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Comprehensive metabolic flux analysis to explain skeletal muscle weakness in COPD

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Abstract

Background & aims—Metabolic characterization of a well-defined group of patients could be a powerful tool in revealing metabolic signatures to explain limb muscle weakness in chronic diseases. Studies are currently limited in Chronic Obstructive Pulmonary Disease (COPD) to the identification of differential amino acid concentrations but lack comprehensive analysis of the flux through relevant muscle function related metabolic pathways.

Methods—In 23 stable patients with moderate to very severe COPD and 19 healthy controls, a comprehensive metabolic flux analysis was conducted by administering an intravenous pulse and primed constant infusion of multiple stable tracers of amino acids known to play a role in muscle health. Blood samples were obtained to calculate production (WBP) and interconversion rates, and plasma concentrations of these amino acids. Lower and upper limb muscle strength, muscle mass, lung function, physical activity level, and disease history and characteristics were assessed.

Results—The COPD group was characterized by lower and upper limb muscle weakness ($P<0.01$) despite preserved muscle mass. Higher values were found in COPD for plasma glutamine, WBP of leucine ($P<0.001$), 3-methylhistidine ($P<0.01$) (marker of enhanced myofibrillar protein breakdown), citrulline ($P<0.05$), and arginine to citrulline conversion ($P<0.05$) (reflecting enhanced nitric oxide synthesis). Plasma concentration of β -hydroxy β -methylbutyrate

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Conflict of interest

The authors have no conflict of interest to declare.

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(HMB with anticatabolic, anabolic and contractile properties), WBP of glycine (precursor of creatine and glutathione), and transcutaneous O₂ saturation explained up to 79% and 65% of the variation in strength of the lower and upper limb muscles, respectively, in COPD.

Conclusions—Comprehensive metabolic flux analysis revealed a homogenous metabolic signature in stable patients with COPD and a specific metabolic profile in those with skeletal muscle weakness.

Keywords

COPD; comprehensive metabolic flux analysis; metabolic signature; muscle weakness; muscle mass; stable tracers; protein and amino acid metabolism

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with many comorbidities and features outside the lung including skeletal muscle dysfunction, negatively influencing morbidity and mortality (1, 2). Proteostasis imbalance and dysregulation of amino acid metabolism may play a critical role in the progression of skeletal muscle dysfunction in COPD as it is related to systemic inflammation, oxidative stress, and protein malnutrition (3, 4). Several changes in concentrations of amino acids that are involved in skeletal muscle protein metabolism and health (mass and contractility) have been identified in plasma, serum and/or urine of COPD patients with quantitative (NMR and MS-based) metabolomics, but confirmed biomarkers in COPD have not yet been reported (4). Increased plasma concentrations of glutamine, 3-methylhistidine and arginine (5–7) and reduced plasma concentrations of glycine (5, 8, 9) were observed in different groups of COPD patients. However, conflicting results were found for phenylalanine and the branched-chain amino acids (BCAA) as increased (5, 9) as well as reduced (5, 8, 10, 11) concentrations were found in COPD patients dependent on their phenotype, nutritional status and/or presence of comorbidities. Although research on biomarkers of patient outcomes in COPD is growing, studies are currently limited to the identification of differential amino acid concentrations. We recently developed a novel multi-stable isotope approach to study the flux through multiple protein and amino acid pathways simultaneously in a single study day and in the same individual (12). This metabolic flux technique is able to provide a holistic picture of potential disturbances in multiple whole body metabolic pathways in addition to changes in plasma amino acid concentrations, while at the same time provides insight in the underlying mechanisms. As changes in the metabolic flux can be present, while plasma concentrations are unaffected, metabolic flux analysis is therefore able to provide deeper insights in the pathogenesis of muscle dysfunction in COPD. To identify whether there is a metabolic signature present in COPD patients with impaired muscle health, we examined in the present study whether skeletal muscle weakness in a well-defined group of COPD patients was associated with a specific (protein and amino acid) metabolic signature, using besides plasma amino concentration analysis, the multi-stable isotope pulse approach. We recruited COPD and healthy control subjects from the MEDIT trial (MEtabolism of Disease with Isotope Tracers), a large controlled and still recruiting trial in healthy and diseased subjects who

were well characterized by their skeletal muscle health (strength and mass) and general- and disease specific features (eg lung function, disease history, comorbidity index, habitual dietary intake, physical activity level). In addition, in each subject, muscle health was associated with the metabolic flux and concentrations of muscle related amino acids including phenylalanine, tau-methylhistidine and glutamine (affecting (muscle) protein breakdown), taurine (defense against free radical-mediated damage after exercise)(13), glycine (precursor of creatine and glutathione)(14), branched-chain amino acids and its metabolites (eg β -hydroxy- β -methylbutyrate), and arginine (precursor of nitric oxide synthesis)(15). Identification of a metabolite signature using a multi-tracer flux approach in a COPD population with muscle dysfunction might guide the development of targeted and more individualized (nutritional) regimens to improve muscle health of these patients.

2. Material and methods

2.1. Subject inclusion

MEDIT included 23 clinically stable patients with a clinical diagnosis of COPD (grade II-IV)(16), and 19 healthy age matched control subjects (Table 1). Recruitment took place through pulmonologist referral and *via* advertisements in the local community. Medical history and medication use were assessed as part of the screening process and transcutaneous oxygen saturation measured using pulse oximetry. Exclusion criteria were pre-existent untreated metabolic or renal disease (including diabetes mellitus requiring daily insulin administration), malignancy, recent surgery, and use of systemic corticosteroids (< 4 weeks before the study day). All patients received bronchodilator treatment, 91% inhalation corticosteroids and 61% were on long-term oxygen therapy. Written informed consent was obtained, and the study was approved by the local Institutional Review Board.

2.2. Study design

All subjects were studied at the Clinical Research Unit of the Center for Translational Research in Aging and Longevity, Texas A&M University, and the study day lasted for approximately 5 hr.

Anthropometrics, body composition and lung function—Body weight and height were measured by a digital beam scale and stadiometer, respectively, and whole body and extremity fat mass (FM) and fat-free mass (FFM) were obtained by dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500/ Version 12.7.3.1 (Bedford, MA)). Measures were standardized for height (kg/m^2 to obtain BMI, FFM index (FFMI), FM index (FMI), and appendicular skeletal muscle index (ASMI). Forced expiratory volume in 1 second and Forced vital capacity was assessed with the highest value from 3 technically acceptable maneuvers being used (17). Peripheral arterial oxygen saturation was measured using a finger pulse oximeter while at rest. If long term oxygen was used by the COPD subject (as part of their daily life requirements), this was continued during the measurement.

