Effect of initiating enteral protein feeding on whole-body protein turnover in critically ill patients

Felix Liebau, Jan Wernerman, Luc JC van Loon, and Olav Rooyackers

ABSTRACT

Background: Critically ill patients are susceptible to protein catabolism. Enteral feeding may ameliorate protein loss, but its effect is not well characterized in terms of protein kinetics.

Objective: We established a method of quantifying the effect of enteral protein feeding on whole-body protein turnover and studied critically ill patients receiving early enteral nutrition.

Design: In a proof-of-concept study, we established, in healthy subjects (n = 6), a method of measuring the effect of continuous enteral protein feeding on whole-body protein turnover by using 13C-phenylalanine (13C-Phe) intrinsically labeled casein by a nasogastric feeding tube and an intravenous 2H5-Phe tracer. The protocol was applied to study critically ill patients (n = 10) during the initial hypocaloric-hyponitrogenous dose of enteral nutrition.

Results: Patients were catabolic with a negative protein balance. The median splanchnic extraction fraction of hourly dietary Phe intake was 92% (range: 86–99%); that is, the availability of dietary Phe in arterial plasma was low. In patients with a stable parenteral amino acid supply (n = 7), the median net protein balance improved during enteral feeding from −8.6 to −5.8 μmol · kg body weight−1 · h−1 (P = 0.018).

Conclusions: Whole-body protein turnover and the contribution of dietary protein can be quantified in critically ill patients by using intravenous and enteral stable-isotope Phe tracers. The whole-body protein balance improved during early hypocaloric-hyponitrogenous enteral protein feeding in these patients. This trial was registered at the Australian New Zealand Clinical Trials Registry as ACTRN12614000333617.

INTRODUCTION

Protein homeostasis is particularly susceptible to critical illness, with patients losing up to 20% of their body protein within weeks (1). A net loss of protein occurs in the presence of increased amino acid (AA) turnover (2). Skeletal muscle appears to be particularly prone to protein loss (1), and loss of muscle mass may be correlated with increased morbidity during and after critical illness. Skeletal muscle loss (3–5) and a caloric deficit in critically ill patients can be mitigated by sufficient nutrition. However, the optimum amount of nutrients, particularly of AAs (5, 6), is debated, and it remains controversial how early nutrition should be given to optimize the outcome (7, 8). There is a consensus that the enteral feeding route should be preferred when the gastrointestinal tract is functional (9, 10). However, providing sufficient energy and nutrients enterally can be challenging because gastric and intestinal motility (11) and nutrient uptake (12) may be affected in critical illness. Intolerance to enteral feeding can result in caloric and protein deficit (13) as well as increased morbidity (14) and mortality (15).

The protein metabolism of critically ill patients can be evaluated by using stable-isotope–labeled AA tracers (16). In the current study, we investigated the effect of early, hypocaloric-hyponitrogenous, continuous enteral protein feeding on whole-body (WB) protein metabolism by using intravenous and enteral Phe tracers. Study nutrition that contained casein intrinsically labeled with a Phe tracer enabled us to separately quantify the contribution of dietary protein. The specific aims of the study were to establish the feasibility of the method and quantify the effect of early enteral protein feeding on WB protein turnover in critically ill patients.

METHODS

The study was conducted in the experimental facility and multidisciplinary intensive care unit (ICU) of Karolinska University Hospital Huddinge, Stockholm, Sweden. Prior approval of the study protocol had been received from the regional ethics review board (Regionala etikprövningsnämnden i Stockholm; registration no. 2009/1647–31/3), and written informed consent was obtained from each study subject or next of kin. This trial was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Stockholm County Council (Regionalt etikprövningsnämnden i Stockholm; registration no. 2009/1647–31/3) and the Regional Ethics Review Board in Stockholm (Regionala etikprövningsnämnden i Stockholm; registration no. 2009/1647–31/3).

Key points

- Critically ill patients are susceptible to protein catabolism.
- Enteral feeding may ameliorate protein loss, but its effect is not well characterized.
- The method of quantifying the effect of enteral protein feeding on whole-body protein turnover was established in healthy subjects.
- The method was applied to critically ill patients receiving early enteral nutrition.
- Whole-body protein turnover improved during early hypocaloric-hyponitrogenous enteral protein feeding.
- The study was conducted in accordance with the principles of the Declaration of Helsinki.

