Ontogeny of methionine utilization and splanchnic uptake in critically ill children

Sascha Verbruggen,1,2,3 Jama Sy,1,2 William E. Gordon,1,2 Jean Hsu,2 Manhong Wu,1,2 Shaji Chacko,2 David Zurakowski,4 Douglas Burrin,2 and Leticia Castillo1,2

1Critical Care Section, Department of Pediatrics, Texas Children’s Hospital, Baylor College of Medicine; 2Children’s Nutrition Research Center, United States Department of Agriculture, Houston, Texas; 3Department of Pediatrics, Erasmus Medical Center, Sophia Children’s Hospital, Rotterdam, The Netherlands; and 4Departments of Anesthesia and Surgery, Children’s Hospital Boston, Harvard Medical School, Boston, Massachusetts

Submitted 2 June 2009; accepted in final form 23 August 2009

Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am J Physiol Endocrinol Metab 297: E1046–E1055, 2009.—To determine the rates of methionine splanchnic uptake and utilization in critically ill pediatric patients we used two kinetic models: the plasma methionine enrichment and the “intracellular” homocysteine enrichment. Twenty four patients, eight infants, eight children, and eight adolescents, were studied. They received simultaneous, primed, constant, intravenous infusions of L-[2H3]methylmethionine and enteral L-[1-13C]methionine. The ratio of [13C]homocysteine to [13C]methionine enrichment was 1.0 ± 0.15, 0.80 ± 0.20, and 0.66 ± 0.10, respectively, for the infants, children, and adolescents, and it was different between the infants and adolescents (P < 0.01). Methionine splanchnic uptake was 63, 45, and 36%, respectively, for the infants, children, and adolescents, and it was different from that in portal venous blood and does not reflect their availability to extraintestinal tissues. This concept has important implications for defining protein and amino acid requirements for nutritional and functional purposes. An extensive catabolism and/or utilization of dietary amino acids in the first pass by the small intestine results in decreased nutritional efficiency and will influence their requirements.

The sulfur amino acids serve important protein and nonprotein functions (16, 18, 36, 37). Methionine, an indispensable sulfur amino acid, serves as the precursor in the synthesis of S-adenosylmethionine, which through the transmethylation pathway is the primary methyl group donor for methylation reactions involved in signal transduction, protein repair, chromatin regulation, and gene silencing (16). Methyl groups are required for biosynthesis of polyamines, cholines, creatine, DNA, and RNA intermediates (16). Homocysteine, the demethylated product of methionine, can be utilized through the transsulfuration pathway and serve as precursor for cystathionine and cysteine, which under physiological conditions is a limiting precursor in the synthesis of the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine; GSH), a major antioxidant with detoxifying and signaling properties that serves a key role in the control of apoptosis and inflammation (13). Alternatively, homocysteine can be remethylated to methionine with betaine or methyltetrahydrofolate as methyl donors (16–18, 36, 37). Therefore, the sulfur amino acids have major functional significance.

It is known that the splanchnic area is greatly affected during critical illness, but the uptake of methionine by the splanchnic area under these conditions has not been determined.

Given the limited knowledge on the nutritional support provided to pediatric critically ill patients, and the nutritional and functional significance of methionine, it is important to examine the quantitative impact of the splanchnic area on methionine availability to the peripheral tissues under conditions of critical illness and whether this is affected by age.

Therefore, the first objective of the study was to determine the rates of methionine first-pass disappearance and oxidation (transsulfuration) by the splanchnic tissues of critically ill children by conducting simultaneous primed, continuous, enteral, and intravenous tracer infusions of L-[1-13C] and L-[2H3]methyl labels of methionine. The second objective was to determine the difference between two kinetic models: the frequently used plasma model that uses plasma homocysteine enrichment and the intracellular model using plasma homocysteine enrichment. Because plasma homocysteine and cystathionine arise from intracellular metabolism of methionine, enrichment of these...
substrates can be used to define intracellular methionine enrichment during an infusion of labeled methionine. Therefore, plasma homocysteine enrichment serves as an indicator of “true” intracellular methionine enrichment (25). The third objective was to ascertain whether the developmental stage from young infants to adolescents affects the rates of methionine splanchnic uptake and its utilization through different metabolic pathways.

MATERIAL AND METHODS

Subjects
These studies were conducted at the Pediatric Intensive Care Unit, Texas Children’s Hospital, Baylor College of Medicine and at the Children’s Nutrition Research Center, U.S. Department of Agriculture. Twenty-four consecutive critically ill children admitted to the Pediatric Intensive Care Unit receiving enteral feedings, supplying complete protein and energy needs as per recommended standard of care, for at least 24 h previous to the study were included. All patients were studied when hemodynamically stable. Three age groups were included the following: infants 0–12 mo, children 1–3 yr, and adolescents 13–18 yr. In the infant group, all patients had diagnosis of respiratory failure, five of them secondary to respiratory syncitial virus bronchiolitis, and two patients had influenza A pneumonia. Another patient underwent a surgical procedure complicated by pneumonia. All infants had been receiving enteral feedings for an average of 8 ± 5 days, and all had received complete feedings for at least 24 h before the study. Six infants required mechanical ventilation, but only two patients required an inspiratory fraction of oxygen >0.6. One patient required vasopressin to maintain blood pressure. In the group of children >1 to 3 yr, the main diagnoses were respiratory failure secondary to parainfluenza or influenza A pneumonia in six patients, septic shock due to methicillin-resistant staphylococci aureus in one patient, and acute lung injury secondary to aspiration pneumonia and viral sepsis in one patient. Six children received mechanical ventilation, and two patients required noninvasive ventilatory support. One child required high frequency oscillatory ventilation, and all others received conventional ventilation. The inspiratory fraction of oxygen was ≤0.6, and none received vasopressors. They had been receiving feedings for an average of 9 ± 3 days, providing complete nutritional support as per current clinical standard for at least 24 h previous to the study. In the adolescent group, the main diagnoses were neurological conditions such as seizures and aspiration pneumonia, postoperative patients from brain tumor resection and acute lung injury, posttraheal reconstruction, and pneumonia. Two previously healthy adolescents had methicillin-resistant staphylococci aureus sepsis. Six were mechanically ventilated on a conventional mode with <0.6 inspiratory fraction of oxygen. Two patients were breathing spontaneously. All adolescents were studied when hemodynamically stable, and none were receiving vasopressors at the time of the study.

