Supplementation of soy protein with branched-chain amino acids alters protein metabolism in healthy elderly and even more in patients with chronic obstructive pulmonary disease\textsuperscript{1–3}

Mariëlle PKJ Engelen, Erica PA Ruten, Carmen LN De Castro, Emiel FM Wouters, Annemie MWJ Schols, and Nicolaas EP Deutz

ABSTRACT
Background: It is often suggested that chronic wasting diseases [eg, chronic obstructive pulmonary disease (COPD)] may benefit from branched-chain amino acid (BCAA) administration via improved protein metabolism.

Objective: The aim was to examine whether adding BCAAs to a soy protein meal would enhance protein anabolism in COPD patients and in healthy elderly persons.

Design: Eight normal-weight COPD patients and 8 healthy control subjects were examined on 2 test days. Simultaneous continuous intravenous infusion of L-[ring-\textsuperscript{2H\textsubscript{5}}]phenylalanine (Phe) and L-[ring-\textsuperscript{2H\textsubscript{5}}]tyrosine tracers was done postabsorptively and at 2 h of ingestion of a maltodextrin soy or maltodextrin soy + BCAA protein meal (rate of ingestion: 0.02 g protein/kg body weight \textsuperscript{-1}·20 min \textsuperscript{-1}) in a crossover design. Together with the meal, oral ingestion of L-[\textsuperscript{13}C\textsubscript{1}]Phe was performed to measure first-pass Phe splanchnic extraction (SPE\textsubscript{Phe}). The endogenous rate of Phe appearance [reflecting whole-body protein breakdown (WbPB)], whole-body protein synthesis (WbPS), and net WbPS (WbPS − WbPB) were calculated. Arterialized venous blood was sampled for amino acid enrichment and concentration analyses.

Results: Soy feeding induced a reduction in WbPB and an increase in WbPS. BCAA supplementation of soy protein resulted in a significantly higher (\(P < 0.05\)) increase in WbPS than did soy protein alone in COPD patients but not in the healthy elderly. BCAA supplementation did not significantly alter the change in WbPB or net WbPS. Furthermore, BCAA supplementation decreased (absolute) SPE\textsubscript{Phe} (\(P < 0.05\)) but did not change the percentage Phe hydroxylation in the splanchnic area, which indicates a BCAA-related reduction in splanchnic protein synthesis.

Conclusion: BCAA supplementation to soy protein enhances WbPS in patients with COPD and alters interorgan protein metabolism in favor of the peripheral (muscle) compartment in healthy elderly and even more in COPD patients.

KEY WORDS Chronic obstructive pulmonary disease, protein feeding, branched-chain amino acid supplementation, whole-body protein turnover, interorgan protein metabolism

INTRODUCTION

Branched-chain amino acids (BCAAs) are essential amino acids that serve as essential substrates and important regulators in the synthesis of body proteins (1). In the past decade, there has been an increased interest in BCAAs as a therapeutic modality to conserve muscle mass via improvements in total body nitrogen metabolism. It is often suggested that chronic wasting diseases, eg, chronic obstructive pulmonary disease (COPD), may benefit from BCAA administration. COPD is increasingly recognized as a chronic metabolic disorder. In addition to changes in basal protein turnover (2–4), alterations in muscle and plasma amino acid profiles have been detected in COPD patients (5–7). Plasma BCAA concentrations were often reduced in COPD, mostly because of a decrease in leucine (Leu) (8). Furthermore, the low plasma Leu concentrations were associated with low values for fat-free mass (6). It is unknown whether BCAA administration in COPD may be of benefit to improve protein metabolism and consequently prevent muscle wasting in these patients.

Consistent evidence is available that intake of casein protein, known for its high intrinsic concentrations of BCAAs, has a larger anabolic effect in healthy subjects than does intake of soy protein (9, 10). We observed that intake of casein protein is not only highly anabolic in the healthy elderly, but also in normal-weight COPD patients (4). Whether the high anabolic effect of casein is related to its large BCAA content or the type of protein per se is unknown.

