Methionine requirement of the enterally fed term infant in the first month of life in the presence of cysteine

Lisha Huang, Jacomine E Hogewind-Schoonenboom, Mariska JA van Dongen, Femke de Groof, Gardi J Voortman, Henk Schierbeek, Jos WR Twisk, Andras Vermes, Chao Chen, Ying Huang, and Johannes B van Goudoever

ABSTRACT

Background: The essential amino acid methionine can be used for protein synthesis but also serves as a precursor for homocysteine and cysteine.

Objective: The objective of this study was to determine the minimal obligatory methionine requirement of infants in the presence of excess cysteine (91 mg·kg⁻¹·d⁻¹) by using the indicator amino acid oxidation (IAAO) method with L-[1-¹³C]phenylalanine as the indicator.

Design: Fully enterally fed term infants <1 mo of age were randomly assigned to methionine intakes that ranged from 3 to 59 mg·kg⁻¹·d⁻¹ as part of an elemental formula. After 1 d of adaptation to the test diet, [¹³C]bicarbonate and L-[1-¹³C]phenylalanine tracers were given enterally. Breath samples were collected at baseline and during isotopic plateaus. The mean methionine requirement was determined by using bisectional regression crossover analysis on the fraction of [¹³C]CO₂ recovery from L-[1-¹³C]phenylalanine oxidation (F[¹³CO₂]). Data are presented as means ± SDs.

Results: Thirty-three neonates (gestational age: 39 ± 1 wk) were studied at 13 ± 6 d. With increasing methionine intakes, F[¹³CO₂] decreased until a methionine intake of 38 mg·kg⁻¹·d⁻¹; additional increases in methionine intake did not affect F[¹³CO₂]. The mean methionine requirement was determined at 38 mg·kg⁻¹·d⁻¹, and the upper and lower CIs were 48 and 27 mg·kg⁻¹·d⁻¹, respectively (P < 0.0001, r² = 0.59).

Conclusions: Although the current recommended methionine intake of 28 mg·kg⁻¹·d⁻¹ is within the CIs of our study, the estimated mean requirement is substantially higher. However, most of the infant formulas provide a methionine intake of 49–80 mg·kg⁻¹·d⁻¹, which is above the upper CI of our study. This trial was registered at www.trialregister.nl as NTR1610.

INTRODUCTION

Methionine is an essential amino acid required for protein synthesis. It is also needed for the biosynthesis of carnitine, which is essential for fatty acid metabolism (1). Methionine is the major methyl donor in mammalian cells and a precursor for polyamine synthesis (2). The transmethylation of methionine leads to homocysteine synthesis. Homocysteine can be remethylated to form methionine or catabolized via the transsulfuration pathway to form cysteine. Cysteine can be incorporated into protein and is also involved in the production of glutathione, taurine, CoA, and inorganic sulfur. Cysteine, glutathione, and taurine play a role in the defense mechanism against oxidative stress.

A deficient intake of methionine not only impairs growth but has also an impact on the sulfur metabolic pathways in the synthesis of its key metabolic intermediates. In contrast, methionine is known as the most toxic amino acid in animals when supplemented in excess (3, 4). Hypermethioninemia and hyperhomocysteinemia were observed in infants who consumed a methionine-fortified formula with a methionine content of 788 mg/L or a high-protein formula that provided 9 g protein·kg⁻¹·d⁻¹ (5, 6). Extreme hypermethioninemia may cause cerebral edema (5). Hyperhomocysteine has been shown to be associated with increased risk of neonatal stroke (7). Because both a deficient and excess intake of methionine have detrimental effects, it is important to determine the methionine requirement to optimize infant nutrition.

Experimental evidence of the methionine requirement of enterally fed infants is scarce. In previous studies with a relatively small number of infants (n = 7–13), the methionine requirement was estimated to be between 27 and 49 mg·kg⁻¹·d⁻¹ (8–11). Because breast milk is considered to be the optimal nutrition for infants ≤6 mo of age, the joint WHO/FAO/UNU expert consultation recommended a methionine intake of 28 mg·kg⁻¹·d⁻¹ on the basis of the average intake of breastfed infants (12). Human milk is known to vary in protein content, whereas the volume ingested also varies on a daily basis. These factors all contribute