Muscle function and Questionnaires—Respiratory muscle function (maximal expiratory pressure (MEP) and inspiratory pressure (MIP)) was assessed by hand-held mouth pressure device (Micro Respiratory Pressure Meter (RPM), MD spiro, Lewiston,

ME). Peak leg force of 5 one-leg reciprocal extensions at 60 degree/sec (Kincom isokinetic dynamometry (Isokinetic International, Chattanooga, TN)), and handgrip force (Vernier dynamometry (Vernier software and Technology, Beaverton, OR) were used as marker of lower and upper muscle strength. Habitual dietary intake was assessed by 24-hour dietary recall, and habitual physical activity level by Physical Activity Scale for the Elderly questionnaire (PASE) (18). The modified medical research council dyspnea scale (mMRC) was used to assess the level of dyspnea, and Charlson index (19) for assessment of associated comorbidities.

Metabolic signature analysis by stable isotope IV continuous infusion and pulse administration—After an overnight fast, one peripheral line was placed in a vein of the lower arm for stable tracer (continuous infusion and pulse) administration and one line in a superficial dorsal vein of the contralateral hand for blood sampling. The hand was placed in a thermostatically controlled hot box (internal temperature: 60°C), a technique to mimic direct arterial sampling (20). After collecting a venous blood sample for baseline enrichment analysis, an intravenous pulse of a cocktail of stable amino acid tracers (Cambridge Isotope Laboratories (Woburn, MA, USA) was administered followed by a primed constant infusion with stable isotope tracers of phenylalanine and tyrosine after 90 min (12). Arterialized-venous blood was sampled at t=2, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 225 and 240 minutes after pulse administration for analysis of tracer enrichments and concentrations of amino acids.

2.3. Biochemical analysis and calculations of metabolic parameters

Arterialized-venous blood was put in Li-heparinized or EDTA tubes, immediately put on ice and centrifuged (4°C, 3120 × g for 5 min) to obtain plasma. Plasma was aliquoted with either 0.1 vol of 33% (w/w) trichloroacetic acid or its residue after evaporation of 0.17 vol of 33% (w/w) 5-sulfosalicylic dihydrate, and stored at −80°C. Tracer enrichments [tracer:tracee ratio (TTR)] and amino acid concentrations were analyzed batch-wise by LC-MS/MS by isotope dilution (12).

Sum of amino acids (sum AA) represents the sum of concentration of all measurable α-amino acids. Sum of branched-chain amino acids (BCAA) is the sum of valine (VAL), leucine (LEU) and isoleucine (ILE). Sum of branched-chain keto acids (BCKA) is the sum of keto-isocaproic acid (KIC), keto-methylvaleric acid (KMV) and ketoisovaleric acid (KIV). The rates of appearance (Ra) of PHE and TYR were calculated to measure whole body production (WBP) from the last hour of the primed constant infusion period, as $Ra = \text{tracer infusion rate} / \text{median TTR}$, and the interconversion as marker of net protein breakdown (21). For all other (pulse) tracers (including tau-methylhistidine, glutamine, taurine, glycine, arginine, branched-chain amino acids and its metabolites, WBP was analyzed by non-compartmental analysis (GraphPad Prism (version 8.0)) and the area under the curve in TTR calculated (12).

The TTR change over time was fitted (22). The TTR decay of the stable tracers with the pulse (DOSE) were fitted with two exponentials: $TTR(t) = a \cdot \exp(-k_1 \cdot t) + b \cdot \exp(-k_2 \cdot t)$ or three exponentials: $TTR(t) = a \cdot \exp(-k_1 \cdot t) + b \cdot \exp(-k_2 \cdot t) + c \cdot \exp(-k_3 \cdot t)$ if that provided

beZer esYmaYon. The area under the curve (AUC) was calculated from the integral of the two or three exponential curve (23). Ra was then calculate as DOSE/AUC. The Ra was calculated as $\text{DOSE}/(a/k_1 + b/k_2)$ for 2 exponentials and as $\text{DOSE}/(a/k_2 + b/k_2 + c/k_3)$ for 3 exponentials. For metabolites from the stable tracers, the change in TTR was fitted as: $\text{TTR}(t) = -a \cdot \exp(-k_1 \cdot t) + b \cdot \exp(-k_2 \cdot t) + c \cdot \exp(-k_3 \cdot t)$ and the integral was calculated to represent the AUC.

The conversion of an amino acid into another was calculated by using Ra of the product amino acid and the ratio between the TTR at plateau or AUC of the TTR from pulse of the product/substrate (23). We determined plasma glucose concentrations using a COBAS c111 semi-automatic analyzer (Gluc2 Kit; Roche Diagnostics®).

2.4. Statistical analysis

Results are expressed as means $\pm$ standard errors (SEs). Population characteristics and baseline measurements were compared using either unpaired Student's *t* test (if data were distributed normally) or Mann-Whitney test with Bonferroni correction. Categorical values were compared using Fisher's exact test. Bivariate Pearson's correlation analysis was used to measure the linear correlation between muscle function, mass and each metabolic variable. Based on the results, we built a best fit multiple linear regression model for each muscle function measure in the COPD group initially including variables with correlation coefficient $p < 0.1$. The variables were selected on the basis of the *q* value (< 0.05) which is the FDR corrected *p* value. Graphpad Prism (Version 8) was used for data analysis and the level of significance was set at $P < 0.05$.

3. Results

The COPD group was characterized by moderate to very severe airflow obstruction and several comorbidities (Charlson Comorbidity index: 2.0 ± 1.1) (Table 1), with cardiovascular disease being the most prevalent (48%). BMI, body composition, and habitual protein intake were not different between the groups (Table 2). COPD patients had lower inspiratory-, handgrip- and leg muscle strength ($P < 0.01$), and tended to be less physically active ($P = 0.09$). ASMI was correlated with handgrip strength (Figure 1a), and max leg extension (Figure 1b). Lower handgrip strength was associated with higher number of exacerbations ($r: -0.52$, $P = 0.01$) and hospitalizations ($r: -0.38$, $p = 0.07$) in preceding year, and lower transcutaneous O_2 saturation ($r: 0.74$, $P < 0.01$) (Figure 5a). Lower leg strength was associated with higher number of exacerbations in past year ($r: -0.27$, $P = 0.07$) and lower physical activity (PASE) ($r: 0.62$, $P < 0.01$) and transcutaneous O_2 saturation levels ($r: 0.67$, $P < 0.01$).

3.1. Metabolism of protein, leucine and its downstream metabolites

The plasma concentrations of phenylalanine and tyrosine, tau-mHIS, BCAAs, branched-chain keto acids (BCKAs), and HMB were not different between the groups. The COPD group was characterized by higher postabsorptive values for WBP of tau-mHIS ($P = 0.01$) and LEU ($P < 0.001$) (Table 3) but comparable values were found for WBP of phenylalanine and tyrosine and its conversion rate. This indicates higher myofibrillar protein breakdown rate

and disturbed LEU metabolism in COPD despite preserved values for whole body protein breakdown and net whole body protein loss.

