Effectiveness of essential amino acid supplementation in stimulating whole body net protein anabolism is comparable between COPD patients and healthy older adults

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Abstract

Background—The development of effective nutritional strategies in support of muscle growth for patients with chronic obstructive pulmonary disease (COPD) remains challenging. Dietary essential amino acids (EAAs) are the main driver of postprandial net protein anabolism. In agreement, EAA supplements in healthy older adults are more effective than supplements with the composition of complete proteins. In patients with COPD it is still unknown whether complete protein supplements can be substituted with only EAAs, and whether they are as effective as in healthy older adults.

Methods—According to a double-blind randomized crossover design, we examined in 23 patients with moderate to very severe COPD (age: 65 ± 2 y, FEV_1: $40 \pm 2\%$ of predicted) and 19 healthy age-matched subjects (age: 64 ± 2 y), whether a free EAA mixture with a high proportion (40%) of leucine (EAA mixture) stimulated whole body net protein gain more than a similar mixture of balanced free EAAs and non-EAAs as present in whey protein (TAA mixture). Whole body net protein gain and splanchnic extraction of phenylalanine (PHE) were assessed by

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Conflict of interest

continuous IV infusion of L-[ring-^{2}H$_5$]-PHE and L-[ring-^{2}H$_2$]-tyrosine, and enteral intake of L-[^{15}N]-PHE (added to the mixtures).

Results—Besides an excellent positive linear relationship between PHE intake and net protein gain in both groups (r=0.84–0.91, $P<0.001$), net protein gain was 42% higher in healthy controls and 49% higher in COPD patients after intake of the EAA mixture compared to the TAA mixture ($P<0.0001$). These findings could not be attributed to the high LEU content, as in both groups net protein gain per gram EAA intake was lower for the EAA mixture ($P<0.0001$). Net protein gain was higher in COPD patients for both mixtures due to a 40% lower splanchnic extraction ($P<0.0001$), but was similarly related to dietary PHE (i.e. EAA) plasma appearance.

Conclusions—In COPD patients, similarly to healthy older adults, free EAA supplements stimulate whole body protein anabolism more than free amino acid supplements with the composition of complete proteins. Therefore, free EAA supplements may aid in the prevention and treatment of muscle wasting in this patient population.

Trial registry—ClinicalTrials.gov; Nos.: NCT01173354 and NCT01172314; URL: www.clinicaltrials.gov

Keywords

essential amino acid; protein turnover; COPD; leucine

1. Introduction

It has been well established that weight and muscle loss in patients with chronic obstructive pulmonary disease (COPD) are independent disease related factors compromising health status. A BMI of < 25 kg/m^2 is already associated with an increased overall risk of mortality in these patients [1], and severe muscle wasting (indicated by a fat-free mass index below the lowest 10th percentile of the general population) is in 26% of cases masked by a normal BMI [2]. As weight gain [1] and higher physical activity level [3] both reduce the overall risk of mortality in these patients, it remains important to develop more effective nutritional strategies that support muscle growth and improved function. Long-term intervention with essential amino acids (EAAs) may have the potential to do both [4].

Our recent data in COPD patients with muscle wasting [5] showed that the milk proteins whey and casein, both high in EAAs, are very effective at stimulating whole body protein anabolism, similar to our findings in normal-weight COPD patients [6, 7]. Importantly, we found an excellent positive linear relationship between EAA intake per kg fat-free mass and whole body protein anabolism in that study [5]. These results are similar to our findings in healthy older adults and in other disease states [8, 9]. It is however still unknown whether the efficacy of dietary EAAs in relation to protein anabolism is similar between patients with COPD and healthy older adults. Previous research in healthy older adults has shown that EAAs are primarily responsible for the stimulation of net muscle protein gain [10, 11], which strongly suggests that the use of only free EAAs instead amino acid mixtures with the composition of complete proteins might be more effective at preventing muscle loss in COPD patients.

The EAA leucine (LEU) has received particular interest because of its unique role in the stimulation of protein translation [12]. In healthy older adults, a higher proportion of LEU as part of a free EAA mixture was required for optimal stimulation of muscle protein synthesis, positively affecting net muscle protein balance [13]. In COPD patients, we found that a low quality soy protein mixture with added free branched-chain amino acids (leucine, isoleucine and valine) improved whole body protein synthesis [14]. On the contrary, free LEU added to hydrolyzed milk protein mixtures neither enhanced whole body protein synthesis, nor improved net protein balance [5]. From the latter study, it remains unclear whether the already high biological value of milk proteins is reason to believe that additional LEU was unnecessary, or whether the presence of non-EAAs in these proteins negated the positive effect of adding LEU.

Therefore, we tested in stable COPD patients whether (1) a supplement with only EAAs stimulated whole body net protein gain more than a supplement with both EAAs and non-EAAs, (2) whether this response was different from healthy older adults, and (3) whether a high proportion of LEU in a supplement with only EAAs is favorable.