1 From the Department of Pediatrics, Division of Neonatology, Medical Centre–Sophia Children’s Hospital, Rotterdam, Netherlands (LH, JEH-S, FdG, GJv, and HS); the Department of Pediatrics, Jeroen Bosch Hospital, ’s-Hertogenbosch, Netherlands (MJAvD); the Departments of Methodology and Applied Biostatistics (JWRT) and Pediatrics (JvBG), VU University Medical Centre, Amsterdam, Netherlands; the Hospital Pharmacy, Erasmus MC, Rotterdam, Netherlands (AV); the Department of Pediatrics, Division of Neonatology (CC) and the Department of Pediatrics, Division of Gastroenterology (YH), Children’s Hospital of Fudan University, Shanghai, China (YH); and the Department of Pediatrics, Academic Medical Centre, Emma Children’s Hospital, Amsterdam, Netherlands (JvBG).

2 Sponsored by Danone. Study formulas were manufactured by SHS UK, and transportation to Shanghai was facilitated by Dumex China. The study sponsors had no influence on the study design, the analysis of the data, or the writing of this manuscript.

3 Address correspondence to JB van Goudoever, Department of Pediatrics, Academic Medical Centre, Emma Children’s Hospital, 9 Meibergdreef, PO Box 22660, 1100 DD Amsterdam, Netherlands. E-mail: h.vangoudoever@amc.nl; or Y Huang, Department of Pediatrics, Division of Gastro-enterology, Children’s Hospital of Fudan University, 399 Wan Yuan Road, 201102 Shanghai, China. E-mail: yhuang815@163.com.

Received November 16, 2011. Accepted for publication February 29, 2012. First published online April 4, 2012; doi: 10.3945/ajcn.111.028779.
to the difficulty of providing an accurate estimation of the intake of a breastfed infant.

The indicator amino acid oxidation (IAAO)4 method is minimally invasive and, therefore, suitable for the determination of the essential amino acid requirements in vulnerable populations including infants (13, 14).

The aim of this study was to determine the minimal obligatory methionine requirement with an excess intake of cysteine of term infants by using the IAAO method.

SUBJECTS AND METHODS

Subjects

Thirty-three neonates admitted to the Neonatal Ward in the Children’s Hospital of Fudan University participated in the study. Each subject was selected for study by using the following criteria: fully enterally fed infants with a gestational age ≥37 wk, birth weight ≥2500 g, and clinically stable with a weight-gain rate ≥5 g · kg⁻¹ · d⁻¹ in the preceding 3 d. Subjects were excluded if they had congenital anomalies, gastrointestinal pathology, or sepsis.

The study was approved by the institutional review boards of the Children’s Hospital of Fudan University, and a statement of no objection was obtained from the Erasmus Medical Centre–Sophia Children’s Hospital. Written consent was obtained from at least one of the parents of each subject by a Chinese-speaking researcher.

Study formula

The study formula used was an elemental formula that was based on free amino acids. The amino acid, fat, carbohydrate, and energy content of the study formula are shown in Table 1. The composition was the same as Neocate formula (SHS International) except for the methionine, phenylalanine, and alanine content. Methionine, which was completely withdrawn from the study formula, was separately added in the form of L-methionine to obtain different amounts of intake. The formula provided a cysteine intake of 91 mg · kg⁻¹ · d⁻¹. This amount was considered to be in excess because it is ≥3 times the intake in a breastfed infant (12). This amount should have minimized the amount of methionine that was metabolized to cysteine via the transsulfuration pathway, which enabled us to determine the minimal obligatory methionine requirement. The phenylalanine intake was kept constant during the study by separately adding L-phenylalanine during the 24-h adaptation period to obtain the same amount as in the Neocate formula (SHS International), and this amount of phenylalanine was given as stable isotope L-[1-¹³C]phenylalanine on the tracer-infusion day. The phenylalanine intake during the study was 166 mg · kg⁻¹ · d⁻¹, which was above the recommended amount of 72 mg · kg⁻¹ · d⁻¹ (12). A generous amount of tyrosine (166 mg · kg⁻¹ · d⁻¹) was provided to ensure that the newly formed [¹³C]tyrosine hydroxylated into ¹³CO₂, which could be measured in expired air (15). This amount of tyrosine was almost twice the recommended intake (12). The nitrogen intake was kept constant for all subjects by the substitution of L-alanine for the methionine that was withdrawn. The caloric intake was kept constant during the study period in all infants. The minerals and trace elements supplied in 100 g formula were as follows—iron: 7.0 mg; calcium: 325 mg; phosphorus: 230 mg; magnesium: 34 mg; sodium: 120 mg; chloride: 290 mg; potassium: 420 mg; manganese: 0.38 mg; iodine: 47 µg; selenium: 11 µg; copper: 380 µg; and zinc: 5.0 mg.

