887±0.025	0.050
50/5 impedance ratio	0.911±0.018	0.922±0.020	0.075
Phase angle (degrees)	4.84±0.89	4.30±0.92	**0.060**

Note: Data are presented as mean values ± standard deviation or count data. FFM, FFMI, FM, FMI, ASM, ASMI, IR, PhA and HGS adjusted for gender and age.

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; IC/TLC, inspiratory capacity/ total lung capacity; , GOLD, global initiative for chronic obstructive lung disease; FFM, fat-free mass; FFMI, FFM index; ASM, appendicular skeletal muscle mass; ASMI, ASM index.

Table 5. Respiratory function, body composition and muscle strength in 180 selected COPD patients with GOLD III and IV, divided into quartiles, according to IR and PhA values

	High IR and low PhA (n.33)	Other patients (n.147)	P value
Age (years)	72.8±7.2	67.9±7.8	**0.001**
Height (cm)	158.9±9.2	162.8±7.8	**0.012**
Weight (kg)	57.8±15.4	67.2±15.5	**0.002**
BMI	23.0±5.9	25.3±5.2	**0.026**
FEV$_1$ (% pred.)	34.8±15.4	31.8±10.1	0.180
IC/TLC	0.17±0.07	0.20±0.10	**0.230**
HGS (kg)	19.1±6.6	27.3±7.4	**<0.001**
FFM (kg)	42.2±6.9	47.2±8.1	**0.001**
Low FFMI (number)	15	40	**0.035**
FM (kg)	15.6±11.3	20.1±9.7	**0.022**
ASM (kg)	19.7±4.3	22.5±4.9	**0.003**
Low ASMI (number)	16	46	**0.049**
250/5 impedance ratio	0.859±0.018	0.809±0.023	**<0.001**
Phase angle	3.47±0.50	4.98±0.680	**<0.001**
Prevalence of malnutrition (%)	39.4%	21.1%	**0.027**
Prevalence of sarcopenia (%)	48.5%	29.9%	**0.041**

Note: Data are presented as mean values ± standard deviation, percentage or count data. FFM, FFMI, FM, ASM, ASMI, impedance ratio, phase angle and handgrip strength adjusted for gender and age.**Abbreviations:** BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; IC/TLC, inspiratory capacity/ total lung capacity; HGS, handgrip strength FFM, fat-free mass; FFMI, FFM index; ASM, appendicular skeletal muscle mass; ASMI, ASM index.

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CHAPTER V

EVALUATION OF BODY COMPOSITION IN COPD PATIENTS USING MULTIFREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS

Abstract

Multifrequency bioelectrical impedance analysis (MF-BIA) is a technique that measures body impedance (Z) at different frequencies (5, 10, 50, 100, and 250 kHz). Body composition may be estimated using empirical equations, which include BIA variables or, alternatively, raw BIA data may provide direct information on water distribution and muscle quality.

The aim of this study was to compare raw MF-BIA data between COPD patients and controls and to study their relationship with respiratory and functional parameters in COPD patients.

MF-BIA was performed (Human Im-Touch analyzer) in 212 COPD patients and 115 age- and BMI-matched controls. Fat-free mass (FFM) and fat mass were estimated from BIA data, and low- to high-frequency (5 kHz/250 kHz) impedance ratio was calculated. Physical fitness, lung function and respiratory muscle strength were also assessed in COPD patients.

After adjusting for age, weight, and body mass index, FFM and the 5/250 impedance ratio were lower in COPD patients (P・0.001) and were negatively affected by disease severity. In both male and female patients, the 5/250 impedance ratio was significantly correlated mainly with age (r=-0.316 and r=-0.346, respectively). Patients with a 5/250 impedance ratio below median value had lower handgrip strength (P<0.001), 6-minute walk distance (P<0.005), respiratory muscle strength (P<0.005), forced expiratory volume in 1 second (P<0.05) and vital capacity (P<0.005). Finally, the 5/250 impedance ratio was reduced (P<0.05) in patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) III and IV (compared to those with GOLD I and II) or a BODE index between 6 and 10 points (compared to those with BODE index between 1 and 5 points).

In conclusion, MF-BIA may be a useful tool for assessing body composition and nutritional status in COPD patients. In particular, the impedance ratio could give valuable information on cellular integrity and muscle quality.

1 Introduction and aims

Nutrition and the evaluation of body composition play an increasingly central role in the diagnosis, assessment and management of COPD. Besides smoking cessation, pharmacological therapies and management of comorbidities, Celli et al. (1) have recently indicated nutritional assessment to be one of the most important topics in COPD.

Several techniques are available to assess body composition in COPD, including anthropometry, bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA) as well as more advanced imaging technologies like computed tomography (CT), high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) (2). The choice of method depends not only on the type of study and the number of compartments to be studied, but also on its applicability in clinical practice (3).

Bioelectrical impedance analysis (BIA) is the bedside method that has been most widely investigated in clinical research, due to its affordability, portability, and ease of use (4). Actually, BIA does not directly measure body composition; estimates of body composition are derived from raw BIA data, such as impedance (Z), resistance, reactance and phase angle, using predictive equations (5). Alternatively, information about water distribution (between intra- and extra-cellular compartments) and cell integrity may be obtained through the electrical properties of body compartments from raw data, such as low to high frequency impedance ratio and phase angle (6-9).

BIA can be applied for estimating body composition using data generated by single-frequency BIA (SF-BIA), multifrequency BIA (MF-BIA) or bioimpedance spectroscopy (BIS) devices (5, 10).

1.1 Single frequency BIA

SF-BIA devices measure impedance variables (i.e, Z, and PhA) at a single frequency, typically 50 kHz.

SF-BIA is useful to estimate a body compartment (i.e. total body water, TBW and FFM) in patients with COPD, using several disease-specific predictive equations (11-14), which include BIA variables. However, general predictive equations and manufacturer's equations have also been frequently used. The choice of a prediction equation (from the abundant literature on the topic) that ideally matches the patient that he or she wishes to assess, is one of several limitations to the application of SF-BIA in the clinical setting (10).

Alternatively, as suggested by a growing number of authors(5, 10), a potentially useful approach to the use of SF-BIA might be to use the raw data generated, in particular, PhA.

Very few data have been published on SF-BIA raw data in COPD, specifically focusing on PhA (15-17). As reported by previous papers, it was shown to be higher in males, negatively correlated with age and positively with BMI and FEV_1 (% predicted) (15). Furthermore, it was shown to be significantly decreased across COPD stages suggesting that lower PhA may be associated with decreased cell integrity (16).

1.2 Multifrequency BIA

MF-BIA devices measure Z at several frequencies, usually in the range between 1-1000 kHz (5). Since at low frequency the current cannot pass through the cell membrane due to its capacitance, low frequency currents are conducted only through extra-cellular water (ECW), whereas high frequency currents penetrate cell membranes and are thus used to estimate total body water (TBW) (18). As a consequence, low to high frequency impedance ratio (i.e Z at 5 kHz / Z at 250 kHz) is a derived MF-BIA variable which provides information on water

distribution between intra and extra-cellular compartments and therefore on body cell mass and muscle quality (6, 9, 19).

In patients with COPD, BIA has usually been used to estimate FFM and body composition through predictive equations. As previously stated, very few studies had focused on raw BIA data in patients with COPD, specifically focusing on SF-BIA data (i.e PhA) and no comparison with healthy subjects has been carried out yet. Furthermore, no data on low to high frequency impedance ratio are available in COPD patients.

The general aim of this study was to evaluate whether MF-BIA is a useful tool for the assessment of nutritional status in COPD patients. More specifically, this study compared MF-BIA between COPD patients and matched controls and investigated the association of MF-BIA with gender, age, weight, BMI and FFM in COPD patients. Also, the relationship of MF-BIA with established markers of physical fitness, lung function and respiratory muscle strength in COPD patients was studied.

2. Methods

2.1 Subjects

Two hundred and sixty patients with COPD, consecutively admitted to the Pulmonary Rehabilitation Section of Clinic Center S.p.A (Naples, Italy) from March 2013 to November 2015, were assessed for eligibility. Forty-eight COPD patients did not meet the general inclusion criteria. Furthermore, a group of age and BMI matched controls, from a larger database was included. Inclusion criteria for both groups were age >50 years and BMI between 20 and 35 kg/m2. For COPD patients a baseline post-bronchodilator Forced Expiratory Volume in 1 second (FEV1)/forced vital capacity (FVC) of <70 was mandatory to be enrolled. Exclusion criteria were related to diagnosis of known respiratory disorders other than COPD, known history of significant inflammatory disease

other than COPD, a COPD exacerbation within 4 weeks of enrolment, and long term (>3 consecutive months) oral corticosteroids at time of inclusion. In addition, the usual criteria of uncontrolled disease (i.e. peripheral oedema or instable heart failure) likely to interfere with the study or impact on subject safety and substance abuse were applied.

Ethical approval was not indicated because all of the tests were done as part of the routine initial assessment, and analyzed retrospectively. De-identified records were used for COPD patients.. The Ethics Committee of Federico II, University of Naples, approved the study protocol for controls.

2.2 Study protocol

Starting at 9.30 AM, COPD patients and control subjects underwent body composition assessment. The same nutritionist always collected all data. For COPD patients, respiratory and other clinical parameters measurements followed, under the supervision of a chest physician. Always for COPD patients, physical fitness tests were also carried out.

2.3 Body composition

Body height and weight were measured using a platform balance (SECA 711+220; Hamburg - Germany), and body mass index (BMI) was calculated as body weight/height2. Body composition was assessed by performing MF-BIA in standardized conditions (i.e. ambient temperature between 23 and 25°C, fast of > 3 h, empty bladder, clean skin surface), using a Human Im-Touch analyzer (© DS Medica S.r.l., Milan, Italy).

Participants were asked to remain in the supine position for at least 10 minutes before starting the measurement, with legs and arms slightly abducted at 30° so there was no contact between the extremities and trunk.

Briefly, the procedure involves the placement of 2 electrodes on the hand (one on the bony protuberance that forms the wrist, i.e. the styloid process of the ulna, and the other just behind the meta-carpals), and 2 electrodes on the foot (one on the ankle placed midline between the medial and lateral malleoli, i.e. the styloid process of the radius, and the other just behind the metatarsals). The procedure was repeated for the left side.

Z was determined at five frequencies (5 kHz, 10 kHz, 50 kHz, 100 kHz and 250 kHz) with an imperceptible electrical current of 800 mA. In addition, bioelectrical impedance index (BI index) and low-to high- frequency impedance ratio were obtained as explained below. BI index was calculated by squared height divided by impedance at 50 kHz representing an established parameter of TBW (20). Z at 5 kHz and Z at 250 kHz are thought to be inversely related to ECW and TBW, respectively. By considering TBW as the sum of ECW+ intra-cellular water (ICW), for the same ECW, an increase of TBW may be interpreted as an increase in ICW. Therefore, as suggested by previous papers (5, 10), the ratio between Z at 5 kHz to Z at 250 kHz (5/250 impedance ratio) is used as an indicator of fluid distribution between intra/extra cellular compartments and muscle quality (10). FFM and FFM index (FFMI kg/m^2 = FFM/height^2) were estimated from Z at 50 kHz using a validated predictive BIA equation (21) in both groups for consistency, and also a disease specific equation in COPD patients only (14). FM and FMI (FMI kg/m^2 = FM/height^2) were calculated as total body weight minus FFM.

2.4 Lung function

All COPD patients performed a baseline post-bronchodilator spirometry and body plethysmography (QBOX® COSMED) according to ATS/ERS standardization (22). FEV$_1$ and FVC were assessed in accordance with the latest GOLD guidelines (23). Vital capacity (VC) and inspiratory capacity (IC) were assessed too. Plethysmographic lung volumes, such as Total Lung Capacity

(TLC), Intra Thoracic Gas Volume (ITGV) and Residual Volume (RV) were assessed. IC/TLC ratio (24) was calculated by dividing TLC−ITGV for TLC, and was used as a marker for hyperinflation, the abnormal increase in the volume of air remaining in the lungs at the end of spontaneous expiration (25).