2. Materials and methods

2.1. Subject inclusion

Twenty-three patients with moderate to very severe airflow obstruction (GOLD stage II-IV) [15], and 19 healthy older adults from the Little Rock, AR area were included between 2009 and 2012 (Supplemental Figure 1). Recruitment took place through pulmonologist referral and local advertising efforts. Medical history and medication use were assessed as part of the screening process. All patients had a clinical diagnosis of COPD and did not experience a respiratory tract infection or exacerbation at least 4 weeks prior to the study. All received bronchodilator treatment, except for two patients who did not receive any maintenance therapy. Furthermore, 18 patients were taking inhalation corticosteroids and nine were on long-term oxygen therapy. Use of systemic corticosteroids one month prior to the study was an exclusion criterion, as well as malignancy, recent surgery and severe unstable endocrine, hepatic or renal disorders. Written informed consent was obtained from all participants, and the Institutional Review Board of the University of Arkansas for Medical Sciences approved the study (UAMS, IRB no. 105558).

2.2. Lung function and anthropometric data

Height and weight were measured using standard procedures. Body composition was assessed using Dual-Energy X-ray Absorptiometry (Hologic QDR 4500). Values were standardized for height. Forced expiratory volume in 1 second (FEV_1) was assessed with the highest value from 3 technically acceptable maneuvers being used [16].

2.3. Study design

Participants were studied on two days (within one week, but one day apart) of six hours each, after an overnight fast (Figure 1). After insertion of a catheter into an antecubital vein, a blood sample was taken to measure natural stable isotope enrichment, glucose and insulin levels (t= −180 min). For the measurement of whole body protein kinetics, we subsequently

started a primed, constant intravenous infusion of L-[ring-^{2}H$_5$]-phenylalanine (L-[ring-^{2}H$_5$]-PHE) (prime: 3.6 μmol/kg; IV infusion: 3.6 μmol/kg/h) and L-[ring-^{2}H$_2$]-tyrosine (prime: 1.14 μmol/kg; IV infusion: 1.14 μmol/kg/h), and L-[ring-^{2}H$_4$]-tyrosine was primed orally (0.31 μmol/kg). L-[^{15}N]-PHE was given orally as well, mixed with each of the free amino acid mixtures (the amount in both mixtures was similar, and equal to 20% of the PHE content in the EAA mixture) for the measurement of splanchnic PHE extraction. A second catheter for arterialized venous blood sampling was placed in a superficial dorsal vein of the contralateral hand or lower arm, and the hand was placed in a thermostatically controlled heated box to mimic direct arterial sampling [17]. Whole body protein breakdown, synthesis, net protein gain (protein synthesis - protein breakdown), and splanchnic PHE extraction were calculated from the plasma isotope enrichment during either postabsorptive steady state or postprandial non-steady state conditions, using previously described equations [5] available in the online supplemental material. Blood was processed and analyzed in batch by LC-MS/MS using routine laboratory procedures [5].

2.4. Composition of the free amino acid mixtures

Participants were given two free amino acid mixtures (Table 1) as a single bolus, according to a double-blind randomized crossover design (randomizer.org). The EAA mixture contained a balanced mixture of solely EAAs similar to the composition of whey protein, enriched with additional leucine (40% w/w EAA content) [9]. The total amino acid (TAA) mixture contained a balanced mixture of EAAs and non-EAAs also similar to the composition of whey protein (24% leucine w/w EAA content). As shown in Table 1 the EAA profile (i.e. distribution) of both mixtures was identical, with the exception of LEU (24 vs. 40%). Healthy controls were also in part recruited for another clinical trial [9], and as such received larger mixtures, but with the same composition as the mixtures provided to the COPD group. As we express the data per kg fat-free mass, the net protein gain response of the control group falls mostly within the range of the COPD group. Therefore, we consider it acceptable to compare the responses of healthy subjects who received more amino acids to those of COPD patients who received less amino acids. Maltodextrin was added to both mixtures to stimulate postprandial insulin secretion, and to thereby achieve a greater whole body net protein gain than attainable with amino acids only [18].

2.5. Statistical analysis

Results were expressed as mean ± standard error. Population characteristics and baseline measurements were compared using either the unpaired Student's t-test or Mann-Whitney test, depending on the distribution of the data. Calculations for the postabsorptive phase of the study were done using the median value for measurements taken at t= −30, −15 and 0 min prior to mixture intake. Postprandial phenylalanine kinetics were expressed as the 3 h area under the curve, derived from all postprandial measurements. For the within group comparison of postprandial phenylalanine kinetics we used either the paired Student's t-test or Wilcoxon matched-pairs signed rank test. Computation of Pearson's correlation coefficient and linear regression analysis was used for the comparison of PHE intake, dietary PHE plasma appearance, and change in PHE concentration with net protein gain. Two-way (Repeated) Measures Analysis Of Variance (two-way RM ANOVA) with "group" and "mixture", or "time" and "mixture" as factors were used to compare differences in plasma

amino acid and insulin concentrations, and net protein gain between mixtures and/or groups and/or over time (during the 3 h postprandial period). We applied Bonferroni post hoc testing to evaluate within-time differences between mixtures. The level of significance was set at $P<0.05$, and Graphpad Prism (version 6.07) was used for data analysis.