Until now, most studies of the metabolic effects of free BCAA ingestion were performed in healthy subjects, examining intake of one of the BCAAs (predominantly Leu) without adding the
other 2 BCAAs, valine (Val) and isoleucine (Ile) (11–14). However, selective Leu administration reduces plasma Val and Ile concentrations (11, 14–16), which is related to an enhanced splanchnic bed uptake (11). This so-called BCAA antagonism was also associated with lower plasma phenylalanine (Phe) and tyrosine (Tyr) concentrations, suggesting that all 3 BCAAs are required for obtaining protein anabolism. There is also evidence that BCAA-enriched formulas or BCAA-supplemented diets are preferred to pure BCAA formulas to obtain maximal protein anabolism, because pure BCAA infusions are able to reduce protein breakdown but do not stimulate protein synthesis, possibly due to the absence of nonessential and essential (other than those provided by the BCAAs) amino acids (17, 18). This suggests that the high anabolic effects of casein protein (9, 10, 16) is related to the high and balanced BCAA concentrations and the presence of nonessential amino acids.

Until now, the exact role of BCAAs in relation to the anabolic capacity of dietary protein in health and disease was uncertain. The purpose of the present study was to examine whether adding BCAAs to a protein meal influences the response in protein metabolism differently in normal-weight patients with COPD compared with the healthy elderly. To prove if coinigestion of the 3 BCAAs in a protein meal improves the anabolic response of a meal, a soy protein meal was studied, because soy protein has a low concentration of BCAAs and the anabolic capacity of soy is inferior to that of other proteins such as casein (9, 10).

**SUBJECTS AND METHODS**

**Subjects**

A group of 8 patients with moderate airflow obstruction [\(\bar{x} \pm SD\) forced expiratory volume in 1 s; 50 ± 4% of predicted value] and 8 healthy, age-matched volunteers (controls) were studied (Table 1). All patients and controls were men. The patients were in clinically stable condition and had moderate COPD stage 2 + 3 according to the established Global Initiative for Chronic Obstructive Lung Disease guidelines (19). The patients were outpatients who attended the hospital for routine control with a chest physician every 6 or 12 mo. Exclusion criteria were malignancy, cardiac failure, recent surgery, and severe endocrine, hepatic, or renal disorder. Also, subjects who were using systemic corticosteroids within 3 mo before the beginning of the study were excluded. The number of present smokers in the COPD and control groups each was 2. The number of former smokers in the COPD and control groups was 5 (average time since smoking cessation: 10.2 y) and 2 (average time since smoking cessation: 12.5 y), respectively. The maintenance treatment of the studied COPD patients consisted of inhaled \(\beta_2\)-agonists, inhaled anticholinergics, inhaled corticosteroids, oral theophylline, or a combination of these. Written informed consent was obtained from all subjects, and the study was approved by the medical ethics committee of the University Hospital Maastricht.

**Study protocol**

The protocol started at 0715 after an overnight fast beginning at 0000. All subjects were in a supine position for 3.5 h. After insertion of a catheter in the right antecubital vein, the first blood sample was taken for baseline measurements. Immediately after, all tracers were primed intravenously or orally, and a constant continuous tracer infusion (80 mL/h) was started by using calibrated pumps (IVAC Corporation, San Diego, CA) until the end of the experiment. Primed and constant infusion of the stable isotopes \(^{1-}\text{ring-}^{2}\text{H}_2\text{Phe}[\text{prime: } 2.19 \mu\text{mol/kg body weight (bw)}; \text{infusion: } 0.066 \mu\text{mol} \cdot \text{kg fat-free mass (FFM)}^{-1} \cdot \text{min}^{-1} \cdot \text{min}^{-1}]\) and \(^{1-}\text{ring-}^{2}\text{H}_2\text{Tyr}[\text{prime: } 0.95 \mu\text{mol/kg bw}; \text{infusion: } 0.022 \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1} \cdot \text{h}^{-1}]\) were given via the antecubital vein catheter. Primed infusion of \(^{1-}\text{ring-}^{2}\text{H}_2\text{Val}[\text{prime: } 30.31 \mu\text{mol/kg bw}]\) was additionally given through the same catheter. \(^{1-}\text{ring-}^{13}\text{C}_2\text{Phe}[\text{prime: } 0.88 \mu\text{mol/kg bw}; \text{infusion: } 0.066 \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1} \cdot \text{min}^{-1}]\) was given orally (75 mL/h) every 20 min during the first 1.5 h in the postabsorptive state and together with the liquid meal during feeding. The stable isotopes were purchased from Cambridge Isotopic Laboratories (Woburn, MA).