The vitamin content of 100 g formula was as follows—vitamin A: 528 µg retinol equivalent; vitamin D: 8.5 µg; vitamin E: 3.3 mg α-tocopherol equivalent; vitamin K: 21 µg; thiamine: 390 µg; riboflavin: 600 µg; niacin: 4.5 mg; vitamin B-6: 520 µg; vitamin B-12: 1.3 µg; pantothenic acid: 2.3 mg; folic acid: 38 µg; vitamin C: 40 mg; and biotin: 26 µg.

Experimental design

The study design was based on the minimally invasive IAAO method (13), which was recently modified to apply in enterally fed infants (14). The advantages of this method are the short adaptation period to the test intake (1 d), the enterally delivered isotopes, and the sampling of expired air without sampling of the amino acid enrichments in plasma or urine. The IAAO method is based on the concept that, when the test amino acid intake is insufficient to meet the requirement, protein synthesis will be limited and all of the amino acids will be oxidized, including the indicator amino acid, which is labeled with a stable isotope. As the dietary intake of the

<table>
<thead>
<tr>
<th>Component</th>
<th>Per 100 g formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>475</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>54</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>23</td>
</tr>
<tr>
<td>Total amino acid (g)</td>
<td>13</td>
</tr>
<tr>
<td>l-Alanine (g)⁴</td>
<td>≥0.61</td>
</tr>
<tr>
<td>l-Arginine (g)</td>
<td>1.08</td>
</tr>
<tr>
<td>l-Asparagine (g)</td>
<td>1.01</td>
</tr>
<tr>
<td>l-Cysteine (g)</td>
<td>0.4</td>
</tr>
<tr>
<td>Glycine (g)</td>
<td>0.95</td>
</tr>
<tr>
<td>l-Histidine (g)</td>
<td>0.62</td>
</tr>
<tr>
<td>l-Isoleucine (g)</td>
<td>0.95</td>
</tr>
<tr>
<td>l-Leucine (g)</td>
<td>1.63</td>
</tr>
<tr>
<td>l-Lysine (g)</td>
<td>1.11</td>
</tr>
<tr>
<td>l-Methionine (g)⁵</td>
<td>0</td>
</tr>
<tr>
<td>l-Phenylalanine (g)⁶</td>
<td>0.20</td>
</tr>
<tr>
<td>l-Proline (g)</td>
<td>1.16</td>
</tr>
<tr>
<td>l-Serine (g)</td>
<td>0.71</td>
</tr>
<tr>
<td>l-Threonine (g)</td>
<td>0.8</td>
</tr>
<tr>
<td>l-Tryptophan (g)</td>
<td>0.32</td>
</tr>
<tr>
<td>l-Tyrosine (g)</td>
<td>0.73</td>
</tr>
<tr>
<td>l-Valine (g)</td>
<td>1.04</td>
</tr>
<tr>
<td>l-Carnitine (g)</td>
<td>0.01</td>
</tr>
<tr>
<td>Taurine (g)</td>
<td>0.03</td>
</tr>
<tr>
<td>l-Glutamine (g)</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Notes

1. Variable amounts of l-alanine were added to the diet depending on the test methionine amount of each infant to maintain an isonitrogenous diet. The study formula contained ≥0.61 g l-alanine/100 g formula.

2. l-Methionine was added separately depending on the test methionine amount.

3. A total of 0.53 g l-phenylalanine/100 g formula was added to the study diet on day 1. An equivalent amount of l-phenylalanine (0.52 g/100 g formula) was given as isotope on day 2.

4. Abbreviations used: APE, atom percentage excess; F ¹³CO₂, fraction of ¹³CO₂ recovery from l-[1-¹³C]phenylalanine oxidation; IAAO, indicator amino acid oxidation.

5. l-Methionine was added separately depending on the test methionine amount.
test amino acid increases, the oxidation rate of the indicator will decrease until the requirement of the test amino acid is met. Once the requirement of the test amino acid is met, an additional increase in its intake will have no additional influence on the oxidation rate of the indicator amino acid. The oxidation of the indicator amino acid can be measured in expired air as \(^{13}\text{C}_2\text{O}_2\).