2.5 Respiratory muscle strength

Maximum inspiratory pressures (MIP) and maximum expiratory pressures (MEP) were measured according to the method described by Black and Hyatt (26). Measurements were obtained in the sitting position with MicroRPM® (CareFusion, Hoechberg, Germany).

2.6 Physical fitness

As measures of physical fitness, handgrip strength (HGS) and 6-minute walk test (6MWT) were performed.

HGS, a surrogate measurement of overall muscle strength (27), was measured at baseline with a digital dynamometer (Dynex, MD systems Inc .Ohio USA). Three measurements were taken for both body sides. The maximum values obtained for each side was considered for statistical analysis (28).

6MWT was performed according to ATS standards (29), during which oximetry was performed at 10-second intervals with a pulse oximeter (Nellcor™ OxiMax N-65; Covidien, Boulder, CO). The 6-minute walk distance (6MWD) was the primary outcome of the 6MWT.

2.7 Other measurements

Breathlessness was measured by using the Medical Research Council (MRC) dyspnoea scale (30). A composite prognostic index, the BODE index (31), was used as surrogate of global disease severity.

2.8 Statistical analyses

Values were reported as means ± standard deviation (SD) unless otherwise specified. Comparisons between COPD patients and controls were conducted by analysis of variance (ANOVA). A general linear model (GLM) was applied for adjusting for age, weight and BMI. Additional analyses were performed in the COPD patients. Correlations were performed between 5/250 impedance ratio and weight, BMI, FFM and FFMI. In order to evaluate the impact of 5/250 ratio on physical fitness and respiratory parameters in COPD, male and female patients were also stratified according to the median value of 5/250 impedance ratio (124.4 for males; 122.7 for females). Subsequently, GLM was performed to investigate the association of the 5/250 impedance ratio with COPD diagnosis and severity. Statistical analysis was performed using SPSS version 20.0 and a *p value* <0,05 was considered significant in all analyses.

3 Results

3.1 Participants

After excluding 48 COPD patients who did not satisfied inclusion and exclusion criteria, 212 patients with stable COPD (144 men and 68 women) and 115 age-and-BMI matched controls (53 men and 62 women) were enrolled in this study. Age and BMI were comparable between COPD patients and controls, while a small difference in terms of weight still existed (table 1).

Participants had a mean age of 69.9±7.6 and 68.6±7.4 years and a mean BMI of 26.4±4.1 and 27.2±2.5 kg/m^2 (COPD and controls, respectively). Within the COPD group, patients had a mean FEV$_1$% predicted of 45.3% and COPD severity ranged from mild to very severe (GOLD I/II/III/IV: 2.5/30.8/37.3/29.4%).

3.2 Body composition in COPD patients compared to controls

In the unadjusted model, both male and female COPD patients had reduced FFM and FFMI compared to controls, while no difference was seen in FM or FMI. After adjusting for age, weight and BMI, FFM remained significantly reduced (and FM became significantly higher) in COPD patients compared to controls (between-group difference 2.33 kg; p<0.001).

With respect to raw MF-BIA data, the 5/250 impedance ratio was found to be significantly lower in COPD patients compared to controls both in males (124.1±4.9 vs 126.8±4.7; p<0.001) and females (122.3±4.1 vs 126.0±3.5; p<0.001), even after adjusting for age, weight and BMI (p<0.001).

3.3 Impedance ratio in COPD patients

Determinants of 5/250 impedance ratio in COPD

The 5/250 impedance ratio was higher (p <0.001) in male patients compared to female patients. The 5/250 impedance ratio was inversely related to age, both in males (r= −0.316; p<0.001) and females (r= −0.346; p<0.005). A significant correlation with weight (r=0.261; p<0.001), BMI (r=0.264; p<0.001), FFM (r=0.287; p<0.001) and FFMI (r=0.286; p<0.001) emerged only in males.

Body composition and clinical characteristics in patients with a low 5/250 impedance ratio

In table 2, COPD patients were stratified according to the median value of the 5/250 impedance ratio. In men, but not in women, lower values of 5/250 impedance ratio were associated with lower BMI (p<0.01), FFMI (p<0.02) and FMI (p<0.05). In addition, a significant reduction in HGS (and 6MWD) was seen both in male patients (p<0.001) and female patients (p<0.001). The same was true for 6MWD (see table 2). All these differences persisted after adjusting for age, weight and BMI. HGS (and 6MWD) was more strongly related to the 5/250 impedance ratio than to FFM or FFMI, both in male patients (r=0.514 vs r=0.399 and vs r=0.251; respectively) and female patients (r=0.660 vs r=0.204 and vs r=-0.204 respectively).

Table 3 shows respiratory parameters of COPD patients stratified according to the median value of the 5/250 impedance ratio. Patients with lower values had significantly lower FEV_1 (*p*=0.049) and VC (*p*=0.005), while no difference was found in any of the static lung hyperinflation markers considered (RV, ITGV, IC/TLC).

Regarding respiratory muscle strength, both MIP (*p*<0.001) and MEP (p=0.001) were significantly reduced in patients with lower 5/250 impedance ratio. Actually, MIP and MEP were more strongly related to 5/250 ratio

(r=0.471 and r=0.411 respectively) (figure 1[a] and 1[b]) than to FFM (r=0.352 and r=0.346) or FFMI (r=0.344 and r=0.306).

Finally, after adjusting for age, weight and BMI, the 5/250 impedance ratio was significantly reduced ($p<0.05$) in patients with GOLD III and IV, compared to those with GOLD I and II. Likewise, lower 5/250 impedance ratio ($p<0.001$) was reported in male as well as female patients with a BODE index between 6-10 points compared to those with an index between 1-5 points, even after adjusting for age, weight and BMI ($p<0.001$).

4. Discussion

The present study indicates that MF-BIA is a valid tool for identifying COPD with poor nutritional status. In particular, the 5/250 impedance ratio was decreased in COPD patients and relates to function and disease severity.

Alteration in nutritional status, which is a systemic effect of COPD, may be assessed using different methods. In particular, BIA is a widely used bedside method, to obtain estimates of body composition (TBW and FFM) using predictive equations (32, 33). In the present study, for consistency we used the same formula (21) to estimate FFM and FM in both COPD patients and controls. In accordance with previous papers (34-36), our study shows that FFM is significantly lower in COPD patients compared to controls. More interestingly, this difference persisted after adjusting for age, weight and BMI. In other words, COPD patients exhibited a lower ratio between lean tissues and adipose tissue, which is a typical feature of sarcopenia (37). Similar findings were obtained when a disease specific equation for COPD patients was employed (14).

Another issue of increasing interest is how to evaluate body composition in the clinical setting using raw BIA variables. In this regards, very few data are so far available for COPD patients; in particular, phase angle, as an index of cell quantity and/or cellular health, was recently shown to be independently associated with measures of physical fitness and disease severity (15, 38). On the other hand, to the best of our knowledge, there are no data on MF-BIA in COPD patients. MF-BIA measures Z at several frequencies: at low frequency, current is conducted only through ECW, whereas at high frequency it can penetrate cell membranes, being associated with TBW (18). Consequently, low to high frequency impedance ratios are raw MF-BIA derived variables that may provide direct information on both water distribution between intra-cellular and extra-cellular compartments and muscle quality (6, 9, 19).

To the best of our knowledge, this is the first study that compared the impedance ratio between COPD and controls. Our results show that the 5/250 impedance ratio is reduced in COPD patients compared to age-and-BMI matched controls (independently of age, weight and BMI) suggesting the presence of a disease-related cellular deterioration.

As far as between-patients variability was considered, age emerged as the most powerful predictor of the 5/250 impedance ratio in both genders, while after adjusting for age, no relationship persisted with weight, BMI or FFM (data not shown). These results were in line with previous papers showing that phase angle significantly declined with age in COPD patients (15).

From a clinical point of view, evaluating the relationship between body composition and body functions is a major issue in the diagnosis, assessment and management of chronic diseases. When COPD patients were stratified according to the median value of the 5/250 impedance ratio, those with a lower 5/250 impedance ratio exhibited poorer physical fitness, as estimated by HGS and 6MWD. Even more interestingly, in both genders, HGS, 6MWD and respiratory muscle strength (MIP and MEP) were more strongly related to the 5/250 impedance ratio than to FFM or FFMI. Furthermore, reduced lung function (ie FEV_1 and VC) was also seen in patients with a 5/250 impedance ratio below median value.

Finally, the reduction of the 5/250 impedance ratio was more pronounced in GOLD stages III/IV compared to I/II, even when age and BMI were taken into account as covariates. Likewise, a reduction of the 5/250 impedance ratio was observed in the patients with a higher BODE index (6-10 vs 1-5 BODE score). Thus, differences in the impedance ratio were related to disease severity.

4.1 Strength and limitations of the study

Taking into account that the experimental protocol was carried out in a single centre and had cross sectional study design, to the best of authors' knowledge,

this is the first study that evaluates impedance ratio. In addition, a quite large sample of COPD patients, with different disease severity and function impairment was studied. The main limitation is related to the fact that FFM was not concurrently measured with a reference technique, such as DXA. However, DXA does not provide information on water distribution between intra-cellular and extra-cellular compartments or muscle quality.

Overall, the results of the present study indicate that FFM and the impedance ratio were decreased in COPD patients, even after adjusting for age, weight, and BMI. The impedance ratio is affected by clinical conditions, being related to physical fitness and lung function. Thus, MF-BIA may be a useful tool for assessing body composition and nutritional status in COPD patients, especially with respect to cellular integrity and muscle quality.

5. Conclusion

Nutritional status is an important determinant of outcome of COPD. The measurement of raw MF-BIA data could be useful as a bedside approach, independently of body composition estimates, by allowing clinicians to identify malnourished patients, even if they are not underweight. Further multicentre studies are needed to better define the role of MF-BIA in assessing changes in nutritional status with time, due to clinical status, rehabilitation and nutritional treatment.

Table 1*: Age and body composition parameters of 212 COPD patients and 115 age-and-BMI matched controls.

	Males (N=197)			Females (N=130)		
	COPD (N=144)	Controls (N=53)	*p* Value	COPD (N=68)	Controls (N=62)	*p* Value
Age (years)	70.3±7.3	68.5±7.8	0.117	69.1±8.2	68.7±7.0	0.752
Height (cm)	165.4±7.0	167.5±6.9	0.063	152.3±6.4	156.9±7.5	**<0.001**
Weight (kg)	71.4±12.6	75.3±9.3	**0.041**	62.7±10.7	68.0±8.4	**0.002**
BMI (kg/m²)	26.0±3.9	26.7±2.4	0.227	27.0±4.6	27.6±2.7	0.383
FFM (kg)	53.0±7.2	57.6±7.4	**<0.001**	40.3±3.6	45.3±5.5	**<0.001**
FFMI (kg/m²)	19.3±1.4	20.5±1.7	**<0.001**	17.4±1.3	18.3±1.3	**<0.001**
FM (kg)	18.4±7.2	17.7±5.3	0.552	22.4±7.8	22.8±5.4	0.780
FMI (kg/m²)	6.7±2.5	6.3±1.9	0.330	9.7±3.3	9.3±2.1	0.373
5/250 IR	124.1±4.9	126.8±4.7	**<0.001**	122.3±4.1	126.0±3.5	**<0.001**

Note: Mean value ± standard deviation.

Abbreviations: BMI, Body Mass Index; FFM, Fat-Free Mass; FFMI, Fat-Free Mass Index; FM, Fat Mass; FMI, Fat Mass Index; 5/250 IR, ratio between impedance at 5kHz and 250 kHz.

Table 2: General characteristics of 212 COPD patients divided by gender and stratified according to the median value of 5/250 impedance ratio (124.4 for men; 122.7 for women).