For sampling arterialized blood, a venous catheter was placed in a dorsal vein of the left hand and the heated box technique (20), a technique to mimic direct arterial sampling, was used. After 1.5 h of stable isotope infusion to reach steady state enrichments, enteral nutrition was started by sip feeding every 20 min for a total duration of 2 h. The test meals involved a liquid soy-based protein meal with and without adding individual BCAAs to it. In this way, the test meal contained an identical composition of the individual BCAAs as was present in casein protein, which is known for its high concentration of BCAAs. Arterialized venous blood samples were taken at 80, 85, 90, 200, 205, and 210 min after the start of infusion. Body composition was measured with the use of Bioelectrical Impedance Spectroscopy (BIS Xitron 4000B; Xitron Technologies, San Diego, CA) to express protein metabolism data per kg FFM. The FFM of the COPD patients was calculated by using a patient’s specific regression equation (21), whereas the FFM of the healthy controls was calculated by using a specific equation for elderly men as described by Dey et al (22).

**Enteral protein meals**

To avoid metabolic changes due to recent modifications of the diet, the subjects were instructed to eat their usual diet for \(\geq 3\) d before the study. On the experimental day, the test meal was

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**TABLE 1**

Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Control group ((n = 8))</th>
<th>COPD group ((n = 8))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>63.1 ± 3.0</td>
<td>68.1 ± 3.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 ± 0.02</td>
<td>1.74 ± 0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.5 ± 3.7</td>
<td>81.8 ± 3.5</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.4 ± 0.9</td>
<td>27.2 ± 0.8</td>
</tr>
<tr>
<td>FFMI (kg/m(^2))^2</td>
<td>19.4 ± 0.9</td>
<td>17.7 ± 0.4</td>
</tr>
<tr>
<td>FEV(_1) (% of predicted)</td>
<td>110 ± 5</td>
<td>50 ± 4(^a)</td>
</tr>
<tr>
<td>DLco (% of predicted)</td>
<td>104 ± 9</td>
<td>78 ± 7(^b)</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>116 ± 5</td>
<td>93 ± 8(^a)</td>
</tr>
<tr>
<td>ITGV (% of predicted)</td>
<td>111 ± 8</td>
<td>146 ± 11(^b)</td>
</tr>
<tr>
<td>TLC (% of predicted)</td>
<td>106 ± 5</td>
<td>115 ± 6</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm \text{SEM}\). COPD, chronic obstructive pulmonary disease; FFMI, fat-free mass index (ie, fat-free mass divided by height); FEV\(_1\), forced expiratory volume in 1 s; DLco, diffusing capacity for carbon monoxide; FVC, forced vital capacity; ITGV, intrathoracic gas volume; TLC, total lung capacity.

\(^2\) A tendency toward a significant group effect was observed, \(P = 0.08\).

\(^a\) Significantly different from the control group (unpaired Student’s \(t\) test), \(P < 0.01\); \(^b\) \(P < 0.05\).
The enrichments [tracer-to-tracee ratios (TTRs)] of the amino acids Phe and Tyr in arterialized-venous plasma were analyzed by a liquid chromatography–mass spectrometry system (LC-MS; Thermoquest LCQ, Veenendaal, The Netherlands) (23). Plasma concentrations of amino acids were measured with the use of a fully automated HPLC (Pharmacia, Woerden, The Netherlands) after precolumn derivatization with 9-fluorenylmethylchloroformate (24).

Plasma glucose, lactate, urea, and ammonia were analyzed spectrophotometrically on a COBAS Mira S (Roche Diagnostica, Hoffman-La Roche, Basel, Switzerland) by standard enzymatic methods (25). The plasma insulin concentration was analyzed with a commercially available electrochemiluminescence immunoassay (Hitachi Modular Analyzer; Roche, Mannheim, Germany).