3. Results

COPD patients had a significantly lower pulmonary function (FEV_1 % of predicted) than healthy older adults (40 ± 2 vs. 95 ± 4, $P<0.0001$), and included n=3 GOLD II patients (albeit $FEV_1 < 60$% of predicted), n=15 GOLD III patients, and n=5 GOLD IV patients. Diffusion capacity (D_{LCO}) from the medical record was available for n=15 COPD patients, of which n=9 had a moderate to severe reduction in diffusing capacity ($D_{LCO} < 60$% of predicted). As previously published [19], gender division, age (64 ± 2 vs. 65 ± 2 y), BMI (27.8 ± 1.1 vs. 26.3 ± 1.0 kg/m^2), and fat-free mass index (17.7 ± 0.6 vs. 17.0 ± 0.5 kg/m^2) were all similar between the controls and COPD patients. Furthermore, COPD patients varied in comorbidities and exacerbation frequency [19]. Fasting glucose (5.7 ± 0.2 vs. 5.2 ± 0.1 mmol/L), insulin (8.0 ± 1.2 vs. 8.7 ± 1.1 μIU/mL), and HOMA-IR (1.16 ± 0.16 vs. 1.22 ± 0.15) were not different between the groups.

3.1. Plasma amino acid and insulin kinetics

Postabsorptive plasma concentration of the three branched-chain amino acids was significantly lower in COPD patients compared to controls (leucine: 71 ± 2 vs. 95 ± 3 μM, isoleucine: 33 ± 1 vs. 44 ± 2 μM, and valine: 141 ± 5 vs. 192 ± 8 μM, $P<0.0001$). As previously described [19], this contributed to an overall lower plasma EAA concentration in COPD patients.

In both groups, postprandial plasma EAA and LEU concentrations (Figure 2) were significantly higher ($P<0.0001$) for the EAA mixture compared to the TAA mixture. Also, the insulin response for the EAA mixture (Figure 3) was significantly higher, albeit small, in both groups (healthy controls: $P=0.0382$, COPD patients: $P=0.0285$).

3.2. Postprandial whole body protein kinetics

Data regarding plasma isotope enrichments can be found in Supplemental Figures 2 and 3. Postabsorptive values for whole body protein turnover did not differ between controls and COPD patients [19]. Figure 4A and Supplemental Figure 4 illustrate that in both groups there was an excellent positive linear relationship between the amount of PHE (r=0.84–0.91, $P<0.001$) and total EAAs (r=0.73–0.81, $P<0.0001$) consumed and net protein gain. Although the relationship between PHE intake and net protein gain differed between the groups, the relationship between the amounts of PHE that appeared from the mixture (dietary PHE plasma appearance) and net protein gain were similar (Figure 4B).

Taking into account the exact size of each mixture (Table 1), whole body net protein gain was 42% and 49% higher after intake of the EAA mixture compared to the TAA mixture in the control group and the COPD group, respectively ($P<0.0001$) (Figure 5A). Per gram of total amino acid intake, COPD patients had a higher net protein gain (i.e. efficiency) than

controls for both mixtures (P<0.0001). Splanchnic extraction was about 40% lower in the COPD group for both mixtures (Table 2 and 3).

In control subjects, the higher net protein gain after intake of the EAA mixture was caused by a greater increase in whole body protein synthesis (P<0.0001) (Table 2). In COPD patients this was explained by a greater increase in protein synthesis (P=0.0027), as well as a larger reduction in protein breakdown (P=0.0043) (Table 3).

Per gram EAA intake, the TAA mixture stimulated net protein gain to a greater extent than the EAA mixture (P<0.0001) (Figure 5B). Per gram *balanced* EAA intake, this difference was smaller, but still significantly lower for the EAA mixture (P<0.0001) (Figure 5C).

4. Discussion

In both healthy older adults and COPD patients, we found that a mixture with only EAAs and additional LEU caused a greater whole body net protein gain than a similar mixture with the amino acid composition of complete proteins. The favorable response to a mixture with EAAs only was supported by an excellent positive linear relationship between PHE (i.e. EAA) intake per kg fat-free mass and net protein gain, but could not be attributed to the high LEU content. COPD patients had an overall higher net protein gain per gram amino acids, associated with a reduced splanchnic extraction. However, based on the dietary PHE plasma appearance net protein gain was comparable to the healthy control group.