Keywords critical illness, nutritional support, stable isotope tracers, whole-body protein turnover, intrinsically isotope-labeled casein

was registered at the Australian New Zealand Clinical Trials Registry as ACTRN12614000333617. In a proof-of-concept phase, the experimental protocol was tested in healthy subjects to ensure that the procedure was workable and sufficient isotopic enrichment of AA tracers in plasma samples could be detected. Subjects for this group were recruited from an institutional database of volunteers and determined to be healthy on the basis of medical history records and physical examinations. In the main study phase, a group of patients was recruited from the ICU and were eligible if they were intubated or tracheostomized, had arterial and central venous catheters in place, and had a clinical indication for the initiation of enteral feeding. Exclusion criteria were defined as age <18 y, prior enteral feeding, milk-protein allergy, ongoing renal replacement therapy or other extracorporeal blood treatment, liver failure, ongoing hemorrhage requiring transfusion, major surgery during the study period, or contraindications to enteral feeding via a nasogastric tube.

### Stable-isotope tracers

Stable-isotope–labeled phenylalanine and tyrosine tracers (L-ring-2H5-Phe, L-ring-2H4-Tyr, and L-3,3-2H2-Tyr) were obtained from Cambridge Isotope Laboratories Inc. and were tested for chemical and isotopic purity. Sterile solutions were prepared by the hospital pharmacy and tested for sterility and nonpyrogenicity before use. Intrinsically labeled milk protein was produced by infusing a lactating cow with L-[1-13C]-Phe. A batch of highly isotopically enriched casein concentrate from the production described in reference 17 was used for this study and was stored at –20°C until use. Chemical and microbiological analyses (NIZO Food Research B.V.) showed the product to be within food-grade specifications with a total protein content of 9.1% of which 97% was chemical and isotopic purity. Sterile solutions were prepared by the hospital pharmacy and tested for sterility and nonpyrogenicity before use. Intrinsically labeled milk protein was produced by infusing a lactating cow with L-[1-13C]-Phe. A batch of highly isotopically enriched casein concentrate from the production described in reference 17 was used for this study and was stored at –20°C until use. Chemical and microbiological analyses (NIZO Food Research B.V.) showed the product to be within food-grade specifications with a total protein content of 9.1% of which 97% casein. The Phe content was 45 mg/g casein (18), and our analysis showed the product to be within food-grade specifications with a total protein content of 9.1% of which 97%

### Experimental protocol: healthy subjects

Healthy subjects were admitted to the study facility after an overnight fast and remained resting in bed throughout the experiment. After a resting period, energy expenditure was measured with indirect calorimetry (DeltaTrac II; Datex Instrumentarium) by using gas sampling from a canopy. A stable reading over a 30-min period was required, and the averaged value of that period was used for additional calculations. A cubital vein of the nondominant arm was cannulated. A modified Allen’s test (19) was performed on the nondominant hand, and the radial artery was cannulated immediately before use. Enteral feeding was given at a dose identical by volume, protein, and carbohydrate contents to that of the feeding formula routinely used at our ICU (Fresubin original; Fresenius Kabi), which contains milk protein as the protein component. Casein concentrate was thawed in aliquots of 60 mL. Maltodextrin for enteral use (Fairst ingredients for initial enteral feeding at the time of the study. A primed continuous intravenous infusion of AA tracers and an intravenous infusion of parenteral nutrition (PN) solution were started simultaneously at a time point defined as zero. The AA tracer priming dose was 0.5 mg L-ring-2H5-Phe kg body weight (BW)⁻¹, 0.15 mg L-ring-2H4-Tyr kg BW⁻¹, and 0.3 mg L-3,3-2H2-Tyr kg BW⁻¹. The continuous infusion dose was 0.5 mg L-ring-2H5-Phe kg BW⁻¹ h⁻¹ and 0.3 mg L-3,3-2H2-Tyr kg BW⁻¹ h⁻¹. PN (containing 23.6 g AAs/L, 67.4 g glucose/L, and 35.4 g soybean oil/L) with a total energy content of 694 kcal/L. Kabiven perifer; Fresenius Kabi) was given at a dose calculated to supply 7% of measured daily energy expenditure per hour and was continued throughout the study period of 510 min, providing 59.5% of daily caloric needs over a period of 8.5 h.

