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Whole body protein anabolism in COPD patients and healthy older adults is not enhanced by adding either carbohydrates or leucine to a serving of protein

Renate Jonker a, Nicolaas E.P. Deutz a, Annemie M.W.J. Schols b, Eugene A. Veley c, Rajesh Harrykissoon d, Anthony J. Zachriad, Mariëlle P.K.J. Engelen a,*

a Center for Translational Research in Aging & Longevity, Dept. of Health and Kinesiology, Texas A&M University, College Station, TX, USA
b NUTRIM School for Nutrition, Toxicology and Metabolism, Dept. of Respiratory Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands
c Dept. of Medicine, Div. of Pulmonary & Critical Care Medicine, Baylor Scott & White Medical Center, College Station, TX, USA
d Center for Pulmonary and Sleep Disorders, College Station Medical Center, College Station, TX, USA

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SUMMARY

Background & aims: Carbohydrates (CHO) and leucine (LEU) both have insulinotropic properties, and could therefore enhance the protein anabolic capacity of dietary proteins, which are important nutrients in preventing muscle loss in patients with Chronic Obstructive Pulmonary Disease (COPD). LEU is also known to activate protein anabolic signaling pathways independent of insulin. Based on our previous findings in COPD, we hypothesized that whole body protein anabolism is enhanced to a comparable extent by the separate and combined co-ingestion of CHO and LEU with protein.

Methods: To disentangle the protein anabolic effects of CHO and/or free LEU when co-ingested with a high-quality protein, we studied 10 patients with moderate to very severe COPD and dyspnea (GOLD: II-IV, mMRC dyspnea scale ≥ 2), at risk for muscle loss, and 10 healthy age- and gender-matched controls. On four occasions, in a single-blind randomized crossover design, each subject ingested a drink containing 0.6 g/kg fat-free mass (ffm) hydrolyzed casein protein with, a) no add-ons (protein), b) 0.3 g/kg ffm CHO (protein + CHO), c) 0.095 g/kg ffm LEU (protein + LEU), d) both add-ons (protein + CHO + LEU). Whole body protein breakdown (PB), protein synthesis (PS), and net protein balance (PS = PB) were measured by IV primed and continuous infusion of L-[ring-2H5]-phenylalanine and L-[13C9,15N]-tyrosine. L-[15N]-phenylalanine was added to the protein drinks to measure splanchnic extraction.

Results: In both groups, whole body PS, PB and net protein balance responses were comparable between the four protein drinks, despite higher postprandial plasma LEU concentrations for the LEU supplemented drinks (P < 0.05), and higher insulin concentrations for the CHO supplemented drinks as compared to the protein only drink (P < 0.05).

Conclusions: Adding CHO and/or LEU to a serving of high-quality protein does not further augment whole body protein anabolism in dyspneic COPD patients at risk for muscle loss or healthy older adults.

Trial registry: ClinicalTrials.gov; No. NCT01734473; URL: www.clinicaltrials.gov.

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1. Introduction

Nutritional interventions with high anabolic effectiveness are clinically important in patients with Chronic Obstructive Pulmonary Disease (COPD) with moderate to severe dyspnea at risk for muscle loss as disturbances in intermediary amino acid and protein metabolism are already present in these normal weight patients [1–3]. Although high-quality proteins [4], and specifically dietary
essential amino acids [5] are very effective in the acute stimulation of protein anabolism in COPD patients, the specific contribution of leucine (LEU) remains controversial.

LEU is of particular interest as it is unique among the essential amino acids for its potential to activate signaling pathways that stimulate muscle protein synthesis (PS) [6,7]. LEU acts on these pathways through insulin dependent as well as independent mechanisms [6,7]. In humans, insulin administration has been suggested to improve net protein balance by inhibiting muscle protein breakdown (PB) [8,9]. LEU and CHO therefore partially work through the same mechanism to promote increases in protein anabolism, namely by stimulation of insulin. In contrast, we observed no anabolic effects of LEU ingestion with a bolus of high-quality protein or dietary essential amino acids in COPD patients [4,5]. However in these COPD studies, carbohydrates (CHO) were always added to the protein/AMINO acid meal. It is particularly important for COPD patients to disentangle the anabolic effects of LEU and CHO, as this patient group is characterized by alterations in LEU metabolism [5,10,11] and glucose intolerance [12,13]. Furthermore, minimizing supplement dose by optimizing the quality is clinically important particularly in dyspeptic COPD patients at risk for muscle loss as it may impair their food intake.