3.2. Metabolism of arginine, glutamine, glycine, taurine (amino acids involved in muscle contractility), and remaining amino acids

In COPD, higher values were found for WBP of citrulline (precursor of arginine) ($P<0.05$), the conversion of arginine to citrulline (reflecting nitric oxide synthesis) ($P<0.05$), and taurine ($P=0.09$) (Table 3), but comparable values were found for WBP of glycine, glutamine, and the remaining amino acids. COPD patients had higher values for plasma glutamine ($531 \pm 15 \mu\text{mol/L}$ vs. $459 \pm 18 \mu\text{mol/L}$, $P<0.01$) and ornithine concentrations ($47 \pm 2 \mu\text{mol/L}$ vs. $39 \pm 2 \mu\text{mol/L}$, $P<0.01$) were also elevated in COPD.

3.3. Relationships between disease characteristics, and muscle strength and metabolism

FEV1 was correlated with WBP GLY ($r:-0.47$, $p<0.05$) but not with muscle strength. Lower transcutaneous O₂ saturation was associated with higher values for WBP of LEU ($r:-0.39$, $p=0.07$), and lower muscle function (MIP ($r:0.52$, $p<0.05$), handgrip strength ($r:0.73$, $p<0.001$) (Figure 5a), leg strength ($r:0.52$, $p<0.05$) (Figure 5b) and plasma HMB ($r:0.43$, $p=0.05$). Furthermore, CRP in the COPD group was significantly correlated with dyspnea scale ($r:0.44$, $p=0.03$), and the metabolic markers of WBP glutamine ($r:0.45$, $p<0.05$), LEU ($r:0.45$, $p<0.05$), tau-mHiS ($r:0.61$, $p<0.01$) and plasma concentration of tau-mHiS ($r:0.58$, $p<0.01$) and ILE ($r:0.50$, $p<0.05$) but not with muscle strength.

3.4. Relationships between muscle strength and metabolism

Handgrip strength

Whole group: Lower handgrip strength was associated with elevated NO synthesis ($r:-0.34$, $P<0.05$), and WBP of citrulline ($r:-0.49$, $P<0.001$), LEU ($r:-0.52$, $P<0.001$), glycine ($r:-0.47$, $P<0.01$) (Figure 3a), and keto-isocaproic acid (KIC) ($r:-0.33$, $P<0.05$), and lower plasma concentrations of the individual and sum of BCAA ($r:0.37-0.41$, $P<0.05$) (Figure 4a) and BCKA ($r:0.32-0.35$, $P<0.05$), PHE ($r:0.38$, $P<0.05$), and HMB ($r:0.43$, $P<0.01$) (Figure 2a).

COPD only: Lower handgrip strength was associated with elevated WBP of glycine (-0.49 , $P<0.05$), glutamine ($r:-0.48$, $P<0.05$), LEU ($r:-0.48$, $P<0.05$), and citrulline ($r:-0.46$, $P<0.05$). Lower handgrip strength was associated with lower plasma concentrations of phenylalanine ($r:0.52$, $P<0.05$), tyrosine ($r:0.46$, $P<0.05$), LEU ($p=0.07$), BCAA ($p=0.07$) (Figure 4a), the individual and sum BCKA ($r:0.51-0.60$, $P<0.05$), and HMB ($r:0.61$, $P<0.01$) (Figure 2a). Plasma HMB and transcutaneous O₂ saturation explained up to 65% of the variation in handgrip strength (Table 4).

Maximal single leg extension

Whole group: Lower leg strength was associated with higher WBP of glycine ($r:-0.49$, $P<0.01$) (Figure 3b), LEU ($r:-0.43$, $p<0.01$), citrulline ($r:-0.46$, $P<0.01$), *de novo* ARG synthesis ($r:-0.36$, $p<0.05$), NO synthesis ($r:-0.35$, $P<0.05$), and plasma concentrations of

the individual and sum BCAA ($r:0.40\text{--}0.49$, $P<0.05$) (Figure 4b), and HMB ($r:0.44$, $P<0.01$) (Figure 2b).

COPD only: Lower leg strength was associated with higher WBP of glycine ($r:-0.64$, $P<0.01$) (Figure 3b), *de novo* ARG synthesis ($r:-0.47$, $p<0.05$), and plasma concentrations of glutamate ($r:0.48$, $P<0.05$), phenylalanine ($r:0.46$, $P<0.05$), tau-MHIS ($r:0.38$, $P=0.09$), the individual and sum BCAA ($r:0.59\text{--}0.61$, $P<0.01$) (Figure 4b), and HMB ($r:0.66$, $P<0.001$) (Figure 2b). Plasma concentrations of HMB, transcutaneous O₂ saturation, and WBP of glycine explained up to 79% of the variation in leg muscle strength (Table 4).

4. Discussion

In the present study, an innovative multi-tracer approach was used in a well defined group of COPD patients to make instant analysis possible of multiple metabolic pathways of amino acids known to relate to muscle health. Despite preserved plasma concentrations of most amino acids, we identified a relatively homogenous metabolic flux profile in patients with stable COPD characterized by lower and upper limb muscle weakness but preserved muscle mass. Furthermore, reduced transcutaneous O₂ saturation but not CRP, as a marker of systemic inflammatory response, was related to muscle dysfunction in these patients.

4.1. Increased myofibrillar protein breakdown and disturbances in leucine and downstream metabolism

Contractile (myofibrillar) protein breakdown (reflected by WBP of tau-methylhistidine) was increased in COPD while net whole body protein catabolism was unchanged, confirming that whole body protein metabolism does not necessarily reflect skeletal muscle protein metabolism (11, 24). The ubiquitin-proteasome pathway is assumed to provide most of the proteolytic activity required for the degradation of myofibrillar protein (25). No relationship, however, was found between increased contractile protein breakdown and reduced upper or lower muscle strength in COPD. The higher contractile protein breakdown despite comparable muscle mass suggests that muscle protein synthesis postabsorptively is also elevated in these patients, indicative of elevated muscle protein turnover. Systemic inflammation, but not reduced transcutaneous O₂ saturation, was highly associated with elevated production and plasma concentration of tau-methylhistidine in COPD.

