Cysteine: a conditionally essential amino acid in low-birth-weight preterm infants? 1–4

Maaike A Riedijk, Ron HT van Beek, Gardi Voortman, Henrica MA de Bie, Anne CM Dassel, and Johannes B van Goudoever

ABSTRACT

Background: Cyst(e)ine can be synthesized de novo from methionine and serine and is, therefore, a nonessential amino acid in human adults. Several studies have suggested that cyst(e)ine might be a conditionally essential amino acid in preterm infants because of biochemical immaturity. No data are available on cyst(e)ine requirements in low-birth-weight (LBW) preterm infants.

Objective: The aim was to determine cyst(e)ine requirements in LBW infants with gestational ages from 32 to 34 wk, measured 1 mo after birth with the use of the indicator amino acid oxidation technique.

Design: LBW infants were randomly assigned to 1 or 2 of the 5 formulas containing graded cystine concentrations (11, 22, 32, 43, or 65 mg cyst(e)ine/100 mL) and generous amounts of methionine. After 24-h adaptation, cyst(e)ine requirement was determined by 13CO2 release from [1-13C]phenylalanine in expired breath. 13CO2 enrichment was measured by isotopic ratio mass spectrometry.

Results: Cyst(e)ine requirement was determined in 25 LBW infants with a mean (±SD) gestational age of 33 ± 1 wk and birth weight of 1.78 ± 0.32 kg. Fractional oxidation of [1-13C]phenylalanine did not differ between the 5 groups.

Conclusions: There is no evidence for limited endogenous cyst(e)ine synthesis in 4-wk-old LBW preterm infants born at gestational ages from 32 to 34 wk. It is safe to conclude that the cyst(e)ine requirement is <18 mg · kg −1 · d −1 providing generous amounts of methionine and that cyst(e)ine is probably not a conditionally essential amino acid in fully enterally fed LBW preterm infants born at 32–34 wk. Am J Clin Nutr 2007;86:1120–5.

KEY WORDS Amino acid requirements, indicator amino acid oxidation, nutrition

INTRODUCTION

Cyst(e)ine is a sulfur-containing amino acid that is not essential in humans. The human body synthesizes cyst(e)ine de novo from methionine, the only essential sulfur-containing amino acid, and serine. Cyst(e)ine has several important metabolic functions. First, like all other amino acids it is involved in growth and protein synthesis. Furthermore, it is a precursor for the tripeptide glutathione, an important intracellular antioxidant. Then, it is also a precursor for the production of taurine, another antioxidant, and sulfate.

Some nonessential amino acids are classified as conditionally essential. These amino acids may become temporarily essential when synthesis during rapid growth or critical illness is insufficient. Cyst(e)ine is believed to be conditionally essential in preterm infants because of biochemical immaturity of the enzyme cystathionase (EC 4.4.1.1) that is involved in cyst(e)ine synthesis (1–3). It is, therefore, important to know the exact cyst(e)ine requirement of preterm infants and also in view of the higher amino acid requirement as a result of rapid growth and development.

Different methods are used to estimate individual amino acid requirements, eg, the nitrogen balance method, growth rate, plasma amino acid patterns, and the factorial approach. The first reports on amino acid requirements in neonates were based on nitrogen balance and weight gain rate (4, 5). Nitrogen balance has several limitations (6), for instance, it requires 7–10 d to adapt to the test diet (7). Because present-day practice will not allow neonates to be maintained on either deficient or excess intakes of amino acids for a minimum of 7 d, no recent requirement studies have used this method in preterm infants.

At present, the factorial approach is used to define amino acid requirements in infants (8). This model is based on fetal protein accretion during normal intrauterine development. Accretion data are derived from body carcass analysis of stillborn preterm infants, some born >100 y ago (9, 10). Not all data are suitable as reference material; gestational age and cause of death were not accurately obtained (11), and, in view of this study, cyst(e)ine content of the body was not determined. The current recommendation for cyst(e)ine requirement for preterm infants, ie, 66–95 mg · kg −1 · d −1 , is based on the minimum and maximum amounts of each amino acid present in the amounts of human milk.

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2 “Cyst(e)ine” is used throughout to designate any undefined combination of cysteine and cystine. The terms “cysteine” and “cystine” are used to designate the specific amino acids.

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Because methionine is the precursor of cyst(e)ine, the methionine intake needs to be taken into account as well. The current estimated methionine requirement for preterm infants is 48–69 mg·kg⁻¹·d⁻¹ (8). To ensure an adequate methionine intake, the study formula supplied an amount of 70 mg methionine on average (8). To ensure an adequate methionine intake, the study formula supplied an amount of 70 mg methionine on average (8). Shoveller et al (12) determined the total sulfur amino acid requirements in enterally fed neonatal piglets when no dietary cysteine was provided. They calculated cyst(e)ine requirements in fully enterally fed low-birth-weight (LBW) infants supplied with an adequate methionine intake. We hypothesized that cyst(e)ine is a conditionally essential amino acid for these infants.