The study was approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from parents or guardians. All patients had drawing and infusing intravascular lines and a postpyloric feeding tube placed for clinical indication. The position of the tube was confirmed in three groups of eight patients each, participated in this study. Each patient required high frequency oscillatory ventilation, and all others received conventional ventilation. The inspiratory fraction of oxygen was ≤0.6, and none received vasopressors. They had been receiving feedings for an average of 9 ± 3 days, providing complete nutritional support as per current clinical standard for at least 24 h previous to the study. In the adolescent group, the main diagnoses were neurological conditions such as seizures and aspiration pneumonia, postoperative patients from brain tumor resection and acute lung injury, posttraheal reconstruction, and pneumonia. Two previously healthy adolescents had methicillin-resistant staphylococci aureus sepsis. Six were mechanically ventilated on a conventional mode with <0.6 inspiratory fraction of oxygen. Two patients were breathing spontaneously. All adolescents were studied when hemodynamically stable, and none were receiving vasopressors at the time of the study.

The study was approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from parents or guardians. All patients had drawing and infusing intravascular lines and a postpyloric feeding tube placed for clinical indication, and all had received full enteral feedings for at least 24 h. All were assessed for severity of disease by the pediatric risk mortality score (PRISM III; Ref. 30), which predicts mortality rates in relation to acuity of disease. High scores indicate higher probability of mortality.

Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure, as well as those requiring renal replacement therapies, were excluded. The demographic characteristics of the patients are shown in Table 1. Because of ethical constraints, studies in healthy infants and children were not conducted.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infants</th>
<th>Children</th>
<th>Adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>0.5±0.3</td>
<td>2.8±1.3</td>
<td>15.0±2.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>5.5±1.5</td>
<td>13.6±3.1</td>
<td>63.3±23</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>3±3</td>
<td>8.0±2.9</td>
<td>6.0±5</td>
</tr>
<tr>
<td>PRISM III</td>
<td>23</td>
<td>30</td>
<td>12</td>
</tr>
</tbody>
</table>

All values are means ± SD. BMI, body mass index; PRISM III, pediatric risk mortality score.

Diets
Patients received nutritional support according to current standard clinical practice. All patients were receiving complete enteral nutrition supplied through continuous 24-h feedings, via a nasojejunal tube placed for clinical indication. The position of the tube was confirmed by X-ray. Protein and energy intake in all groups was directed by the attending physician(s) as per standard clinical care in collaboration with the Nutrition Service. In the infant group, the formulas used were Enfamil (Meadjohnson, Atlanta, GA) in four patients, Prossobee (Meadjohnson) in one patient, Goodstart (Nestle, Oakland CA) in one patient. Pregestamill (Meadjohnson) in one patient, and Portagen (Meadjohnson) in one patient. In the children’s group, all patients received Pediasure (Abbott Laboratories, Grove City, OH). In the adolescent group, two patients received Ensure (Abbott Laboratories), four patients received Jevity (Abbott Laboratories), and two patients received Osmolite (Abbott Laboratories). The protein and specific methionine and cysteine content of the formulas supplied were recorded, and they are shown in Table 2. Average protein intakes were 2.3 ± 1.0, 2.0 ± 0.3, and 1.4 ± 0.6 g·kg⁻¹·day⁻¹, while energy intakes were 87.6 ± 28, 66.1 ± 9.7, and 37.1 ± 15.5 kcal·kg⁻¹·day⁻¹, respectively, for the infants, children, and adolescents. Sulfur amino acid intakes were variable in the infant group due to the variable content of sulfur amino acids in the formulas used, while similar formulas were used among children and adolescents, as shown in Table 2. The total sulfur amino acid (methionine and cysteine) intakes were 70.9 ± 40, 72.3 ± 8, and 50.1 ± 19.9 mg·kg⁻¹·day⁻¹, respectively, for infants, children, and adolescents and above the Institute of Medicine (15) recommended dietary intakes of 43, 28, and 22 mg·kg⁻¹·day⁻¹ for infants, children, and adolescents.

Materials
L-[¹³C]methionine (99 atom%), L-[²H₃]methylmethionine (99 atom%), and NaH¹³CO₃ were purchased from Cambridge Isotopes (Andover, MA).

Tracer Study Protocol
The experimental design is shown in Fig. 1. Twenty-four patients distributed in three groups of eight patients each, participated in this study. Each patient received one tracer infusion study after they had received complete, continuous, enteral feedings for at least 24 h. On the day of the study, priming doses of L-[¹³C]methionine at 5 μmol/kg and NaH¹³CO₃ at 0.8 μmol/kg were administered by the nasojejunal tube simultaneously with the enteral feedings. Concurrently, L-[²H₃]methylmethionine was intravenously primed at 2.5 μmol/kg through a preexisting indwelling intravenous catheter placed for clinical indication. These doses were immediately followed by a 9-h, continuous nasojejunal infusion of the L-[¹³C]methionine tracer at 5 μmol·kg⁻¹·h⁻¹ and intravenous infusion of L-[²H₃]methylmethionine at 2.5 μmol·kg⁻¹·h⁻¹, infused by means of calibrated infusion pumps (Gemini PC-2TX infusion pump; Alaris Medical System, San Diego, CA).

The tracers were prepared in sterile physiological saline solution by the Research Pharmacy at Texas Children’s Hospital and filtered.
through a 0.22-micron filter. Aliquots of the infused solution were collected at the end of the tracer study for determination of infusate concentration. Exact tracer infusion doses were determined from the analysis of the amino acid concentrations in the infusates.