	COPD male patients (N=144)			COPD female patients (N=68)		
	5/250 IR Below median value (n.72)	5/250 IR Above median value (n.72)	p Value*	5/250 IR Below median value (n.34)	5/250 IR Above median value (n.34)	p Value*
Age (years)	71.7±7.6	69.0±6.8	**0.029**	71.3±8.2	67.1±7.6	**0.030**
Weight (kg)	68.7±14.6	74.0±13.3	**0.012**	61.7±17.4	64.5±10.1	0.269
BMI (kg/m^2)	25.2±3.8	26.9±3.9	**0.009**	26.4±4.0	27.6±4.7	0.246
FFMI	19.0±2.0	19.7±1.9	**0.018**	17.4±2.4	17.5±1.4	0.719
FMI	6.3±2.5	7.1±2.2	**0.045**	10.2±6.9	10.1±3.4	0.979
HGS (Kg)	25.7±5.7	33.0±7.0	**<0.001**	16.1±3.3	22.0±4.9	**<0.001**
6MWD (m)	0; 348	300; 434	**0.002**	0; 60	240; 349	**0.030**

Note: Mean value ± standard deviation. Median value and interquartile range for 6MWD.

Abbreviations: 5/250 IR, ratio between impedance at 5kHz and 250kHz; BMI, Body Mass Index; FFMI, Fat-Free Mass Index; FMI, Fat Mass Index; HGS, handgrip strength – dominant side; 6MWD, 6 Minute Walk Distance.

Table 3: Respiratory parameters of 212 COPD patients stratified according to the median value of 5/250 impedance ratio (124.4 for men; 122.7 for women).

COPD patients (N=212)

	5/250 Below median value (n.106)	5/250 Above median value (n.106)	p Value*
FEV$_1$ (% predicted)	42.52±18.3	47.7±19.3	**0.049**
VC (% predicted)	66.0±17.8	73.3±19.0	**0.005**
RV (% predicted)	243.1±66.7	258.9±68.6	0.093
ITGV (% predicted)	183.3±49.1	195.1±48.9	0.081
IC/TLC	0.3±0.8	0.2±0.3	0.498
MIP (cmH$_2$O)	49.9.5±17.7	61.7±20.8	**<0.001**
MEP (cmH$_2$O)	72.2±24.2	84.5±27.3	**0.001**
MRC dyspnoea scale	3.8±0.4	3.6±0.5	**0.037**

Note: Mean value ± standard deviation.

Abbreviations: 5/250: impedance ratio between Z at 5kHz and Z at 250 kHz.; FEV$_1$: Forced Espiratory Volume in 1° Second; FVC: Forced Vital Capacity; VC: Vital Capacity; RV: Residual Volume; ITGV: Intrathoracic gas volume; IC: Inspiratory Capacity; TLC: Total Lung Capacity; MIP: Maximal Inspiratory Pressure; MEP: Maximal Espiratory Pressure

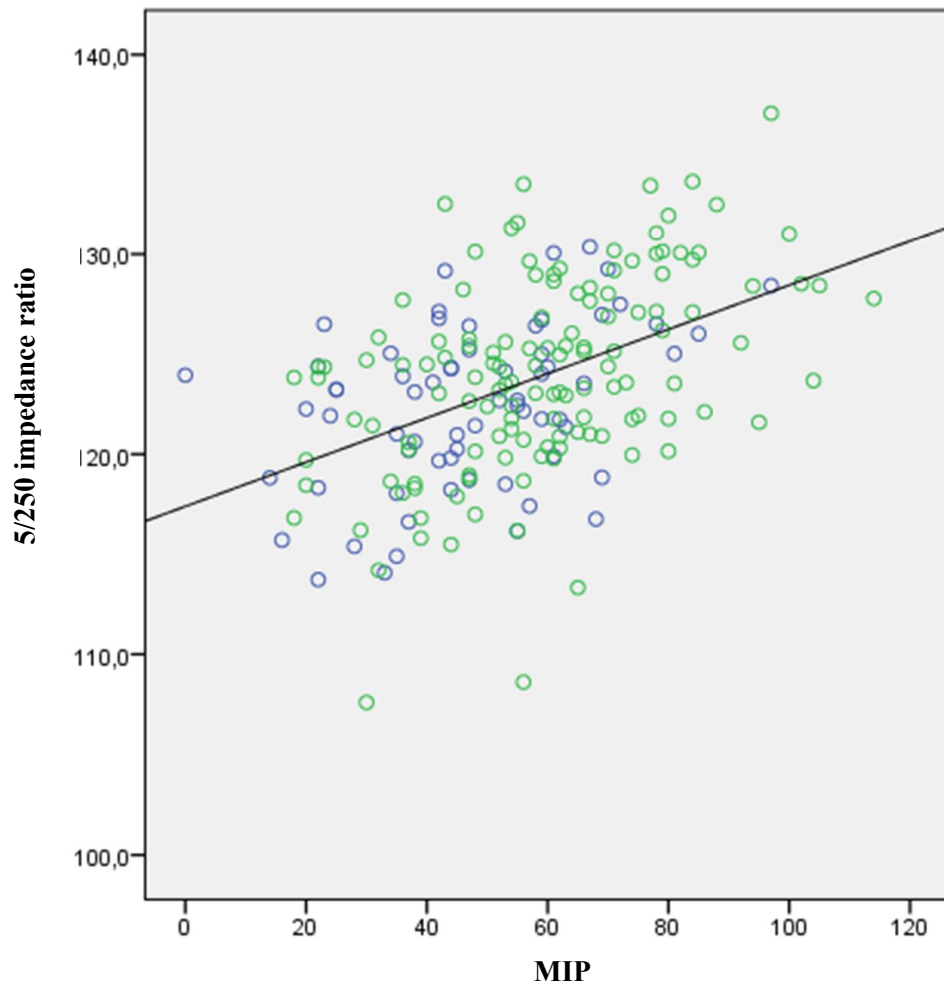**Figure 1**. Correlation plot of MIP with 5/250 impedance ratio in 212 COPD patients (r = 0.471; p<0.001)
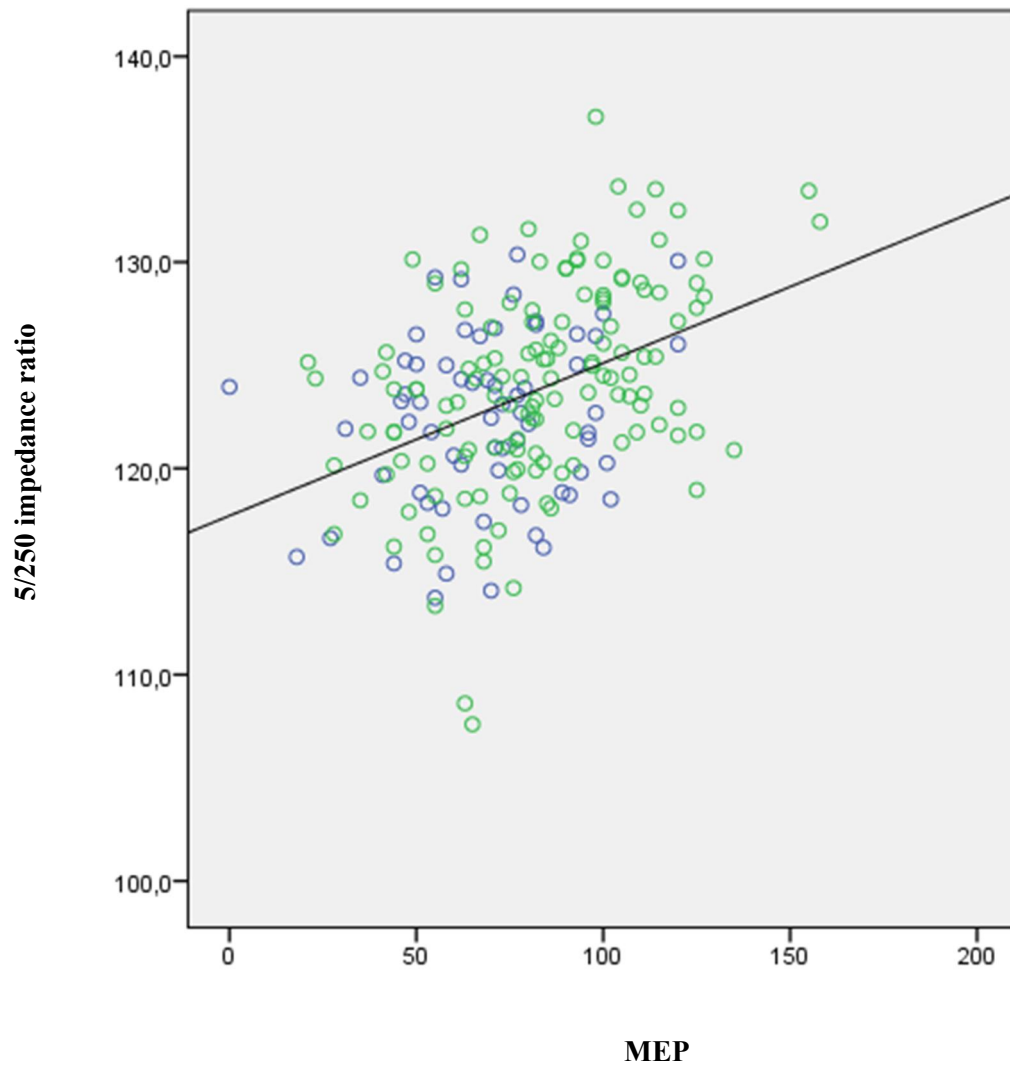
Figure 2. Correlation plot of MEP with 5/250 impedance ratio in 212 COPD patients (r = 0.411; p<0.001)

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CHAPTER VI

ASSOCIATION BETWEEN MUSCLE STRENGTH AND BIOELECTRICAL IMPEDANCE ANALYSIS IN COPD

Submitted for publication

Abstract

Loss of fat free mass (FFM) and reduced muscle strength, which are both highly prevalent in chronic obstructive pulmonary disease (COPD), may be evaluated in the clinical setting with single frequency bioelectrical impedance analysis (SF-BIA) and handgrip strength (HGS) test, respectively. In addition to BIA-derived body composition estimates, phase angle (PhA) is a raw BIA variable, considered as a potential marker of muscle quality. The aim of the present study was to assess whether PhA is a predictor of HGS and respiratory muscle strength in COPD patients, possibly stronger than anthropometric variables and BIA-derived body composition estimates.

Two-hundred and thirty-seven COPD patients (161 M, 76 F) underwent respiratory, anthropometric, SF-BIA (Human Im-Touch analyser, © DS Medica S.r.l., Milan, Italy), HGS and respiratory muscle strength (maximum inspiratory/expiratory pressure−MIP/MEP) measurements. FFM was estimated using three disease-specific BIA equations. BIA variables and HGS were calculated as the mean of dominant and non-dominant body sides.

Linear correlation analysis showed that in COPD patients the association of HGS with PhA ($p < 0.001$) was stronger compared to those with anthropometric variables and FFM estimates. In multiple regression analysis, PhA emerged as an independent predictor of HGS in males (adjusted $R2 = 0.502$; $p < 0.001$) and females (adjusted $R2 = 0.426$; $p < 0.001$). With respect to respiratory muscle strength, a stronger association was also found between MIP and PhA ($r = 0.456$ in males and $r = 0.421$ in females; $p < 0.001$) and, only in males, between MEP and PhA ($r = 0.450$; $p < 0.001$), compared to anthropometrics and BIA-derived FFM estimates.

In conclusion, as a raw variable, phase angle is the best predictor of handgrip strength in COPD patients, possibly providing more useful

information than BIA-derived FFM for the assessment of muscle quality beyond the use of body composition equations.

1. Introduction and aims

Skeletal muscle abnormalities are highly prevalent extra-pulmonary consequences in COPD patients (1, 2) in terms of loss of fat free mass (FFM)(3) and decreased muscle strength (4).

A decrease of FFM (atrophy) (2) was observed in 4 to 35% COPD patients and may occur independently of preserved or even increased body mass index (BMI) (5, 6).