4.1. Whole body net protein gain

EAAs are primarily responsible for the stimulation of net muscle protein gain [11]. Although we did not measure specifically muscle protein turnover rates, a greater whole body net protein gain after intake of the EAA mixture is likely to result in a greater net muscle protein gain as well. Whether the extent of net muscle gain differs between healthy older adults and COPD patients due to potential disease related factors remains to be studied. The crucial role of dietary EAAs is further emphasized by the excellent positive linear relationship between EAA intake and whole body net protein gain, in agreement with our previous findings in COPD [5], Cystic Fibrosis [8], and lung cancer patients [9]. Although we used relatively small mixtures in our study, with an EAA content comparable to 7 and 15 g of whey protein, a recent study in healthy older adults showed the same linear relationship for EAA intake levels up to ~90 g [20]. It is yet to be determined whether specifically muscle protein follows the same pattern. However, that protein intake beyond the upper limit for muscle protein synthesis [20–22] in healthy older adults does not limit whole body net protein gain [20], suggests that net muscle protein anabolism above a certain protein intake level is driven by a reduction in muscle protein breakdown. Assuming a 4% contribution of PHE to protein [23], we can convert net protein gain from μmol PHE/kg fat-free mass to g protein/kg fat-free mass. With that, the intake of 1 g of the EAA mixture in comparison to the TAA mixture resulted in approx. 0.25 g greater net protein gain in the control group (0.6 g vs. 0.85 g protein/3h), and 0.43 g greater net protein gain in the COPD group (0.9 g vs.1.3 g protein/3h), for an average person with 50 kg fat-free mass. The higher efficiency in COPD patients as shown in Figure 4A was related to a lower splanchnic extraction.

4.2. Role of leucine

In the current study, the specific anabolic properties ascribed to LEU [12] did not translate into an improved whole body net protein gain in either healthy older adults or COPD patients. One could even argue that the added LEU was slightly counterproductive. Also, in our previous study in COPD [5] we observed that additional LEU when given in combination with hydrolyzed milk proteins did not change whole body protein turnover. From that study we can now conclude that it was not the presence of non-EAA that masked a potentially beneficial effect of LEU. Also, the small differences in digestion and/or absorption kinetics of the hydrolyzed proteins and free LEU were apparently not important. On the contrary, in another group of healthy older adults, it was the additional LEU that was needed in combination with an EAA mixture similar to ours to optimally stimulate skeletal muscle protein anabolism [13]. Thus, LEU may exert its effects specifically on skeletal muscle protein.

This being said, we are skeptical towards the additional use of LEU as a single free amino acid instead of the combined use of all EAAs to stimulate skeletal muscle protein anabolism, especially at greater intake levels. In support, a 21-day study in neonatal pigs showed that the use of more EAAs in the form of milk protein was better than the use of more LEU to achieve an increase in lean mass [24]. Furthermore, the available long-term intervention studies in humans that used LEU supplementation have not shown an increase in muscle mass [25, 26].

After we expressed net protein gain in this study per gram *balanced* EAAs (thereby correcting for the high LEU content), the EAA mixture still had a lower efficacy per gram EAAs. We first considered the possibility of a leucine-induced branched-chain amino acid antagonism where plasma valine and isoleucine concentrations fall below baseline values [27] and limit net protein gain. However, our data were unsupportive (Supplemental Figure 5), and thus the amounts of valine and isoleucine in the EAA mixture were sufficient to prevent a branched-chain amino acid antagonism. Possibly, there was a small anabolic effect of the non-EAAs that made up the difference in net protein gain per gram *balanced* EAAs between the EAA and TAA mixtures.

4.3. Splanchnic extraction of phenylalanine

We found a reduced splanchnic extraction of PHE in COPD patients. This is in line with our previous findings for PHE and other amino acids in this condition [6, 7], and similarly related to a higher net protein gain [6, 7]. The effect of the splanchnic area on protein turnover in COPD patients is intriguing, as weight loss and muscle atrophy are common phenomenon in COPD [2]. Possibly, there are more or higher catabolic events in between meals that negate the more positive protein balance following meals.

The lower splanchnic extraction of amino acids in COPD could be related to changes in the gut and/or liver. However, less extraction by the liver seems unlikely, as the inflammatory process in COPD stimulates the production of acute phase proteins [28, 29]. Use of inhaled corticosteroids, which was 78% in our COPD group, can significantly reduce the acute

phase response [30], and may explain why we did not see an increase in the concentration of C-reactive protein in our study.

For several reasons we are inclined to think that the gut plays a crucial role in the altered splanchnic extraction in COPD patients. It was recently discovered that COPD patients are characterized by an increased permeability of the small intestine and colon [31], similar to heart failure patients [32]. Furthermore, dietary intake induced hypoxia [33] with episodes of intestinal oxygen deprivation, and "inflammatory organ cross-talk" [34] are potential mechanisms that could induce COPD related intestinal inflammation and damage. It is, however, still unknown whether this also indicates changes in amino acid absorption capacity. We could not find a difference in splanchnic extraction between COPD patients with or without supplemental oxygen (data not shown). Another factor that supports involvement of the gut, is the presence of metabolic acidosis in COPD due to the development of stable hypercapnia [35]. Metabolic acidosis is a process known to reduce protein turnover in the splanchnic area [36]. Finally, all COPD patients except for three in this study used inhalation beta-agonists as a maintenance medication. Use of beta-agonists is known to reduce gut mass in favor of increased muscle anabolism [37–39], and in that sense may even provide COPD patients some degree of protection from muscle atrophy. It is clear that more COPD research is needed aimed at the intrinsic factors that contribute to alterations in splanchnic amino acid extraction and an enhanced net anabolic response to feeding.