Low transcutaneous O₂ saturation in the studied COPD group, however, contributed to the elevated WBP of LEU observed in COPD, in line with our previous data (26). LEU is one of the branched-chain amino acids that play an important role in protein degradation and synthesis and a source of nitrogen for synthesis of other amino acids and nucleotides. Although absolute plasma levels were comparable in both groups, low plasma BCAA concentrations in COPD were associated with reduced values for appendicular skeletal muscle index and muscle weakness. Factors negatively affecting plasma BCAA concentrations are increased skeletal muscle amino acid oxidation and enhanced systemic inflammation *via* stimulating muscle BCAA catabolism, and increased activity of muscular branched-chain keto acid dehydrogenase (27). Activation of this enzyme and muscle protein catabolism is an essential source of providing glucose through gluconeogenesis when in the

fasted state (28). In line, WBP of LEU was significantly correlated with plasma CRP levels in the studied COPD group.

Skeletal muscle tissue is the major site of amino acid catabolism and the source of the branched-chain amino acid derived keto-acids (BCKA). Isoleucine, valine and LEU are catabolized by the enzyme branched chain aminotransferase (regulated by acetyl CoA) to α -keto-methylisovalerate, α -keto-isovalerate, and α -keto-isocaproate (KIC), respectively. The first two keto acids, but not KIC, are converted to propionyl CoA, a precursor for the TCA cycle intermediate succinyl CoA. We found that upper muscle strength in COPD was significantly associated with plasma concentrations of all 3 keto acids. Although plasma HMB levels was not reduced in COPD on whole group level, we found a strong positive correlation between all measures of muscle function and plasma HMB, in line with previous studies in young and older adults (29). HMB is formed by oxidation of KIC in the liver cytosol, and less than 5% of dietary LEU is oxidized into HMB (30). HMB is effective in reducing muscle protein breakdown and increasing protein synthesis rates (31, 32). HMB given as a dietary supplement increases strength, power and aerobic performance in healthy adults (33) and positively affects muscle contractility in chronic diseases by stimulating cholesterol synthesis and proliferation of satellite cells (necessary for tissue repair), excitation-contraction coupling and mitochondrial biogenesis (34). HMB as part of oral nutritional supplementation resulted in reduced mortality and improved nutritional status in malnourished, older, hospitalized adults (including COPD patients) (35) and increased muscle mass and strength as compared to pulmonary rehabilitation program alone in bronchiectasis (36). As plasma HMB concentration significantly contributed to the variation of upper and lower muscle strength in COPD, we suggest a role for HMB intervention in the treatment of muscle dysfunction in these patients.

4.2. Enhanced nitric oxide production rates and reduced glutamine clearance

WBP of citrulline was elevated in COPD, in line with our previous findings using a primed constant and continuous infusion protocol (37), likely to contribute to the observed elevated nitric oxide synthesis rate in COPD by supporting higher ARG requirements. Low grade systemic inflammation enhances higher nitric oxide synthesis demands needed for airway smooth-muscle relaxation (38). Furthermore, during concentric muscle activity, nitric oxide synthesis increases force development, maximal shortening velocity, and maximal power of muscle fibers and isolated muscles through activation of the nitric oxide synthesis soluble guanylyl cyclase-cycle GMP pathway (39). Our data show that muscle weakness in COPD cannot be explained by impaired nitric oxide synthesis availability as observed in heart failure which was due to overall decline in endothelial nitric oxide synthase (NOS) activity (including skeletal muscle) (40). Our COPD patients tended to have elevated plasma glucose levels ($p=0.06$) which is important in light of the previous finding that changes in plasma levels of NO metabolites or precursors may not reflect nitric oxide production by skeletal muscle in patients with reduced insulin sensitivity (41).

During exercise, glutamate plays a central role in energy provision because it participates in the tricarboxylic acid and the purine nucleotide cycle. WBP of glutamate was unaffected in the COPD group, in line with previous data (42), although a significant relation was

observed between leg strength and plasma glutamate. Previously we observed that a reduced muscle glutamate concentration in COPD was associated with early lactate production during exercise (43). Furthermore, skeletal muscle is the principal organ of the synthesis of glutamine from glutamate, which is exported to tissues such as the liver, gut, kidney and immune cells. Our novel intravenous pulse approach (12) makes it possible to measure precisely the glutamine production rate in COPD, as the large pool size of glutamine prohibits an accurate assessment using continuous infusion methods (44). WBP of glutamine was unaltered in COPD group whereas plasma glutamine concentration was elevated, in line with previous data in COPD GOLD stage IV (5). This indicates a reduced glutamine clearance rate which is indicative of a reduced glutamine disposal capacity by the liver, kidney, gut or immune system.

4.3. Alterations in glycine and taurine metabolism

WBP and plasma concentration of glycine were unaltered in the studied COPD, although serum glycine and creatine concentrations were previously found to be reduced in emphysema (5) suggesting disrupted creatine synthesis and glycine degradation. A highly significant association was found in COPD between WBP of glycine and muscle weakness and lower lung function. Besides that glycine is a precursor for creatine and glutathione, it has the potential to attenuate muscle wasting during dieting or fasting and protect muscles in a variety of wasting conditions including cancer cachexia and sepsis (14) *via* suppressing the production of reactive oxygen species and the expression of genes associated with muscle inflammation and macrophage infiltration (45). Furthermore, we found in COPD an elevated WBP of taurine which is an important amino acid in the defense against free radical-mediated damage after exercise (13). Furthermore, it has a regulatory effect on ion channels, calcium homeostasis, oxidative stress and control of membrane excitability. Taurine supplementation has been used to ameliorate performance and muscle strength during aging and to reduce atrophy due to disuse (39).

In the present study, we found that besides transcutaneous O₂ saturation level, the plasma concentration of the downstream metabolite of LEU namely HMB and production of glycine (for lower limb muscle strength) were contributing to > 65% of the variation in skeletal muscle strength in COPD. Although a significant correlation was found between muscle strength and muscle mass, physical activity level, and rates of exacerbation and hospitalization in the previous year, these variables as well as habitual daily dietary protein intake, (lung) disease severity, and comorbidity index did not significantly contribute to the variation in muscle strength in COPD. Transcutaneous O₂ saturation was correlated with respiratory and upper and lower limb muscle strength as well as with plasma HMB. More research needs to be performed to unravel the underlying mechanisms in which systemic hypoxia negatively affects muscle function by affecting metabolism of HMB.

