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 $^{13}$C-phenylalanine ($^{13}$C-Phe) intrinsically labeled casein by a nasogastric feeding tube and an intravenous $^{2}$H$_5$-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 $\mu$mol · 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. Am J Clin Nutr 2015;101:549–57.

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

INTRODUCTION

Metabolic alterations are common in critically ill patients. 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)$^2$ 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 obtained from each study subject or next of kin. This trial was registered at the Australian New Zealand Clinical Trials Registry as ACTRN12614000333617. Am J Clin Nutr 2015;101:549–57.

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3 Abbreviations used: AA, amino acid; BW, body weight; EN, enteral nutrition; ICU, intensive care unit; PN, parenteral nutrition; WB, whole body.

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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 was 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.

**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 Instrumentum) 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 under infiltration anesthesia. A nasogastric feeding tube (8 French, 3-mL dead space; Compat, Nestlé Healthcare Nutrition) was placed under surface anesthesia, and its intragastric position was verified by air insufflation with epigastric auscultation. For a timeline of nutrition, tracer infusion, and sampling, see **Figure 1**. The experimental protocol was designed to imitate our clinical procedures 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 AAa/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 immediately before use. Enteral feeding was given at a dose identified 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 (Fairing Sports Nutrition) was prepared as an aqueous solution of 230 g/1000 mL H2O. Separate infusion pumps for casein concentrate and maltodextrin solution were connected to the nasogastric tube by a 3-way stopcock. Infusion was started at 150 min at rates of 8.3 mL/hh⁻¹ and 11.7 mL/h = 2.73 g/h maltodextrin and was continued throughout the study period.

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 10-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

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**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 1
Anthropometric and clinical characteristics of critically ill patients (*n* = 10) receiving hypocaloric-hyponitrogenous, continuous enteral protein feeding

<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>
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<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>
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<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 inguinal 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>
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<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>356</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>14</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>
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<td>75</td>
<td>22</td>
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<td>&gt;365</td>
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<tr>
<td>15</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>16</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>17</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>18</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>

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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 analytical 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-2H5-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</td>
<td>PN + EN</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...
and one woman 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, respectively), 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-[\(^{13}\)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 unweighted moving averages for individual subjects over \( n = 5 \) consecutive time points and computing means for healthy subjects and patients, respectively.

**FIGURE 2** Isotopic enrichment of L-[\(^{13}\)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 the EN to the EN + PN time point. The direction of change between EN and EN plus PN time points was consistent in all patients except those with an unstable AA supply (Figure 4). The splanchnic extraction fraction of Phe in patients with a stable AA supply was 92% (86–98%).

### 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 L-[1$^{13}$C]-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 L-[1$^{13}$C]-Phe enrichment in patients’ plasma was slow with an onset from ~2 h after the start of the casein 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

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**TABLE 3**

Parenteral amino acid supply of critically ill patients receiving hypocaloric-hyponitrogenous, continuous enteral protein feeding.

<table>
<thead>
<tr>
<th>Patient</th>
<th>g AA · kg BW before ICU admission$^{-1} · h^{-1}$</th>
<th>T = 0 min</th>
<th>T = 120 min</th>
<th>T = 240 min</th>
<th>T = 360 min</th>
<th>T = 480 min</th>
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<td>0.035</td>
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<tr>
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<tr>
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<tr>
<td>15</td>
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<td>19</td>
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</tr>
</tbody>
</table>

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

2Patient with variation >50% of the maximum value during the duration of the experiment.
### Table 4

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

<table>
<thead>
<tr>
<th>Healthy (n = 6)</th>
<th>All patients (n = 10)</th>
<th>Patients with stable AA supply (n = 7)</th>
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<tbody>
<tr>
<td></td>
<td>PN</td>
<td>PN + EN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td><strong>Protein breakdown, 2h</strong></td>
<td></td>
<td>52.5 (44.5–59.8)</td>
</tr>
<tr>
<td><strong>Protein net balance</strong></td>
<td></td>
<td>50.0 (–3.1 to +10.9)</td>
</tr>
<tr>
<td><strong>Splanchnic extraction, %</strong></td>
<td>NA</td>
<td>83 (60–93)</td>
</tr>
</tbody>
</table>

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 (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-hyponitrogenous 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 (29, 30) as well as hepatic metabolism 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; \(^{12}\)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; \(^{1}\)H\(_2\)-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\(_3\)-Phe tracer) (38) is potentially relevant to the critical care setting.
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 intravenous 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 patients are per se a very heterogeneous population, and it may be problematic to generalize results from any specific cohort. The patients 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 received, 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
retained in 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.

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REFERENCES