From a clinical perspective, low FFM, independently of BMI and fat mass, may have adverse effects on health status (7), increasing the frequency or severity of acute exacerbations of the disease, and being a strong predictor of mortality (8, 9).

Bioelectrical impedance analysis (BIA) provides estimates of FFM by means of predictive equations which include BIA variables and frequently other variables such as age and weight (in some cases gender) (10). As an alternative, the use of raw BIA variables, such as phase angle (PhA) or impedance ratio (IR), has gained popularity in the clinical setting, in order to evaluate the extracellular/intracellular distribution of water, body cell mass and muscle quality (10-16).

Higher PhA, which is a directly-measured (raw) BIA variable expressed in degrees (17), suggests greater cell quantity or better muscle quality, while a smaller PhA suggests cellular loss or reduced cellular integrity (18). Preliminary data in COPD showed that PhA significantly decreased across COPD stages (19). Furthermore, it was found to be negatively correlated with age and positively with BMI and FEV_1 (% predicted) (12).

Decreased muscle strength (or weakness) is also common in COPD, affecting both lower and upper limb muscles (1), possibly due to a number of

specific reasons such as deconditioning, inflammation, malnutrition, oxidative stress, hypoxemia, in addition to a loss of muscle mass (2).

Lower limb muscle strength, which is usually evaluated by measuring isometric knee extension strength (i.e. the quadriceps strength), is reduced by 20 to 30% in COPD patients (2), and is considered to be a strong predictor of both exercise capacity and mortality (20).

Similarly, handgrip strength (HGS), which is related to the strength of forearm and hand muscles, was found to be reduced in malnourished/sarcopenic COPD patients (21-25), being inversely associated with their quality of life and mortality (26, 27).

Despite the obvious interest in evaluating changes in both body composition and skeletal muscle abnormalities − for example, for defining nutritional phenotypes(9) − only few studies (28-33) have provided data on the relationship between upper and lower limbs muscle strength and FFM. Patients with depleted FFM (measured by BIA) were previously found to have lower HGS (29, 30). More specifically, preliminary data evidenced a significant correlation between HGS and BIA-derived FFM (31, 32) Nevertheless, the relation between muscle strength and raw BIA variables has so far been poorly evaluated in COPD (12, 15). Furthermore, to the best of our knowledge only three papers have focused on the relationship between respiratory muscle strength and body composition (34-36), none of which took into consideration raw BIA variables.

With this perspective, the major aim of this study was to assess retrospectively the relationship between HGS and raw single frequency BIA in COPD patients. We also tested the hypothesis that raw single frequency BIA (SF-BIA) variables are stronger predictors of HGS than anthropometrics and body composition BIA estimates (e.g., FFM). The secondary aim was to analyse the relationship between maximum inspiratory and expiratory muscle strength and SF-BIA variables.

2. Methods

2.1 Subjects

Patients with COPD, consecutively admitted to the Pulmonary Rehabilitation Section of Clinic Center S.p.A (Naples, Italy) from March 2013 to May 2016, were assessed for eligibility. Inclusion criteria were age >50 years and a baseline post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) < 0.7. Exclusion criteria were related to diagnosis of known respiratory disorders other than COPD, known history of significant inflammatory disease other than COPD and a COPD exacerbation within 4 weeks of enrolment. The Ethics Committee of the "Federico II" University of Naples approved the research protocol and all patients gave informed consent to participate in the study.

2.2 Lung function

All COPD patients performed a baseline post-bronchodilator spirometry and body plethysmography (QBOX® COSMED) according to American Thoracic Society (ATS)/European Respiratory Society (ERS) standardization (37). FEV_1 and FVC were assessed in accordance with the latest GOLD guidelines (38).

Patients were classified into four stages according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. If $FEV_1 \geq 80\%$ of the predicted, patients were classified as GOLD stage I (mild), if $50\% \geq FEV_1 > 80\%$ of the predicted, as GOLD stage II (moderate), $30\% \geq FEV_1 > 50\%$ of the predicted as GOLD stage III (severe), and if <30% of the predicted as GOLD stage IV (very severe).

2.3 Body composition

Body weight and body height were measured to the nearest 0.1 kg and 0.5 cm respectively, using a mechanical column scale (SECA 711+220; Hamburg - Germany), and BMI was calculated as body weight/height.^{2} Body composition was assessed by performing BIA in standardized conditions (i.e. ambient temperature between 23 and 25°C, fast of > 3 h, empty bladder, supine position for at least 10 minutes before starting the measurement), using a Human Im-Touch analyzer (© DS Medica S.r.l., Milan, Italy). In addition, after cleaning skin surface, patients were asked to lay with legs and arms slightly abducted at 30° so there was no contact between the extremities and trunk.

A standard tetra-polar technique was used, with measuring electrodes placed on the anterior surface of the wrist and ankle, and injecting electrodes placed on the dorsal surface of the hand and the foot, respectively. Z and phase angle were obtained at 50 kHz for both dominant (D) and non-dominant (ND) sides with an imperceptible electrical current of 800 mA. In addition, bioelectrical impedance index (BI index) was calculated as squared height divided by impedance at 50 kHz, representing an established parameter of total body water (TBW) (39). Phase angle, expressed as a degree, provides information on hydration status, cellular mass and quality (17, 18). FFM and FFM index (FFMI kg/m^2 = FFM/height^2) were estimated from Z at 50 kHz using three different BIA equation (40-42). Fat mass (FM) was calculated as total body weight minus FFM and FMI (kg/m^2) as FM/height^2.

2.4 Muscle strength

HGS, a surrogate measurement of overall muscle strength (43), was measured at baseline with a digital dynamometer (Dynex, MD systems Inc .Ohio USA). Three measurements were taken for both body sides (dominant and non

dominant). Mean value of the two body sides was indicated as whole body HGS (or HGS). The maximum values were considered for statistical analysis (44).

Respiratory muscle strength was measured as maximum inspiratory pressures (MIP) and maximum expiratory pressures (MEP), according to the method described by Black and Hyatt (45). Measurements were obtained in the sitting position with MicroRPM® (CareFusion, Hoechberg, Germany).

2.5 Other measurements

Breathlessness was measured using the Medical Research Council (MRC) dyspnoea scale (46). A composite prognostic index, the BODE index (47), was used as surrogate of global disease severity.

2.6 Statistical analysis

Whole body data were obtained by calculating the mean of the measurements taken for D and ND body sides. Values were reported as mean ± standard deviation (SD) unless otherwise specified.

Pearson correlation coefficient was used to evaluate the association between variables, a general linear model was applied for adjusting data for age, weight and BMI.

The McNemar's test was used to compare the prevalence of low muscle mass according to the different FFM equations. Statistical analysis was performed using SPSS version 20.0 and a p value <0.05 was considered significant in all analyses.

3. Results

Two hundred and thirty-seven patients (161 males and 76 females) with stable COPD were studied. The majority of patients had moderate to severe impaired lung function, as 3.4% of patients were classified in GOLD stage I, 30.4% in

GOLD stage II, 39.7% in GOLD stage III, and 26.6% in GOLD stage IV. Characteristics of the whole group of patients were shown in table 1.

3.1 SF-BIA data

As far as raw SF-BIA data are concerned, BI-indexes were significantly lower by about 23.3% in females than males ($p<0.001$), while PhA was higher (by 6.0%) in males ($p=0.034$). PhA was inversely correlated ($p<0.001$) with age, both in males ($r=-0.281$) and females ($r=-0.202$), and directly correlated with height ($r=0.188$; $p=0.017$), weight ($r=0.317$; $p<0.001$) and BMI ($r=0.282$; $p<0.001$) only in males. In a multivariate analysis age ($p<0.001$), BMI ($p=0.02$) and FEV_1 measured ($p<0.001$) emerged as simultaneous significant predictors of PhA in males, while height and weight did not (overall adjusted $R^2=0.220$, $p<0.001$). On the contrary, in females, the only significant determinant of PhA was FEV_1 measured (overall adjusted $R^2=0.049$, $p=0.031$).

A significant decrease of PhA across GOLD stages was reported only in males (5.37±0.89 degrees, 5.00±0.92 degrees and 4.82±0.99 degrees in I+II, II and IV, respectively; $p=0.02$). Furthermore an inverse correlation with BODE index was also reported, both in males ($r=-0.343$, $p<0.001$) and females ($r=-0.358$, $p=0.006$).

With regards to body composition, FFM was estimated by using three different equations. Whichever predictive equation used, FFM and FFMI were clearly significantly higher and percentage of body fat lower in males compared to females (table 2).

In both genders, FFM and FFMI estimates significantly differed depending upon the equation used. Mean FFM (and, consequently, FFMI) was the highest when the Rutten equation was used and the lowest when the Kyle equation was used.

3.2 Relationship between muscle strength and body composition

With respect to simple correlation analysis, the association between HGS and demographic and anthropometric parameters was more evident in males than in females. In males, HGS (p<0.001) was inversely related to age, and directly to height, weight and BMI, while in females a significant relationship emerged only for height (p<0.001) and less consistently for age (p=0.07). With respect to respiratory muscle strength, a significant relationship emerged in males between MIP and weight, BMI, and FEV_1 (r=0.424) (similar relationship emerged with MEP); while in females a significant relationship was found between MIP and weight and FEV_1 (r=0.347), MEP and weight and BMI.

Indeed, FFM tended to be more strictly associated with HGS (r between 0.472 and 0.480, in males, and between 0.235 and 0.302, in females), than demographic and anthropometric variables. The association with HGS was clearly weaker (i.e. r below 0.3) for FFMI, independently of the equation used (data not shown).

In addition, our findings showed that BI indexes were positively and more strongly correlated with HGS compared to BIA-derived FFM estimates (quite more strongly in males than in females) (table 3). Similar data were obtained from the two sides separately. Interestingly, the association was even stronger for PhA (r=0.584, p<0.005 and r=0.635, p<0.005 in males and female, respectively−figure 1).

Similar results were obtained for respiratory muscle strength. Indeed, PhA was more strongly related to MIP and MEP compared to BI index and, more interestingly, compared to FFM estimates (table 3), in males. In females, a positive and moderate relation emerged only with respect to MIP (but not to MEP).

Multiple regression model was applied in order to identify the best predictors of HGS, first considering demographic and anthropometric variables,

plus FEV_1 as potential determinants. In males, age (p<0.001), height (p<0.001), weight (p<0.001), BMI (p<0.001) and FEV_1 (p=0.002) entered into the model with a coefficient of determination (R^2) of 0.333. In females, only height entered into the model (age, weight, BMI and FEV_1 were excluded) with a R^2 of 0.280. Similar findings were obtained when D and ND sides were considered separately (data not shown).

When included together in the regression model, age, FFM and FM (FEV_1 was excluded) all emerged as determinants of HGS in all models, except for age and FM in females when the Steiner equation was used. Adjusted R^2 was 0.397, 0.388 and 0.412 in males, and 0.221, 0.043 and 0.201 in females for Rutten, Steiner and Kyle equation, respectively.

Finally, age FFM, raw BIA variables and FEV_1 were taken into consideration. PhA and BI index emerged as predictors of HGS (table 4), together with age only in males. The R^2 of the regression were 0.502 and 0.426 in males and females, respectively. In practical terms, HGS was almost 3 kg higher for each degree of PhA increased in both genders (1 degree roughly corresponds to 1 SD). Similar results emerged when dominant and non-dominant sides were considered separately (data not shown).

Similarly, a multiple regression analysis was applied in order to identify the best predictors of MIP and MEP, with age FFM, raw BIA variables and FEV_1 as independent variables. As shown in table 4, PhA and BI index emerged as predictors of both MIP and MEP in males, while in females, only PhA emerged as predictor of MIP, (p<0.001 for all analysis), but not of MEP.