### Calculations

The sum of amino acids represents the sum of measurable α-amino acids (glutamine, glycine, threonine, histidine, citrulline, alanine, taurine, arginine, α-amino butyric acid, Tyr, Val, methionine, Ile, Phe, tryptophan, Leu, ornithine, and lysine), and BCAA represents the sum of the 3 branched-chain amino acids Val, Leu, and Ile.

All metabolic data were determined under steady-state conditions. The TRR of Phe and Tyr reached an isotopic steady state within 1.5 h of infusion in the postabsorptive state and within 2 h of feeding (data not shown) in both groups.

1. In the postabsorptive state and 2 h after the start of enteral intake of the soy or soy + BCAA meals, whole-body protein synthesis (WbPS) is calculated by subtracting hydroxylation of Phe to Tyr from the whole-body rate of disappearance [which equals the whole-body rate of appearance (Ra) under steady-state] of Phe (which equals the infusion rate/TTR Phe with a mass of +5 in plasma) (3).

Splanchnic extraction (SPEPhe) represents the fraction (in %) of ingested Phe taken up by the gut and liver during its first pass and metabolized via oxidation or protein synthesis. SPEPhe is calculated as (26, 27)

$$\text{SPEPhe} = \left[ 1 - \left( {Ra_{\text{H}_{2}}\text{Phe}}/{Ra_{\text{H}_{3}}\text{Phe}} - \text{Phe} \right) \right] \times 100\%$$

(1)

where $Ra_{\text{H}_{2}}\text{Phe}$ and $Ra_{\text{H}_{3}}\text{Phe}$ represent whole-body Ras of Phe calculated from the intravenous $^{2}$H$_{5}$Phe and intragastric $^{13}$C-Phe isotopes, respectively. The absolute splanchnic extraction (ASPE) of Phe from the meal (in nmol·kg FFM$^{-1}$·min$^{-1}$) can be calculated by multiplying SPEPhe by the infusion rate of Phe with the meal.

### Table 2

Total intake of phenylalanine and the 3 branched-chain amino acids (BCAAs) via the soy and soy + BCAA meal in the chronic obstructive pulmonary disease (COPD) and control groups

<table>
<thead>
<tr>
<th>Control group</th>
<th>Soy</th>
<th>Soy + BCAA</th>
<th>COPD group</th>
<th>Soy</th>
<th>Soy + BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td></td>
<td></td>
<td>Soy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmol·kg FFM$^{-1}$·min$^{-1}$</td>
<td>284 ± 14</td>
<td>282 ± 14</td>
<td>333 ± 9</td>
<td>335 ± 9</td>
<td></td>
</tr>
<tr>
<td>Leucine$^{2,4}$</td>
<td>538 ± 27</td>
<td>857 ± 43</td>
<td>632 ± 17</td>
<td>1018 ± 29</td>
<td></td>
</tr>
<tr>
<td>Isoleucine$^{2,4}$</td>
<td>322 ± 16</td>
<td>553 ± 28</td>
<td>379 ± 10</td>
<td>657 ± 18</td>
<td></td>
</tr>
<tr>
<td>Valine$^{2,4}$</td>
<td>379 ± 19</td>
<td>715 ± 36</td>
<td>445 ± 12</td>
<td>850 ± 24</td>
<td></td>
</tr>
</tbody>
</table>

All values are $\bar{x} \pm$ SEM. Two-factor repeated-measures ANOVA was used to test group and protein effect.

1 A significant protein-by-group interaction was observed, $P < 0.01$.

2 A significant group effect was observed, $P < 0.01$.

3 A significant group effect was observed, $P < 0.001$.

4 A significant protein-by-group interaction was observed, $P < 0.01$.

Prepared to contain soy or the identical amount of soy to which the BCAAs Leu, Val, and Ile were added to equal the amount found in casein, which is generally known to have a high amount of BCAAs (Leu: 9.33 g/100g protein; Ile: 6.06 g/100g protein; Val: 6.95 g/100g protein). The meals were ingested orally at 4 °C until use.