At times −30 and 0 min, two baseline blood samples were obtained from a preexistent intravascular catheter (arterial line) placed for clinical indication, followed by several samples at 500, 520, and 540 min for the infants; 480, 500, 520, and 540 min for the children; and 460, 480, 500, 520, and 540 for the adolescents. For determination of blood $^{13}$CO$_2$ enrichment, it was ensured that air entered neither the blood drawing nor the collecting tube during the blood transfer. Samples of 0.5 ml were transferred from the collection syringe into a 3-ml sodium-heparin-coated, capped, evacuated tube and were maintained at room temperature. Samples were then processed as previously described (38). For the plasma isotopic enrichment of the methionine tracers given and of derived homocysteine, blood samples were kept on ice immediately centrifuged, and plasma was then withdrawn and stored at −80°C until used for analysis.

**Measurement of CO$_2$ Production Rates**

In the mechanically ventilated patients, the rates of CO$_2$ production ($\dot{V}CO_2$; ml/min) were measured with a respiratory monitor (Cosmo; Respironics, Wallingworth, CT). The device was calibrated and directly connected to the endotracheal tube. Continuous measurements were obtained during the tracer study, and the average value was recorded. This respiratory monitor is routinely used as per standard clinical practice in ventilated patients to adequately monitor pulmonary function. For the spontaneously breathing patients, $\dot{V}CO_2$ was determined by indirect calorimetry using a Vmax Encore (Vyassis Healthcare, Yorbalinda CA), calorimeter, connected to a plastic canopy, which was placed over the head and chest of the patients. None had air leaks through the chest tubes.

**Analytical Methods**

Analyses of blood samples for $^{13}$CO$_2$ enrichment were all conducted as previously described (38). In brief, the carbon dioxide was liberated from the blood bicarbonate by adding 2 ml of 85% (vol/vol) phosphoric acid into the evacuated tube and the contents vortexed. The evacuated tube was then backfilled with nitrogen to bring it to atmospheric pressure and let stand overnight. The liberated carbon dioxide was transferred to a plain, non-silicon-coated 15-ml venoject tube, which was subsequently backfilled with nitrogen to bring it to atmospheric pressure. The $^{13}$CO$_2$ enrichment was then measured by isotope ratio mass spectrometry (ThermoQuest Finnigan Delta mass XL Isotope Ratio Mass Spectrometer coupled with Gasbench II; Bremen, Germany; Ref. 38). The plasma enrichment of the methionine tracers given was determined as previously described (25). In brief, the plasma methionine and homocysteine isotopic enrichments were measured by tandem liquid chromatography-mass spectrometry. Plasma methionine and homocysteine were converted to their 5-(dimethylamino)-1-naphthalene sulfonamide derivatives and analyzed on a triple quadrupole mass spectrometer (TSQ Quantum Ultra; Thermo Fisher Scientific, San Jose, CA), equipped with an electrospray ionization source, a Survey pump (Thermo Fisher Scientific), and an HTC PAL autosampler (Leap Technologies, Carrboro, NC). The ions were then analyzed by selected reaction monitoring mode. The transitions observed were precursor ions m/z 383, 384, and 386 to product ion m/z 170 for methionine and precursor ion m/z 426 and 427 to product ion m/z 170 for homocysteine. Instrumental control, data acquisition and analysis were done by XCalibur (version 2.0) software package (Thermo Fisher Scientific).

**Calculations**

**Plasma fluxes.** We used two models to estimate plasma fluxes, splanchnic uptake and oxidation (transferrulation) of methionine. In the first model, we used plasma methionine enrichment as the precursor. In the second model, the plasma enrichment of $[^{13}$C]homocysteine intracellularly formed from transmethylation of the L-$[^{13}$C]methionine infused was used. This is the intracellular model. The methyl group of L-$[^{3}$H$_3$]methylmethionine is lost during transmethylation; therefore, intracellular homocysteine enrichment derived from $[^{3}$H$_3$]methylmethionine tracer is estimated from the ratio of L-$[^{13}$C]homocysteine to L-$[^{13}$C]methionine ($^{13}$Chcy/$^{13}$Cmeth), as previously described (25). Hence, all calculations were obtained

**Table 2. Enteral nutritional support**

<table>
<thead>
<tr>
<th></th>
<th>kcal·kg$^{-1}$·day$^{-1}$</th>
<th>Glucose, g·kg$^{-1}$·day$^{-1}$</th>
<th>Protein, g·kg$^{-1}$·day$^{-1}$</th>
<th>Lipids, g·kg$^{-1}$·day$^{-1}$</th>
<th>Methionine, mg·kg$^{-1}$·day$^{-1}$</th>
<th>Cysteine, mg·kg$^{-1}$·day$^{-1}$</th>
<th>Total Sulfur Amino Acid Intake, mg·kg$^{-1}$·day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1 yr ($n = 8$)</td>
<td>87.6±28</td>
<td>9.3±3.4</td>
<td>2.3±1.0</td>
<td>4.4±1.5</td>
<td>51.1±34.8</td>
<td>19.9±12.4</td>
<td>70.9±40.0</td>
</tr>
<tr>
<td>&gt;1–3 yr ($n = 8$)</td>
<td>66.1±9.7</td>
<td>6.8±2.0</td>
<td>2.0±0.3</td>
<td>2.5±0.4</td>
<td>55.2±8.0</td>
<td>17.2±2.6</td>
<td>72.3±8.2</td>
</tr>
<tr>
<td>13–18 yr ($n = 8$)</td>
<td>37.1±15.5</td>
<td>4.0±1.8</td>
<td>1.4±0.6</td>
<td>1.4±1.0</td>
<td>39.2±16.2</td>
<td>11.0±3.9</td>
<td>50.1±19.9</td>
</tr>
</tbody>
</table>

All values are means ± SD.
with the plasma methionine and homocysteine enrichments (plasma and intracellular models).

Plasma methionine fluxes obtained with the enteral L-[13C]methionine or the intravenous L-[3H]methylmethionine tracers were calculated using the steady-state isotope dilution equation and a simplified single-pool model (27) as follows:

\[ R_a (\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = I \times [(E_E/E_P) - 1] \] (1)

where \( R_a \) is the rate of appearance, \( I \) is the rate of enteral \([13C]\)methionine or intravenous \([3H]\)methylmethionine tracers infused \((\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\), \( E_E \) is the enrichment of the labeled methionine tracer (99%), and \( E_P \) is the mean plasma isotopic enrichment of the \([13C]\)methionine for the plasma model or \([3H]\)homocysteine for the intracellular model. For the intracellular model of the \([3H]\)methylmethionine tracer, the \([4H]\)methylmethionine tracer was used for the plasma model.

Splanchnic uptake. The splanchnic uptake of dietary methionine was estimated, as previously described (9). In brief, the plasma amino acid fluxes obtained with the enteral and intravenously administered tracers and the fraction of labeled tracer taken up in a first pass through the splanchnic area before reaching the systemic circulation can be determined as follows (9):

\[ R_a (3H)\text{methylmeth} = I_V \times [(E_{EV}/E_{PV}) - 1] \] (2)

where the rate of appearance or plasma flux form the intravenous \([3H]\)methylmethionine tracer is obtained as in Eq. 1.

The rate of appearance or plasma flux from the enteral \([13C]\)methionine tracer \((R_a \([13C]\text{meth})\) is determined from the enteral tracer’s plasma enrichment also as in Eq. 1:

\[ R_a \([13C]\text{meth}) = I_E \times [(E_{EV}/E_{PV}) - 1] \] (3)

Because an unknown fraction \((f)\) of the enteral tracer’s plasma fraction may be taken up by the first pass through the splanchnic tissues before reaching the systemic plasma pool, the actual enteral tracer infusion rate into the plasma is \(IE \times (1 - f)\). Hence, \(IE \times (1 - f)\) is the actual rate of isotopic tracer infusion into the sampled plasma compartment, and the true plasma methionine flux as estimated from the enteral tracer is:

\[ R_a \([13C]\text{meth}) = I_E \times (1 - f) \times [(E_{EV}/E_{PV}) - 1] \] (4)

For estimates of the first pass disappearance of methionine within the splanchnic region, the following relationship stands:

\[ I_V \times [(E_{EV}/E_{PV}) - 1] = I_E \times (1 - f) \times [(E_{EV}/E_{PV}) - 1] \] (5)

Rearranging and solving for \((1 - f)\)

\[ (1 - f) = \frac{I_V \times [(E_{EV}/E_{PV}) - 1]}{I_E \times [(E_{EV}/E_{PV}) - 1]} \] (6)

Therefore, the fraction of labeled tracer taken up during the first pass through the splanchnic tissues before reaching the systemic circulation can be solved as the ratio of the intravenous and enteral plasma methionine fluxes.

\[ (1 - f) = R_a \frac{I_V \times (3H)\text{methylmeth}}{R_a \times E \times (13C)\text{meth}}} \] (7)

and by rearrangement

\[ f = 1 - \frac{R_a \times I_V \times (3H)\text{methylmeth}}{R_a \times E \times (13C)\text{meth}}} \] (8)

where, the unknown fraction \(f\) 1 minus the ratio of plasma flux of the intravenous tracer over the plasma flux of the enteral administered tracer. The fraction of the tracer taken up in a first pass should be the same as the fraction of unlabeled dietary amino acid that is taken up in a first pass through splanchnic tissues. Therefore, the first pass disappearance of dietary methionine may be estimated by multiplying dietary methionine intake by the fraction of tracer taken up during the first pass.

Splanchnic methionine oxidation (transulfuration of homocysteine). The rate of \(13CO_2\) appearance from methionine oxidation (Meth ox: \(\mu\)mol-kg\(^{-1}\)-h\(^{-1}\)) corresponds to irreversible methionine loss through transulfuration (TS). Hence, TS has been assumed to be equivalent to methionine oxidation (25, 31). For determination of methionine oxidation in critically ill children using the enteral L-\([13C]\)methionine tracer, it was necessary to establish a factor to be used for the retention of \(13CO_2\) liberated from enteral supplied methionine, during its oxidative decarboxylation via \(\alpha\)-ketobutyrate (19, 37). For this purpose, we used previous data by our group obtained in enterally fed critically ill children receiving a primed, constant infusion of NaH\(^{13C}\)O\(_3\) that showed bicarbonate recovery of 69.80% (38). Although the bicarbonate tracer in the latter studies was provided by the parenteral route (38), there is no difference on bicarbonate recovery when the tracer is given through the enteral vs. the parenteral routes (34). Therefore the mean value of 69.80 was used to estimate methionine oxidation in the patients. Methionine oxidation (TS) was then calculated as follows:

\[ \text{meth ox} = TS = (V\text{CO}_2 \times 13CO_2) = (V\text{CO}_2/69.80)/E_p \] (9)

where methionine oxidation \((\mu\)mol-kg\(^{-1}\)-h\(^{-1}\)) is determined by \(V\text{CO}_2\) measured in milliliters per minute, obtained by using a respiratory monitor or indirect calorimetry and converted to millimoles per hour by multiplying by 60 min and dividing by 22.4, which is the number of \(I\) in 1 mole of an ideal gas at standard temperature and pressure to convert milliliters to millimoles per minute; \(V\text{CO}_2\) is obtained by multiplying \(V\text{CO}_2\) by blood \(13CO_2\) enrichment from the \([13C]\)methionine tracer infused and corrected by 69.80, the bicarbonate retention factor obtained in critically ill children enterally fed (38), and \(E_p\) is the plasma enrichment of methionine for the plasma model or enrichment of homocysteine for the intracellular model.

Methionine balance. The methionine balance (mg·kg\(^{-1}\)-day\(^{-1}\)) was estimated as follows:

\[ \text{methionine balance} = \text{meth intake} - \text{meth ox} \] (10)

where intake is estimated from dietary methionine and cysteine over the 24-h period and the intake of tracer infused. Cysteine is accounted for because it spares methionine (7, 14, 19, 31). The methionine tracer contribution, although minimal, was also estimated in the intake.

Statistical Analyses

Continuous variables were tested for normality using the Kolmogorov-Smirnov goodness-of-fit statistic, and no significant departures were found, indicating that means ± SD are appropriate descriptive statistics. Two-way ANOVA was used to assess differences between the plasma and intracellular models for enrichment, fluxes, splanchnic uptake and oxidation within each age group and the \([4H]\text{Chcy}/[13C]\text{meth}\) ratio, plasma fluxes, and splanchnic uptake oxidative and nonoxidative disposal between groups (infants, children and adolescents). A mixed-model ANOVA approach was applied to maximize statistical power and to properly handle the within-subject correlation, since each patient had measurements obtained from both plasma and intracellular models. Two-tailed \(P < 0.05\) was considered statistically significant with Bonferroni correction to adjust for multiple comparisons and protect against false positive results due to multiple testing. Statistical analysis was performed using the SPSS software package (version 16.0, SPSS, Chicago, IL).

RESULTS

As shown in Table 3 the plasma isotopic enrichment in the critically ill infant group was 0.057 ± 0.001 for \([13C]\)methionine (plasma) and 0.056 ± 0.01 for \([13C]\)homocysteine (intracellular), and there was no difference \((P = 0.77)\) between the
plasma and the intracellular models. In contrast, in the critically ill children there was a significant difference (P < 0.05) between [13C]methionine and [13C]homocysteine enrichment, with values of 0.087 ± 0.02 and 0.067 ± 0.01, respectively. Likewise, the plasma and intracellular enrichment models were different (P < 0.01) in the critically ill adolescent group with values of 0.11 ± 0.01 and 0.073 ± 0.01, respectively, for [13C]methionine and [13C]homocysteine. Hence, the ratio of [13C]homocysteine to [13C]methionine (hcy/meth) was 1.0 ± 0.15, 0.80 ± 0.20, and 0.66 ± 0.10, respectively, for the infants, children, and adolescents, and it was different between the infants and adolescents (P < 0.01) but not between the infants and children or the children and adolescents. The plasma isotopic enrichments for the intravenously infused L-[13C]methionine and blood 13CO2 enrichments are also shown in Table 3. Plateau isotopic enrichment was achieved in the three patient groups, but a difference was observed again between the plasma and intracellular models in the children (P < 0.05) and adolescent (P < 0.05) groups. From these enrichments, the rates of appearance of methionine obtained with both tracers were estimated.

The rates of appearance or plasma methyl methionine and [13C]methionine obtained with the plasma and the intracellular models for the three patient groups are shown in Fig. 2, A and B. In the infant group, there was no difference (P > 0.5) in the [13C]carbon methionine fluxes between the plasma and the intracellular models; both yielded rates of appearance of 85.4 ± 18 and 86.0 ± 14 μmol·kg⁻¹·h⁻¹ (means ± SD), suggesting that there was no significant intracellular dilution of the [13C]methionine label. In contrast, in the children group the plasma [13C]methionine rates of appearance were 54.4 ± 12 and 69.7 ± 5 μmol·kg⁻¹·h⁻¹, respectively, and significantly different (P < 0.05) for the plasma and the intracellular models. Likewise in the adolescent group rates of appearance were 39.5 ± 3 vs. 63.7 ± 8 μmol·kg⁻¹·h⁻¹, respectively, for the plasma and intracellular models and significantly different (P < 0.001) among the models. The plasma fluxes obtained with homocysteine as the precursor were 22 and 38% higher, respectively, for children and adolescents, indicating a significant intracellular dilution of methionine enrichment in these age groups. The plasma [13C]methionine fluxes obtained with either model were higher in the infants compared with children (P < 0.05) and adolescents (P < 0.001).

The methionine rates of appearance obtained with the intravenous [13C]methionine tracer were 29.7 ± 8.3 and 30.3 ± 8.2; 28.7 ± 8.5 and 38.7 ± 14; and 25.5 ± 6 and 40.5 ± 10 μmol·kg⁻¹·h⁻¹, respectively, for the plasma and intracellular models in the infants, children, and adolescents. While in the infant group there was no difference in the methyl fluxes.
obtained with either model, in the children and adolescent groups the methylmethylionine fluxes obtained with the intracellular model yield 25.6 (P < 0.05) and 37% (P < 0.01) higher values, respectively.

We did not infuse intravenous [13C]methionine in these patients; thus for the intracellular model of the intravenously infused [1H3]methylmethylionine, we used the 13Chcy/13Cmethyl ratio obtained with the enteral [13C]methionine tracer, as previously described (25). We cannot determine if the 13Chcy/13Cmethyl ratio is similar when the tracers are given by the intravenous (25) vs. the enteral route in critically ill children. However, studies in the piglet model (33) suggest that the 13Chcy/13Cmethyl ratio is 0.738 when the tracers are given intravenously and 0.795 when given intragastrically, and therefore they appear to be comparable.

The methylmethylionine splanchnic uptake for the three groups using both models and estimated as described in MATERIALS AND METHODS is shown in Fig. 3. Both models yield the same fractional first-pass disappearance of methylionine. Interestingly, the infant group had the highest fractional methylmethylionine splanchnic uptake of 0.63 ± 0.14 compared with values of 0.45 ± 0.19 and 0.36 ± 0.13 for children (P < 0.01) and adolescents (P < 0.001), respectively.

The absolute first-pass disappearance or dietary methylmethylionine splanchnic uptake in the infant group was 9.4 μmol·kg⁻¹·h⁻¹ and highly variable due to the different methylionine content in the infant formulas used, whereas in the children and adolescents the dietary methylmethylionine splanchnic uptake was ∼6.7 and 3.6 μmol·kg⁻¹·h⁻¹, respectively.

Table 3 also summarizes the results for methylionine oxidation (transulfuration), the blood 13CO₂ enrichment (atom percent excess × 10⁻³), and the rates of 13CO₂ production (V13CO₂; μmol·kg⁻¹·h⁻¹). In the infant group, the rates of methylionine oxidation (transulfuration) were −23.5 μmol·kg⁻¹·h⁻¹ with either the plasma or intracellular models and not different from each other. In addition, the plasma model yielded comparable values of methylionine oxidation among infants, children, and adolescents. However, the plasma model compared with the intracellular model underestimated methylionine oxidation rates by 32% (P < 0.05) in the children and by 44% (P < 0.01) in the adolescents. Hence, the model used had an impact on the children and adolescents but not on the infant group. Furthermore, although there was not statistical difference in the rates of methylionine oxidation obtained with the intracellular homocysteine model between the infants, children, and adolescents (P = 0.077), biologically there was a difference in methylionine oxidation rates of 16 μmol·kg⁻¹·h⁻¹ between the infants and adolescents.