4. Discussion

The main finding of the present study is that in COPD patients, raw SF-BIA variables (BI index and PhA) are strong predictors of muscle strength, as expressed by HGS, and to a more significant extent compared to age, height, weight, BMI and body composition estimates derived from BIA (i.e. FFM) . As an additional finding, we have also observed that, in the same way, MIP and MEP tended to be more strictly related to raw BIA data than to other potential predictors, as above mentioned.

As recently outlined (9), different metabolic phenotypes may be described in COPD patients, by assessing body composition and muscle strength, such as muscle wasting, sarcopenia, sarcopenic-obesity and obesity. Actually, loss of skeletal muscle mass and weakness are highly prevalent in COPD, being associated with decreased physical activity, exercise intolerance, poor quality of life, and higher morbidity and mortality (2, 27).

FFM and, more specifically, skeletal muscle mass, can be assessed in terms of quantity and quality (i.e., body cell mass and cellular integrity). With this perspective, raw BIA variables (BI index and PhA) are receiving increasing attention, beyond using them in body composition equations, as suggested by a growing number of authors (10, 14, 18, 48). For instance, phase angle is thought to provide direct information on cellular mass and muscle quality (18, 49).

Very few data have been published on PhA in COPD (12, 15, 19). It was previously shown to be higher in males, negatively correlated with age and positively with BMI and FEV_1 (% predicted) (12). Furthermore, it was shown to be significantly decreased across COPD stages possibly because of the decreased cell integrity (19). We extended these observations (12, 19) by examining determinants of PhA separately in males and females, finding a significant decrease of PhA across GOLD stages in males, a direct correlation

with FEV_1 in both genders, an inverse correlation with age and a direct correlation with BMI only in males.

Furthermore, in a multivariate analysis, PhA, age, FEV_1 and BMI emerged as significant predictors of PhA in males, and only FEV_1 in females.

In addition, it should be noted that underweight male patients (BMI<21 kg/m^2) have lower values of PhA compared to those with a higher BMI (4.49±0.98 degrees vs 5.10±0.90 degrees; p<0.001). No difference was found in underweight female patients compared to those with a normal weight. Further investigations are needed in the future, in order to better understand whether the occurrence of this between-genders difference is real and due to a physiological gender dichotomy, and to relate it with nutritional status.

Loss of skeletal muscle strength is another feature of muscle dysfunction, and a major systemic consequence of COPD (2). According to the European Working Group on Sarcopenia in Older People (EWGSOP), low skeletal muscle strength can be diagnosed by measuring HGS, a good and reliable surrogate for more complicated measures of muscle strength, such as isokinetic quadriceps muscle strength (50).

In agreement with previous studies showing reduced HGS in COPD (51), low HGS (<30 kg in males and <20 kg in females, according to EWGSOP), has been found in 63.4% and 71.1% of our male and female COPD patients, respectively.

Taking into consideration the scarcity of data, the primary aim of the present study was to evaluate the relationship of muscle strength (in terms of both HGS and respiratory muscle strength) and raw BIA variables, estimates of body composition and other potential predictors.

Indeed, very few studies have provided limited data on the relationship between muscle strength and body composition in COPD (none as primary endpoint), showing that patients with depleted FFM had lower HGS (29-32).

Furthermore, only one paper has already been published on the association between quadriceps strength and PhA (12).

Simple correlation analysis shows that HGS is more strongly related to raw BIA data and, in particular, to PhA, compared to anthropometric variables and FFM estimates, demonstrating that PhA may represent a novel marker of cellular mass and muscle quality.

In order to identify the best predictor of muscle strength, we have analysed several potential determinants of HGS (age, FEV_1, BIA-derived FFM estimates, BI index and PhA), finding an inverse relation between HGS and age only in males, in the way that older male patients are more prone to have lower HGS values (−0.27 kg for each year).

Furthermore, raw BIA variables emerged as significant predictors of HGS, while FFM estimates and FEV_1 did not. In particular, HGS directly increases by 0.25 kg for each cm^2/ohm of BI index and 2.83 kg for each degree of PhA at 50 kHz, in males. In females, a similar association was found between HGS and PhA, in the way that HGS increases by 3.09 kg for each degree of PhA at 50 kHz. Nevertheless, differences emerged with respect of the association with BI index, between genders.

Interestingly, when considered simultaneously in the regression model, age, FEV_1 and BIA-derived FFM estimates were excluded.

Respiratory muscle strength is another objective functional measure with prognostic potential in COPD patients (52). In particular, a single study has shown that MIP was associated with PhA in cancer patients (53), while to the best of our knowledge no data are available in COPD. Actually, we have found a positive association between respiratory muscle strength and raw SF-BIA data (PhA and BI index). A possible explanation to this association could be the reduced mass of the diaphragm muscle and chest wall or reduced mechanical efficiency of the respiratory muscles in COPD patients with poorer body

composition (54). The association between respiratory muscle strength and raw BIA variables was stronger compared to those with other potential predictors (anthropometrics and BIA-derived FFM estimates). Furthermore, PhA emerges as an independent predictor of both MIP and MEP in males, and only of MIP in females, highlighting that PhA, as raw SF-BIA variable, represents a novel marker of cellular function and muscle quality (also in terms of muscle strength).

Some limitations of the study must be acknowledged. Firstly, this is a single centre study, which, does however, include COPD patients referred for a pulmonary rehabilitation program, from several hospitals and chest physicians in Campania (number of residents = 5,850,850), South Italy.

Secondly, although BIA is a reliable method for measuring FFM and FFMI in population studies (48), no reference technique for body composition (e.g. dual-energy X-ray absorptiometry) was available. However, given the advantages of BIA, it has great potential for measuring body composition in large populations, being less expensive, and more easily accessible than other methods in clinical settings. On the other hands, three disease specific BIA equations were considered in order to obtain estimates of FFM. Furthermore, it should be noted that muscle strength as well as body composition measurements have been taken for both body sides, separately, in clinically stable patients. All measurements are highly repeatable and reproducible. To the best of author's knowledge, this is the first study, which takes into consideration raw SF-BIA data as independent predictors of handgrip strength.

In conclusion, the present study demonstrates that PhA, as raw BIA variable, is an independent predictor of muscle strength, more strongly associated to HGS and respiratory muscle strength, compared to and BIA-derived FFM estimates and, even more to anthropometric parameters, such as height, weight and BMI. The results of this study suggest that the assessment of raw BIA variables may have a role in the overall nutritional assessment in

COPD. Nevertheless, longitudinal studies are needed to examine whether these variables can predict risk of functional decline in these patients.

5. Tables and figures

Table 1. Demographic parameters and raw BIA variables by genders in stable COPD patients.

	Males (n=161)	Females (n=76)	P
Age (years)	70.4±7.5	68.7±7.8	NS
Height (cm)	165.2±7.0	152.7±6.3	<0.001
Weight (kg)	69.9±16.8	64.7±13.7	0.021
BMI (kg/m²)	25.5±5.5	27.8±5.8	0.004
FEV_1 (% pred.)	41.1±18.2	49.8±20.3	0.001
FEV_1/FVC	54.9±8.4	58.2±7.7	0.005
Impedance (Ohm)	546±103	600±82	<0.001
Bioimpedance index (cm^2/Ohm)	51.6±9.8	39.6±6.3	<0.001
Phase angle (degrees)	5.1±1.0	4.8±0.9	0.034
HGS (kg)	27.8±7.1	18.4±4.6	<0.001
MIP (mmH_2O)	56.4±21.0	47.6±18.1	0.002
MEP (mmH_2O)	79.8±26.7	71.9±21.3	0.029

Note: Data are presented as mean ± standard deviation. Bioimpedance index, phase angle and HGS expressed as mean of the two body sides.

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HGS, handgrip strength, MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure.

Table 2. BIA-derived body composition, as estimated with different equations, in clinically stable COPD patients.

	Males (n=161)	Females (n=76)	P
FFM $_{Rutten}$ (kg)	50.4 ± 7.2^{ab}	38.4 ± 5.1^{b}	<0.001
FFM $_{Steiner}$ (kg)	47.3 ± 7.7^{ac}	38.3 ± 4.9^{c}	<0.001
FFM $_{Kyle}$ (kg)	46.1 ± 6.3^{bc}	36.1 ± 4.7^{bc}	<0.001
FFMI $_{Rutten}$ (kg/m^2)	18.5 ± 2.1^{ab}	16.5 ± 1.9^{b}	<0.001
FFMI $_{Steiner}$ (kg/m^2)	17.3 ± 2.5^{ac}	16.5 ± 2.0^{c}	<0.010
FFMI $_{Kyle}$ (kg/m^2)	16.9 ± 1.9^{bc}	15.5 ± 1.8^{bc}	<0.001
Body fat $_{Rutten}$ (%)	26.0 ± 8.7^{ab}	39.5 ± 6.5^{b}	<0.001
Body fat $_{Steiner}$ (%)	30.9 ± 7.4^{ac}	39.6 ± 7.2^{c}	<0.001
Body fat $_{Kyle}$ (%)	32.3 ± 8.2^{bc}	43.1 ± 6.4^{bc}	<0.001

Note: Data are presented as mean ± standard deviation.

P value < 0.05 for significant differences.

[a] = p<0.001 between Rutten and Steiner equation

[b] = p<0.001 between Rutten and Kyle equation

[c] = p<0.001 between Steiner and Kyle equation

Abbreviations: FFM,fat-free mass; FFMI, fat-free mass index; ASMM, appendicular skeletal muscle mass, ASMMI, appendicular skeletal muscle mass index.

Table 3. Correlation between body composition and muscle strength in clinically stable COPD patients (161 males and 76 females).

	Males			Females		
	HGS	MIP	MEP	HGS	MIP	MEP
Age	−0.419**	−0.056	−0.076	−0.209	−0.176	−0.085
Height	0.385**	0.073	0.097	0.538**	0.086	0.156
Weight	0.373**	0.342**	0.307**	0.113	0.245*	0.443**
BMI	0.255**	0.358**	0.300**	−0.069	0.223	0.395*
FFM $_{Rutten}$	0.472**	0.385**	0.342**	0.301**	0.178	0.320*
FFM $_{Steiner}$	0.472**	0.423**	0.366**	0.235**	0.173	0.299*
FFM $_{Kyle}$	0.480**	0.410**	0.353**	0.302**	0.193	0.317*
BI index	0.505**	0.447**	0.378**	0.291**	0.182	0.276*
PhA	0.584**	0.456**	0.450**	0.635**	0.421**	0.127

Note: Data are presented as coefficients correlation (r). Bioimpedance index, phase angle and HGS expressed as mean of the two body sides.

*$p<0.05$; **$p<0.005$

Abbreviations: BMI, body mass index; FFM, fat-free mass; BI index, bioelectrical impedance index; PhA, phase angle; HGS, handgrip strength; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure.

Table 4. Multivariate stepwise regression with HGS, MIP and MEP as dependent variables for male (M) and female (F) patients, separately.

Males

HGS	= 19.6	+ 0.25 × BI-index	+ 2.83 × PhA	− 0.27 × age	R²=0.502	SEE=5.02
	(5.2)	(0.04)	(0.47)	(0.06)		
MIP	= −16.9	+ 0.69 × BI-index	+ 7.46 × PhA	NS	R²=0.292	SEE=17.02
	(9.2)	(0.16)	(1.59			
MEP	= −5.8	+ 0.68 × BI-index	+ 10.07 × PhA	NS	R²=0.248	SEE=23.16
	(12.1)	(0.20)	(2.09)			

Females

|						
HGS	= − 1.9	+ 0.14 × BI-index	+ 3.09 × PhA	NS	R²=0.426	SEE=3.37
	(0.5)	(0.06)	(0.45)			
MIP	= 5.3	NS	+ 8.83 × PhA	NS	R²=0.165	SEE=16.51
	(11.1)		(2.28)			
MEP	= 4.0	+ 0.92× BI-index	NS	NS	R²=0.167	SEE=19.4
	(17.7)	(0.36)				

Note: HGS as a mean of the two body sides. Standard errors of the regression coefficients in brackets.