In conclusion, as previous identification of differential amino acids did not confirm plasma amino acid biomarkers in COPD(4), analysis of the relevant metabolic pathways is required to provide deeper insights in the pathogenesis of muscle dysfunction in COPD. The use of a comprehensive innovative multi-tracer pulse approach allows for simultaneous assessment of a large set of metabolic pathways and therefore provides in depth metabolic insight on an

individual level and during a single study visit. The present study revealed a relatively homogenous metabolic signature in COPD which is characterized by an upregulated myofibrillar protein breakdown, citrulline, LEU and taurine production and nitric oxide synthesis, and a specific metabolic profile in those patients with skeletal muscle dysfunction namely disturbances in glycine and HMB metabolism. Further research is warranted whether the upregulated production rates of glycine, nitric oxide, and taurine are compensatory mechanisms to preserve/prevent further deterioration of muscle health in COPD, and whether the strong association between muscle weakness and low BCAA and HMB plasma concentrations reflect rather a cause or consequence of reduced muscle health in COPD. Whether changes in metabolic fluxes in time could predict future reduction of skeletal muscle function or COPD progression deserves further investigation. More mechanistic data can even be obtained when the stable tracer pulse method is combined with muscle biopsies to measure both fractional protein breakdown and synthesis rates, and when more stable tracers are added to the pulse. Limitations of this study are that we did not perform the pulse approach with the phenylalanine and tyrosine stable tracers, and the relatively small group sizes. As stable isotope studies are able to measure whole body production rates of proteins and amino acids very accurately using LC-MS/MS technology, a sample size of 15 per group is often sufficient to compare (diseased and healthy) groups(12, 37, 46–48) although low for correlation analysis. The practical limitation is that the tracer approach cannot be used easily as a biomarker selection technique due to (tracer and analytical) costs and accessibility. Although confirmed plasma amino acid biomarkers in COPD have not yet been reported(4), the consistently elevated glutamine concentration among studies in COPD and its scientific and clinical significance deserves further investigation. Furthermore, this multi-tracer flux approach needs to be applied to a larger group of COPD patients to enable identification of metabolite signatures that might distinguish COPD patients with different comorbidities, and pulmonary and body composition phenotypes so that nutritional regimens can be developed and evaluated to improve muscle health in COPD. Ultimately Identification of the metabolic signature of disease related systemic features of COPD like skeletal muscle dysfunction is of critical importance to provide the required insights to develop targeted and more individualized (nutritional) therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ARG	arginine
ASMI	appendicular skeletal muscle index
BCAA	branched-chain amino acid
BCKA	branched-chain keto acid
CIT	citrulline
COPD	chronic obstructive pulmonary disease
FFM	fat-free mass
HMB	3-hydroxy-3-methylbutyrate
KIC	α -ketoisocaproic acid
LEU	leucine
mHIS	methylhistidine
PHE	phenylalanine
Ra	rate of appearance
TYR	tyrosine
WBP	Whole body production rate

REFERENCES

1. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare R, et al. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2014;189(9):e15–62. [PubMed: 24787074]
2. Vilaro J, Ramirez-Sarmiento A, Martinez-Llorens JM, Mendoza T, Alvarez M, Sanchez-Cayado N, et al. Global muscle dysfunction as a risk factor of readmission to hospital due to COPD exacerbations. *Respiratory medicine*. 2010;104(12):1896–902. [PubMed: 20541383]
3. Min T, Bodas M, Mazur S, Vij N. Critical role of proteostasis-imbalance in pathogenesis of COPD and severe emphysema. *Journal of molecular medicine*. 2011;89(6):577–93. [PubMed: 21318260]
4. Ran N, Pang Z, Gu Y, Pan H, Zuo X, Guan X, et al. An Updated Overview of Metabolomic Profile Changes in Chronic Obstructive Pulmonary Disease. *Metabolites*. 2019;9(6):111.
5. Ubhi BK, Riley JH, Shaw PA, Lomas DA, Tal-Singer R, MacNee W, et al. Metabolic profiling detects biomarkers of protein degradation in COPD patients. *European Respiratory Journal*. 2012;40(2):345–55. [PubMed: 22183483]
6. Ubhi BK, Cheng KK, Dong J, Janowitz T, Jodrell D, Tal-Singer R, et al. Targeted metabolomics identifies perturbations in amino acid metabolism that sub-classify patients with COPD. *Molecular BioSystems*. 2012;8(12):3125–33. [PubMed: 23051772]
7. Singh B, Jana SK, Ghosh N, Das SK, Joshi M, Bhattacharyya P, et al. Metabolomic profiling of doxycycline treatment in chronic obstructive pulmonary disease. *Journal of Pharmaceutical and Biomedical Analysis*. 2017;132:103–8. [PubMed: 27697570]

8. Engelen MP, Wouters EF, Deutz NE, Menheere PP, Schols AM. Factors contributing to alterations in skeletal muscle and plasma amino acid profiles in patients with chronic obstructive pulmonary disease. *Am J Clin Nutr.* 2000;72(6):1480–7. [PubMed: 11101475]
9. Wang C, Li J-X, Tang D, Zhang J-Q, Fang L-Z, Fu W-P, et al. Metabolic changes of different high-resolution computed tomography phenotypes of COPD after budesonide–formoterol treatment. *International journal of chronic obstructive pulmonary disease.* 2017;12:3511. [PubMed: 29255358]
10. Novotna B, Abdel-Hamid M, Koblizek V, Svoboda M, Hejduk K, Rehacek V, et al. A pilot data analysis of a metabolomic HPLC-MS/MS study of patients with COPD. *Adv Clin Exp Med.* 2018;27(4):531–9. [PubMed: 29943523]
11. Rutten EP, Franssen FM, Engelen MP, Wouters EF, Deutz NE, Schols AM. Greater whole-body myofibrillar protein breakdown in cachectic patients with chronic obstructive pulmonary disease. *Am J Clin Nutr.* 2006;83(4):829–34. [PubMed: 16600935]
12. Deutz NEP, Thaden JJ, Ten Have GAM, Walker DK, Engelen M. Metabolic phenotyping using kinetic measurements in young and older healthy adults. *Metabolism: clinical and experimental.* 2018;78:167–78. [PubMed: 28986165]
13. Spriet LL, Whitfield J. Taurine and skeletal muscle function. *Curr Opin Clin Nutr Metab Care.* 2015;18(1):96–101. [PubMed: 25415270]
14. Koopman R, Caldwell MK, Ham DJ, Lynch GS. Glycine metabolism in skeletal muscle: implications for metabolic homeostasis. *Curr Opin Clin Nutr Metab Care.* 2017;20(4):237–42. [PubMed: 28375879]
15. Luiking YC, Ten Have GA, Wolfe RR, Deutz NE. Arginine de novo and nitric oxide production in disease states. *American journal of physiology Endocrinology and metabolism.* 2012;303(10):E1177–89. [PubMed: 23011059]
16. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. *Am J Respir Crit Care Med.* 2017;195(5):557–82. [PubMed: 28128970]
17. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med.* 1999;159(1):179–87. [PubMed: 9872837]
18. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. *Journal of clinical epidemiology.* 1993;46(2):153–62. [PubMed: 8437031]
19. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *Journal of clinical epidemiology.* 1994;47(11):1245–51. [PubMed: 7722560]
20. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism: clinical and experimental.* 1981;30(9):936–40. [PubMed: 7022111]
21. Engelen MP, Safar AM, Bartter T, Koeman F, Deutz NE. High anabolic potential of essential amino acid mixtures in advanced nonsmall cell lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2015;26(9):1960–6.
22. Wolfe RR, Chinkes DL. *Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis.* New York: Wiley, New York, New York; 2005 1–274 p.
23. Mason A, Engelen M, Ivanov I, Toffolo GM, Deutz NEP. A four-compartment compartmental model to assess net whole body protein breakdown using a pulse of phenylalanine and tyrosine stable isotopes in humans. *American journal of physiology Endocrinology and metabolism.* 2017;313(1):E63–E74. [PubMed: 28270442]
24. Deutz NE, Wagenmakers AJ, Soeters PB. Discrepancy between muscle and whole body protein turnover. *Curr Opin Clin Nutr Metab Care.* 1999;2(1):29–32. [PubMed: 10453327]
25. Hasselgren PO, Fischer JE. Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. *Ann Surg.* 2001;233(1):9–17. [PubMed: 11141219]
26. Engelen MP, De Castro CL, Rutten EP, Wouters EF, Schols AM, Deutz NE. Enhanced anabolic response to milk protein sip feeding in elderly subjects with COPD is associated with a reduced