Study nutrition was prepared individually for each subject. Baseline venous blood samples were drawn in duplicate before tracer priming doses were given. Additional blood samples were drawn from the arterial cannula at 10-min intervals from 120 to 150 min, at 15-min intervals from 165 to 465 min, and at 30-min intervals from 480 to 510 min. After completion of the experiment, catheters were removed, local hemostasis was obtained, and study subjects were discharged after an observation period.

### Experimental protocol: ICU patients

Patients received standard care according to institutional routines, including respiratory support, analgesic and/or sedative drug regimens, invasive monitoring, and circulatory support as ordered by the treating physicians. Intravenously, i.v., intravenous; 13C-Phe, L-[1-13C] labeled phenylalanine.

#### FIGURE 1
Timeline of sampling (upper rows) and nutrition/amino acid tracer infusion schedule (lower rows) of healthy volunteers and critically ill patients receiving early enteral feeding. D2-Tyr, L-3,3-2H2 labeled tyrosine; D5-Phe, L-ring-2H5 labeled phenylalanine; i.v., intravenous; 13C-Phe, L-[1-13C] labeled phenylalanine.
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Main diagnoses/procedures</th>
<th>Sex</th>
<th>Age, y</th>
<th>BW pre, kg</th>
<th>BW ICU, kg</th>
<th>Height, m</th>
<th>Start, h</th>
<th>APACHE 2</th>
<th>SOFA</th>
<th>CRP, mg/L</th>
<th>LOS, d</th>
<th>VD, d</th>
<th>Survival, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Mesenterial embolism, small bowel resection</td>
<td>F</td>
<td>75</td>
<td>75</td>
<td>85</td>
<td>1.58</td>
<td>84</td>
<td>26</td>
<td>8</td>
<td>206</td>
<td>7</td>
<td>7</td>
<td>314</td>
</tr>
<tr>
<td>10</td>
<td>Community acquired pneumonia, ARDS</td>
<td>M</td>
<td>70</td>
<td>81</td>
<td>84</td>
<td>1.70</td>
<td>47</td>
<td>25</td>
<td>8</td>
<td>162</td>
<td>8</td>
<td>7</td>
<td>&gt;365</td>
</tr>
<tr>
<td>11</td>
<td>Sepsis, wound infection, pneumonia</td>
<td>M</td>
<td>65</td>
<td>88</td>
<td>90</td>
<td>1.72</td>
<td>56</td>
<td>24</td>
<td>10</td>
<td>138</td>
<td>41</td>
<td>23</td>
<td>&gt;365</td>
</tr>
<tr>
<td>12</td>
<td>Incarcerated inguiinal hernia, ileus, sigmoidostomy</td>
<td>M</td>
<td>75</td>
<td>75</td>
<td>83</td>
<td>1.65</td>
<td>34</td>
<td>37</td>
<td>11</td>
<td>325</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>13</td>
<td>Community acquired pneumonia, COPD</td>
<td>M</td>
<td>71</td>
<td>64</td>
<td>66</td>
<td>1.67</td>
<td>44</td>
<td>40</td>
<td>3</td>
<td>57</td>
<td>22</td>
<td>21</td>
<td>&gt;365</td>
</tr>
<tr>
<td>16</td>
<td>Hypoxic heart arrest, COPD, pneumonia, sepsis</td>
<td>F</td>
<td>72</td>
<td>95</td>
<td>95</td>
<td>1.73</td>
<td>39</td>
<td>31</td>
<td>7</td>
<td>356</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>Acute interstitial pneumonitis, respiratory failure</td>
<td>M</td>
<td>79</td>
<td>87</td>
<td>86</td>
<td>1.67</td>
<td>76</td>
<td>19</td>
<td>3</td>
<td>30</td>
<td>6</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>18</td>
<td>Abdominal lymph node dissection, biliary peritonitis</td>
<td>F</td>
<td>78</td>
<td>53</td>
<td>58</td>
<td>1.53</td>
<td>87</td>
<td>22</td>
<td>6</td>
<td>204</td>
<td>6</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>19</td>
<td>Aspiration pneumonia, COPD, rectum cancer</td>
<td>M</td>
<td>65</td>
<td>64</td>
<td>71</td>
<td>1.70</td>
<td>65</td>
<td>35</td>
<td>12</td>
<td>210</td>
<td>9</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>Interstitial lung disease, hypertension, chronic pain</td>
<td>F</td>
<td>75</td>
<td>58</td>
<td>62</td>
<td>1.62</td>
<td>55</td>
<td>25</td>
<td>6</td>
<td>104</td>
<td>8</td>
<td>7</td>
<td>28</td>
</tr>
</tbody>
</table>