Based on our previous findings in COPD, we hypothesized in the present study that whole body protein anabolism is enhanced to a comparable extent by the separate and combined co-ingestion of CHO and LEU with protein. Furthermore, we tested whether the observations are COPD specific or also present in healthy control subjects. To examine this, we studied the response effect of administering a serving of high-quality protein with or without the addition of free LEU and/or CHO on whole body protein metabolism and glucose and insulin kinetics in COPD patients with moderate to severe dyspnea at risk for muscle loss as compared to healthy older adults, using stable isotope tracer methodology.

2. Subjects and methods

2.1. Subjects

The study population consisted of 10 older adults with a clinical diagnosis of moderate to very severe airflow obstruction (grades II-IV), according to the established Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [14], moderate to severe dyspnea (mMRC dyspnea scale ≥ 2), and 11 healthy participants of similar age and gender. We recruited participants through pulmonologist referral and local advertising. We assessed medical history and medication use as part of the screening process. COPD patients were in clinically stable condition and not suffering from a respiratory tract infection or exacerbation of their disease ≤ 4 weeks before the study. As maintenance therapy, 9 patients received bronchodilator treatment, 8 patients received inhalation corticosteroids, and 7 patients were on long-term oxygen therapy. Eight patients compared to 4 controls were treated for hypertension, 5 patients compared to 2 controls were treated for depression and/or anxiety, and 2 patients versus 2 controls received medication for dyslipidemia. The use of systemic corticosteroids ≤1 month before the study was an exclusion criteria, as well as malignancy, recent myocardial infarction, recent surgery and severe endocrine, hepatic or renal disorders. We obtained written informed consent from all participants, and the Texas A&M University Institutional Review Board approved the study (no. 2012-0561).

2.2. Lung function and anthropometric data

We measured forced expiratory volume in 1 s (FEV1) and forced vital capacity in all participants, with the highest value from ≥3 technically acceptable maneuvers being used. Spirometric reference values were those of a US population [15]. We measured height and weight using a stadiometer and beam scale, respectively. We assessed body composition, including whole body fat-free mass (FFM), by dual-energy X-ray absorptiometry (Hologic QDR 4500).

2.3. Study design

We studied all participants on four experimental test days (≥1 day apart and ≤ 2 days per week) within a time period of 1 month (Fig. 1). Test days started in the early morning after an overnight fast, and lasted 5.5 h, during which participants were in a supine position. After insertion of a catheter into an antecubital vein, the first blood sample was taken to measure natural background isotope enrichment. Consecutively, we started a primed, constant intravenous infusion of L-[ring-2H5]-phenylalanine ([2H5]-PHE: prime = 270 μmol; infusion = 270 μmol × h⁻¹) and L-[U-13C9,15N]-tyrosine ([U-13C9,15N]-TYR: prime = 8.5 μmol; infusion = 8.5 μmol × h⁻¹) to assess whole body protein metabolism. At the same time, we gave an oral bolus dose of L-[ring-2H5]-tyrosine to prime the plasma pool ([2H5]-TYR: prime = 25.5 μmol). We also placed a second catheter for arterialized venous blood sampling in a superficial dorsal vein of the contralateral hand or lower arm. The hand was placed in a thermostatically controlled heated box, a technique to mimic direct arterial sampling [16]. We obtained arterialized-venous blood samples at 70, 80, and 90 min after the start of infusion for the measurement of isotope enrichment values and plasma concentrations of amino acids, glucose and insulin in the postabsorptive state, and at 15, 30, 45, 60, 90, 120, 150, 180, 210

![Fig. 1](https://example.com/fig1.png)

Fig. 1. Study design. All participants were studied during four experimental test days (≥1 day apart and ≤2 days per week) within a time frame of one month. The responses to four different protein drinks were examined according to a randomized cross-over design. Time points t = −20, −10 and 0 min were used to analyze postabsorptive protein metabolism. Postprandial protein kinetics were measured by using the time points t = 0–240 min in order to calculate the area under the curve between 0 and 4 h.
and 240 min after intake of the protein drinks for similar post-prandial assessments.