- splanchnic extraction of multiple amino acids. *Clin Nutr.* 2012;31(5):616–24. [PubMed: 22682082]
27. Nawabi MD, Block KP, Chakrabarti MC, Buse MG. Administration of endotoxin, tumor necrosis factor, or interleukin 1 to rats activates skeletal muscle branched-chain alpha-keto acid dehydrogenase. *J Clin Invest.* 1990;85(1):256–63. [PubMed: 2404025]
 28. Cynober L, Harris RA. Symposium on branched-chain amino acids: conference summary. *J Nutr.* 2006;136(1 Suppl):333S–6S. [PubMed: 16365109]
 29. Kuriyan R, Lokesh DP, Selvam S, Jayakumar J, Philip MG, Shreeram S, et al. The relationship of endogenous plasma concentrations of beta-Hydroxy beta-Methyl Butyrate (HMB) to age and total appendicular lean mass in humans. *Experimental gerontology.* 2016;81:13–8. [PubMed: 27108182]
 30. Engelen M, Deutz NEP. Is beta-hydroxy beta-methylbutyrate an effective anabolic agent to improve outcome in older diseased populations? *Curr Opin Clin Nutr Metab Care.* 2018;21(3):207–13. [PubMed: 29406417]
 31. Giron MD, Vilchez JD, Salto R, Manzano M, Sevillano N, Campos N, et al. Conversion of leucine to beta-hydroxy-beta-methylbutyrate by alpha-keto isocaproate dioxygenase is required for a potent stimulation of protein synthesis in L6 rat myotubes. *Journal of cachexia, sarcopenia and muscle.* 2016;7(1):68–78.
 32. Wilkinson DJ, Hossain T, Limb MC, Phillips BE, Lund J, Williams JP, et al. Impact of the calcium form of beta-hydroxy-beta-methylbutyrate upon human skeletal muscle protein metabolism. *Clin Nutr.* 2018;37(6 Pt A):2068–75. [PubMed: 29097038]
 33. Kraemer WJ, Hatfield DL, Volek JS, Fragala MS, Vingren JL, Anderson JM, et al. Effects of amino acids supplement on physiological adaptations to resistance training. *Medicine and science in sports and exercise.* 2009;41(5):1111–21. [PubMed: 19346975]
 34. Holecek M Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. *Journal of cachexia, sarcopenia and muscle.* 2017;8(4):529–41.
 35. Deutz NE, Matheson EM, Matarese LE, Luo M, Baggs GE, Nelson JL, et al. Readmission and mortality in malnourished, older, hospitalized adults treated with a specialized oral nutritional supplement: A randomized clinical trial. *Clin Nutr.* 2016;35(1):18–26. [PubMed: 26797412]
 36. Oliveira G, Oliveira C, Dona E, Palenque FJ, Porras N, Dorado A, et al. Oral supplement enriched in HMB combined with pulmonary rehabilitation improves body composition and health related quality of life in patients with bronchiectasis (Prospective, Randomised Study). *Clin Nutr.* 2016;35(5):1015–22. [PubMed: 26522923]
 37. Jonker R, Deutz NE, Erbland ML, Anderson PJ, Engelen MP. Alterations in whole-body arginine metabolism in chronic obstructive pulmonary disease. *Am J Clin Nutr.* 2016;103(6):1458–64. [PubMed: 27146652]
 38. Malerba M, Radaeli A, Olivini A, Damiani G, Ragnoli B, Montuschi P, et al. Exhaled nitric oxide as a biomarker in COPD and related comorbidities. *Biomed Res Int.* 2014;2014:271918. [PubMed: 24719850]
 39. Kaminski HJ, Andrade FH. Nitric oxide: biologic effects on muscle and role in muscle diseases. *Neuromuscul Disord.* 2001;11(6–7):517–24. [PubMed: 11525879]
 40. Katz SD, Khan T, Zeballos GA, Mathew L, Potharlanka P, Knecht M, et al. Decreased activity of the L-arginine-nitric oxide metabolic pathway in patients with congestive heart failure. *Circulation.* 1999;99(16):2113–7. [PubMed: 10217650]
 41. Krause M, Rodrigues-Krause J, O'Hagan C, De Vito G, Boreham C, Susta D, et al. Differential nitric oxide levels in the blood and skeletal muscle of type 2 diabetic subjects may be consequence of adiposity: a preliminary study. *Metabolism: clinical and experimental.* 2012;61(11):1528–37. [PubMed: 22683098]
 42. Rutten EP, Engelen MP, Castro CL, Wouters EF, Schols AM, Deutz NE. Decreased whole-body and splanchnic glutamate metabolism in healthy elderly men and patients with chronic obstructive pulmonary disease in the postabsorptive state and in response to feeding. *J Nutr.* 2005;135(9):2166–70. [PubMed: 16140893]

43. Engelen MP, Schols AM, Does JD, Gosker HR, Deutz NE, Wouters EF. Exercise-induced lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2000;162(5):1697–704. [PubMed: 11069799]
44. Van Acker BA, Hulsewe KW, Wagenmakers AJ, Deutz NE, Van Kreel BK, Halliday D, et al. Absence of glutamine isotopic steady state: implications for the assessment of whole-body glutamine production rate. *Clin Sci (Lond)*. 1998;95(3):339–46. [PubMed: 9730854]
45. Ham DJ, Murphy KT, Chee A, Lynch GS, Koopman R. Glycine administration attenuates skeletal muscle wasting in a mouse model of cancer cachexia. *Clin Nutr*. 2014;33(3):448–58. [PubMed: 23835111]
46. Engelen MP, Com G, Luiking YC, Deutz NEP. Enhanced De Novo Arginine Synthesis And Protein Turnover In Pediatric Cystic Fibrosis Patients With Nutritional Failure. *American Journal of Respiratory and Critical Care Medicine*. 2012;185:A2471.
47. Engelen MP, Com G, Wolfe RR, Deutz NE. Dietary essential amino acids are highly anabolic in pediatric patients with cystic fibrosis. *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society*. 2013;12(5):445–53. [PubMed: 23357545]
48. Engelen M, Klimberg VS, Allasia A, Deutz NEP. Major surgery diminishes systemic arginine availability and suppresses nitric oxide response to feeding in patients with early stage breast cancer. *Clin Nutr*. 2018;37(5):1645–53. [PubMed: 28826699]

FIGURE 1.