— Proportion or median (range)

6 M/4 F 73.5 (65–79) 75 (53–95) 83.5 (58–95) 1.67 (1.53–1.73) 55.5 (34–87) 25.5 (19–40) 7.5 (3–12) 183 (30–356) 8 (4–41) 7 (4–23) 24 (4 to <365)

1APACHE 2, Acute Physiology and Chronic Health Evaluation score version 2; ARDS, adult respiratory distress syndrome; BW ICU, body weight on day of experiment; BW pre, body weight before intensive care unit admission; COPD, chronic obstructive lung disease; CRP, serum C-reactive protein on day of study; ICU, intensive care unit; ID, identifier; LOS, length of intensive care unit stay; SOFA, Sequential Organ Failure Assessment; Start, time point of experiment start after intensive care unit admission; Survival, length of survival from intensive care unit admission; VD, days on ventilator with tracheal intubation or tracheostomy.
required. No invasive procedures were performed beyond those clinically indicated. Arterial and central venous catheters that were in place were used for blood sampling and infusion, respectively. The correct positioning of previously placed nasogastric tubes (8 mL dead space) was verified by revising recent chest radiographs and by air insufflation and epigastric auscultation. Ongoing intravenous infusion regimens were not altered, and ongoing PN schemes were continued as clinically indicated with a target of 4.5% of estimated daily energy expenditure per hour. Patients received parenterally either Kabiven perifer (see above) or Kabiven (containing 33.1 g AAs/L, 97.5 g glucose /L, and 39.0 g soybean oil/L with a total energy content of 877 kcal/L; Fresenius Kabi). Some patients also received infusions of l-alanyl-l-glutamine (Dipeptiven; Fresenius Kabi) or glucose. Enteral nutrition other than study nutrition was not given.

To minimize the variability introduced by acute changes in body composition through fluid accumulation (20), intravenous tracer doses were based on the last-known BW before ICU admission if that weight differed from the patient’s current BW. The protocol for enteral feeding, tracer infusion, and blood sampling was identical to that for healthy subjects.

Samples and analytic techniques

For isotopic analysis and the analysis of AA concentrations, 3-mL blood samples were taken in refrigerated EDTA-coated vacuum tubes, stored on ice, and processed within 60 min. Plasma was obtained by centrifugation at 4°C, and samples were immediately frozen at −80°C. For isotopic analysis by gas chromatography-mass spectrometry, samples were prepared as described (21), and measurements made at a m/z of 336 for Phe, 337 for 13C-Phe, 341 for ring-2H3-Phe, 466 for Tyr, 468 for 3,3-2H2-Tyr, and 470 for ring-2H4-Tyr. Plasma AA concentrations were analyzed by HPLC as described (21).

Patient data

Data acquired from hospital records and the ICU patient data management system included patients’ anthropometric data, diagnoses, Acute Physiology and Chronic Health Evaluation 2 scores on the day of ICU admission, Sequential Organ Failure Assessment score on the day of the experiment, serum C-reactive protein, ICU nutrition protocol, ICU length of stay, number of days on ventilator with tracheal intubation or tracheostomy, drug doses, and length of survival.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 6)</th>
<th>Patients (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PN + EN</td>
<td>P</td>
</tr>
<tr>
<td>PN</td>
<td>PN + EN</td>
<td>P</td>
</tr>
<tr>
<td>Essential AAs, μmol/L</td>
<td>1248 (940–1566)</td>
<td>1382 (1070–1539)</td>
</tr>
<tr>
<td>Nonessential AAs, μmol/L</td>
<td>1839 (1478–2072)</td>
<td>1900 (1364–2281)</td>
</tr>
<tr>
<td>Phenylalanine, μmol/L</td>
<td>96 (83–130)</td>
<td>114 (80–132)</td>
</tr>
<tr>
<td>Leucine, μmol/L</td>
<td>145 (116–190)</td>
<td>154 (126–169)</td>
</tr>
</tbody>
</table>

1All values are medians; ranges in parentheses. Values were averaged from measurements at time points 120–150 min for PN and 480–510 min for PN + EN. Essential AAs represent His, Thr, Val, Met, Trp, Phe, Ile, Leu, and Lys; nonessential AAs represent Glu, Asn, Ser, Gin, Gly, Arg, Ala, and Tyr. P values for within-group comparisons were determined by Wilcoxon’s matched-pairs test. AA, amino acid; EN, enteral nutrition; PN, parenteral nutrition.