During the study, all infants received a fluid intake of \(\sim 150\ \text{mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\), a caloric intake of \(108\ \text{kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\), and an amino acid intake equal to the protein intake of \(\sim 2.96\ \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\). Infants were randomized to one of the graded test intakes of methionine, which ranged from 3 to 59 mg \cdot \text{kg}^{-1} \cdot \text{d}^{-1}.\) To maximize the power of the breakpoint analysis used in our method, a wide range of methionine intakes with approximately equal number of intakes above and below the expected breakpoint was chosen (16). Because the methionine requirement was expected to approximate the methionine content in human milk, we studied an equal number of intakes above and below the expected requirement of \(28\ \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) (12).

Each study took place over a 31-h period, whereby each infant received one of the test intakes. After 24 h of consumption of the study formula, tracers were administered on day 2 for 7 h. In the adaptation period, the feeding regimen changed to an hourly bottle-feeding during the tracer infusion until the end of the study. On the tracer day, a nasogastric tube was placed for tracer infusion. Infants received a primed (14 mol/kg) continuous (9 mol \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) enteral infusion of \(^{13}\text{C}\)bicarbonate [sterile pyrogen free, 99% \(^{13}\text{C}\) atom percentage excess (APE; Cambridge Isotopes)] for 3 h to quantify individual \(^{13}\text{CO}_2\) production rates (17).

Phenylalanine was used as the indicator. After the \(^{13}\text{C}\)bicarbonate infusion was stopped, a primed (34 mol/kg) continuous (27 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\) enteral infusion of \(\text{L-}[1-^{13}\text{C}]\)phenylalanine (99% \(^{13}\text{C}\) APE; Cambridge Isotopes) was started and lasted for 4 h. Syringes were weighted before and after the study to determine the exact amount of tracers that were given to infants. The tracer infusion day is depicted in Figure 1.

**Sample collection and analysis**

Breath samples were obtained by using the direct nasopharyngeal sampling method described by van der Schoor et al (18). Briefly, a 6F gastric tube (6 CH Argyle; Sherwood Medical) was placed 1–1.5 cm into the nasopharynx, and the end-tidal breath was taken slowly with a syringe. Collected air was transferred into 12 mL sterile, nonsilicon-coated evacuated glass tubes (Van Loenen Instruments) and was stored at room temperature until analysis. Two duplicated baseline samples were obtained before the start of tracer infusion. Duplicated breath samples were obtained at 15-min intervals during an isotopic plateau of \(^{13}\text{C}\) bicarbonate between 105 and 180 min. Seven duplicated samples were obtained every 10 min during an isotopic plateau of \(\text{L-}[1-^{13}\text{C}]\)phenylalanine between 360 and 420 min (Figure 1).

\(^{13}\text{C}\) isotopic enrichment in breath samples was analyzed by using an infrared isotope analysis technique (Helifan; Analytic Fischer Instruments) (19). The \(^{13}\text{C}\) enrichment was expressed as the APE above baseline.

**Calculations**

The isotopic steady state was represented by a plateau in \(^{13}\text{CO}_2\). Plateaus were determined by using visual inspection and were confirmed by using regression analysis as a slope not significantly different from zero.

The estimated body \(^{13}\text{CO}_2\) production rate (mmol \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) was calculated as described previously (14, 17). The fraction of \(^{13}\text{CO}_2\) recovery from \(\text{L-}[1-^{13}\text{C}]\)phenylalanine oxidation (\(\text{F}^{13}\text{CO}_2\)) as a percentage was calculated by using the following equation (20):

\[
\text{F}^{13}\text{CO}_2 = \frac{\text{IE}_{\text{PHE}} \times i_b}{(\text{P}_{\text{PHE}} \times \text{IE}_B) \times 100\%}
\]

where \(\text{IE}_{\text{PHE}}\) is the \(^{13}\text{C}\) isotopic enrichment in expired air during \(\text{L-}[1-^{13}\text{C}]\)phenylalanine infusion (APE), \(i_b\) is the infusion rate of \(^{13}\text{C}\)bicarbonate (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\), \(i_{\text{PHE}}\) is the infusion rate of \(\text{L-}[1-^{13}\text{C}]\)phenylalanine (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\), and \(\text{IE}_B\) is the \(^{13}\text{C}\) isotopic enrichment in expired air during \(\text{L-}[1-^{13}\text{C}]\)bicarbonate infusion.