Dependent variables: Age, FFM, BI index, PhA, FEV_1.

FFM and FEV_1 were excluded from all models

Abbreviations: BI index, bioelectrical impedance index; PhA, phase angle; HGS, handgrip strength; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; SEE, standard error of estimate.

Figure 1. Correlation plot between PhA at 50 kHz and HGS in males (r=0.584) and females (r=0.635), separately (p<0.005).
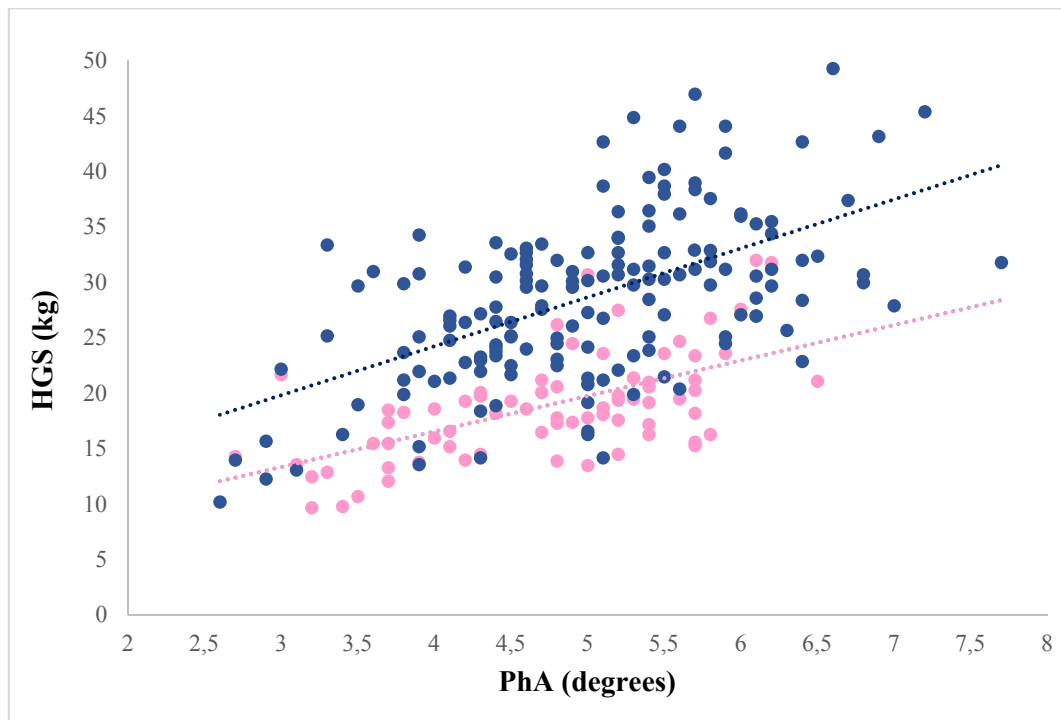
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CHAPTER VII

ASSESSMENT OF VISCERAL ADIPOSE TISSUE USING DUAL-ENERGY X-RAY ABSORPTIOMETRY IN COPD

Abstract

Visceral adipose tissue (VAT) was shown to be increased in patients with chronic obstructive pulmonary disease (COPD) compared to control subjects with comparable body mass index (BMI). The aims of the present study were to determine the relation of VAT by dual-energy x-ray absorptiometry (DEXA) in patients with COPD by disease severity, BMI, other indices of body composition and static lung volumes.

294 COPD patients admitted for rehabilitation were studied. Lung function, static lung volumes and body composition (i.e. BMI, waist circumference, fat-free mass, fat mass and fat distribution between android and gynoid fat mass) were assessed before entering pulmonary rehabilitation. VAT was estimated within the android region by using DEXA. Patients were stratified for gender, BMI (cutoff of 25 kg/m) and GOLD stage. To assess the impact of VAT on lung volumes, patients were also stratified for VAT less and above 50th percentile.

Both male and female patients with more severe airflow limitation had significantly lower VAT values, but these differences disappeared after stratification for BMI. VAT was significantly and strongly correlated with other body composition parameters (all *p < 0.001*). Patients with moderate to severe airflow limitation and lower VAT had increased static lung hyperinflation and lower diffusing capacity for carbon monoxide. Nevertheless, multivariate stepwise regression models including for BMI, age, gender and forced expiratory volume in 1 s (FEV) as confounders did not confirm an independent role for VAT on static lung hyperinflation and diffusion capacity.

In conclusion, after stratification for BMI, VAT is comparable in moderate to very severe COPD patients. Furthermore, BMI and demographics, but not VAT, were independent predictors of static lung hyperinflation and diffusing capacity in COPD.

1. Introduction and aims

Although defined by the presence of persistent airflow limitation (1), alterations in body weight and body composition contribute to disease severity in patients with chronic obstructive pulmonary disease (COPD). Indeed, low body mass index (BMI) (<21 kg/m^2) is related to poor exercise tolerance (2), low fat-free mass (FFM) (3) and co-morbid osteoporosis (4). Furthermore, it is incorporated in the prognostic assessment of COPD (5). Otherwise, high BMI (≥30 kg/m^2) is related to increased dyspnoea (6), reduced weight-bearing exercise performance (7) and systemic inflammation in COPD (3). At the same time, reductions in lung volumes have been consistently reported in obese COPD patients (8).

In addition to the discriminating role of BMI in the clinical phenotyping of COPD patients, alterations in body composition, i.e. the distribution between FFM and fat mass (FM), are common and have additional impact on the burden of disease. In more detail, visceral adipose tissue (VAT) is receiving increasing attention in COPD, since it was shown that elderly subjects with obstructive lung disease have increased visceral adipose area in comparison with control subjects with comparable BMI (9). Also, there is increasing evidence for a role of VAT in the pathophysiology of systemic inflammation (10) and cardiovascular morbidity in COPD (10, 11).

Typically, VAT is assessed by computer tomography (CT) or magnetic resonance imaging (MRI), but these measurements are expensive, time-consuming and not widely available. Alternatively, dual-energy x-ray absorptiometry (DEXA) is a commonly used device with lower radiation dose, alternative to estimate body composition in COPD and was recently introduced as a validated method to quantify VAT (12, 13). However, no clinical studies investigating VAT with DEXA was carried out yet in COPD patients. Thus, associations between VAT assessed by this method and other traditional markers of body composition in COPD was still unknown. Also, the impact of VAT per se on lung volumes was not previously studied in COPD.

Therefore, the aims of the present study were: 1) to quantify the amount of VAT measured by DEXA in patients with COPD in relation to disease severity and BMI; 2) to explore the relationships between VAT and other indices of body composition and 3) to study the impact of VAT on static lung volumes. In the present study, we hypothesized that VAT does significantly and negatively influence static lung volumes and hyperinflation in patients with COPD.

2. Methods

2.1 Subjects

Data were abstracted from the Integrated Knowledge System based on BioXM™ (Biomax Informatics AG, Munich, Germany) of 294 patients with a primary diagnosis of COPD (post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio of $\leqslant 0.70$ and post-bronchodilator FEV_1 <80% predicted) who were evaluated during the initial assessment of a comprehensive pulmonary rehabilitation (PR) program at CIRO Horn (The Netherlands) between August 2014 and May 2015 (14). Exclusion criteria were: a history of asthma, a1-antitrypsin deficiency, lung cancer, any previous lung surgery, instable inflammatory or endocrine diseases, acute myocardial infarction within the last 6 months, any known bone disease other than osteoporosis, treated malignant disease within the last 5 years and an exacerbation of COPD within the last 4 weeks. Ethical approval was not indicated because all of the tests were done as part of the routine initial assessment (14), and analyzed retrospectively. The Board of Directors of CIRO approved the use of de-identified patients' records.

2.2 Lung function measurement

As part of the routine 3-day initial assessment of integrated health status, all patients performed post-bronchodilator spirometry (Masterlab®; Jaeger, Würzburg, Germany). FEV_1 and FVC were assessed in accordance with the

latest GOLD guidelines (1). Inspiratory vital capacity (IVC) was assessed too. Plethysmographic lung volumes, such as Total Lung Capacity (TLC), Intra Thoracic Gas Volume (ITGV), Expiratory Reserve Volume (ERV) and Residual Volume (RV) were assessed. Inspiratory Capacity(IC)/TLC ratio (15) was calculated by dividing TLC−ITGV for TLC, and was used as a marker for hyperinflation (defined as an abnormal increase in the volume of air remaining in the lungs at the end of spontaneous expiration (16)). Diffusing capacity of the lung for carbon monoxide (DLCO) was measured using the single-breath method. All obtained values are expressed as percentages of the predicted value, by comparison with age and sex-specific reference values (17).

2.3 Body composition

Body height was measured to the nearest 0.5 cm with a wall-mounted stadiometer. Body weight was assessed to the nearest 0.1 kg using a weighing scale, and BMI was calculated as weight/height^2. A total body scan was performed by DEXA using a Lunar Prodigy® system (GE Healthcare - enCORE v14, Madison, WI, USA). Subjects lay supine on the DEXA table with arms adequately separated from the trunk and were instructed to remain still throughout the scanning procedure. After analysis of the whole body scan, a quadrilateral box was manually drawn around the L1–L4 region of interest (abdomen) bounded inferiorly by the horizontal line identifying L4/L5 vertebral space and superiorly by the horizontal line identifying the T12/L1 vertebral space. Scans were displayed with an adjustment of the gray scale, so that all of the soft tissue in the designated area was included. From the total body scan, body composition was assessed: bone mineral content (BMC), lean mass (LM), FFM (LM plus BMC). FFMI was calculated by dividing FFM by height^2, and FM by subtracting FFM from total weight. The base of the android region (figure 1A - shown in blue) sits immediately above the pelvis and is in height equal to 20% of the distance from the pelvis to the chin. The android and gynoid (figure 1A - shown in red) regions are separated by a distance equal to 1.5 times

the height of the android region, while the height of the gynoid region is double that of the android one. VAT was estimated within the android region (12) (figure 1B number 1).

2.4 Statistical Analysis

Values are reported as means ± SD with the exception of continuous variables, presented as median and interquartile range (IQR). In order to quantify the amount of VAT in relation to disease severity, patients were stratified for gender, BMI (cut-off of 25 kg/m^2) and GOLD stages (I+II, III, IV). Since the proportion of patients with mild airflow limitation referred for PR is limited (18), these patients were grouped with those with moderate airflow limitation. Furthermore, in order to study the impact of VAT on lung volumes, both men and women were divided in two subgroups according to VAT 50th percentile (2183.0 g for men and 912.5 g for women).

Statistical analysis was performed using SPSS version 20.0. Comparisons across GOLD stages were performed using ANOVA with post-hoc Tukey-Kramer tests for multiple comparisons. Subsequently, multivariate stepwise linear regression analysis was performed to investigate a relationship between VAT and selected indices of lung hyperinflation. A *p value* <0.05 was considered significant in all analyses.

3. Results

3.1 Subjects characteristics

Patients were characterized by moderate to very severe airflow obstruction (GOLD I/II, 44.9%; GOLD III, 38.1%; GOLD IV, 17.0%). 154 (52.4%) were men (median age 67 years, IQR 13 years; median BMI 26.9 kg/m^2, IQR 8.6 kg/m^2) and 140 (47.6%) were women (median age 61 years, IQR 13 years; median BMI 25.3 kg/m^2, IQR 8,1 kg/m^2). The median number of pack years smoked was 40 (IQR 24) 30.3% of patients were current smokers and 63.6% of

patients were former smokers. 25.5% of patients used long-term oxygen therapy.