### RESULTS

In the proof-of-concept phase, experiments were started in n = 7 healthy subjects. One subject was excluded from analysis because no arterial access could be established, which left n = 6 subjects for data analysis. There were 5 men

WB protein kinetics

WB Phe kinetics were calculated at baseline (time points: 120–150 min; only PN) and at the end of the enteral feeding period [480–510 min; PN plus enteral nutrition (EN); see Figure 1]. For each of these periods, values of isotopic enrichment were averaged from 4 samples taken at 10-min intervals. Variables of steady state WB Phe kinetics were calculated as described (21, 22) by using the equations defined in reference 23. With the omission of intermediary calculations, the model yielded values for:

1) WB protein breakdown (equal to the WB endogenous rate of appearance of Phe calculated from the WB rate of appearance of Phe minus contributions from PN and EN).

2) WB conversion of Phe to Tyr (Qpt).

3) WB rate of disappearance of Phe (Rd).

4) WB protein synthesis (equal to Rd minus Qpt).

5) WB net protein balance (equal to WB protein synthesis minus WB protein breakdown).

6) Splanchnic uptake of dietary Phe (22) (expressed as a percentage of hourly total dietary Phe intake and termed splanchnic extraction fraction representing the fraction of dietary Phe that is retained in the splanchnic organs, which, thus, does not reach the systemic circulation).

Values for WB kinetics were normalized to BW before ICU admission and expressed as μmol · kg BW⁻¹ · h⁻¹.

### Statistics

A sample-size calculation was not done because the effect size was unknown and could not be estimated from available knowledge. Descriptive statistics are reported as medians (ranges). Within-groups comparisons were done by using Wilcoxon’s matched-pairs test with Statistica software (version 10; StatSoft Scandinavia AB).
and one women with a median (range) age of 26.5 y (21–43 y),
BW of 80 kg (60–106 kg), and height of 1.87 m (1.70–1.95 m).

In the main study phase, experiments were started in \( n = 12 \)
patients but were cancelled in 2 patients when continuation
was deemed inappropriate (because of transfer to another
hospital and a decision to discontinue critical care, re-
spectively), which left \( n = 10 \) patients for data analysis.
Anthropometric and clinical characteristics of these patients
are shown in Table 1. Medication included proton-pump
inhibitors for stress ulcer prophylaxis in all but one patient
(patient 12).

Plasma AA concentrations are shown in Table 2. The time
course of recovery of L-[1\(^{13}\)C]-Phe from dietary casein into
arterial plasma in individual subjects is shown in Figure 2.
Figure 3 was derived from the same data by calculating un-
weighted moving averages for individual subjects over \( n = 5 \)
consecutive time points and computing means for healthy sub-
jects and patients, respectively.

![Figure 2](https://academic.oup.com/ajcn/article-abstract/101/3/549/4569409)

**Figure 2** Isotopic enrichment of L-[1\(^{13}\)C]-Phe from dietary intrinsically labeled casein in arterial plasma of \( n = 6 \) healthy volunteers (A) and \( n = 10 \)
critically ill patients (B) receiving early enteral feeding. Data are shown for individual subjects and medians (dashed line). APE, atom percent excess.
Because the dosing of PN was not standardized in this study protocol, all patients’ infusion schemes were reviewed in 2-h intervals. The total caloric supply from PN during the experiment was 5.2 kcal/kg BW (2.3–9.1 kcal/kg BW), which corresponded to 14.6 kcal·kg BW⁻¹·24 h⁻¹ (6.5–25.7 kcal·kg BW⁻¹·24 h⁻¹). Substantial variability in intravenous AA supply during the experiments was shown in 3 patients (Table 3). Because WB protein breakdown and synthesis of critically ill patients are sensitive to variations of substrate supply (21), values for WB protein metabolism were analyzed separately for the entire cohort and for the subgroup of patients with a stable AA supply (n = 7). Results for parameters of WB Phe kinetics are shown in Table 4. In patients with a stable AA supply, we found decreases in protein breakdown, protein synthesis, and Phe conversion, whereas the net protein balance improved from