4.4. Study limitations

The measurement of blood gasses could potentially have given us more insight in the relation between hypoxia, metabolic acidosis and splanchnic extraction. Secondly, the comparison of data regarding whole body protein kinetics between the healthy controls and COPD patients would have been facilitated by the use of mixtures similar in size. The greater load of dietary amino acids in controls resulted in a less complete absorption of dietary PHE after 3 h (Supplemental Figure 2D and 3D), and therefore to some extent this contributed to a higher value for splanchnic extraction in this group. However, as our previous studies have also shown that splanchnic extraction of amino acids is lower in COPD patients [6, 7], we expect that our current findings are not primarily due to methodological issues. Based on our comparable studies in cystic fibrosis [8] and lung cancer [9], the number of subjects included in this study was greater than the number required. However, with a group of 23 COPD patients we were able to perform within group comparisons for other previously published primary outcomes [19].

In conclusion, our data indicate that a more optimal stimulation of whole body protein anabolism in COPD patients can be achieved by supplements that contain a balanced mixture of EAAs only, instead of both EAAs and non-EAAs. As supplements often become replacements due to their effect on satiety, supplements with only EAA may be a more energy efficient means to promote muscle gain in COPD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

List of abbreviations

COPD   chronic obstructive pulmonary disease

EAA   essential amino acid

PHE   phenylalanine

TAA   total amino acid

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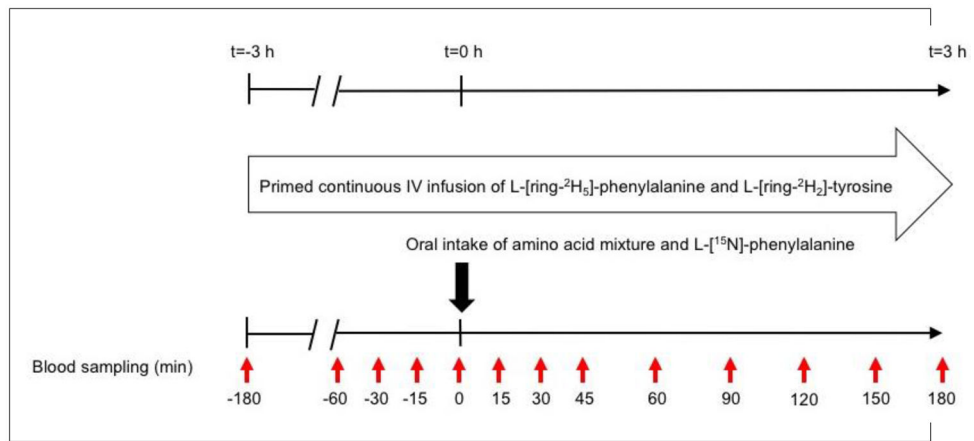