2.4. Protein drinks

Participants received one out of four protein drinks each test day, according to a single-blind randomized cross-over design. Protein drinks were ingested orally and consisted of 0.6 g/kg ffm hydrolyzed casein protein (PepToPro® hydrolyzed caseinate, True Nutrition, Vista, CA, USA) with, a) no add-ons (protein), b) 0.3 g/kg ffm CHO (protein + CHO), c) 0.095 g/kg ffm leucine (protein + LEU), d) both add-ons (protein + CHO + LEU). CHO were given in the form of maltodextrin. We dissolved all components in non-caloric soda (6 mL/kg ffm), and added 5.45 mg/kg ffm L-[15N]-phenylalanine ([15N]-PHE) (i.e. 10% of PHE content) to each of the drinks for the measurement of splanchnic PHE extraction. For an average person of 50 kg FFM, this would mean that the bolus meal contained 30 g hydrolyzed caseinate with/without 15 g CHO and the addition of free LEU increased the total LEU content from 20 to 40% (from ~3 to 7.7 g) of the essential amino acid content.

2.5. Sample processing and biochemical analysis

We collected arterialized-venous blood samples in tubes with lithium heparin or EDTA (Sarstedt, Nümbrecht, Germany), and immediately put them on ice to minimize enzymatic reactions. We centrifuged the blood (5 min, 8000 g, 4 °C) and put a portion of the plasma in 50% trichloroacetic acid matrices for deproteinization. We instantly froze the plasma and stored it at −80 °C until further analyses.

Samples were analyzed in batch. We determined isotope tracer enrichments and concentrations on a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/MS-MS) system (QTrap 5500 MS (AB Sciex, Foster City, CA) with ExpressHT-Ultra LC (Eksigent Div., AB Sciex, Foster City, CA) [4]. Enrichment was expressed as tracer (labeled substance)/tracee (unlabeled substance) ratio (TTR), and corrected for natural background abundance (cTTR). We determined plasma glucose concentrations using a COBAS c111 semiautomatic analyzer (Gluc2 Kit; Roche Diagnostics®), and plasma insulin concentrations by enzyme linked immunosorbent assay (Alpco®).

2.6. Calculations

For the assessment of plasma amino acid, glucose and insulin concentrations, we calculated the 4-h postprandial area under the curve (AUC) per individual, using the postabsorptive concentration as baseline reference. The AUC was then divided by time to obtain the average increase in postprandial plasma concentration.

We calculated whole body PHE and TYR appearance rates from the isotope enrichment values of [6,5H]-PHE and [13C9,15N]-TYR. In the postabsorptive state, enrichments were at isotopic steady state, and we calculated whole body PS, PB and net protein balance (PS – PB) using the standard steady state isotope dilution equations [4]. As we provided a bolus meal, the enrichments were not in steady state during the 4 h feeding period. The tracers were infused by primed continuous infusion and the response to the bolus meal was assessed by calculating the area under the curve between 0 and 4 h, taking into consideration that the uptake of food is not complete after 4 h [17]. During the 4-h postprandial state, we calculated whole body PS, PB, net protein balance and splanchnic PHE extraction using previously published steady state equations [18], adapted for the current non-steady state conditions [17]:

$$\text{RaPHE} = \frac{F(\text{PHE} \text{ring} - 2H_5)}{\text{AVG cTTR}(\text{PHE} \text{ring} - 2H_5)}$$

$$\text{RaTYR}[13C_9, 15N] = \frac{F(\text{TYR}[13C_9, 15N])}{\text{AVG cTTR}(\text{TYR}[2H_4, 15N])}$$