3.2 VAT across GOLD stages

The amount of VAT separated for men and women is shown in figure 2A. In each GOLD subgroup VAT was greater in men than in women (in all GOLD stages: *p< 0.001)*. Furthermore, patients with more severe airflow obstruction had significantly lower values of VAT, both in men (GOLD I/II: 2501.1 g ± 1141.7 g; GOLD III: 2246.4 g ± 1488.5 g; GOLD IV: 1697.2 g ± 1350.1 g, p =0.031) and women (GOLD I/II: 1259.7 g ± 938.0 g; GOLD III: 1160.0 ± 929.8 g, GOLD IV: 653.0 ± 442.8 g, p =0.015). Figure 2B shows the amount of VAT in the three different GOLD subgroups after stratification for BMI: as it could be expected, patients with a BMI$\geq$25 kg/m^2, had consistently higher values of VAT, compared with those with a BMI<25 kg/m^2 (p<0.001), both in men and women. After stratification for gender and BMI, differences in VAT across the three GOLD subgroups disappeared (figure 2B).

3.3 Relationships of VAT with other indices of total and central adiposity

The amount of VAT was significantly and strongly correlated with BMI, waist circumference, FM, FMI, android fat mass and gynoid fat mass (figures 3 A-F). The strength of correlations was higher in male than in female patients. Android fat mass was the most strongly correlated with VAT (r^2=0.919 in men; r^2=0.857 in women), followed by waist circumference (r^2=0.831 in men; r^2=0.757 in women), FM (r^2=0.858 in men; r^2=0.694 in women), FMI (r^2=0.894 in men; r^2=0.653 in women), BMI (r^2=0.826 in men; r^2=0.641 in women) and gynoid fat (r^2=0.746 in men; r^2=0.467 in women).

3.4 Impact of VAT on lung volumes

Besides disease severity, patients were stratified for VAT less and above the 50th percentile. Patients with moderate to severe airflow limitation and lower

VAT levels had increased ITGV and ERV and reduced IC/TLC (table 1) compared to those with higher VAT levels. There was no significant difference for FEV_1 (% pred), FEV_1/FVC (%) or IVC (% pred), or for lung volumes in GOLD IV patients.

The same trend was observed in both genders, although it was less pronounced in female than in male patients (data not shown). DLCO was significantly increased in patients with greater VAT in all GOLD categories ($p=0.002$ in GOLD I+II; $p=0.003$ in GOLD III; $p=0.015$ in GOLD IV). The same finding was true when considering male and female patients separately.

3.5 Determinants of hyperinflation

In order to investigate whether VAT itself is a predictor of lung hyperinflation, independently from BMI, age, gender and FEV_1 (L), stepwise regression was performed and results are shown in table 2A. Considering IC/TLC (%) as dependent variable (and FEV_1 as the best predictor), the r^2 of the regression model was 0.701. Only FEV_1 (L), BMI, age and gender are included in the final equation and the multiple regression was significant ($p<0.001$). VAT was excluded from the model.

Similar results were obtained when choosing ITGV (L) as dependent variable (table 2B). Only BMI, gender, FEV_1 (L) and age were significant determinants, the r^2 was 0.465 and the regression was significant ($p<0.001$). VAT was again excluded from the model.

Concerning diffusing capacity, stepwise regression results showed that FEV_1 (L), age, gender and BMI were significant determinants of DLCO (L) in the way that younger male patients and patients with higher BMI and higher FEV_1 (L), have also higher DLCO values (table 2C). Nevertheless, VAT was again excluded from the model. The r^2 of the regression model was high (0.532), and the regression was significant ($p<0.001$).

4. Discussion

The present study reported several novelties regarding the research concerning the involvement of the adipose tissue in the pathology of COPD. First, it showed that VAT is comparable across GOLD stages for airflow limitation if BMI is taken into account. Second, traditional markers of total and central adiposity correlated very well with VAT assessed by DEXA. Third, VAT is not an independent predictor of static lung hyperinflation in COPD, while demographics and BMI are.

4.1 VAT in COPD

Furutate et al. (19) investigated VAT in patients with COPD using CT. They observed that VAT was higher in COPD compared to controls with comparable BMI, and that VAT tended to increase with COPD progression, although not all differences were statistically significant. In addition, the authors found that, in patients with severe emphysema, VAT was retained despite the absence of obesity. In the same study, BMI, waist circumference, FFMI, FMI and SAT were shown to decrease with increasing emphysematous severity. Besides the fact that we did not relate VAT to emphysema severity, the differences in ethnicity (Furutate included a Japanese sample, the present study population were all caucasians) between studies makes comparison difficult, but our results indicates that VAT is comparable across GOLD stages, after stratification for BMI.

4.2 Assessment of VAT in COPD

Several methods can be used for measuring VAT. The most commonly used imaging technique is abdominal CT, one of the current gold standards for quantifying VAT (20-24). However, because of the radiation dose involved, the high costs and the heavily utilized clinical equipment requirement, CT is not optimal as screening method for VAT. MRI has also been used for VAT

measurement (25, 26). It avoids the radiation dose involved with CT measurements but still requires an expensive and time-consuming procedure as well as heavily utilized clinical equipment. DEXA is another suitable clinical reference method for the assessment of body composition. It measures the differential attenuation of two different energy level x-rays as they pass through the body and subdivides soft tissue into bone, lean and fat compartments. New software was recently proposed to quantify VAT in the android region by DEXA (12, 13), with several advantage relative to CT and MRI, such as lower costs, lower x-ray exposure and short-scanning time (27-30).

4.3 Correlates of VAT

To the best of author's knowledge, no data have been previously published with respect of the relationships between VAT measured by DEXA and other indices of body composition in COPD patients. In the present study we find a strong and significant correlation of VAT with BMI, waist circumference, FM, FMI as well as android and gynoid fat mass, in both genders but more strongly in males (likely due to their higher amount of VAT compared with females). As reported by Kaul et al., DEXA is a valid tool for quantification of VAT and other adipose tissue regions in the clinical setting (12), but more studies are needed in COPD in order to validate this method against CT, which is one of the current gold standards for quantifying VAT.

4.4 Hyperinflation in COPD

To the best of the authors' knowledge, this study is the first that carefully evaluated VAT by DEXA in relation to static lung volumes in a large group of patients with moderate to very severe COPD. O'Donnell et al.(31) examined the relationship between increasing BMI and plethysmographic lung volumes in a population with airflow obstruction. It was shown that lung hyperinflation decreased exponentially with increasing BMI. However, the potential impact of fat distribution on lung volumes was not investigated. In the present study,

patients with greater VAT seemed to have lower ITGV, ERV, RV and higher IC/TLC and DLCO without a difference in FEV_1, FEV/FVC or IVC in most GOLD stages (and most pronounced in early GOLD stage). This finding appears to confirm that the major effect of obesity is on lung volumes, with no direct effect on airway obstruction (32). The results of the multiple stepwise regression analysis confirmed a predicted role of FEV_1, BMI, gender and age on lung hyperinflation but no independent role of VAT for any of the lung volume parameters. This finding is somewhat surprising, since VAT is located in the abdominal region below the respiratory compartment. Future studies have to seek out what parameters of obesity are related to lung hyperinflation. Probably SAT in the abdominal and thoracic region could be worth considering.

In health, increasing BMI is known to be associated with increasing diffusing capacity, possibly reflecting the increased pulmonary blood volume in obesity (33, 34). In the current study, a relationship was confirmed between increasing BMI and DLCO in COPD patients. Nevertheless, no significant predictive role of VAT on DLCO was found.

4.5 Strength and limitations of the study

the major strength of this study was that it is the first study, which used DEXA in order to measure VAT in COPD patients. Additionally, a large population with moderate to very severe disease and careful clinical characterization was enrolled in the study. Nevertheless, various limitations of the study have to be considered. Firstly, there was no age-matched healthy control group included in the present study. Previous studies however already suggested that COPD patients have increased VAT by CT scan compared to matched controls. Secondly, no gold standard reference method (such as CT) was available and future studies need to validate the DEXA device for VAT. As mentioned in the introduction however, this was not the aim of the present study.

5. Conclusions

DEXA provides lots of useful information in clinical practice. Not only is it the gold standard method for evaluating bone mineral density and discriminate patients with and without osteoporosis, but it is also a useful method for the assessment of other clinically relevant body composition parameters, among which VAT.

In conclusion, after stratification for BMI, VAT is comparable across GOLD stages in moderate to very severe COPD patients. VAT measured by DEXA is strongly correlated to other traditional and most commonly used body composition parameters, such as BMI, waist circumference as well as android and gynoid fat mass. BMI and demographics, but not VAT, were independent predictors of static lung hyperinflation and diffusing capacity in COPD.

6. Tables and figures

Table 1. General and clinical parameters in 294 COPD patients stratified for GOLD stages and divided in two groups, based on VAT 50th percentile (2183 g for men; 912.5 g for women).

	GOLD I-II (N=132)			GOLD III (N=112)			GOLD IV (N=50)		
	VAT<50th	VAT>50th	P value	VAT<50th	VAT>50th	P value	VAT<50th	VAT>50th	P value
Age (years)	65.3 ± 10.9	64.9 ± 9.2	0.856	65.1 ± 10.4	65.7 ± 6.8	0.697	59.6 ± 7.5	59.1 ± 10.5	0.868
BMI (kg/m²)	24.0 ± 3.5	31.9 ± 4.4	<0.001	21.6 ± 2.9	30.5 ± 4.8	<0.001	20.7 ± 3.4	28.1 ± 4.5	<0.001
FFMI (kg/m²)	16.7 ± 2.4	18.7 ± 2.6	<0.001	15.5 ± 1.7	17.9 ± 2.5	<0.001	14.6 ± 1.4	16.9 ± 2	<0.001
FMI (kg/m²)	7.5 ± 2.0	13.2 ± 3.0	<0.001	6.4 ± 2.4	12.7 ± 3.1	<0.001	6.2 ± 2.6	11.3 ± 2.9	<0.001
Gynoid Fat (g)	3322.2 ± 1005.5	5517.6 ± 1314.4	<0.001	2770.4 ± 1031.5	5148.4 ± 1572.9	<0.001	2810.2 ± 1251.9	4737.4 ± 1397.2	<0.001
Android Fat (g)	1797.3 ± 769.0	4120.8 ± 1110.6	<0.001	1428.9 ± 736.2	3997.5 ± 1274.8	<0.001	1336.6 ± 760.1	3641.8 ± 1513.6	<0.001
FEV₁ (% pred)	70.6 ± 20.6	68.9 ± 13.5	0.572	38.5 ± 5.5	39.6 ± 5.7	0.306	23.4 ± 5.0	25.2 ± 3.1	0.181
FEV₁/FVC (%)	46.8 ± 11.3	49.4 ± 9.6	0.160	31.5 ± 6.7	33.4 ± 7.4	0.157	22.9 ± 4.4	26.2 ± 7.3	0.050
IVC (% pred)	115.9 ± 19.5	108.1 ± 18.7	0.021	96.3 ± 17.0	93.0 ± 15.9	0.289	79.8 ± 15.4	78.4 ± 15.0	0.750
ITGV (% pred)	132.9 ± 27.6	112.5 ± 21.9	<0.001	163.6 ± 33.1	146.0 ± 27.1	0.003	193.4 ± 35.1	174.6 ± 37.6	0.090
ERV (% pred)	132.6 ± 46.4	106.9 ± 44.9	0.002	130.6 ± 40.1	112.2 ± 35.1	0.012	106.4 ± 29.8	90.4 ± 35.9	0.103
RV (% pred)	134.0 ± 33.7	116.1 ± 26.9	0.001	178.3 ± 40.3	159.3 ± 32.6	0.008	234.5 ± 61.8	212.9 ± 48.8	0.226
IC/TLC (%)	37.9 ± 8.2	42.6 ± 7.2	0.001	26.6 ± 7.7	30.2 ± 6.5	0.008	20.3 ± 5.4	24.0 ± 5.2	0.027
DLCO (% predicted)	54.8 ± 15.9	65.5 ± 22.6	0.002	42.7 ± 12.9	50.6 ± 12.5	0.003	33.6 ± 13.1	45.1 ± 16.4	0.015

Values reported as mean ± standard deviation

BMI, body mass index; FFM, fat-free mass index; FMI, fat mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; IVC max, inspiratory vital capacity; ITGV, intra-thoracic gas volume, ERV, expiratory reserve volume; RV, residual volume; IC, inspiratory capacity; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide.