The fraction of plasma methylionine flux utilization for oxidative (TS) and nonoxidative disposal and the proportion of plasma methylionine flux originating from protein breakdown are shown in Fig. 4. The pattern of enteral methylionine utilization was different in the infants compared with the adolescents. In the infants, 26.7 ± 9.8% of plasma methylionine flux was utilized for oxidative disposal (transulfuration) and 73.3 ± 9.8% for nonoxidative disposal (P < 0.01). In contrast, in the adolescents 60.5 ± 21.2% was utilized for oxidative disposal and 39.5 ± 16.6% for nonoxidative disposal, and there were intermediate values of 40.7 ± 16.6 and 59.3 ± 16.6%, respectively, for the fraction of plasma methylionine flux utilization for oxidative and nonoxidative disposal in the children. There was no difference in the pattern of methylionine flux utilization among the children and adolescents, or children and infants. However, there was a significant difference in the pattern of methylionine utilization between infants and adolescents, with a greater proportion of methylionine flux utilized for nonoxidative disposal in the infants compared with adolescents (P < 0.01) and conversely, greater utilization for oxidative disposal in adolescents compared with infants (P < 0.01). Most of the plasma methylionine flux originated from protein breakdown in all age groups despite that they were receiving standard enteral nutritional intake.

The methylionine balance for the three age groups is shown in Fig. 5. All groups were in negative balance with significant differences observed between infants and adolescents (P < 0.01) and infants and children (P < 0.01).

Plasma amino acids and glutathione concentrations obtained in our patients and compared with reference values (22, 28) are shown in Table 4. Only the values that were abnormal or close to the abnormal range are reported. Among the essential amino acids, histidine and tryptophan were lower in all age groups and methylionine appeared to be increased in the children and adolescents. For the nonessential amino acids, cysteine appeared to be lower in all groups. Glutamine, glycine, and serine appeared to be in the lower range only in the adolescent group. The children and adolescents appear to have lower taurine values. All other nonreported amino acid values were within normal range. Plasma homocysteine appeared to be within normal range and plasma glutathione appeared to be lower in all groups compared with reference values. However, plasma glutathione has limited value because the major pool of glutathione is intracellular.

DISCUSSION

We investigated in vivo aspects of methylionine metabolism in three different age groups of critically ill children. We used the [13C]- and [1H3]methylmethylionine tracers by the enteral and parenteral routes, respectively. We acknowledge that both tracers have different metabolic fates, and given that they were not simultaneously administered by the same route, the splanchnic rates of transmethylation and remethylation could not be determined. It would have been difficult to obtain a second tracer study day using the alternate route of tracer administration (intravenous [13C]methylmethylionine and enteral...
production of glutathione, taurine, and H2S (36) or remethyl-
 homocysteine, which may be transulfurated to cysteine with
 betaine or methyltetrahydrofolate as donors (18, 36).

Increased transmethylation rates will render
 is transmethylation to homocysteine and transulfuration to
 piglet model that the methionine main metabolic fate in the gut
 these critically ill patients. It has been demonstrated in the
 observations suggest that in critically ill infants there is a
 greater utilization of dietary methionine in the splanchnic
 tissues of sick infants compared with sick adolescents and that
 the rates of splanchnic methionine utilization appear to
decrease in the more mature individuals. Greater utilization rates
 of methionine in the splanchnic tissues of infants may be due
 to increased demand for synthesis of methionine substrates in
 these critically ill patients. It has been demonstrated in the
 piglet model that the methionine main metabolic fate in the gut
 is transmethylation to homocysteine and transulfuration to
 cysteine (33). Increased transmethylation rates will render
 methyl groups available for the synthesis of polyamines, cre-
tate, and DNA methylation and will increase production of
 homocysteine, which may be transulfurated to cysteine with
 production of glutathione, taurine, and H2S (36) or remethyl-
 ated to methionine for further availability of methyl groups,
 with betaine or methyltetrahydrofolate as donors (18, 36).

The intestine is a highly active metabolic tissue, and due to
its high proliferation rate, the intestinal and colonic mucosa
have a special demand for polyamines (24). These low molecu-
lar weight cations have major regulatory roles on tissue
growth and differentiation. Additionally, the use of broad
spectrum antibiotics will result in changes in intestinal flora,
which is an important contributor to intestinal polyamines. The
lack or limited polyamines, creatine, and choline content in the
formulas supplied to critically ill patients may also impose a
greater demand in the splanchnic tissues for the synthesis of
these compounds (4, 8, 18, 23) and, therefore, the need for
greater availability of methyl groups.

Interestingly, the elevated need for methionine precursor in
the splanchnic tissues appears to decrease at later develop-
tal stages, as demonstrated by the lower rates of methionine
splanchnic uptake in adolescents. Hence, there appears to be
ontogeny of methionine splanchnic uptake. The expression of
amino acid transporter systems (5, 6) and spatiotemporal pat-
terns of enzymatic expression may explain the ontogeny ob-
erved for amino acids and nutrients in the developing mammal
(32, 35).

In the infant population, there was no difference between
the enrichments of 13C methionine and [13C]homocysteine and,
therefore, no difference in the plasma fluxes obtained with the
plasma or the intracellular models, suggesting that there was
minimal or no intracellular dilution of the [13C]methionine
tracer enterally infused. In addition, there was no difference
in the rates of transulfuration (methionine oxidation) ob-
tained with either model, and ~27% of the plasma methio-
nine flux was used for transulfuration to cysteine, while 73% was
used for nonoxidative disposal, which involves the
synthesis of protein and methionine derived compounds. How-
ever, the absolute rates of methionine transulfuration of ~23
µmol·kg−1·h−1 were significantly higher than the dietary methi-
nine splanchnic uptake of 9.4 µmol·kg−1·h−1, indicating that
dietary methionine is insufficient to maintain transulfuration rates,
and, therefore, plasma methionine from protein breakdown has
to be utilized under these conditions. This is confirmed by the
fact that ~89% of the [13C]methionine flux originates from
protein breakdown and that these critically ill infants are in a
negative methionine balance of about −11.4 mg·kg−1·day−1.

