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Original Study

Increased Myogenic and Protein Turnover Signaling in Skeletal Muscle of Chronic Obstructive Pulmonary Disease Patients With Sarcopenia

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A B S T R A C T

Background: Sarcopenia was recently recognized as an independent condition by an *International Classification of Diseases, Tenth Revision, Clinical Modification* code, and is a frequently observed comorbidity in chronic obstructive pulmonary disease (COPD). Muscle mass is primarily dictated by the balance between protein degradation and synthesis, but their relative contribution to sarcopenia is unclear.
Objective: We aimed to assess potential differential molecular regulation of protein degradation and synthesis, as well as myogenesis, in the skeletal muscle of COPD patients with and without sarcopenia.
Methods: Muscle biopsies were obtained from the vastus lateralis muscle. Patients with COPD were clustered based on sarcopenia defined by low appendicular skeletal muscle mass index (nonsarcopenic COPD, n = 53; sarcopenic COPD, n = 39), and compared with healthy nonsarcopenic controls (n = 13). The mRNA and protein expression of regulators and mediators of ubiquitin-proteasome system (UPS), autophagy-lysosome system (autophagy), and protein synthesis were analyzed. Furthermore, mRNA expression of myogenesis markers was assessed.
Results: UPS signaling was unaltered, whereas indices of UPS regulation (eg, FOXO1 protein; p-FOXO3/FOXO3), autophagy signaling (eg, LC3BII/I; p-ULK1[Ser757]/ULK1), and protein synthesis signaling (eg, AKT1; p-GSK3B/GSK3B; p-4E-BP1/4E-BP1) were increased in COPD. These alterations were even more pronounced in COPD patients with sarcopenia (eg, FOXO1 protein; p-FOXO1/FOXO1; LC3BII/I; p-ULK(Ser555); p-AKT1/AKT1; AKT1; p-4E-BP1). Furthermore, myogenic signaling (eg, MYOG) was increased in COPD despite a concomitant increase of myostatin (MSTN) mRNA expression, with no difference between sarcopenic and nonsarcopenic COPD patients.
Conclusion: Together with elevated myogenic signaling, the increase in muscle protein turnover signaling in COPD, which is even more prominent in COPD patients with sarcopenia, reflects molecular alterations associated with muscle repair and remodeling.

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Sarcopenia is defined as the loss of function in the presence of loss of muscle mass, and was recently recognized as an independent condition by an *International Classification of Diseases, Tenth Revision, Clinical Modification* code.[1] Apart from an age-associated decline in muscle mass, sarcopenia also encompasses the loss of muscle mass due to diseases such as chronic obstructive pulmonary disease (COPD).[2] In COPD patients, sarcopenia is a frequently observed comorbidity, affecting exercise capacity,[3,4] quality of life,[3] and survival.[5–7] Although muscle mass maintenance is considered to be primarily dictated by the balance between protein degradation and protein synthesis rates (ie, protein turnover), their relative contribution to imbalanced protein turnover during muscle mass loss remains unclear.

To provide a first insight into the potential drivers of this imbalance between muscle protein synthesis and breakdown in COPD patients, several studies assessed the molecular regulation of skeletal muscle protein turnover in COPD, and nearly all point to both increased protein degradation signaling[8–14] and increased protein synthesis signaling.[10,11,15] These findings are in accordance with the reported increase in whole body protein turnover rate in COPD as assessed by stable isotopes.[16] It was previously suggested that increased protein synthesis signaling in COPD may serve to maintain muscle mass in the presence of an elevated protein degradation rate.[11] However, such a compensatory mechanism to prevent muscle atrophy appears insufficient, because the prevalence of sarcopenia in COPD patients is high (ie, 12%–33%).[17–19] Although there are some data available on protein turnover signaling in sarcopenic COPD patients,[8,9,11] these studies are either limited in sample size or focus solely on either protein degradation or protein synthesis signaling. Furthermore, the role of myogenesis in muscle mass maintenance is frequently overlooked or incompletely assessed.

