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Original article

Whole body protein turnover in critically ill patients with multiple organ failure

Olav Rooyackers*, Ramin Kouchek-Zadeh, Inga Tjäder, Åke Norberg, Maria Klaude, Jan Wernerman

Department of Anesthesiology and Intensive Care Medicine at Karolinska University Hospital Huddinge and Karolinska Institutet, Stockholm, Sweden

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SUMMARY

Background & aims: To evaluate the effect of nutrition therapy on protein turnover in critically ill patients isotopically labeled amino acids can be used. Here parallel measurements using ^{13}C-leucine and $^{2}H_5$-phenylalanine were performed to evaluate if one tracer was to be preferred.
Methods: As a reference group, healthy volunteers ($n = 8$) were studied in the postabsorptive state and during parenteral nutrition delivery. ICU patients with multiple organ failure ($n = 8$) were studied during parenteral nutrition delivery only.
Results: For the volunteers, the net protein balances changed from negative to positive during parenteral nutrition delivery (compared to the postabsorptive state) when evaluated with leucine and phenylalanine ($P < 0.0001$). For phenylalanine this change was attributable to an increased protein synthesis ($P < 0.0001$), while for leucine the change was attributable to a decreased protein degradation ($P < 0.0001$). For the patients, only measured during parenteral nutrition delivery, the estimates by the two amino acid tracers agreed, showing a protein balance not statistically significantly different from zero. The whole body protein turnover was higher than that of the healthy volunteers during parenteral nutrition delivery. In the patients, the net protein balance correlated positively to the amount of amino acids given.
Conclusions: Critically ill patients with multiple organ failure have an increased protein turnover. The findings in the healthy volunteers indicate that the use of the two different amino acid tracers in parallel in future studies should be considered.

1. Introduction

The wasting and sarcopenia of critically ill patients in the intensive care unit (ICU) is widely recognized.[1–3] Nutritional therapy is the primary effort to attenuate these losses. However recent studies have seriously questioned the way we are feeding these patients.[4,5] Part of this debate is around the amount of amino acids/proteins patients should be given.[6] The effect of protein nutrition has traditionally been evaluated using nitrogen balance measurements.[7] However, to be valid, this technique requires a prolonged metabolic steady state and that the subject is adapted to the level of both calories and nitrogen intake.[8,9] These prerequisites are rarely, if ever, met in the ICU setting.

To enable evaluation of the effects of nutrition and other means of therapy on protein wasting and sarcopenia in the ICU, other tools are needed. The most obvious technique to estimate protein metabolism is by utilizing isotopically labeled amino acids to assess whole body protein turnover. Underlying assumptions for a steady state are less rigorous in terms of time as compared to the nitrogen balance technique. Furthermore, the nitrogen economy may be divided into protein synthesis, protein degradation and amino acid oxidation, giving more information about the underlying mechanisms.

However, using isotopically labeled amino acids for such measurements is not without problems. Essential amino acids, which are not endogenously produced, are needed. Ideally, the amino acid should not be metabolized, just reused in protein metabolism or excreted. Unfortunately such an amino acid does not exist, so assumptions need to be made to compensate for the metabolic degradation or oxidation. Traditionally most studies have used ^{13}C labeled leucine. However, for this method a correction factor to compensate for incomplete recovery of the ^{13}C label from the bicarbonate pool is used when calculating leucine oxidation rates.[10] The assumption that this recovery factor is the same in all

* Corresponding author. Dept of Anesthesiology and Intensive Care Medicine, K32, Karolinska University Hospital Huddinge, 14186 Huddinge, Sweden.
E-mail address: olav.rooyackers@ki.se (O. Rooyackers).

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patients on mechanical ventilation with different ventilator settings is questionable.[11] We therefore wanted to compare protein turnover measurements using the ^{13}C-leucine with an alternative method using $^{2}H_5$-phenylalanine which is not dependent on this bicarbonate recovery factor for measuring oxidation rates. In the latter the oxidation of the phenylalanine is estimated by the rate of metabolism of phenylalanine to tyrosine.

In this study the two different isotopically labeled amino acids were used to study healthy volunteers in the postabsorptive and during parenteral nutrition delivery and critically ill patients with multiple organ failure during parenteral nutrition delivery only. Protein turnover in critically ill patients have been studied before, but very limited data is available for patients with multiple organ failure especially during nutritional therapy, since most previous studies have been performed in the fasted state for both the patients and the controls. The primary aim was to compare the measurements of whole body protein turnover obtained by ^{13}C-leucine and $^{2}H_5$-phenylalanine and possibly establish if one amino acid should be preferred over the other when studying critically ill patients. The secondary aim was to establish whole body protein turnover rates in patients with multiple organ failure during parenteral nutrition delivery in the ICU.