Table 2A. Multivariate stepwise regression with IC/TLC (%) as dependent variable

Model	Standardized Coefficients	p
(Constant)		<0.001
FEV_1 (L)	0.710	<0.001
BMI	0.289	<0.001
Age	-0.091	<0.05
Gender*	-0.092	<0.001

Note: Independent variables: VAT, BMI, age, gender and FEV_1. Adjusted $R^2 = 0.697$. VAT was excluded from the model. *gender=0 for women; =1 for men.

Abbreviations: IC/TLC, inspiratory capacity/total lung capacity; FEV_1, forced expiratory volume in 1second; BMI, body mass index; VAT, visceral adipose tissue.

Table 2B. Multivariate stepwise regression with ITGV (L) as dependent variable

Model	Standardized Coefficients	p
(Constant)		<0.001
FEV1 (L)	0.428	
Age	-0.232	<0.001
Gender*	0.239	<0.001
BMI	0.302	<0.001

Note: Independent variables: VAT, BMI, age, gender and FEV_1. Adjusted $R^2 = 0.525$. VAT was excluded from the model. *gender=0 for women; =1 for men.

Abbreviations: ITGV, intra-thoracic gas volume; FEV_1, forced expiratory volume in 1second; BMI, body mass index; VAT, visceral adipose tissue.

Table 2C. Multivariate stepwise regression with DLCO (L) as dependent variable

Model	Standardized Coefficients	p
(Constant)		<0.001
BMI	-0.385	<0.001
Gender*	0.505	<0.001
FEV_1 (L)	-0.391	<0.001
Age	-0.133	<0.005

Note: Independent variables: VAT, BMI, age, gender and FEV_1. Adjusted R^2 = 0.458. VAT was excluded from the model. *gender=0 for women; =1 for men.

Abbreviations: DLCO, diffusing capacity of the lung for carbon monoxide; FEV_1, forced expiratory volume in 1second; BMI, body mass index; VAT, visceral adipose tissue.

Figure 1A. A model of total body DEXA scan. The android region is highlighted in blue and the gynoid region is highlighted in red (refer to text for further explanations).
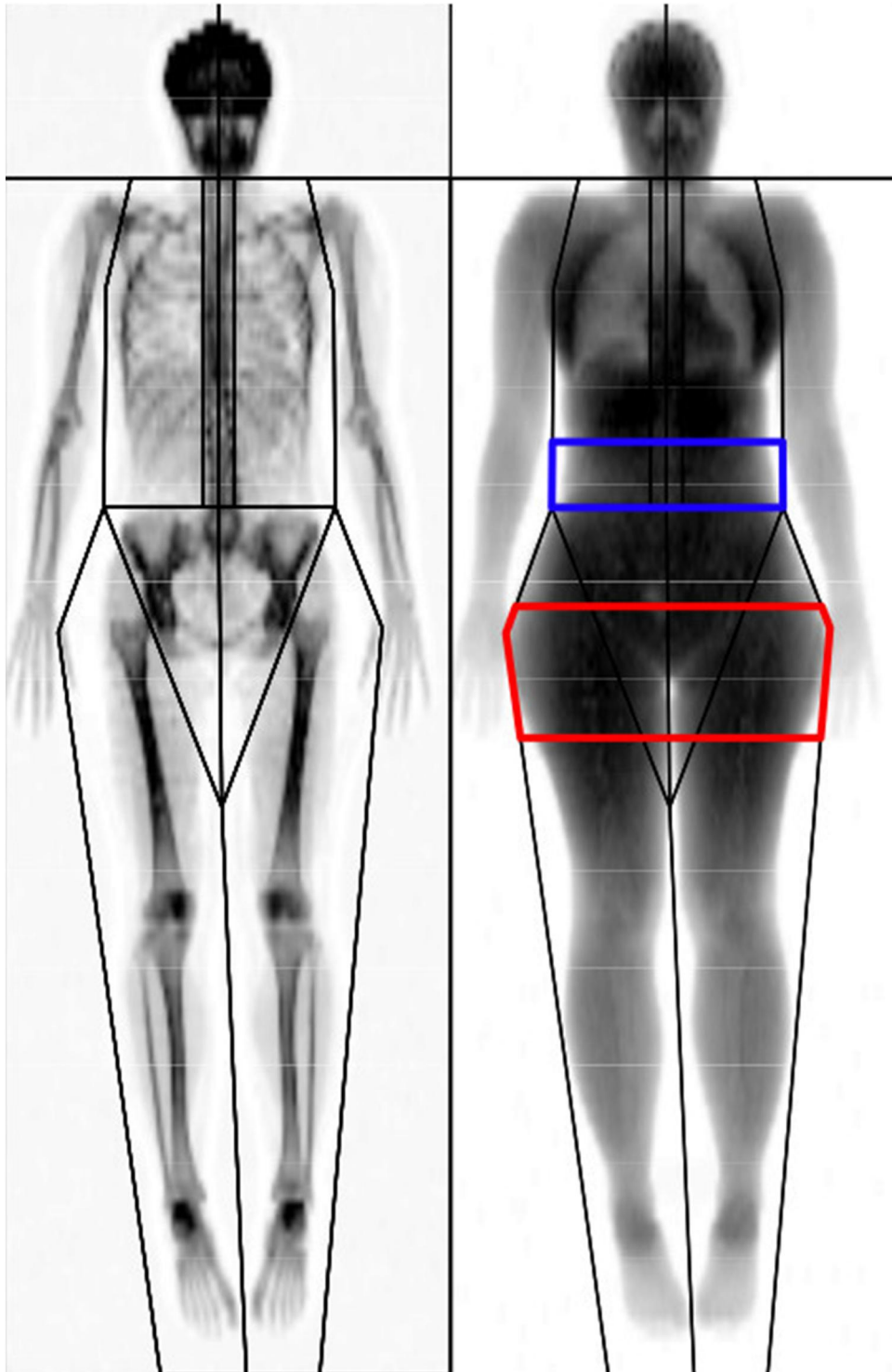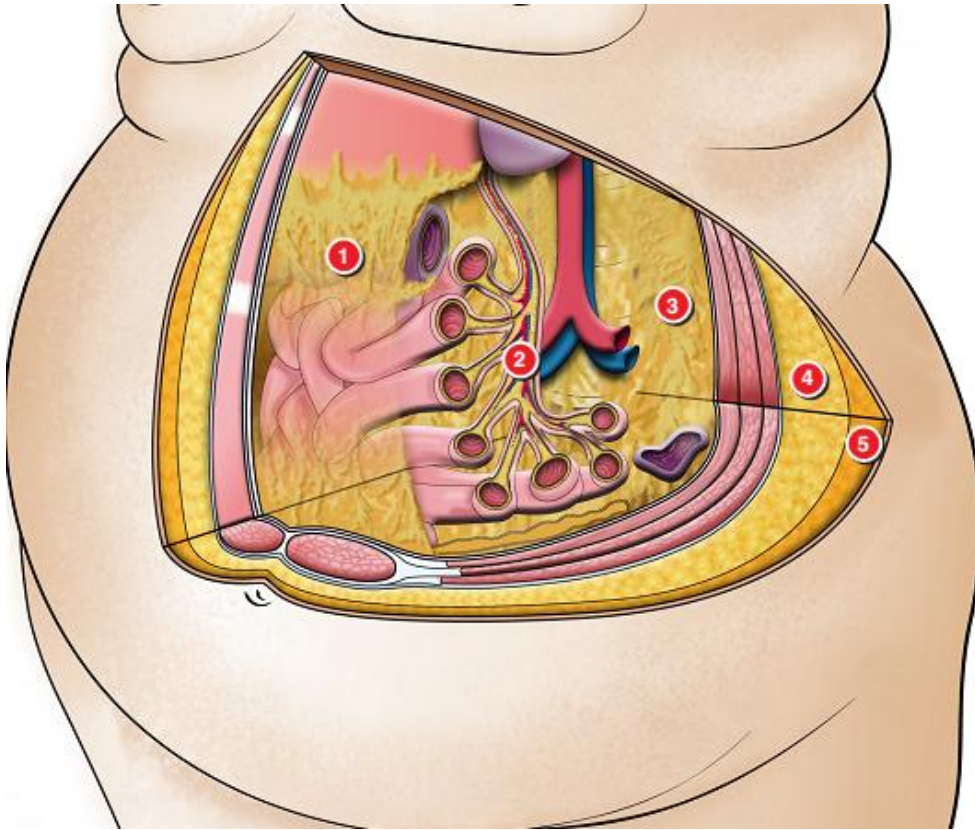
Figure 1B. Representation of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) within the android region

Note: VAT is shown in figure as the sum of 1) omental adipose tissue, 2) mesenteric adipose tissue and 3) retroperitoneal adipose tissue. 4) Deep subcutaneous adipose tissue and 5) superficial subcutaneous adipose tissue are part of the **SAT**.

7. References

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CHAPTER VIII

FINAL CONSIDERATIONS

COPD is a complex syndrome. Besides the assessment of pulmonary function and respiratory symptoms, several extra-pulmonary manifestations and comorbidities need to be considered in the individualised management of patients. In this perspective, this thesis provides a detailed overview of the assessment of nutritional status and body composition in COPD patients, especially in relation with respiratory function and muscle strength.

More specifically, the first study presented in this thesis shows that bioelectrical impedance analysis (BIA) has been widely used in COPD, for the assessment of body composition. However, no guidelines have so far specified how this technique could be applied in order to stratify COPD patients into nutritional phenotypes such as malnutrition and sarcopenia.

Given the lack of information on the prevalence of malnutrition (as defined by the European Society of Parenteral and Enteral Nutrition, ESPEN) and sarcopenia (according to the European Working Group on Sarcopenia in Older People, EWGSOP) the second study of the present thesis focused on the assessment of these two conditions, highlighting their association with patients' clinical characteristics. Furthermore, the results of the present study show that sarcopenia is more prevalent in malnourished patients compared to non-malnourished and, within the group of malnourished patients, in those with inflammation (cachectic).

In the third study of the present thesis, multifrequency BIA (MF-BIA) has emerged as a useful bedside approach, independently of body composition estimates (i.e. fat-free mass), for identifying patients with poor nutritional status. More specifically, this study pointed out that raw MF-BIA variables are altered in COPD patients compared to healthy controls and correlate well with respiratory and muscle function.

Actually, as resulted from the fourth study presented, raw BIA variables are strong predictors of muscle strength, both in terms of peripheral and respiratory muscle strength, and to a more significant extent compared to general

anthropometric parameters and body composition estimates derived from BIA (i.e. FFM). The assessment of raw BIA variables may have a role in the overall nutritional assessment in COPD.

Finally, the fifth study of the present thesis aimed to investigate the association of abdominal visceral adipose tissue (VAT) − measured by dual-energy X-ray absorptiometry (DEXA) − with disease severity and lung hyperinflation, finding out no differences in moderate to very severe COPD patients, after stratification for BMI.

Concluding, BIA represents a valuable method to estimate body composition in the clinical setting. Theoretically, more accurate methods exist to assess muscle mass, e.g. computed tomography, magnetic resonance imaging or DEXA, but are expensive, and often hardly accessible in some health settings.

Several applications of BIA may be encouraged. One is to apply BIA data to a validated equation for predicting FFM or appendicular skeletal muscle mass (ASM) and then to normalize them to height in order to create standardized measures that might be used to diagnose malnutrition or sarcopenia. As an alternative, our findings add to evidence regarding clinical applications of BIA beyond use in body composition equations. As direct measures, raw BIA data (e.g. impedance ratio and phase angle) provides additional information on cellular mass and muscle quality, independently of whole-body volumes/masses.

Although future research is needed in order to identify optimal preventive and personalized treatment strategies, the results of the five studies of the present thesis confirm the need for prevention strategies and suggest possible tools for the implementation of effective approaches targeting specific populations.