TABLE 3
Parenteral amino acid supply of critically ill patients receiving hypocaloric-hyponitrogenous, continuous enteral protein feeding1

<table>
<thead>
<tr>
<th>Patient</th>
<th>ID</th>
<th>$g$ AA · kg BW before ICU admission⁻¹ · h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 0 min</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>T = 120 min</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>T = 240 min</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>T = 360 min</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>T = 480 min</td>
<td>0.035</td>
<td></td>
</tr>
</tbody>
</table>

1Values represent the total hourly amino acid supply from intravenous nutrition including $\alpha$-alanyl-$\alpha$-glutamine. AA, amino acid; BW, body weight; ICU, intensive care unit; ID, identifier.

DISCUSSION

We studied the feasibility of a stable-isotope-tracer method of measuring WB protein turnover and the effect of early, hypocaloric-hyponitrogenous, continuous enteral protein feeding on WB protein breakdown and synthesis. In a proof-of-concept study in healthy volunteers, we showed that the protocol was workable, and sufficient isotopic enrichment of tracer AA could be detected in plasma. We proceeded to investigate critically ill patients receiving a low dose of EN at the initiation of enteral feeding. The use of different Phe tracers by the intravenous and enteral routes allowed us to quantify the contribution of dietary protein to WB turnover and calculate the splanchnic extraction fraction of dietary Phe. Major findings were that, in patients, a large fraction of dietary Phe was retained in the splanchnic circulation, and the initiation of enteral protein feeding yielded a detectable, although small, improvement of the WB protein balance. Patients and healthy subjects in our study differed substantially in age (24), morbidity, and nutritional status, and therefore, we did not make direct comparisons between the 2 groups.

Dietary casein

One advantage of the use of intrinsically labeled casein is that it is presumably chemically similar to the protein component in ICU feeding formula (designated “milk protein”; detailed information not available). Casein is known to be readily digested and absorbed in healthy subjects; e.g., after oral bolus feeding, an early rise of dietary Phe in blood plasma is seen with a peak at ~0.5 h after intake (25). The digestion and absorption kinetics of AAs from casein differ from those of free AAs or AAs from whey protein (26). It is unclear whether such differences are relevant in critically ill patients receiving proton-pump inhibitors, which can be assumed to interfere with pH-dependent casein precipitation in the stomach.

Phe from dietary casein

In the time course of $\alpha$-[1-13C]-Phe enrichment, large relative variations between consecutive time points were noted both in measurements for individual subjects and in means for each group. Although unadjusted values were used for kinetic calculations, data shown in Figure 3 allow a better visualization of the process. The rise in $\alpha$-[1-13C]-Phe enrichment in patients’ plasma was slow with an onset from ~2 h after the start of the infusion (Figure 3), and enrichment remained near zero throughout the experiment in several patients (Figure 2B). Although the slow onset could partly be explained by dead space of the nasogastric tube and the lack of a priming bolus in our protocol, alterations of gastrointestinal motility, nutrient absorption, and splanchnic-organ metabolism must also be considered. Delayed gastric emptying and slow intestinal motility cause intolerance to enteral feeding, occur with a high prevalence in critical illness (11, 15), and may offer an explanation for

FIGURE 3 Mean (±SEM) isotopic enrichment of $\alpha$-[1-13C] Phe from dietary intrinsically labeled casein in arterial plasma of healthy volunteers (n = 6, shown as circles) and critically ill patients (n = 10, shown as squares) receiving early enteral feeding. Unweighted moving averages were calculated for individual subjects over n = 5 consecutive measurements. APE, atom percent excess.
### TABLE 4

Whole-body protein kinetics in healthy subjects and critically ill patients receiving parenteral compared with parenteral plus hypocaloric-hypo nitrogenous, continuous enteral protein feeding