Figure 1.
Study design. All participants were studied twice within one week (one day apart) during a 6-hour infusion protocol, and received two different free amino acid mixtures according to a randomized crossover design.
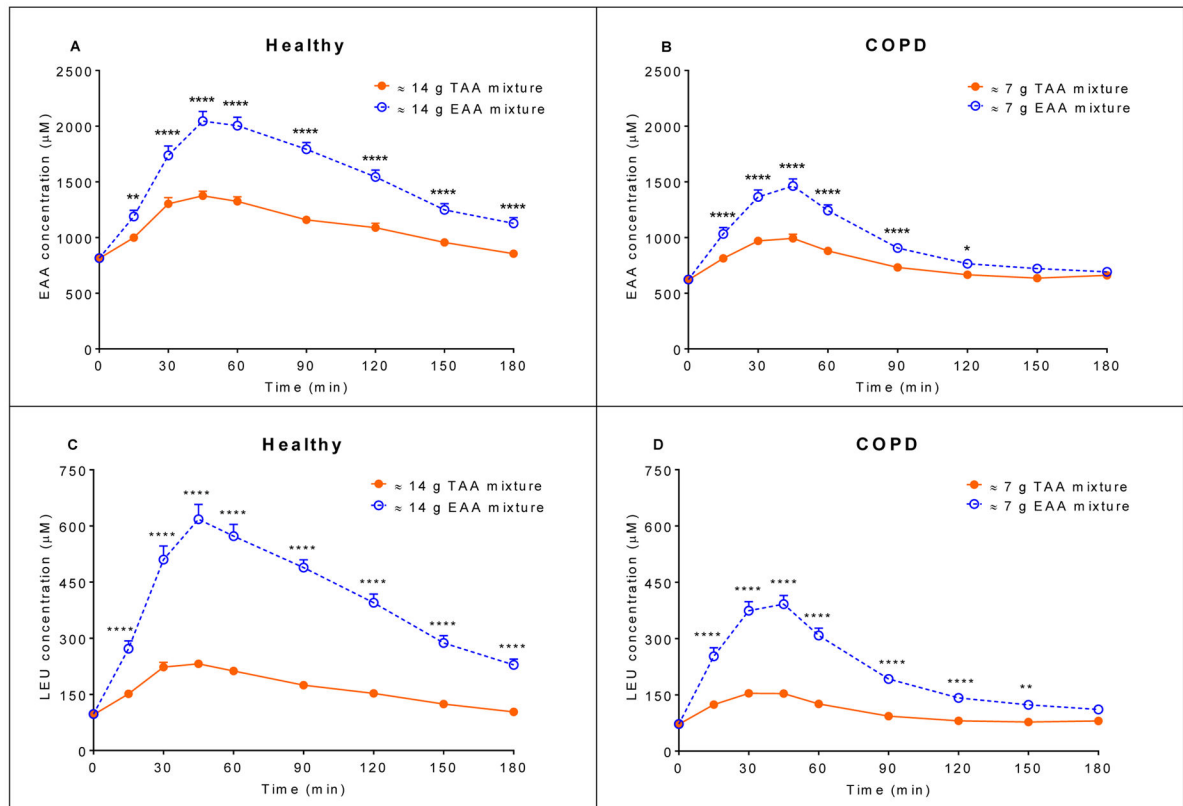
Figure 2.
Mean (± SE) (**A**) plasma EAA concentration after intake of the 14 g TAA and EAA mixtures in healthy controls (n=19) (**B**) plasma EAA concentration after intake of the 7 g TAA and EAA mixtures in COPD patients (n=23) (**C**) plasma LEU concentration after intake of the 14 g TAA and EAA mixtures in healthy controls (n=19) (**D**) plasma LEU concentration after intake of the 7 g TAA and EAA mixtures in COPD patients (n=23). Statistics were done using two-way repeated measures analysis of variance with "time" and "mixture" as factors used to compare differences between mixtures and over time. In both groups, and for the EAA as well as the LEU concentration: time effect, $P<0.0001$, mixture effect, $P<0.0001$, and time x mixture interaction, $P<0.0001$. EAA mixture significantly different from TAA mixture, $^{****}P<0.0001$, $^{**}P<0.01$, $^{*}P<0.05$. TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA). EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU). EAA: essential amino acid. LEU: leucine.
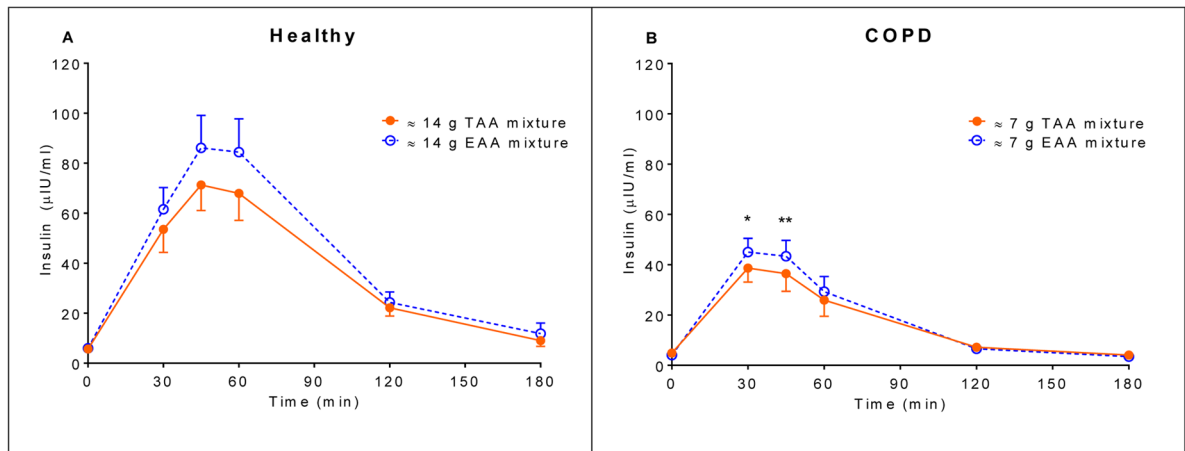
Figure 3.
Mean (± SE) (**A**) plasma insulin concentration after intake of the 14 g TAA and EAA mixtures in healthy controls (n=19) (**B**) plasma insulin concentration after intake of the 7 g TAA and EAA mixtures in COPD patients (n=23). Statistics were done using two-way repeated measures analysis of variance with "time" and "mixture" as factors used to compare differences between mixtures and over time. Healthy controls: time effect, $P<0.0001$, mixture