where RaPHE [ring-2H₅] and RaTYR [13C₉, 15N] (µmol × kg ffm⁻¹ × h⁻¹) represent the average total systemic Ra of PHE and TYR calculated from the intravenous infusion of PHE [ring-²H₅] and TYR [1⁵C₉, 1⁵N] respectively. F represents the infusion rate (µmol × kg ffm⁻¹ × h⁻¹) of PHE [ring-²H₅] and TYR [1⁵C₉, 1⁵N]. AVG (average) of cTTR (PHE [ring-²H₅]) is calculated as the AUC of cTTR (PHE [ring-²H₅]) divided by 240 (time in min). AVG cTTR (TYR [1⁵C₉, 1⁵N]) is the AUC of cTTR (TYR [1⁵C₉, 1⁵N]) divided by 240 (time in min).

$$\text{Ra PHE}[15N] = \frac{\text{tracer in drink}(\text{PHE}[15N])}{\text{AUC cTTR}(\text{PHE}[15N])}$$

where Ra PHE [¹⁵N] was recalculated per hour (µmol × kg ffm⁻¹ × h⁻¹) and represents the average total systemic Ra of PHE calculated from the oral intake of PHE [¹⁵N].

$$\text{SPE} = 1 - \frac{\text{RaPHE}[\text{ring} - 2H_5]}{\text{RaPHE}[15N]}$$

$$\text{Exogenous RaPHE} = \text{Total PHE intake} \times \frac{\text{RaPHE}[\text{ring} - 2H_5]}{\text{RaPHE}[15N]}$$

$$\text{PB} = \text{RaPHE}[\text{ring} - 2H_5] - \text{exogenous RaPHE}$$

where SPE-PHE is the splanchnic extraction of PHE and exogenous Ra PHE (µmol × kg ffm⁻¹ × h⁻¹) represents the average systemic appearance of PHE from the drink, and PB is equal to endogenous RaPHE (µmol × kg ffm⁻¹ × h⁻¹). Total PHE intake is the intake of unlabeled PHE and labeled PHE [¹⁵N] with the drink.

$$\text{Hydroxylation PHE} = \frac{\text{RaTYR}[13C_9, 15N]}{\text{AVG cTTR} - \text{TYR}[\text{ring} - 2H_4]} \times \frac{\text{AVG cTTR} - \text{PHE}[\text{ring} - 2H_5]}{\text{PHE}[15N]}$$

$$\text{PS} = \frac{\text{Ra PHE}[15N]}{\text{Hydroxylation PHE}} - \text{hydroxylation PHE}$$

$$\text{Net protein balance} = \text{PS} - \text{PB}$$

where hydroxylation of PHE (µmol × kg ffm⁻¹ × h⁻¹) represents the conversion PHE into TYR by the enzyme PHE hydroxylase. AVG cTTR-TYR [²H₄] is AUC of cTTR-TYR [²H₄] divided by 240 (time in min). AVG cTTR-PHE [²H₅] is AUC of cTTR-PHE [²H₅] divided by 240 (time in min). Net protein gain is present when net protein balance >0 and net protein loss when net protein balance <0.

2.7. Statistical analysis

Results are expressed as means ± standard errors (SEs). We compared clinical characteristics of the study populations using either the unpaired Student’s t-test or Fisher’s exact test, depending on the variable. Whole body insulin resistance was assessed using the computerized homeostasis model assessment of insulin resistance (HOMA-IR) (version 2.2.3) [19]. We used the median value of
data collected at time points $t = -20, -10$ and $0$ min to compare assessments in the postabsorptive state. Postprandial protein kinetics were measured by using the time points $t = 0–240$ min to calculate the area under the curve between 0 and 4 h. AUC was divided by time to obtain an average. As the uptake of food is not complete after 4 h [17], the postprandial calculations are approximations. In the postabsorptive state, PHE hydroxylation was similar to the rate of net protein balance. We imputed missing values with the median for one subject who did not complete the test day to the rate of net protein balance. We imputed missing values with the median for one subject who did not complete the test day with the protein + CHO drink to be able to include this subject in the repeated measurement analysis. For BMI, FEV$_1$ (% of predicted), glucose concentration ($P < 0.0001$) and protein drink effect ($P < 0.0001$) were significant, and there was a tendency toward a higher HOMA index for IR in COPD patients ($P = 0.0516$). The average postprandial plasma glucose concentration increases in response to the four protein drinks were dependent on the protein drink, but not on the group. When comparing the four protein drinks within groups, the glucose concentration increases between the two CHO supplemented drinks were comparable, and higher compared to the two protein drinks without CHO ($P < 0.001$) (Fig. 3A).