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n = 6)</th>
<th>All patients (n = 10)</th>
<th>Patients with stable AA supply (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PN + EN</td>
<td>PN + EN</td>
<td>PN</td>
</tr>
<tr>
<td>Protein breakdown, μmol/kg/h</td>
<td>52.5 (44.5–59.8)</td>
<td>62.5 (97–116.8)</td>
<td>62.6 (97–116.8)</td>
</tr>
<tr>
<td>Protein synthesis, μmol/kg/h</td>
<td>56.1 (56.1–56.1)</td>
<td>10.3 (10.3–25.9)</td>
<td>10.3 (10.3–25.9)</td>
</tr>
<tr>
<td>Protein net balance, μmol/kg/h</td>
<td>56.2 (49.6–68.2)</td>
<td>55.2 (44.4–59.2)</td>
<td>55.2 (44.4–59.2)</td>
</tr>
<tr>
<td>Protein net balance, %</td>
<td>+4.3 (+1.3 to +8.4)</td>
<td>+5.2 (+2.3 to +7.0)</td>
<td>+5.2 (+2.3 to +7.0)</td>
</tr>
<tr>
<td>Splanchnic extraction, %</td>
<td>NA</td>
<td>NA</td>
<td>83 (60–91)</td>
</tr>
</tbody>
</table>

**TABLE 4 Notes:**
- All values are medians; ranges in parentheses.
- P-values for within-group comparisons were determined by Wilcoxon’s matched pairs test. AA, amino acid; EN, enteral nutrition; NA, not applicable; PN, parenteral nutrition.

**Parameters of WB metabolism of critically ill patients react to changes in the intravenous AA supply:**

Parameters of WB metabolism of critically ill patients react to changes in the intravenous AA supply, which we found in 3 patients, confounded the effects of EN and invalidated the steady state assumption underlying the model used for calculations. Therefore, we analyzed patients with a stable AA supply separately (Table 4). In critical illness, both protein synthesis and breakdown are increased, and protein losses occur predominantly through increased breakdown (39). Earlier patient studies focused on the effect of PN and showed that a higher intravenous supply of AA mitigated WB protein catabolism (2). We measured WB protein turnover in critically ill patients in 2 previous studies. In one study (16), Leu and Phe tracers were used to quantify WB protein turnover in parenterally fed ICU patients. Patients were shown to have increased turnover compared with that of healthy subjects and a zero net balance. In the other study (21), varying doses of PN in patients on a neurosurgical ICU were studied, and it was shown that hypocaloric-hypo nitrogenous PN resulted in a lower protein net balance than did full PN. Data on the effect of enteral feeding on WB protein turnover in critical illness are apparently scarce. In healthy humans, WB and splanchnic protein synthesis is stimulated by a mixed meal, and this effect is dependent on the presence of AAs in the diet (40). Our data showed that protein breakdown, protein synthesis, and Phe conversion all declined during the initial dose of EN, whereas the net protein balance improved in patients. During our findings. Although near-complete protein digestion and AA absorption is postulated in healthy subjects, it is unclear how these processes are affected in critically ill patients. Decreased intestinal AA absorption has been shown in septic rats (27) and in a small number of critically ill patients (28) and could have contributed to the low systemic availability of dietary Phe seen in our patients. Finally, dietary AA undergo a substantial splanchnic first-pass metabolism, which fuels intestinal metabolism and AA cycling including hepatic protein synthesis (31). The splanchnic first-pass metabolism of Phe can be quantified as a splanchnic extraction fraction. We showed a value of 92% of hourly intake after 6 h of low-dose continuous feeding in previously fasted patients (Table 4). In contrast, values in healthy humans have been measured as 26–32% (continuous intragastric infusion; $^2$H$_5$-Phe tracer) (32), 58% (repeated meals; $^{13}$C-Phe tracer) (33), and 53% (6 h cumulative after bolus feeding of L-[1-$^{13}$C]-Phe intrinsically labeled casein) (18). The splanchnic organ metabolism of AA may be dependent on the amount of enteral supply. The splanchnic extraction fractions of lysine and threonine are dose-dependent (preterm infants; full EN compared with PN plus EN) (34, 35). Phe splanchnic extraction was shown to be dose-dependent in one study (healthy adults; repeated meals; $^2$H$_5$-Phe tracer) (36), but not in another (elderly men; 4 h cumulative after bolus feeding of L-[1-$^{13}$C]-Phe intrinsically labeled casein) (37). Thus, the high value shown in our patients may have in part reflected a physiologic response to low dietary supply. Finally, the finding of a dose-dependent increase of splanchnic extraction fraction in experimental endotoxemia (healthy adults; hepatic vein sampling; intravenous $^2$H$_5$-Phe tracer) (38) is potentially relevant to the critical care setting.