effect, $P=0.0382$, and no time x mixture interaction. COPD patients: time effect, $P<0.0001$, mixture effect, $P=0.0290$, and time x mixture interaction, $P=0.0167$. EAA mixture significantly different from TAA mixture, **$P<0.01$, *$P<0.05$. TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA). EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU).
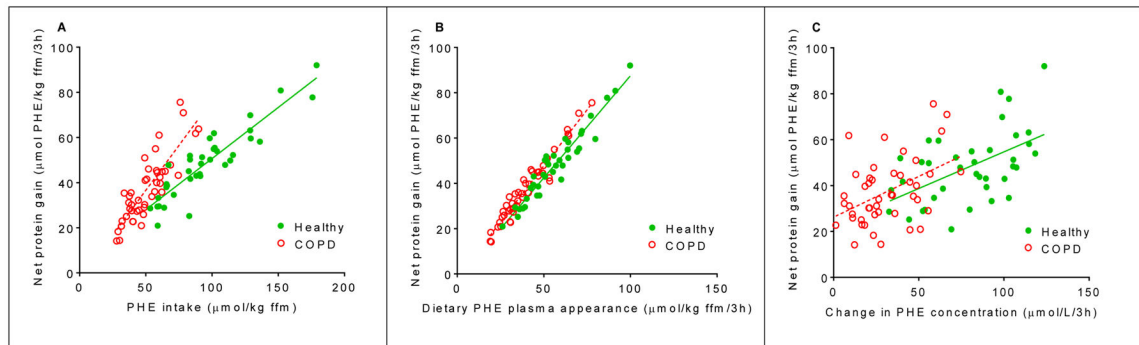
Figure 4.
Correlation after intake of the TAA and EAA mixtures between whole body net protein gain and (**A**) PHE intake, in healthy controls (n=19) and COPD patients (n=23). Healthy: net protein gain = 0.45 × + 5.42, r=0.91, P<0.0001. COPD: net protein gain = 0.79 × EAA intake – 3.1, r=0.85, P<0.0001. Slopes are significantly different between groups, P<0.0001. (**B**) Dietary PHE plasma appearance after splanchnic extraction, in healthy controls (n=19) and COPD patients (n=23). Healthy: net protein gain = 0.90 × EAA intake – 2.58, r=0.96, P<0.0001. COPD: net protein gain = 0.99 × EAA intake – 1.81, r=0.97, P<0.0001. Slopes are similar between groups. (**C**) Change in PHE concentration, in controls (n=19) and COPD patients (n=23). Healthy: net protein gain = 0.32 × EAA intake + 22.76, r=0.53, P=0.0006. COPD: net protein gain = 0.36 × EAA intake + 24.93, r=0.45, P=0.0007. Slopes are similar between groups. Statistics were done using linear regression analysis and computation of Pearson's correlation coefficient. Net protein gain, dietary PHE plasma appearance and change in PHE concentration are expressed as the 3h postprandial area under the curve. TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA). EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU). PHE: phenylalanine. Net protein gain = protein synthesis – protein breakdown.
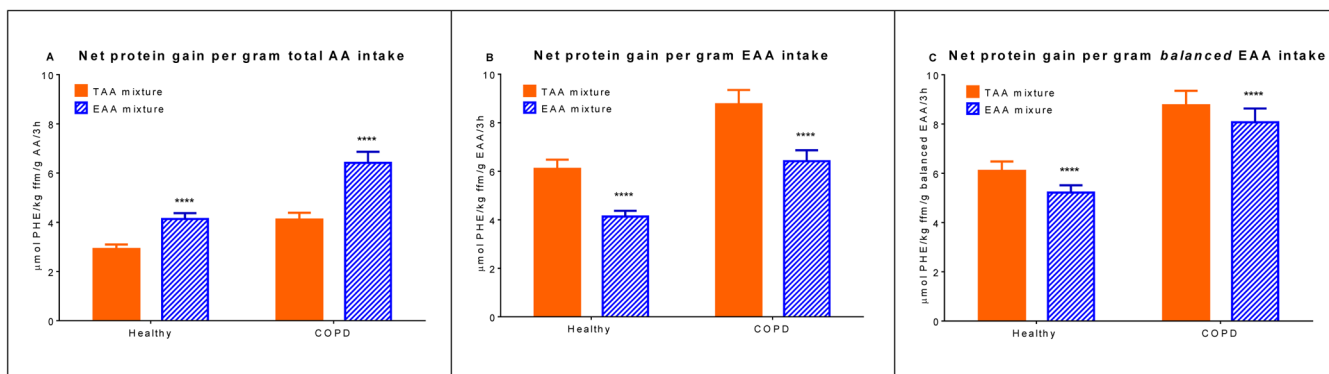
Figure 5.