Scatter plot of appendicular lean mass index and handgrip strength (Fig 1a) and leg muscle strength (Fig 1b) in the COPD group (closed circles) and the healthy control group (open circles). Data were analyzed with 2-tailed tests of significance by measuring bivariate Pearson's correlation coefficients. The regression lines are as follows:

Figure 1a: Healthy: Handgrip strength = $28.91 * \text{appendicular lean mass index} + 62.6$ ($r:0.67$, $P<0.01$; slope significant from zero, $P<0.01$); COPD: Handgrip strength = $16.9 * \text{appendicular lean mass index} + 98.03$ ($r:0.37$, $P=0.14$; slope tended to be significant from zero, $p=0.08$). Slopes are not significantly different ($P=0.33$).

Figure 1b: Healthy: One leg extension = $52.3 * \text{appendicular lean mass index} - 49.49$ ($r:0.66$, $P<0.01$; slope significant from zero, $P<0.01$); COPD: One leg extension = $33.2 * \text{appendicular lean mass index} + 1.819$ ($r:0.54$, $P<0.01$; slope significant from zero, $P<0.05$). Slopes are not significantly different ($p=0.33$).

FIGURE 2.

Scatter plot of plasma HMB concentration and handgrip strength (Figure 2a) and one leg extension (Figure 2b) in the COPD group (closed circles) and the healthy control group (open circles). Data were analyzed with 2-tailed tests of significance by measuring bivariate Pearson's correlation coefficients and linear regression analysis. The regression lines are as follows:

Figure 2a: Healthy: plasma HMB = $-0.0028 \times \text{handgrip strength} - 0.3428$ ($r:0.36$, ns; slope not significant from zero); COPD: plasma HMB = $0.0043 \times \text{handgrip strength} - 1.029$ ($r:0.62$, $P<0.01$; slope significant from zero, $P<0.01$). Slopes are not significantly different ($P=0.12$).

Figure 2b: Healthy: plasma HMB = $0.0023 \times \text{leg muscle strength} + 0.739$ ($r:0.34$, ns; slope not significant from zero); COPD: plasma HMB = $0.0082 \times \text{leg muscle strength} + 0.7429$ ($r:0.66$, $P<0.01$; slope significant from zero, $P<0.01$). Slopes are significantly different ($P<0.05$).

FIGURE 3.

Scatter plot of whole body production of glycine and handgrip strength (Figure 3a) and one leg extension (Figure 3b) in the COPD group (closed circles) and the healthy control group (open circles). Data were analyzed with 2-tailed tests of significance by measuring bivariate Pearson's correlation coefficients and linear regression analysis. The regression lines are as follows:

Figure 3a: Healthy: WBP glycine = $-0.373 \times \text{handgrip strength} + 304.2$ ($r: -0.40$, $P=0.09$; slope significant from zero, $P=0.09$); COPD: WBP glycine = $-0.748 \times \text{handgrip strength} + 528.8$ ($r: -0.49$, $P<0.05$; slope significant from zero, $P<0.05$). Slopes are not significantly different.

Figure 3b: Healthy: WBP glycine: $y = -0.429 \times \text{one leg extension} + 168.1$ ($r: -0.34$, ns; slope not significant from zero); COPD: WBP glycine = $-1.163 \times \text{leg muscle strength} + 300.6$ ($r: -0.64$, $P<0.01$; slope significant from zero, $P<0.01$). Slopes are significantly different ($P<0.05$).

FIGURE 4.

Scatter plot of plasma concentration of BCAA and handgrip strength (Figure 4a), and one leg extension (Figure 4b) in the COPD group (closed circles) and the healthy control group (open circles). Data were analyzed with 2-tailed tests of significance by measuring bivariate Pearson's correlation coefficients and linear regression analysis. BCAA: sum of branched-chain amino acids. The regression lines are as follows:

Figure 4a: Healthy: plasma BCAA = $0.4036 * \text{handgrip strength} + 227.7$ ($r:0.40$, ns; slope not significant from zero); COPD: plasma BCAA = $0.5995 * \text{handgrip strength} + 193.5$ ($r:0.38$, $P=0.08$; slope not significant from zero). Slopes are not significantly different ($P=0.053$).

Figure 4b: Healthy: plasma BCAA = $0.2486 * \text{one leg extension} + 253.8$ ($r:0.46$, $P=0.07$; slope not significant from zero); COPD: plasma BCAA = $0.7442 * \text{one leg extension} + 155.6$ ($r:0.61$, $P<0.01$; slope significant from zero, $P<0.01$). Slopes are not significantly different ($p=0.07$).

FIGURE 5.

Scatter plot of transcutaneous O₂ saturation (SaO₂) and handgrip strength (Figure 5a), and one leg extension (Figure 5b) in the COPD group (closed circles) and the healthy control group (open circles). Data were analyzed with 2-tailed tests of significance by measuring bivariate Pearson's correlation coefficients and linear regression analysis. The regression lines are as follows:

Figure 5a: COPD: SaO₂ = 0.0431 * handgrip strength + 83.41 (r:0.73, $P < 0.001$; slope significant from zero, $p < 0.001$).

Figure 5b: COPD: SaO₂ = 0.0343 * one leg extension + 84.98 (r:0.67, $P < 0.01$; slope significant from zero, $P < 0.01$).