**WB protein turnover**

Parameters of WB metabolism of critically ill patients react to changes in the intravenous AA supply (21). Substantial variability in the parenteral AA supply, which we found in 3 patients, confounded the effects of EN and invalidated the steady state assumption underlying the model used for calculations. Therefore, we analyzed patients with a stable AA supply separately (Table 4). In critical illness, both protein synthesis and breakdown are increased, and protein losses occur predominantly through increased breakdown (39). Earlier patient studies focused on the effect of PN and showed that a higher intravenous supply of AA mitigated WB protein catabolism (2). We measured WB protein turnover in critically ill patients in 2 previous studies. In one study (16), Leu and Phe tracers were used to quantify WB protein turnover in parenterally fed ICU patients. Patients were shown to have increased turnover compared with that of healthy subjects and a zero net balance. In the other study (21), varying doses of PN in patients on a neurosurgical ICU were studied, and it was shown that hypocaloric-hypo nitrogenous PN resulted in a lower protein net balance than did full PN. Data on the effect of enteral feeding on WB protein turnover in critical illness are apparently scarce. In healthy humans, WB and splanchnic protein synthesis is stimulated by a mixed meal, and this effect is dependent on the presence of AAs in the diet (40). Our data showed that protein breakdown, protein synthesis, and Phe conversion all declined during the initial dose of EN, whereas the net protein balance improved in patients. During
the course of the experiment, we showed no obvious other 
changes relevant to nutrition or metabolism; thus, the 
improvement in protein balance may well have represented an effect 
of enteral feeding. The significance of the decrease in WB protein 
synthesis is not clear. However, note that methods that use in-
travenous AA tracers do not give a complete picture of WB 
protein synthesis in that they only allow the measurement of 
synthesis from plasma precursors. Therefore, any metabolic 
effects dependent on the uptake of AA from the intestinal lumen, 
enterocyte-to-lumen AA cycling, or intracellular AA cycling 
would not be detectable. Thus, the stimulation of splanchnic-
region protein synthesis or other regional effects of feeding may 
have occurred that we were unable to measure.

Limitations

We recognize limitations to the generalizability of our findings. 
First, even though highly enriched intrinsically labeled casein was 
used, the enrichment of dietary L-[1-13C]-Phe in arterial plasma 
remained low overall even in healthy subjects, which resulted in 
a low signal-to-noise ratio for results derived therefrom. Second, 
the small size and highly heterogeneous character of our patient 
sample made any generalizations questionable. Critically ill pa-
tients are per se a very heterogeneous population, and it may be 
problematic to generalize results from any specific cohort. The pa-
tients in this study may have been at particularly high risk of protein 
catabolism because they were old, had high severity-of-illness scores 
and a high 1-y mortality, were in an early phase of illness, and re-
ceived, on average, a relatively low amount of parenteral AA.

Conclusions

We have shown that WB protein turnover and the contribution of 
dietary protein can be measured by using intravenous and enteral 
stable-isotope Phe tracers in critically ill patients receiving a low dose 
of EN. Patients had high severity-of-illness scores and a poor 1-y 
survival and were in a catabolic state. The initiation of hypocaloric-
hyponitrogenous, continuous enteral feeding by using a mixture of 
protein and carbohydrate correlated with a detectable improvement 
of WB protein balance, although a large fraction of dietary Phe was

![FIGURE 4 A-D. Changes in parameters of whole-body Phe kinetics in healthy volunteers (n = 6) and critically ill patients (n = 10) receiving parenteral compared with parenteral plus enteral feeding. Values are expressed in μmol · kg BW⁻¹ · h⁻¹. Dashed lines refer to patients with substantial variability in parenteral amino acid supply. EN, enteral nutrition; PN, parenteral nutrition.](https://academic.oup.com/ajcn/article-abstract/101/3/549/4569409)
retain the splanchnic circulation. While it remains unclear whether improved protein balance affects the outcome in critical illness, these findings show that early enteral protein supply, even in the initial phase, may be beneficial in terms of protein metabolism.

We gratefully thank Viveka Gustafsson, Kristina Kilsand, Gunilla Herman, and Lena Nyström for expert nursing assistance and Eva Nejman, Christina Hebert, and Towe Jakobsson for expert technical assistance.

The authors’ responsibilities were as follows—FL, JW, and OR: designed the research; FL and OR: conducted the research, analyzed data, and had primary responsibility for the final content of the manuscript; LJvCvL: provided essential materials (13C-Phe intrinsically labeled casein); FL, JW, LJvCvL, and OR: wrote the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES


