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Original article

24-Hour protein, arginine and citrulline metabolism in fed critically ill children – A stable isotope tracer study

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SUMMARY

Background & aims: The reference method to study protein and arginine metabolism in critically ill children is measuring plasma amino acid appearances with stable isotopes during a short (4–8 h) time period and extrapolate results to 24-h. However, 24-h measurements may be variable due to critical illness related factors and a circadian rhythm could be present. Since only short duration stable isotope studies in critically ill children have been conducted before, the aim of this study was to investigate 24-h appearance of specific amino acids representing protein and arginine metabolism, with stable isotope techniques in continuously fed critically ill children.

Methods: In eight critically ill children, admitted to the pediatric (n = 4) or cardiovascular (n = 4) intensive care unit, aged 0–10 years, receiving continuous (par)enteral nutrition with protein intake 1.0–3.7 g/kg/day, a 24-h stable isotope tracer protocol was carried out. L-[ring-$^{2}H_5$]-phenylalanine, L-[3,3-$^{2}H_2$]-tyrosine, L-[5,5,5-$^{2}H_3$]-leucine, L-[guanido-$^{15}N_2$]-arginine and L-[5-^{13}C-3,3,4,4-$^{2}H_4$]-citrulline were infused intravenously and L-[^{15}N]-phenylalanine and L-[1-^{13}C]leucine enterally. Arterial blood was sampled every hour.

Results: Coefficients of variation, representing intra-individual variability, of the amino acid appearances of phenylalanine, tyrosine, leucine, arginine and citrulline were high, on average 14–19% for intravenous tracers and 23–26% for enteral tracers. No evident circadian rhythm was present. The pattern and overall 24-h level of whole body protein balance differed per individual.

Conclusions: In continuously fed stable critically ill children, the amino acid appearances of phenylalanine, tyrosine, leucine, arginine and citrulline show high variability. This should be kept in mind when performing stable isotope studies in this population. There was no apparent circadian rhythm.

Clinical trial register: NCT01511354 on clinicaltrials.gov.

1. Introduction

The acute stress response in critical illness is associated with a protein catabolic state, which in critically ill children in turn is associated with increased morbidity and mortality [1]. Protein requirements to achieve anabolism in critically ill children are increased as compared to healthy children, but unfortunately not well defined [2,3]. Also, the requirements of specific amino acids may be increased. Arginine is especially of interest, because it has functions in the immune system and is the precursor of nitric oxide

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List of abbreviations

AA	amino acid
Arg2	L-[guanido-$^{15}N_2$]-arginine
Cit5	L-[5-^{13}C-3,3,4,4-$^{2}H_4$]-citrulline
CV	Coefficient of variation
CVICU	Cardiovascular intensive care unit
EN	Enteral
IV	Intravenous
Leu1	L-[1-^{13}C]leucine
Leu3	L-[5,5,5-$^{2}H_3$]Leucine
Phe1	L-[^{15}N]-phenylalanine
Phe5	L-[ring-$^{2}H_5$]-phenylalanine
PICU	Pediatric intensive care unit
Ra	Rate of appearance
TTR	Tracer-to-tracee ratio
Tyr2	L-[3,3-$^{2}H_2$]Tyrosine
WbPB	Whole body protein breakdown
WbPBal	Whole body protein balance
WbPS	Whole body protein synthesis

(NO). As sepsis and critical illness are considered arginine-deficient states, arginine supplementation might be beneficial [4]. However scarce data exist on arginine metabolism and NO synthesis in critically ill children.

The reference method to study protein and arginine metabolism is considered to be stable isotope tracer techniques with measurement of amino acid appearances [4,5]. Usually an experimental protocol of 4–8 h is used of which the results are extrapolated to 24-h metabolism for recommendations of daily protein intake [6–9] or insight in arginine metabolism [10]. Studies with 24-h designs, which are more representative to metabolism during an entire day, have been conducted in healthy adults and comprised both a fasted and a fed state [11–13]. These studies showed a circadian pattern [11–13], with e.g. 53% higher phenylalanine appearances in the fasted than in the fed state [13]. In healthy neonates, a circadian rhythm is found in plasma amino acid concentrations [14], with 40% higher amino acid levels during day time as compared to night time.