Phenylalanine flux was not obtained. As shown in our previous study, the test amino acid intake has no effect on the phenylalanine flux (14).

**Statistical analysis**

Descriptive data are expressed as means \(\pm\) SDs. Determination of the methionine requirement (ie, the breakpoint) was performed by using a biphasic linear regression crossover model (21). With the biphasic linear regression analysis, the regression equation was split into 2 parts. For the first part, an intercept and slope were estimated, whereas for the second part, the slope was restricted to zero. Therefore, the estimated intercept of the second line was equal to the breakpoint. The model with the best fit on the basis of the highest \(r^2\) was selected. The 95% CIs were calculated. \(P < 0.05\) was considered significant. Analyses were performed with STATA software (version 11; StataCorp LP).

The power analysis could not be performed. We aimed to study 20–35 infants, which was greater than the number of infants used in studies in parenterally fed infants by using the same approach with an intravenous administration of the tracer (22–24).

**RESULTS**

**Subject characteristics**

Thirty-three term neonates participated in the study. The neonates were studied at a methionine intake that ranged between 3 and 59 mg \cdot \text{kg}^{-1} \cdot \text{d}^{-1}. Subject characteristics are summarized in Table 2. All subjects were growing well before entering the study. The mean (\(\pm\)SD) weight gain rate 3 d before the study was \(13 \pm 5\ \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\). The mean (\(\pm\)SD) energy intake was \(109 \pm 1\ \text{kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\). The nitrogen intake was equivalent to a protein intake of \(3.0 \pm 0.1\ \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\). Infants were

---

**Figure 1.** Schematic overview of tracer infusion day. Arrows indicate times that breath samples were taken.
clinically stable and considered healthy because they were discharged on the study day or the day after. Primary reasons for admissions were unconjugated hyperbilirubinemia ($n = 15$), pneumonia with a negative blood culture ($n = 6$), asphyxia ($n = 4$), infection suspicion with a negative blood culture ($n = 5$), wet lung ($n = 1$), observation that was due to uterine bleeding ($n = 1$), and pending results of toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex virus ($n = 1$), which were negative. Intravenous antibiotics (penicillins and/or cephalosporins) were given to 28 of the 33 infants.

13CO2 enrichments during [13C]bicarbonate infusion

The baseline 13CO2 enrichment was $-17.04 \pm 0.94$ Pee Dee Belemnite. The mean 13CO2 enrichment at isotopic plateau during [13C]bicarbonate infusion was 0.0380 $\pm$ 0.0032 APE. The corresponding mean CO2 production rate was 23.44 $\pm$ 2.04 mmol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$. The mean 13CO2 enrichment at the isotopic plateau and their corresponding CO2 production rate of each infant were plotted against the methionine intake (Figure 2).

L-[1-13C]phenylalanine oxidation

The mean 13CO2 enrichment at isotopic plateau during L-[1,13C]phenylalanine infusion was 0.0198 $\pm$ 0.0024 APE. These 13CO2-enrichment values and the F13CO2 were plotted against

### TABLE 2

Subject characteristics and protein and energy intakes before the study of infants who participated in the study ($n = 33$)

<table>
<thead>
<tr>
<th>Values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>3.3 $\pm$ 0.4</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39 $\pm$ 1</td>
</tr>
<tr>
<td>Age on study day (d)</td>
<td>13 $\pm$ 6</td>
</tr>
<tr>
<td>Weight on study day (kg)</td>
<td>3.5 $\pm$ 0.4</td>
</tr>
<tr>
<td>Weight gain before study (g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$)</td>
<td>13 $\pm$ 5</td>
</tr>
<tr>
<td>Sex (F:M)</td>
<td>9:24</td>
</tr>
<tr>
<td>Protein intake before the study (g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$)</td>
<td>2.5 $\pm$ 0.4</td>
</tr>
<tr>
<td>Energy intake before the study (kcal $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$)</td>
<td>108 $\pm$ 14</td>
</tr>
</tbody>
</table>