Mean (± SE) whole body net protein gain after intake of the TAA and EAA mixtures in healthy controls (n=19) and COPD patients (n=23) (**A**) per gram total AA intake (**B**) per gram EAA intake (**C**) per gram *balanced* EAA intake (=EAA intake minus additional LEU). Statistics were done using two-way measures analysis of variance with "group" and "mixture" as factors used to compare differences between groups and mixtures. Net protein gain per gram total AA intake: group effect, $P<0.0001$, mixture effect, $P<0.0001$, and no group x mixture interaction. Net protein gain per gram EAA intake: group effect, $P=0.0005$, mixture effect, $P<0.0001$, and no group x mixture interaction. Net protein gain per gram *balanced* EAA intake: group effect, $P=0.0004$, mixture effect, $P<0.0001$, and no group x mixture interaction. Net protein gain is expressed as the 3h postprandial area under the curve. EAA mixture significantly different from TAA mixture, ****$P<0.0001$. TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA). EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU). AA: amino acid. EAA: essential amino acid. PHE: phenylalanine. Net protein gain = protein synthesis – protein breakdown.

Table 1

Composition of the TAA and EAA mixture per study group[1]

	Healthy		COPD	
	TAA mixture[2]	EAA mixture[3]	TAA mixture	EAA mixture
Histidine	0.256 (4)	0.386 (4)	0.128 (4)	0.193 (4)
Isoleucine	0.714(11)	1.252 (11)	0.357 (11)	0.626 (11)
Leucine	1.562 (24)	2.690 (24)	0.781 (24)	1.345 (24)
Lysine	1.582 (25)	2.674 (24)	0.791 (25)	1.337 (24)
Methionine	0.358 (6)	0.654 (6)	0.179 (6)	0.327 (6)
Phenylalanine	0.458 (7)	0.794 (7)	0.229 (7)	0.397 (7)
Threonine	0.830 (13)	1.428 (13)	0.415 (13)	0.714 (13)
Valine	0.652 (10)	1.140 (10)	0.326 (10)	0.570 (10)
Sum balanced EAA	6.412 (100)	11.018 (100)	3.206 (100)	5.509 (100)
Extra leucine	0.000	2.886	0.000	1.443
Sum leucine	1.562	5.576	0.781	2.788
Sum BCAA[4]	2.928	7.968	1.464	3.989
Sum EAA	6.412	13.904	3.206	6.952
Sum NEAA[5]	7.000	0.000	3.487	0.000
Sum amino acids	13.412	13.904	6.693	6.952
Maltodextrin	30.000	30.000	15.000	15.000

[1] Values are in gram (% of balanced EAA content).

[2] TAA: total amino acid;

[3] EAA: essential amino acid;

[4] BCAA: branched-chain amino acid (includes isoleucine, leucine and valine);

[5] NEAA: non-essential amino acid (includes alanine, arginine, aspartic acid, glutamic acid, glycine, proline, serine, and tyrosine). The amino acid mixtures were prepared, packaged, coded and analyzed by Ajinomoto Co., Inc.

Table 2

Whole body postprandial phenylalanine kinetics in healthy older adults[1]

	TAA mixture[2]	EAA mixture[3]
Protein breakdown	154 ± 5	158 ± 5
Protein synthesis	193 ± 6	215 ± 6 ****
Hydroxylation	19 ± 1	22 ± 1 ***
Net protein gain	39 ± 2	57 ± 3 ****
Splanchnic extraction	39 ± 2	41 ± 2

[1] Values are mean ± SE. Values are expressed as the 3h postprandial area under the curve in µmol PHE/kg fat-free mass, except for splanchnic extraction which is expressed as a percentage.

[2] TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA);

[3] EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU). Protein breakdown = endogenous phenylalanine rate of appearance. Protein synthesis = phenylalanine rate of appearance – phenylalanine hydroxylation. Net protein gain = protein synthesis – protein breakdown. Statistics were performed using either the paired Student's t-test (if data were distributed normally) or Wilcoxon matched-pairs signed rank test. Significant difference between TAA and EAA mixture,

*** $P<0.001$,

**** $P<0.0001$.

Table 3

Whole body postprandial phenylalanine kinetics in COPD patients[1]

	TAA mixture[2]	EAA mixture[3]
Protein breakdown	171 ± 6	164 ± 5 **
Protein synthesis	200 ± 7	209 ± 7 **
Hydroxylation	16 ± 1	17 ± 1 **
Net protein gain	29 ± 2	45 ± 3 ****
Splanchnic extraction	23 ± 3	22 ± 3

[1] Values are mean ± SE. Values are expressed as the 3h postprandial area under the curve in μmol PHE/kg fat-free mass, except for splanchnic extraction which is expressed as a percentage.

[2] TAA mixture: total amino acid mixture (balanced mixture of EAA and non-EAA);

[3] EAA mixture: essential amino acid mixture (balanced mixture of EAA and additional LEU). Protein breakdown = endogenous phenylalanine rate of appearance. Protein synthesis = phenylalanine rate of appearance – phenylalanine hydroxylation. Net protein gain = protein synthesis – protein breakdown; Statistics were performed using either the paired Student's t-test (if data were distributed normally) or Wilcoxon matched-pairs signed rank test. Significant difference between TAA and EAA mixture,

** $P < 0.01$,

**** $P < 0.0001$.