The fact that the enrichments obtained with the intracellular
homocysteine and the plasma methionine models do not differ
in the infant population, despite significant protein breakdown
and expected intracellular dilution, suggests that there is major
compartmentation of methionine pools in the splanchnic
tissues in this age group. The mechanism(s) whereby this com-
partmentation occurs is beyond the scope of this investigation.
However, nuclear magnetic spectroscopy of subcellular frac-
tions in splanchnic tissues may help to better understand the
underlying mechanisms (21).

In contrast, in the children and adolescent patients there
were clear differences in methionine enrichments between the
plasma and the intracellular models, and the ratio of 13Chcy/13Cmeth
of 0.80 in the children and 0.66 in the adolescents
were closer to the values of 0.80 reported in healthy adult
women (12) and 0.58 reported in healthy men (25), suggesting
that there was significant intracellular dilution of labeled me-
thionine. Therefore, there was a marked difference in plasma
[13C]methionine fluxes in children and adolescents when ob-

Fig. 4. Percentage of splanchnic methionine flux (Q) utilized for nonoxidative
disposal (NOD), oxidative disposal [transulfuration (TS)], and methionine flux
derived from protein breakdown (PB) in critically ill infants, children, and
adolescents.

[3H3]methylmethionine) in these critically ill children. This
limits the scope of our results but does not impact on our
conclusions. Our data showed that the first pass disappearance
or splanchnic uptake of methionine was ~63% and signifi-
cantly higher in the critically ill infant population compared
with children and adolescents and almost double the value of
~33% estimated from various studies in healthy adults (7, 19,
31). There are no comparable values on methionine splanchnic
uptake in healthy children, infants, or adolescents, but our
observations suggest that in critically ill children there is a
greater utilization of dietary methionine in the splanchnic
tissues of sick infants compared with sick adolescents and that
the rates of splanchnic methionine utilization appear to
decrease in the more mature individuals. Greater utilization rates
of methionine in the splanchnic tissues of infants may be due
to increased demand for synthesis of methionine substrates in
these critically ill patients. It has been demonstrated in the
piglet model that the methionine main metabolic fate in the gut
is transmethylation to homocysteine and transulfuration to
cysteine (33). Increased transmethylation rates will render
methyl groups available for the synthesis of polyamines, cre-
tate, and DNA methylation and will increase production of
homocysteine, which may be transulfurated to cysteine with
production of glutathione, taurine, and H2S (36) or remethyl-
ated to methionine for further availability of methyl groups,
with betaine or methyltetrahydrofolate as donors (18, 36).

The intestine is a highly active metabolic tissue, and due to
its high proliferation rate, the intestinal and colonic mucosa
have a special demand for polyamines (24). These low molecu-
lar weight cations have major regulatory roles on tissue
growth and differentiation. Additionally, the use of broad
spectrum antibiotics will result in changes in intestinal flora,
which is an important contributor to intestinal polyamines. The
lack or limited polyamines, creatine, and choline content in the
formulas supplied to critically ill patients may also impose a
greater demand in the splanchnic tissues for the synthesis of
these compounds (4, 8, 18, 23) and, therefore, the need for
greater availability of methyl groups.

Interestingly, the elevated need for methionine precursor in
the splanchnic tissues appears to decrease at later develop-
mental stages, as demonstrated by the lower rates of methionine
splanchnic uptake in adolescents. Hence, there appears to be
ontogeny of methionine splanchnic uptake. The expression of
amino acid transporter systems (5, 6) and spatiotemporal pat-
terns of enzymatic expression may explain the ontogeny ob-
erved for amino acids and nutrients in the developing mammal
(32, 35).

In the infant population, there was no difference between
the enrichments of 13C methionine and [13C]homocysteine and,
therefore, no difference in the plasma fluxes obtained with the
plasma or the intracellular models, suggesting that there was
minimal or no intracellular dilution of the [13C]methionine
tracer enterally infused. In addition, there was no difference
in the rates of transulfuration (methionine oxidation) ob-
tained with either model, and ~27% of the plasma methio-
nine flux was used for transulfuration to cysteine, while 73% was
used for nonoxidative disposal, which involves the
synthesis of protein and methionine derived compounds. How-
ever, the absolute rates of methionine transulfuration of ~23
µmol·kg−1·h−1 were significantly higher than the dietary methi-
nine splanchnic uptake of 9.4 µmol·kg−1·h−1, indicating that
dietary methionine is insufficient to maintain transulfuration rates,
and, therefore, plasma methionine from protein breakdown has
to be utilized under these conditions. This is confirmed by the
fact that ~89% of the [13C]methionine flux originates from
protein breakdown and that these critically ill infants are in a
negative methionine balance of about −11.4 mg·kg−1·day−1.

The fact that the enrichments obtained with the intracellular
homocysteine and the plasma methionine models do not differ
in the infant population, despite significant protein breakdown
and expected intracellular dilution, suggests that there is major
compartmentation of methionine pools in the splanchnic
tissues in this age group. The mechanism(s) whereby this com-
partmentation occurs is beyond the scope of this investigation.
However, nuclear magnetic spectroscopy of subcellular frac-
tions in splanchnic tissues may help to better understand the
underlying mechanisms (21).

In contrast, in the children and adolescent patients there
were clear differences in methionine enrichments between the
plasma and the intracellular models, and the ratio of 13Chcy/13Cmeth
of 0.80 in the children and 0.66 in the adolescents
were closer to the values of 0.80 reported in healthy adult
women (12) and 0.58 reported in healthy men (25), suggesting
that there was significant intracellular dilution of labeled me-
thionine. Therefore, there was a marked difference in plasma
[13C]methionine fluxes in children and adolescents when ob-

Fig. 5. Methionine balance in critically ill infants, children, and adolescents.
tained with the intracellular vs. the plasma model. Likewise, the rates of methionine oxidation (transulfuration) obtained with the oral [13C]methionine tracer were 31 and 43% higher, respectively, in children and adolescents when the intracellular model was used. Therefore, the plasma model underestimated in vivo, whole body oxidation rates of methionine when the tracer was given by the enteral route.