Table 1 -

General and clinical characteristics of the COPD and healthy populations

	Healthy (n=19)	COPD (n=23)	P value
Age (years)	64.0 (2.0)	68.8 (2.1)	0.08
Gender (Male/Female)	10/9	11/12	0.44
Body Weight (kg)	81.0 (3.6)	83.1 (4.8)	0.72
Height (m)	1.68 (0.02)	1.68 (0.01)	0.72
Body Mass Index (kg/m ²)	28.5 (1.0)	29.4 (1.4)	0.63
Charlson comorbidity index (score)	0.24 ± 0.20	2.04 ± 0.24	< 0.001
Pulmonary function and COPD related measures			
FEV ₁ (% of predicted) ³	94.3 (3.3)	41.2 (3.0)	< 0.001
FVC (% of predicted) ⁴	91.1 (2.9)	57.5 (3.2)	< 0.001
FEV ₁ /FVC (ratio)	0.94 (0.04)	0.50 (0.03)	< 0.001
D _{LCO} (% of predicted) ^{5#}	-	41.6 (3.7)	-
Years since initial diagnosis	-	10.4 (1.2)	-
Self-reported years of COPD related symptoms	-	11.7 (1.5)	-
mMRC dyspnea grade ⁶	-	2.4 (0.2)	-
Number of exacerbations in the past year	-	1.4 (0.3)	-
Transcutaneous O ₂ saturation	97.4 (0.3)	92.8 (0.7)	< 0.001
O ₂ use (yes/no)	0/20	14/9	< 0.001
Glucose (mmol/L)	5.5 (0.1)	5.9 (0.2)	0.06
CRP (mg/L)	1.9 (0.4)	4.3 (0.9)	0.03

Values are mean (SE) except for O₂ use. D_{LCO}: diffusing capacity for carbon monoxide. FEV₁: Forced Expiratory Volume in one second. FVC: Forced Vital Capacity. mMRC: modified medical research council dyspnea scale. O₂: supplemental oxygen, CRP: C-reactive protein. Statistics are by unpaired t-test, bold is p<0.001.

Table 2 -

Body composition and muscle function characteristics

	Healthy (n=19)	COPD (n=23)	P value
Body Composition			
Lean mass (kg)	50.3 (3.0)	49.4 (2.4)	0.80
Lean mass extremities (kg)	21.0 (1.4)	20.0 (1.0)	0.51
Fat mass (kg)	27.2 (2.5)	32.7 (3.5)	0.23
Fat mass trunk (kg)	15.3 (1.2)	16.3 (1.9)	0.38
Fat mass index (kg/m ²)	9.7 (1.0)	11.6 (1.2)	0.28
Fat-free mass (kg)	52.7 (3.1)	51.3 (2.2)	0.68
Fat-free mass index (kg/m ²) ²	18.4 (0.8)	18.1 (0.6)	0.75
Appendicular skeletal muscle mass (kg)	21.0 (1.5)	20.0 (0.9)	0.51
Appendicular skeletal muscle index (kg/m ²) ³	7.3 (0.4)	7.1 (0.3)	0.51
Android fat (%) ⁴	37.4 (2.5)	37.3 (2.5)	0.98
Gynoid fat (%) ⁴	34.4 (2.5)	36.0 (1.7)	0.54
Fat % android/gynoid (ratio)	1.02 (0.04)	1.04 (0.05)	0.88
Muscle function			
Inspiratory muscle strength (cm H ₂ O)	101.1 (11.8)	68.7 (5.3)	0.006
Expiratory muscle strength (cm H ₂ O)	106.1 (5.9)	101.9 (5.5)	0.71
Maximal handgrip strength (N)	273.3 (15.8)	217.3 (11.9)	0.004
Maximal leg extension force (N)	335.3 (28.0)	234.8 (17.5)	0.003
Other			
PASE (score)	150.5 (22.7)	109.4 (12.7)	0.08
Daily protein intake per kg fat-free mass	1.04 (0.07)	0.99 (0.12)	0.76

Values are mean (SE). Statistics are by unpaired t-test.

²Fat-free mass index = (lean mass + bone mineral content)/height².

³Appendicular skeletal muscle index = (lean mass legs + lean mass arms)/height².

⁴Android fat and gynoid fat correspond to central and peripheral fat distribution, respectively. COPD: chronic obstructive pulmonary disease. PASE: physical activity questionnaire for the elderly.

Table 3 -

Whole body production rates of amino acids and their interconversions

	Healthy (n=20)	COPD (n=23)	P value
Arginine	130.3 (4.3)	114.2 (7.3)	0.71
Citrulline	11.6 (0.6)	14.2 (0.8)	0.03
Glutamate	185.8 (23.3)	211.6 (19.5)	0.46
Glutamine	364.4 (49.8)	316.5 (27.5)	0.38
Glycine	199.7 (13.1)	233.1 (17.9)	0.15
Hydroxyproline	6.1 (0.9)	9.5 (0.5)	0.59
Keto-isocaproic acid	10.2 (0.8)	24.7 (1.2)	0.21
Leucine	80.0 (5.7)	117.2 (7.2)	< 0.0001
Phenylalanine	96.1 (5.1)	90.0 (2.3)	0.23
tau-Methylhistidine	0.47 (0.04)	0.81 (0.1)	0.01
Taurine	21.0 (1.8)	27.1 (2.3)	0.09
Tyrosine	65.6 (4.9)	63.2 (2.1)	0.63
Arginine to citrulline conversion rate (NO production)	4.2 (0.6)	7.2 (0.6)	0.04
Citrulline to arginine conversion rate (ARG <i>de novo</i> production)	14.3 (1.3)	14.1 (1.2)	0.96
Glutamate to glutamine conversion rate	43.5 (8.2)	35.6 (6.2)	0.47
Phenylalanine to tyrosine conversion rate (net protein breakdown)	5.2 (0.7)	4.8 (0.4)	0.73

Values are mean $\pm$ SE and expressed as $\mu\text{mol/kg ffm/h}$ Statistics are by unpaired t-test. COPD: chronic obstructive pulmonary disease

Table 4a -

Multiple Linear Regression model with upper limb muscle strength (handgrip strength) in the COPD group

	Coefficients	SE	t	P value	q value
Intercept	--749.2	232.8	3.22	0.0048	0.076
Transcutaneous O ₂ saturation (%)	9.78	2.61	3.75	0.0015	0.047
HMB plasma concentration (μmol/l)	20.90	8.75	2.39	0.028	0.0294
R²= 0.65, P-value: <0.0001					

COPD: chronic obstructive pulmonary disease

Table 4b -

Multiple Linear Regression model with lower limb muscle strength (one leg extension) in the COPD patients

	Coefficients	SE	t	P value	q value
Intercept	-478.5	237.8	2.012	0.043	0.0301
HMB plasma concentration (μmol/l)	31.39	9.61	3.26	0.005	0.0105
Transcutaneous O ₂ saturation (%)	7.20	2.54	2.84	0.012	0.0126
WBP Glycine (μmol/kg ffm/h)	-0.20	0.11	-2.33	0.079	0.0415

 $R^2 = 0.79$, P -value < 0.0001

WBP: whole body production, HMB: 3-hydroxy-3-methylbutyrate. COPD: chronic obstructive pulmonary disease