A comprehensive analysis of myogenic and protein turnover regulation in COPD patients with and without sarcopenia would provide further insight into the underlying skeletal muscle pathology, potentially providing new targets for intervention. In the present study, we therefore aimed to assess potential differential regulation of protein degradation and synthesis, as well as myogenesis, through analysis of an extensive panel of molecular regulators and mediators of myogenic and protein turnover signaling in the skeletal muscle of COPD patients with and without sarcopenia compared with healthy controls.

Materials and Methods

Study Design and Participants

The skeletal muscle molecular profiles of patients from 2 prospective cohort studies were analyzed. The study performed in Maastricht was registered at www.trialregister.nl as NTR1402, written informed consent was obtained from all participants, and the study was approved by the Maastricht University Medical Centre+ (Maastricht, the Netherlands) ethical review board (08-2-059). The study design was previously published.[20] The study performed in Golnik was registered at www.clinicaltrials.gov as NCT02550808, written informed consent was obtained from all participants, and the study was approved by the Slovenian National Medical Ethics Committee (Ljubljana, Slovenia). The study design was previously published.[21] Only data from baseline (ie, before pulmonary rehabilitation) measurements were used in the current study. All included patients were in a stable disease state, free from exacerbations in the 4 weeks before start of the study protocol.

Participants were excluded from the current analysis if the muscle biopsy or appendicular skeletal muscle mass index (ASMI) measurement was missing. Sarcopenia was solely defined according to cutoffs for ASMI (<7.23 kg/m^2 for men; <5.76 kg/m^2 for women)[22]; see Figure 1. One participant without COPD was classified as sarcopenic and was therefore excluded from the analyses, yielding a study population of 13 healthy controls and 92 COPD patients.

Pulmonary and Physical Function

Spirometry was used to obtain forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and their ratio (FEV_1/FVC) according to the European Respiratory Society guidelines.[23] Patients were classified by disease severity based on Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage.[24] Physical function was assessed by peak load, determined by an incremental load cycling test as previously described.[25]

Anthropometry and Body Composition

Body mass index (BMI) was calculated as body mass/height^2 (kg/m^2). Whole body dual-energy x-ray absorptiometry (Hologic QDR Series Explorer bone densitometer; Hologic Inc, Marlborough, MA) was used to assess total and appendicular (ie, arms and legs) fat mass and fat-free mass. Fat-Free Mass Index (FFMI) was calculated as fat-free mass/height^2 (kg/m^2). ASMI was calculated as appendicular skeletal muscle mass/height^2 (kg/m^2).

Muscle Biopsy and Analyses

Biopsies were obtained from the vastus lateralis muscle of the dominant leg by needle biopsy, at least 20 hours after the last exercise test. Muscle tissue was snap-frozen in liquid nitrogen, and stored at −80°C. Tissue processing and molecular analyses were performed collectively.

Reverse Transcriptase–Quantitative Polymerase Chain Reaction and Western Blotting

Molecular analyses were performed in biopsies from all participants. After removal of technical outliers, which varied between different analyses, the total sample size for protein and mRNA targets included 10 to 13 controls, 50 to 53 nonsarcopenic patients with COPD, and 38 to 39 sarcopenic COPD patients.

Details on the procedures and the exact sample size per analyzed target are provided in the supplementary methods.

Statistics

Differences among nonsarcopenic patients with COPD, sarcopenic patients with COPD, and controls were tested by 1-way analysis of variance with Bonferroni post hoc comparisons in case of a significant group effect. Furthermore, differences between patients with COPD and controls were tested using independent *t* test. Relevant results of this comparison are presented in the figures. Analyses were performed using SPSS Statistics (version 22.0; IBM Corp, Armonk, NY). A *P* value less than .05 was considered statistically significant.

Results

Participant Characteristics

Based on gender-specific cutoffs for ASMI,[22] 39 COPD patients (42%) were sarcopenic, whereas 53 COPD patients (58%) and all control participants were nonsarcopenic. Patient characteristics are presented in Table 1. Although the sarcopenic COPD group contained slightly more men, groups did not differ significantly in sex distribution and age. BMI was lower in sarcopenic than in nonsarcopenic COPD patients, but controls did not differ from either COPD subgroup. Besides ASMI, FFMI was lower in sarcopenic COPD patients than in nonsarcopenic COPD