However, it is not known whether appearances of amino acids, representing protein and arginine metabolism, in critically ill children during continuous feeding show a circadian rhythm. Therefore, we also do not know whether short duration tracer protocols (4–8 h) represent 24-h protein and arginine metabolism in children. We anticipated that children at the pediatric intensive care unit (PICU) have an altered day–night cycle as a result of artificial light exposure, continuous care, sedation and hormonal changes related to the acute stress response [15,16]. We hypothesized that circadian rhythms are attenuated or completely lost in critically ill children.

The objective of the present study was to investigate the pattern of and variability in 24-h appearance of specific amino acids, representing protein and arginine metabolism, in continuously fed critically ill children admitted to the PICU or Cardiovascular ICU (CVICU), using stable isotope tracer methodology with hourly enrichment measurements.

2. Materials and methods

2.1. Subjects and setting

Between May 2008 and December 2010 children aged <18 years admitted to the PICU or CVICU of Arkansas Children's Hospital, Little Rock, AR, were included. Inclusion criteria were: arterial line and multi-lumen central venous line (or two peripheral venous catheters) in place; continuous total parenteral nutrition or continuous enteral feeding with standard nutrition appropriate for age and weight; no planned major changes or interventions (such as surgery) from enrollment to completion of study period; hemodynamic stable condition (with or without continuous inotropic medication) defined as ≤1 boluses of volume resuscitation for hypotension in 24-h; no significant loss of plasma/blood from wounds or drains, that may influence the results of the study, no chylothorax; written informed consent by parent(s) or legally authorized representatives. Exclusion criteria were: congenital/acquired metabolic or endocrine disorders or hepatic or renal failure or anuria or oliguria; gastrointestinal obstructions or any condition that causes malabsorption; active gastro-intestinal bleeding; fluid restriction hindering ability to administer stable isotope tracer fluids.

Z-scores for weight-for-age were determined using 2000 Centers of disease control and prevention (CDC) growth charts (http://www.cdc.gov/growthcharts/zscore.htm).

2.2. Study design

A 24-h stable isotope tracer protocol with hourly blood sampling was conducted to investigate the pattern and variability of kinetics of phenylalanine and leucine, representing protein metabolism, and arginine and citrulline. The tracer protocol is schematically depicted in Fig. 1. It was started after enteral drip-feeding and/or continuous

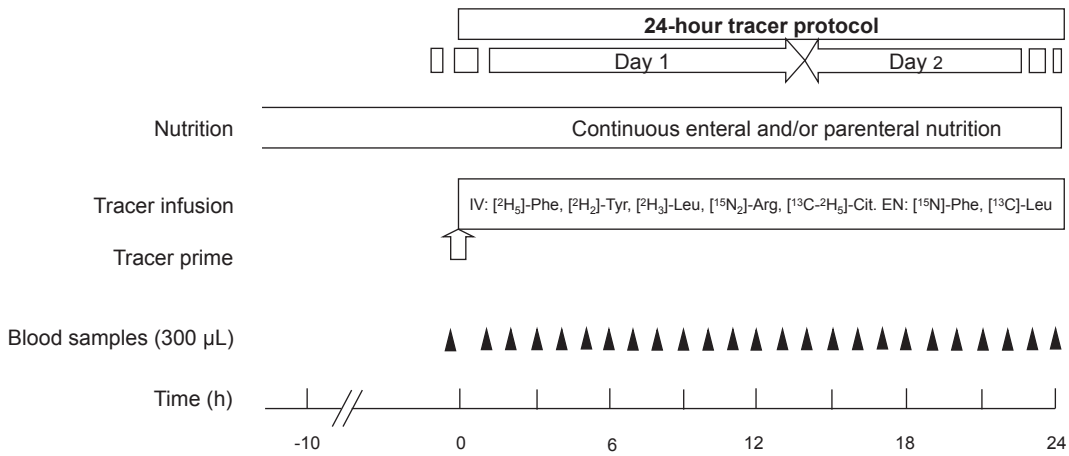

Fig. 1. Study design. Study design of 24-h stable isotope tracer protocol, as used in 8 critically ill children. Black triangles indicate blood samples, the white arrow indicates administration of tracer prime.

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