*All values are means $\pm$ SDs.*

FIGURE 2. A: Mean 13CO2 enrichment at the isotopic plateau during enteral [13C]bicarbonate infusion of each infant plotted against the methionine intake ($n = 33$). B: CO2 production rate of each infant plotted against the methionine intake ($n = 33$). APE, atom percentage excess.
methionine intakes, as shown in Figure 3. As the methionine intake increased, F\text{13CO}_2 decreased. This negative correlation was shown between F\text{13CO}_2 and methionine intakes. Additional increases in methionine intake did not affect the F\text{13CO}_2. With the use of a biphasic linear regression crossover model, the breakpoint was estimated to be 38 mg \cdot kg^{-1} \cdot d^{-1} (P < 0.0001, r^2 = 0.59). The upper CI was 48 mg \cdot kg^{-1} \cdot d^{-1}, and the lower CI was 27 mg \cdot kg^{-1} \cdot d^{-1}. APE, atom percentage excess; F\text{13CO}_2, fraction of \text{13CO}_2 recovery from \text{L-[1-13C]phenylalanine oxidation.}

**DISCUSSION**

The minimal obligatory methionine requirement of enterally fed term infants was estimated to be 38 mg \cdot kg^{-1} \cdot d^{-1} by using the IAAO method. This value is comparable with the estimates of 32–49 mg \cdot kg^{-1} \cdot d^{-1} determined by Snyderman et al (8). In the study of Snyderman et al (8), the methionine requirement was determined in 7 infants with postnatal ages between 2 wk and 2 mo by using weight-gain rates and nitrogen retention. The study diet used by Snyderman et al (8) was an elemental diet that provided a cysteine intake of 64 mg \cdot kg^{-1} \cdot d^{-1}. Fomon et al (9–11) reported a series of studies with soy-protein formulas with or without a methionine supplement fed to infants over a period of several months. Eight to 13 infants were included in each study diet. The adequacy of the diet and, thus, the adequacy of sulfur amino acid intakes were estimated by measurement of growth, serum chemical indexes, and nitrogen retention. Fomon et al (9–11) concluded that for female infants, a diet with a methionine content of 35 mg/100 kcal was considered adequate; however, a methionine intake of 39 mg/100 kcal failed to meet the requirement for male infants, 56 d old. Although our study was not designed to detect sex differences in the methionine requirement, the average requirement estimates by Fomon et al (9–11) were consistent with our results. Limitations of previous studies were the relative small numbers of subjects studied and the methods used. Growth rates and
nitrogen balance might not be the most sensitive and accurate methods for estimating amino acid requirements.

Courtney-Martin et al (22) determined the methionine requirement in parenterally fed postnatal neonates by using the IAAO method to be 49 mg · kg⁻¹ · d⁻¹. The diet was devoid of cysteine. To compare their estimates with ours, the route of nutrition intake and the sparing effect of cysteine on the methionine requirement need to be taken into account. Experiments with human fetal tissues showed the lack of activity of cystathionase (25, 26), which is the enzyme involved in the final step in the cysteine-synthesis pathway. However, clinical studies showed the capability of the transsulfuration of methionine to cysteine in term and preterm neonates (27–29) and, thereby, support the evidence that cysteine has a sparing effect on the methionine requirement. The amount of cysteine for sparing the methionine requirement is ~33% in infants (30), ~55% in school-age children (31), 60–89% in adults (32, 33), and ~40% in piglet studies (34). In a series of experiments, Shoveller et al (34, 35) compared the methionine requirement and cysteine-sparing capacity in piglets that were parenterally and enterally fed. The authors showed that the parenteral methionine requirement was ~70% of the enteral requirement, and the dietary cysteine reduced the methionine requirement by an equal proportion in both feeding routes of ~40%. With the use of these fractions, the parenteral methionine requirement of 49 mg · kg⁻¹ · d⁻¹ with a diet devoid of cysteine in neonates determined by Courtney-Martin et al (22) could be converted to an enteral methionine requirement with an excess of cysteine as in our study. The predicted requirement would be 42 mg · kg⁻¹ · d⁻¹. This amount is nearly the same as our estimated requirement of 38 mg · kg⁻¹ · d⁻¹.

The current essential amino acid recommendations are based on the average intake of an exclusive breastfed infant (12). The estimated methionine intake in the first month of life is 28 mg · kg⁻¹ · d⁻¹, which is lower than our estimated mean requirement. At least 4 explanations might contribute to this finding.