Irrespective of the model used, the rates of methionine transulfuration (oxidation) in the infants, children, and adolescents were higher than the dietary sulfur (methionine and cysteine) amino acid intake. In addition, the fraction of methionine flux utilized for methionine transulfuration, and therefore irreversible carbon losses, significantly increased with age, from 27% in the infants to 61% in the adolescents. Conversely, the infants utilized a higher proportion of methionine for nonoxidative disposal.

Taken together, these data suggest that the metabolic fate and utilization of methionine differ in the critically ill pediatric population depending on age and that there is a greater contribution of plasma methionine for transulfuration in the adolescents, which may reflect a greater need in this age group for transulfuration substrates, such as cysteine, glutathione, taurine, and H2S, while there is a lesser utilization for methylated substrates. Conversely, in the young infants the opposite holds true and the group of children had intermediate values. Our infant population was 6 mo of age in average, fluctuating from 1 to 11 mo, but provided that the same physiological processes take place in the younger newborn and premature infant populations, the decreased methionine transulfuration and therefore limited glutathione availability in young infants may contribute to make young infants more susceptible to oxidative injury and necrotizing enterocolitis, which is seldom observed in older children.

It is known from animal data that nutrition and age influence enzymatic capacity and therefore metabolic pathways. Finkelstein (17) found in the rat model that the hepatic content of the methionine catabolizing enzymes methionine adenosyltransferase III (EC 2.5.1.6), cystathionine β-synthase (EC4.2.1.22; CBA), and γ-cystathionase (EC 4.4.1.1.) increase with age and in response to higher dietary protein or methionine, or both.

It is also known that transulfuration flux is increased by oxidative stress, whereas antioxidants decrease it (36). However, there was no difference in the severity of disease or in the acid base status among our patients, although this could have been limited by our sample size. The redox regulation of the TS pathway may occur at the level of cystathionine β-synthase, which contains a heme group that may serve as a sensor of the oxidative environment (1, 3). There is a tissue-specific metabolic distribution of methionine cycle enzymes (17, 18), and the liver, kidney, small intestine, and pancreas contain both cystathionine β-synthase and γ-cystathionase, key enzymes in the transulfuration pathway, which produces glutathione. Hence, the splanchnic tissues in mature animals, in addition to the kidney, have the most rapid turnover of glutathione (26). Furthermore, it has been shown that hypoxia may affect remethylation by altering methionine adenosyl transferase expression (2) and that these changes are comparable to those of a methyl-deficient diet (11). However, none of our patients were hypoxic during the study or in the immediate preceding days.

Regardless of the rates and fate of methionine utilization, these critically ill patients were in significantly negative balance despite receiving complete enteral feedings as per current clinical standards, demonstrating that our understanding of enteral nutritional support in critically ill patients is rather limited.

The oxidative irreversible loss of carbon moiety during transulfuration is consistent with a lesser efficiency in methionine utilization of critically ill children and adolescents and probably a greater dietary need for sulfur amino acids. On the contrary, in young infants there is a greater utilization of methionine for nonoxidative disposal and, therefore, synthesis of protein and methylated compounds, which may impose a greater need for neomethylation, and therefore folate, betaine, and vitamin B12. These observations are supported by the significantly negative methionine balance observed in the adolescents compared with the infants and children. Specific measurements of transmethylation and remethylation in the splanchnic tissues of critically ill children remain to be determined.

Jahoor et al. (20) estimated methionine kinetics in malnourished, edematous and nonedematous children, slightly older than our infant population, using intravenous tracers. Although the malnourished population is physiologically different from the critically ill population, these authors

<table>
<thead>
<tr>
<th>Essential</th>
<th>Infants</th>
<th>Children</th>
<th>Adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>13.4 ± 3.2 (61–91)</td>
<td>19.4 ± 10.8 (61–91)</td>
<td>18.6 ± 7.0 (77–107)</td>
</tr>
<tr>
<td>Methionine</td>
<td>40.5 ± 7.8 (14–38)</td>
<td>47.5 ± 13.2 (13–22)</td>
<td>42.9 ± 2.2 (20–34)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>21.8 ± 10.5 (34–73)</td>
<td>23.0 ± 11.9 (35–73)</td>
<td>29.5 ± 13.3 (54–93)</td>
</tr>
</tbody>
</table>

Values are means ± SD in micromoles. Nonreported amino acids were within normal range. Reference values in parenthesis are expressed as 10th–90th quantile from Ref. 22. *Values from Ref. 28.
found that methionine transulfuration was maintained despite slower methionine turnover, and as a consequence, less methionine was available for protein synthesis and synthesis of methionine-derived compounds. It is important to underscore that methionine metabolism occurs at the intracellular level and therefore the homocysteine intracellular model is more accurate, while the plasma model understimates methionine kinetics in populations other than young infants.

In summary, we investigated for the first time methionine splanchnic uptake in critically ill infants, children, and adolescents using the plasma and intracellular kinetic models, and we found that there is ontogeny on splanchnic uptake and methionine utilization in critically ill children, characterized by a significant compartmentation of methionine metabolism in young infants, with a greater utilization of methionine for nonoxidative disposal, while in the adolescents methionine splanchnic uptake was lower and there was greater methionine utilization for transulfuration. The methionine balance was negative in all age groups, indicating that current sulfur amino acid intakes provided to critically ill pediatric patients as per current standard care are insufficient to maintain sulfur amino acid balance and probably functional needs. The plasma methionine enrichment model underestimated methionine kinetics in children and adolescents.

ACKNOWLEDGMENTS

We thank the patients and families at Texas Children’s Hospital for selfless contribution to these studies.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-62363, National Institute of Child Health and Human Development Grant T32 HD-007445, and Division of Research Resources Grant M01-RR-00188; USDA/ARS Cooperative Agreement and Kidney Diseases Grant DK-62363, National Institute of Child Health and Human Development.

DISCLOSURES

This work was supported by a research grant from the Amino Acid Research Program (3ARP; Ajinomoto).

REFERENCES