The average postprandial plasma insulin concentration increases in response to the four protein drinks were dependent on the protein drink ($P < 0.0001$), but not on the group. When comparing the four protein drinks within groups, the insulin concentration increases were comparable for the two protein drinks to which we added CHO, and higher compared to the two protein drinks without CHO ($P < 0.0001$) (Fig. 3B). The insulin concentration increases were not significantly different between the protein + CHO drinks. Only in COPD patients, the protein + LEU drinks increased the insulin concentration to such an extent that we did not observe a significant difference from the protein + CHO drink. Supplemental Fig. 3 shows the plasma glucose and insulin concentration responses over time in both groups.

3. Results

We enrolled in total 29 participants. While one participant completed only three out of four test days, 20 participants completed the entire study (Supplemental Fig. 1). The COPD patients were significantly different from healthy age matched controls for lung function ($P < 0.0001$) and smoking history ($P < 0.001$) (Supplemental Table 1). On group level, BMI, FEV$_1$ (% of predicted) and protein drink effect ($P = 0.0516$) were significant, and there was a tendency toward a higher HOMA index for IR in COPD patients ($P = 0.0516$). The average postprandial plasma glucose concentration increases in response to the four protein drinks were dependent on the protein drink, but not on the group. When comparing the four protein drinks within groups, the glucose concentration increases were comparable for the two protein drinks to which we added CHO, and higher compared to the two protein drinks without CHO ($P < 0.0001$) (Fig. 3B). The insulin concentration increases were not significantly different between the protein + CHO drinks. Only in COPD patients, the protein + LEU drinks increased the insulin concentration to such an extent that we did not observe a significant difference from the protein + CHO drink. Supplemental Fig. 3 shows the plasma glucose and insulin concentration responses over time in both groups.

3.2. Postprandial plasma glucose and insulin concentration changes

In the postabsorptive state, we found no differences in plasma glucose and insulin levels or HOMA index for IR between the groups (Supplemental Table 1), although there was a tendency toward a higher HOMA index for IR in COPD patients ($P = 0.0516$). The average postprandial plasma glucose concentration increases in response to the four protein drinks were dependent on the protein drink, but not on the group. When comparing the four protein drinks within groups, the glucose concentration increases between the two CHO supplemented drinks were comparable, and higher compared to the two protein drinks without CHO ($P < 0.001$) (Fig. 3A).
essential amino acids [5] are very effective in the acute
4.1. Leucine and insulin
PB, or improve net whole body protein anabolism.
Despite differences in plasma LEU and insulin responses, we found
who are at risk for muscle loss, as compared to healthy older adults.
weight older adults with moderate to severe COPD and dyspnea
without the addition of free leucine and/or carbohydrates in normal
mass. LEU: leucine.

3.3. Whole body protein turnover responses
Data regarding plasma isotope enrichments can be found in
Fig. 4. In the healthy group, a significant time effect was found for L-
[ring-2H4]-PHE, L-[15N]-PHE, L-[U-13C6, 15N]-TYR, L-[ring-2H4]-TYR
(P < 0.0001) and time-by-protein drink interaction for L-[ring-2H4]-
PHE, L-[15N]-PHE, L-[ring-2H4]-TYR (P < 0.0001), L-[U-13C6, 15N]-
TYR (P = 0.07). In the COPD group, a significant time effect was
found for L-[ring-2H4]-PHE, L-[15N]-PHE, L-[U-13C6, 15N]-TYR, L-
[ring-2H3]-TYR (P < 0.0001) and time-by-protein drink interaction
for L-[15N]-PHE (P < 0.0001). No protein drink effects were
observed for any of the measures in the COPD and control groups.
Postabsorptive whole body PS and PB were comparable between
controls and COPD patients (Supplemental Table 2). Also, no sig-
nificant difference in postabsorptive net protein balance was
observed between the groups.