First, a part of the difference may be caused by the elemental formula we used, whereas the recommendations do not discriminate between whole-protein–based, partially hydrolyzed, or elemental formulas. All of these formulas are on the market for infants. A recent report showed that an elemental diet provides an average of 17% less protein substrate per gram of free amino acids than does a protein-bound diet. This difference is due to the release of a water molecule when a peptide bond is formed (36).

Second, amino acid use and, therefore, retention were shown to be different depending on the rates of protein and amino acid digestion (37, 38). Metges et al (37) showed that the oxidation rate was 22% higher and the nonoxidative disposal was 38% lower when free amino acids were ingested compared with a protein diet. Therefore, we might have overestimated the actual requirement. However, the use of a diet that was based on free amino acids provided us with the ability to vary in-test amino acid intakes while keeping the other amino acids constant. Future studies with an intrinsically labeled protein are required to evaluate this issue.

Third, the composition of human milk shows remarkable variation and is influenced by many factors, such as the gestational age at parturition, stage of lactation, nutritional and status of the mother. The protein content in human milk declines markedly during lactation (39, 40). The recommendations take into account the decline in protein intake by the breastfed infant but not for the change in the whey:casein ratio and, thus, the change in amino acid composition (41).

Last, the protein digestibility and amino acid bioavailability in human milk are different from that in formula. Therefore, the gross amino acid composition of human milk may not necessarily reflect the amino acid–requirement profile of infants who consumed infant formula. An European Society for Paediatric Gastroenterology, Hepatology and Nutrition–coordinated international expert group stated that “the composition of human milk can provide some guidance for the composition of infant formulae, but gross compositional similarity is not an adequate determinant or indicator of the safety and nutritional adequacy of infant formulae” (42). The results of our current study provide more scientific knowledge of amino acid needs of infants fed an infant formula, which is necessarily to improve infant nutrition.

A limitation of our study is that we performed the study in hospitalized infants. Although the infants were recovered from their illnesses, 11 of 33 infants were in (a possible) postinfectious state. Because inflammation along with increased oxidative stress might deplete the liver glutathione pool by increase glutathione usage, the liver glutathione pool might be depleted in these infants. The liver glutathione pool can be restored by increasing the cysteine content in the diet (43). Therefore, we assumed that the current health status would not affect the estimated methionine requirement significantly because cysteine was supplied in excess and glutathione synthesis depends mainly on the availability of cysteine (44).

Another issue in our study is the extensive antibiotic use in our study population. Antibiotic treatment has a major impact on the bacterial flora in the gastrointestinal tract (45), and it is possible that the requirement was met not only by the diet but also by the de novo synthesis by the gastrointestinal bacterial flora (46). However, the bacterial contribution to amino acid requirements is still unclear. Therefore, the impact of antibiotic use on the estimated requirement is unknown.

In conclusion, the minimal obligatory methionine requirement is determined to be 38 mg · kg⁻¹ · d⁻¹ for term neonates fed an amino acid based formula provided with an excess of cysteine. Current infant formulas provide excess methionine (49–80 mg · kg⁻¹ · d⁻¹) when 150 mL · kg⁻¹ · d⁻¹ is consumed (47). The results of our current study provide more scientific knowledge of amino acid needs of infants fed an infant formula, which is necessary to improve infant nutrition.

We especially thank the infants and their parents who participated in this study. We thank all the doctors and nurses in the neonatal ward at the Children’s Hospital of Fudan University for their support and help with patient recruitment.

The authors’ responsibilities were as follows—LH, JEH-S, and MJAvD: conducting of experiments; LH, FdG, and JBvG: study design; LH, MJAvD, HS, JWRT, and JBvG: data interpretation and analysis; GJV: sample analysis; AV: preparation and testing of stable-isotope solutions; FdG, CC, YH, and JBvG: consultation; LH and JBvG: writing of the manuscript; JBvG: primary responsibility for final content of the manuscript; and all authors: reading and approval of the final manuscript. The study sponsors had no influence on the study design, analysis of the data, or writing of this manuscript. LH, JEH-S, FdG, and JBvG received a grant for research from Danone. MJAvD, GJV, HS, JWRT, AV, CC, and YH had no conflicts of interest.

REFERENCES


34. Shoveller AK, Brunton JA, House JD, Pencharz PB, Ball RO. Dietary cysteine reduces the methionine requirement by an equal proportion in both parenterally and enterally fed piglets. J Nutr 2003;133:4215–24.
46. Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr 2000;130:18575–64S.