The enrichments [tracer-to-tracee ratios (TTRs)] of the amino acids Phe and Tyr in arterialized-venous plasma were analyzed by a liquid chromatography–mass spectrometry system (LC-MS; Thermoquest LCQ, Veenendaal, The Netherlands) (23). Plasma concentrations of amino acids were measured with the use of a fully automated HPLC (Pharmacia, Woerden, The Netherlands) after precolumn derivatization with 9-fluorenylmethylchloroformate (24).

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### Calculations

The sum of amino acids represents the sum of measurable α-amino acids (glutamine, glycine, threonine, histidine, citrulline, alanine, taurine, arginine, α-amino butyric acid, Tyr, Val, methionine, Ile, Phe, tryptophan, Leu, ornithine, and lysine), and BCAA represents the sum of the 3 branched-chain amino acids Val, Leu, and Ile.

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Splanchnic extraction (SPEPhe) represents the fraction (in %) of ingested Phe taken up by the gut and liver during its first pass and metabolized via oxidation or protein synthesis. SPEPhe is calculated as (26, 27)

$$\text{SPEPhe} = \left[ 1 - \left( {Ra_{\text{H}_{2}}\text{Phe}}/{Ra_{\text{H}_{3}}\text{Phe}} - \text{Phe} \right) \right] \times 100\%$$

(1)

where $Ra_{\text{H}_{2}}\text{Phe}$ and $Ra_{\text{H}_{3}}\text{Phe}$ represent whole-body Ras of Phe calculated from the intravenous $^{2}$H$_{5}$Phe and intragastric $^{13}$C-Phe isotopes, respectively. The absolute splanchnic extraction (ASPE) of Phe from the meal (in nmol·kg FFM$^{-1}$·min$^{-1}$) can be calculated by multiplying SPEPhe by the infusion rate of Phe with the meal.
Whole-body Ra of Phe, not coming from Phe in protein given by the diet [ie, endogenous Phe (Ra Phe endo)], is calculated by subtracting the corrected Phe intake from the whole-body Phe Ra, as represented in the following formulas:

Corrected Phe intake = dietary Phe intake ×
\[1 - (\text{SPE Phe} \times 0.01)\] = Ra Phe feeding \(\text{(2)}\)

Ra Phe endo = Ra Phe feeding - corrected dietary Phe intake \(\text{(3)}\)

Whole-body protein breakdown (WbPB) = Ra Phe endo \(\text{(4)}\)

net WbPS = WbPB - WbPB \(\text{(5)}\)

**Statistical analysis**

Results are expressed as means ± SEs. The mean value of the measures of protein kinetics and the concentrations of amino acids at the time points 80, 85, and 90 min was used as the postabsorptive state, and that calculated from 200, 205, and 210 min as the fed state. If data failed the normality or equal variance test, they were log-transformed where appropriate. The unpaired Student’s \(t\) test was used to determine differences in general characteristics between the control and COPD groups. The 2-factor repeated-measures analysis of variance (ANOVA; general linear model, SPSS for WINDOWS version 12; SPSS Inc, Chicago, IL) with interaction was performed with a group (control and COPD) and protein (soy and soy + BCAA) effect to test the effects on the change from postabsorptive \(t = 0 \text{ h}\) to the prandial \(t = 2 \text{ h}\) after the start of enteral intake of the soy or soy + BCAA feedings state on protein kinetics and the concentration of plasma amino acids and metabolites. Furthermore, the 2-factor repeated-measures ANOVA with interaction was performed with a group (control and COPD) and protein (soy and soy + BCAA) effect to test the total intake of Phe and the 3 BCAAs and to test the effects on splanchnic extraction of Phe, Ra Phe feeding, and percentage Phe hydroxylation from Tyr at 2 h after the start of enteral intake of the soy or soy + BCAA meals. If there was a significant protein-by-group interaction, the paired samples \(t\) test was used to evaluate the protein effect within each group. The level of significance was set at \(P < 0.05\).

**RESULTS**

Age, height, body weight, and body mass index did not differ significantly between the COPD patients and the healthy elderly control subjects (Table 1). FFM index tended to be lower in the COPD group than in the healthy group \(P = 0.08\). The COPD patients were characterized by moderate airflow obstruction. In the control group, all lung function values were within the normal range.

**Plasma metabolites**

In the postabsorptive state, plasma concentrations of urea and ammonia (Table 3) were not significantly different between the COPD and control groups, but there was a tendency toward higher values for insulin and glucose in the COPD group \(P = 0.08\). Feeding resulted in an increase in insulin and glucose \(P < 0.001\) and in a decrease in urea \(P < 0.01\) independent of the type of protein used. In the COPD group, the increase in glucose tended to be higher \(P = 0.07\) than in the control group, whereas the decrease in urea tended to be lower \(P = 0.07\). There was no significant protein-by-group interaction for insulin, glucose, urea, or ammonia. Lactate (data not shown) was not significantly different in the postabsorptive state between the groups but was increased after feeding \(P < 0.05\).

**Splanchnic extraction**

SPE\(_\text{Phe}\) \(2 \text{ h}\) after the start of enteral intake of the soy or soy + BCAA meal (Figure 1) was lower in the COPD group than in the control group \(P < 0.05\) and lower after the soy + BCAA meal than after the soy meal. ASPE of Phe (ASPE\(_\text{Phe}\)) was lower after the soy + BCAA than after the soy feeding \(P < 0.05\). No significant protein-by-group interaction was observed for either SPE\(_\text{Phe}\) or ASPE\(_\text{Phe}\).

**Whole-body protein turnover**

In the postabsorptive state, significant higher baseline values were found for WbPS (Table 4) and whole-body protein breakdown (WbPB, which equals Ra Phe endo; \(P < 0.05\); Table 4 and

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Plasma concentrations in arterialized-venous blood in the postabsorptive state and during enteral feeding with the soy or soy + branched-chain amino acids (BCAAs) meal†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
</tr>
<tr>
<td></td>
<td>((n = 8))</td>
</tr>
<tr>
<td></td>
<td>Soy</td>
</tr>
<tr>
<td></td>
<td>Soy + BCAA</td>
</tr>
<tr>
<td>Insulin (mU/L)²</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>Glucose (mmol/L)²</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Urea (mmol/L)²</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Ammonia (µmol/L)²</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>² All values are (\bar{x} ± \text{SEM}). Data show postabsorptive values (Postabs) and values at 2 h of enteral soy or soy + BCAA feeding. COPD; chronic obstructive pulmonary disease. Two-factor repeated-measures ANOVA was used to test for group and protein effects on the change from postabsorptive to prandial (2 h of enteral soy or soy + BCAA feeding) state. No significant interaction was observed between group and protein.</td>
<td></td>
</tr>
<tr>
<td>² There was a tendency toward higher postabsorptive values for insulin and glucose in the COPD group than in the control group, (P = 0.08).</td>
<td></td>
</tr>
<tr>
<td>² There was a tendency toward a group effect for the change from postabsorptive values to 2 h during enteral feeding, (P = 0.07).</td>
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</tbody>
</table>

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Figure 2) in the COPD group than in the control group, indicating an elevated basal protein turnover. Feeding resulted in an increase in WbPS and in a reduction of WbPB \((P < 0.001)\). A significant \((P < 0.05)\) protein-by-group interaction was observed for the increase in WbPS, and a tendency \((P = 0.06)\) toward a protein-by-group interaction was observed for the reduction in WbPB. The increase in WbPB was significantly greater \((P < 0.05)\) after soy + BCAA feeding than after the soy feeding in the COPD group but not in the control group. The reduction in WbPB was not significantly different between the soy and soy + BCAA feedings in the COPD or control groups.

Baseline net WbPS was not significantly different between the COPD and control groups. A trend toward a larger anabolic response to feeding in the COPD group than in the control group and a positive anabolic effect of adding BCAAs to soy protein.

The plasma concentration of Phe was not significantly different between the COPD and control groups in the postabsorptive state. Feeding increased Phe concentrations \((P < 0.001)\), and the increase was higher in the COPD than in the control group \((P < 0.05)\).

In Figure 2, total Wb Ra of Phe (WbRa Phe), stratified into Ra of Phe coming from the diet (Ra Phe feeding) and that from the endogenous (nonfeeding) compartment (Ra Phe endo, which equals WbPB and WbRa Phe in the postabsorptive state) is shown. A tendency toward a protein-by-group interaction was present for the increase in WbRa Phe \((P = 0.07)\) and for the reduction in Ra Phe endo \((P = 0.06, \text{ see also Table 4})\). The increase in WbRa Phe tended to be higher after the soy + BCAA meal than after the soy meal in the COPD group \((P = 0.07)\). The reduction in Ra Phe endo was not significantly different after the soy and soy + BCAA feedings in either the COPD or control groups. A group \((P < 0.05)\) and protein \((P < 0.05)\), but no interaction, effect was present for Ra Phe feeding. The higher Ra Phe feeding in the COPD group agrees with the higher total intake of Phe observed in these patients (Table 2), which is related to the relatively lower FFM in the COPD group \((P = 0.06)\) and to the fact that the meal was given on the basis of body...
The higher Ra Phe feeding observed after the soy + BCAA feeding agrees with the lesser effect of BCAA supplementation on splanchnic extraction of Phe.

In Figure 3, the percentage of Phe hydroxylation from Tyr on whole-body level (Figure 3A) and in the splanchnic area (Figure 3B) is shown in the COPD and control groups 2 h after the start of enteral intake of the soy or soy + BCAA meals. No significant group effect or protein-by-group interaction was observed for the percentage Phe hydroxylation at either the whole-body level or the splanchnic area. There was a tendency ($P = 0.06$) toward a lower percentage Phe hydroxylation at the whole-body level after the soy + BCAA feeding than after the soy feeding.

**DISCUSSION**

In the present study, supplementation of BCAA to soy feeding resulted in a significant increase in WbPS in the COPD group but not in the healthy control group. This suggests that supplementation of BCAA could be of benefit in improving whole-body protein metabolism in COPD patients but not in the healthy elderly.

Also, there is evidence supporting a beneficial role for BCAAs on protein metabolism in the literature. Most available studies have studied the BCAA supply to preserve body proteins in subjects who are in a negative energy or protein balance due to reduced dietary intake (28, 29) or after a period of bed rest (30, 31). In these studies, enhanced nitrogen retention, as well as positive effects on whole-body or muscle protein synthesis rates, was observed. Clinical data are available evaluating the effect of BCAA-enriched solutions in chronic heart failure (32), surgery (33), diabetes (34), and hypercatabolic diseases [eg, liver cirrhosis (35)]. Although the results are not always consistent because of the different study designs and the variable amount and duration of BCAAs supplied, for the most part, positive effects were observed on nitrogen balance.

Interestingly, the positive metabolic effects of BCAA supplementation to soy protein were observed in the normal-weight COPD patients with slightly reduced FFM levels ($P = 0.06$) but with preserved plasma BCAA concentrations. In addition to an elevated basal protein turnover in COPD, which agrees with previous data (3), it is likely that COPD patients are also characterized by an increased BCAA turnover, making them more metabolically responsive to BCAA supplementation. Future studies are needed to test this hypothesis.

An adequate protein metabolic response to feeding is of importance in chronic wasting diseases such as COPD. In the present study, there was a tendency toward a higher protein anabolic response to feeding in the COPD group than in the healthy control group. This enhanced anabolic response to feeding was also previously observed in COPD patients after casein
feeding with the use of the same study design and study populations (4) and may be explained by the lower splanchnic extraction of Phe and the higher total protein and Phe intake via the meals in the COPD group than in the control group, related to the lower FFM in the COPD group ($P < 0.06$).