Changes from baseline for PS and PB in response to the four
protein drinks were dependent on the group, with greater increases
for PS (P < 0.0001) and smaller decreases in PB (P = 0.0448) in the
COPD group (Fig. 5A, B). For net protein balance we observed no
differences between the groups (Fig. 5C). Moreover, SPE was lower
in the COPD group (P = 0.0244), without any within group differ-
ences between the four protein drinks (Supplemental Table 3). In
both groups, changes from baseline for PS, PB, and net protein
balance were comparable between all four protein drinks.

4. Discussion
In the present study, we examined whole body protein meta-
bolism in response to a serving of high-quality protein with or
without the addition of free leucine and/or carbohydrates in normal
weight older adults with moderate to severe COPD and dyspnea
who are at risk for muscle loss, as compared to healthy older adults.
Despite differences in plasma LEU and insulin responses, we found
in both groups that co-ingestion of free LEU and/or CHO with a
serving of high-quality protein did not further enhance PS, reduce
PB, or improve net whole body protein anabolism.

4.1. Leucine and insulin
Although high-quality proteins [4], and specifically dietary
essential amino acids [5] are very effective in the acute
stimulation of protein anabolism in COPD patients, the specific
contribution of LEU remains unclear as CHO were always a part of
the protein/amino acid meals. Furthermore, this patient group is
often characterized by alterations in LEU metabolism [5,10,11] and
glucose intolerance [12,13]. Previous findings were based on half
the amount of the same high-quality protein and a similar amount
of CHO as provided in the present study. The current study con-
forms and extends previous findings regarding the absence of an
anabolic effect of LEU co-ingestion to a high-quality protein in
COPD patients and age matched controls [4]. The addition of free
LEU (together with isoleucine and valine) to a low-quality soy
protein, however, was associated with a stimulation of whole
body PS in COPD patients [13], but not in healthy controls. In that
study, the addition of free LEU was also associated with a reduc-
tion in splanchnic amino acid extraction, which we did not observe
in the present study.

Based on previous studies in healthy older adults, reporting
increases in mixed muscle protein and myofibrillar muscle FSR
after LEU co-ingestion [20–22], we expected to find an increase in
whole body PS. Interestingly, the study that reported the highest
increase in FSR by LEU co-ingestion did not observe an increase in
whole body PS [22], suggesting that increases in muscle FSR could
be paired with reductions in PS elsewhere in the body.

LEU and CHO partially work through the same mechanism
to promote increases in protein anabolism, namely by stimulation of
insulin, whereas LEU also directly activates anabolic signaling [6,7].
In the present study, we observed in both groups that CHO co-
ingestion with protein or protein + LEU stimulated the insulin
response. According to the insulinotropic properties of LEU [6,23],
which are at least in part ascribed to various metabolic processes
within the pancreatic β-cell [24], we observed that the leucine dose
induces an insulin response just not above that of the protein and/
cho load.

The higher plasma insulin concentrations associated with the
co-ingestion of CHO did not result in significant increases in
whole body PS or protein anabolism in either group. In line, other
studies have also shown that CHO co-ingestion does not further
stimulate protein synthesis [25–29]. These findings are also in
line with studies showing that plasma insulin concentrations
higher than those obtained via protein or amino acid intake alone
do not stimulate muscle FSR [27,28,30]. Insulin however appears
to primarily act on protein breakdown [9]. An insulin induced
Fig. 4. Mean (±SE) plasma cTTR in healthy controls (n = 11) for L-[ring-2H5]-PHE (A1), L-[15N]-PHE (B1), L-[U-13C9, 15N]-TYR (C1), and L-[ring-2H4]-TYR (D1), before and after intake of 4 different protein drinks. Protein drinks were ingested orally (at time = 0 min) and consisted of 0.6 g/kg ffm hydrolyzed casein protein with, a) no add-ons (protein), b) 0.3 g/kg
reduction in muscle PB was previously observed in young adults for plasma insulin concentrations up to 30 μU/mL. As the protein only intake already resulted in peak insulin concentrations of ~20–25 μU/mL (Supplemental Fig. 5), it is possible that a maximum reduction in whole body PB was already established.