2. Materials and methods

Healthy volunteers ($n = 8$) and critically ill patients with multiple organ failure on mechanical ventilation in the ICU ($n = 8$) were enrolled in the study. For patients the inclusion criteria were (i) receiving parenteral nutrition only, (ii) receiving an oxygen fraction of <50%, and (iii) >18 years old. Exclusion criteria were (i) any order to withhold treatment, or (ii) absence of informed consent. The main characteristics of the patients are given in Table 1. The healthy volunteers were not matched to the patients for age. The study protocol was approved by the regional ethics committee of Stockholm and informed consent was obtained from all subjects after explaining the experimental procedure and the risks involved, both orally and in writing. For the patients the informed consent was obtained from a close relative.

Both volunteers and patients were investigated by indirect calorimetry, using a Deltatrac Metabolic Monitor (Datex-Ohmeda, Helsinki, Finland), to assess their resting energy expenditure. For volunteers, this was done in the postabsorptive state after 60 min of rest and again at the end of the intervention period; a ventilated hood was used for air collection. For the patients this was done in the fed state after 60 min of rest attaching the indirect calorimeter to the ventilator.

The experimental protocol involved measurement of whole body protein turnover differentiating between whole body protein synthesis, whole body protein degradation and protein oxidation. Two different isotopically labeled amino acids were used. For the volunteers the measurement procedure was performed twice; first in the postabsorptive state (overnight fast) and after that during parenteral nutrition delivery when being given 20% of the measured caloric content (Kabiven P, Fresenius-Kabi, Bad-Homburg, Germany) in a peripheral vein. The parenteral nutrition contains 17.9 mM leucine and 14.2 mM phenylalanine. The experimental protocol is illustrated in Fig. 1. The ICU patients received parenteral nutrition only according to standard clinical practice, aiming at 20–25 kcal kg^{-1} day^{-1}. The study was designed as a pragmatic study and therefore no changes in the ongoing therapy were made.

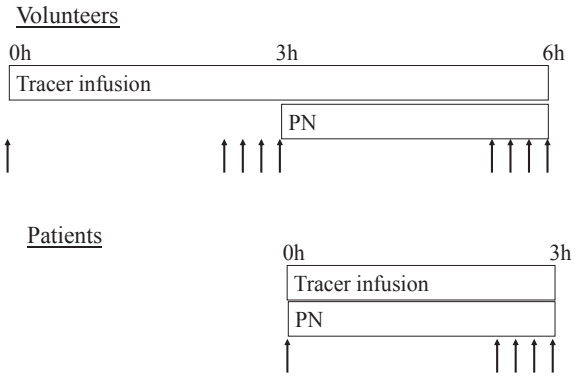

Fig. 1. The experimental protocol used. The time pattern for the primed constant infusion of tracers and of parenteral nutrition (PN) is indicated. Vertical arrows indicate blood sampling.

The whole body protein turnover measurements were performed employing isotopically labeled leucine and phenylalanine in parallel. Volunteers and patients received a primed continuous infusion of the labeled L-amino acids. The prime consisted of ring-$^{2}H_5$-phenylalanine (0.5 mg kg^{-1}), ring-$^{2}H_4$-tyrosine (0.15 mg kg^{-1}), 3,3-$^{2}H_2$-tyrosine (0.3 mg kg^{-1}), 1-^{13}C-leucine (0.9 mg kg^{-1}) and ^{13}C-sodium-bicarbonate 0.2 mg kg^{-1}), and was immediately followed by a continuous infusion of ring-$^{2}H_5$-phenylalanine (0.5 mg kg^{-1} h^{-1}), 3,3-$^{2}H_2$-tyrosine (0.3 mg kg^{-1} h^{-1}) and 1-^{13}C-leucine (1.0 mg kg^{-1} h^{-1}). All isotopes were obtained from Cambridge Isotopes Inc. (Cambridge, MA, USA) and sterile solutions were prepared and tested for sterility and pyrogens by the hospital pharmacy. Labeled amino acids were given intravenously for 150 min to obtain an isotopic steady state after which 4 samples were taken during 30 min. For the volunteers, the infusion continued and an additional infusion of parenteral nutrition was started for another 3 h. A second sampling period of 30 min took place at the end of this 3 h period. The ICU patients were only studied during a 3 h period with sampling in the last 30 min. Blood samples were taken from an artery. Plasma was obtained by centrifugation and frozen at −80 °C until analyses. Breath samples for $^{13}CO_2$ measurements were taken from the DeltaTrac Metabolic Monitor at

Table 1
Patient characteristics.

Diagnosis	Age (y)	BW (kg)	APACHE	Days in ICU	SOFA	REE (kCal day)
Acute infectious endocarditis	61	65	23	7	8	2480
Traumatic subdural bleeding	54	52	15	4	5	1560
Gastrointestinal bleeding	75	72	6	2	7	1560
Septic shock	59	100	22	3	16	2500
Encephalitis	69	85	36	61	7	1800
Pneumonia/sepsis	62	60	23	2	7	1530
Candida sepsis	68	71	29	1	4	1780
Septic shock	76	75	23	6	7	1770

APACHE: APACHE II score taken on admission day. Days in the ICU when studied. SOFA: sequential organ failure assessment on day of the study. REE: Resting energy expenditure measured by indirect calorimetry.

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