Differences in the magnitude of protein anabolism among protein sources with different, but also with similar, concentrations of BCAAs are present. In the present study, a tendency toward a positive effect of BCAA supplementation to soy protein WbPS was found on the increase in net. Despite the fact that individual BCAAs were added to the soy meal to equal the BCAA concentrations present in casein, net WbPS after the soy + BCAA feeding remained lower than those observed after casein feeding in a similar protocol (4) in both groups (COPD: $172 \pm 29$ nmol·kg FFM$^{-1}$.min$^{-1}$ compared with $226 \pm 16$ nmol·kg FFM$^{-1}$.min$^{-1}$; healthy elderly: $120 \pm 12$ nmol·kg FFM$^{-1}$.min$^{-1}$ compared with $156 \pm 22$ nmol·kg FFM$^{-1}$.min$^{-1}$), indicating that the anabolic response to casein protein remains higher than after soy protein when BCAAs are added. It is well accepted that casein and soy protein generally differ in the speed of amino acid absorption [the slow compared with fast concept (36, 37)]; casein is more slowly absorbed and digested than is soy, and after intake of casein, the increase in the plasma amino acid concentrations is less than that observed after soy intake (17). However, a different absorption rate between the casein and soy protein is not responsible for the observed differences in the anabolic response, because both proteins were given in a “continuous” way by intake of frequent small meals (every 20 min). In accordance, the increase in the plasma Phe concentration after intake of the soy meal was similar to that observed after intake of the casein meal (4), suggesting that the release of amino acids in the circulation was identical between the proteins. With a comparable rate of amino acid absorption, it is possible to more specifically evaluate the quality of the amino acid composition of casein and soy protein. The quality of casein protein was superior to that of soy protein, because net WbPS was lower after the soy feeding even when BCAAs were added than after the casein feeding. This suggests that the high anabolic effect of casein protein in both the
COPD and healthy elderly groups is not related to its high concentrations of BCAAs per se.

**Interorgan protein metabolism**

To increase protein synthesis in the periphery, high amino acid availability is important (38, 39). Soy protein feeding increased WbPS in both the COPD and healthy control groups. Interestingly, coinjection of BCAAs and soy protein resulted in an enhanced increase in WbPS in the COPD group but not in the control group. The increased WbPS after BCAA feeding observed in the COPD group can partly be explained by the fact that adding BCAAs reduced the absolute splanchnic extraction of Phe in the COPD group more than did soy alone, resulting in an enhanced amino acid availability in the circulation, as shown by a higher Ra Phe feeding after the soy + BCAA feeding. Because the increase in Phe concentration was not significantly different between the protein meals in the COPD group, this indicates that the increased amino acid release in the circulation was immediately balanced by an increased Phe use, probably for peripheral protein synthesis.

Interestingly, BCAA addition to soy protein resulted in a reduction of absolute splanchnic extraction of Phe in both groups, whereas the percentage of Phe hydroxylation in the splanchnic area was unaltered, suggesting that splanchnic protein synthesis was decreased. Despite this reduction, the increase in WbPS after BCAA supplementation was not significantly different from that after soy feeding alone in the healthy elderly but was even larger in the COPD group. This suggests that BCAA supplementation has a positive effect on protein synthesis rate in the periphery in the healthy elderly and even more so in COPD patients. In agreement with our findings, previous studies showed that BCAAs have specific stimulatory effects on signaling pathways involving translation of mRNA that result in enhanced protein synthesis (13, 40). In contrast to muscle, there was no effect of Leu on the overall protein synthesis in the liver (29), indicating that BCAAs escape liver metabolism. These observations together with our findings suggest that BCAA supplementation alters interorgan protein metabolism in favor of the peripheral (ie, muscle) compartment.

In conclusion, adding free BCAAs to a soy meal did not further improve whole-body protein metabolism in the healthy elderly but enhanced WbPS in patients with COPD. BCAA supplementation reduced splanchnic protein synthesis in both groups, suggesting a positive effect on protein synthesis in the periphery in the healthy elderly and even more so in COPD patients. This altered interorgan protein metabolism after BCAA supplementation in favor of the peripheral (muscle) compartment may particularly be of importance in COPD patients to prevent or delay loss of skeletal muscle mass during the course of the disease. It remains to be determined whether this positive response to BCAA feeding is also present in weight-losing patients with COPD. Furthermore, the positive metabolic response to BCAA supplementation in normal-weight COPD patients suggests an enhanced need for BCAA-enriched nutrition in this group. Future studies are needed that carefully examine the specific needs of the individual BCAAs in both normal-weight and weight-losing COPD patients to optimize their anabolic capacity to feeding.

MPKJE was involved in the study design, data collection, data analysis, and writing of the manuscript. EPAR and CLNDC were involved in the data collection. EFMW and AMWJS were involved in the study design and reviewing of the manuscript. NEPD was involved in the study design, data analysis, and reviewing of the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

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