4.2. Healthy versus COPD

Nutritional approaches that provide an optimal stimulation of muscle protein anabolism are of clinical importance even in normal weight COPD patients with a poor appetite or severe dyspnea. These patients are at risk for muscle loss as disturbances in intermediary amino acid and protein metabolism are present independent of their nutritional status [1–3]. Furthermore, minimizing supplement dose by optimizing the quality is clinically important particularly in dyspneic COPD patients as feeding induced dyspnea may impair their food intake. In this study, lung function in the COPD group was severely impaired, all had moderate to severe dyspnea, 7 out of the 10 patients were dependent on supplemental oxygen, and 5 of them had elevated fasting glucose concentrations ≥5.6 mmol/L. For those reasons, we were surprised not to find a lower FFM and appendicular skeletal muscle index on whole group level in the COPD patients compared to the controls. Appendicular skeletal muscle index was below the cut-off for sarcopenia (<7.23 kg × m⁻² for men; <5.67 kg × m⁻² for women) [31] in 6 of the studied COPD patients versus only in one control subject.

Previously we have established that the use of the oral tracer of 15N-Phe and unlabeled tracee (dietary PHE) display the same digestion and absorption characteristics for protein mixtures consisting of hydrolyzed proteins [4]. In COPD patients, we found a tendency towards a greater stimulation of whole body protein anabolism [5]. Furthermore, plasma levels were not elevated in the prandial state for amino acids including LEU [1]. In that study despite lower SPE in COPD, no differences were found in plasma amino acid levels during whey sip feeding between the groups. A feeding reducing effect was not present for endogenous rate of appearance (reflecting protein breakdown) in COPD for LEU like it was present for PHE. Moreover, plasma levels were not elevated in the prandial state for both amino acids suggesting immediate oxidation of LEU or incorporation into protein.

In conclusion, we did not observe any individual or combined anabolic effects on whole body protein metabolism of CHO and LEU when co-ingested with a serving of high-quality protein in patients with moderate to severe COPD and dyspnea or healthy older adults. Whether this is also the case for COPD patients characterized by recent involuntary weight loss or muscle wasting is of interest for future studies.

Author contributions

RJ, ND and ME had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. RJ, ND, and ME designed and conducted the extraction of amino acids by the portal drained viscera, as observed in this study and likewise in our other studies in COPD [1,3–5] is related to higher peripheral essential amino acid availability (i.e. appearance), and subsequently greater protein anabolism [5].

It remains unclear whether the group differences in PHE vs. LEU concentration responses indicates compromised LEU oxidation, as previously disturbances in LEU metabolism and a discrepancy among the individual BCAAs have been observed in COPD [5,10,11], and/or greater PHE to TYR conversion in these patients. Accurate measurement of LEU oxidation might be difficult in COPD due to air trapping and CO₂ retention in the lungs. The reduced splanchnic extraction (SPE) has been a consistent finding in many of our previous studies resulting in a greater net protein balance. The reduced SPE we previously observed was present for PHE but also for many amino acids including LEU [1]. In that study despite lower SPE in COPD, no differences were found in plasma amino acid levels during whey sip feeding between the groups. A feeding reducing effect was not present for endogenous rate of appearance (reflecting protein breakdown) in COPD for LEU like it was present for PHE. Moreover, plasma levels were not elevated in the prandial state for both amino acids suggesting immediate oxidation of LEU or incorporation into protein.
research. RJ, ND, and ME were involved in the data analysis and writing of the manuscript. AS reviewed the manuscript. EV, RH, and AZ were involved in the recruitment of study participants.

Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clinu.2018.08.006.

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