### SUBJECTS AND METHODS

#### Subjects

Subjects eligible for the study were LBW infants with gestational age (GA) between 32 and 34 wk, admitted to the neonatal department of the Amphia Hospital, Breda; Vlietland Hospital, Vlaardingen; or Deventer Hospital, Deventer (all: the Netherlands). The infants needed to be clinically stable during the study, and those with any congenital or gastrointestinal disease were excluded. All tolerated full enteral feeding and were partly fed through a nasogastric feeding tube and partly bottle-fed. The study protocol was approved by The Central Committee on Research Involving Human Subjects (CCMO) and the Erasmus MC Institutional Review Board. Written and informed consent was obtained from both parents of each subject.

#### Study formula

We used 5 study formulas that contained graded cyst(e)ine concentrations: 11, 22, 32, 43, and 65 mg cysteine/100 mL (Xcys/Neocate; Nutricia Nederland BV, Zoetermeer, the Netherlands/SHS International, Liverpool, United Kingdom). Infants were enrolled in the study when they tolerated full enteral feeding (>150 mL·kg⁻¹·d⁻¹). Except for the cyst(e)ine concentration, the 5 formulas did not differ in amino acid composition (Table 1), and consequently the nitrogen content increased slightly from 0.37 mg nitrogen/100 mL (formula 1) to 0.38 mg nitrogen/100 mL (formula 5). The graded cyst(e)ine concentrations of the study formulas were based on the current estimated cyst(e)ine requirement for preterm infants (8), which is 66–95 mg·kg⁻¹·d⁻¹, and is based on the minimum and maximum amounts of each amino acid present in the amounts of human milk protein corresponding to the recommended minimum and maximum protein contents of 3.0 g/120 kcal and 4.3 g/120 kcal, respectively. We decided to test 3 cyst(e)ine intakes above and 2 cyst(e)ine intakes below the estimated cyst(e)ine requirements for formula-fed preterm infants. Methionine content was similar for all formulas and was supplied generously.

#### Study design and tracer protocol

Cyst(e)ine requirement was measured ≈1 mo after birth (range: 35–37 wk postmenstrual age). Infants were randomly assigned to at least one of the study formulas. The study diet was initiated 24 h before start of the oxidation study, and the dietary intake was not changed until the tracer protocol was finished. All subjects received 170 mL formula · kg⁻¹ · d⁻¹ to ensure that all essential amino acids other than cyst(e)ine were in excess.

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### Table 1

Amino acid profiles of the study formulas

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Formula 1</th>
<th>Formula 2</th>
<th>Formula 3</th>
<th>Formula 4</th>
<th>Formula 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine (mg/170 mL)</td>
<td>166</td>
<td>166</td>
<td>166</td>
<td>166</td>
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<tr>
<td>L-Arginine (mg/170 mL)</td>
<td>292</td>
<td>292</td>
<td>292</td>
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<td>292</td>
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<tr>
<td>L-Aspartic acid (mg/170 mL)</td>
<td>274</td>
<td>274</td>
<td>274</td>
<td>274</td>
<td>274</td>
</tr>
<tr>
<td>L-Cystine (mg/170 mL)</td>
<td>18</td>
<td>36</td>
<td>54</td>
<td>72</td>
<td>109</td>
</tr>
<tr>
<td>L-Glycine (mg/170 mL)</td>
<td>259</td>
<td>259</td>
<td>259</td>
<td>259</td>
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<td>L-Histidine (mg/170 mL)</td>
<td>168</td>
<td>168</td>
<td>168</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>L-Isoleucine (mg/170 mL)</td>
<td>266</td>
<td>266</td>
<td>266</td>
<td>266</td>
<td>266</td>
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<tr>
<td>L-Leucine (mg/170 mL)</td>
<td>435</td>
<td>435</td>
<td>435</td>
<td>435</td>
<td>435</td>
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<tr>
<td>L-Lysine (mg/170 mL)</td>
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<td>L-Methionine (mg/170 mL)</td>
<td>70</td>
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<td>L-Proline (mg/170 mL)</td>
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<td>L-Phenylalanine (mg/170 mL)</td>
<td>197</td>
<td>197</td>
<td>197</td>
<td>197</td>
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<td>L-Serine (mg/170 mL)</td>
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<td>L-Threonine (mg/170 mL)</td>
<td>215</td>
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<td>L-Tryptophan (mg/170 mL)</td>
<td>88</td>
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<td>L-Tyrosine (mg/170 mL)</td>
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<td>L-Valine (mg/170 mL)</td>
<td>282</td>
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<tr>
<td>L-Asparagine (mg/170 mL)</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>L-Citrulline (mg/170 mL)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Carnitine (mg/170 mL)</td>
<td>3</td>
<td>3</td>
<td>3</td>
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</tr>
<tr>
<td>L-Taurine (mg/170 mL)</td>
<td>8</td>
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</tr>
<tr>
<td>L-Glutamine/glutamate (mg/170 mL)</td>
<td>371</td>
<td>371</td>
<td>371</td>
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</tr>
</tbody>
</table>

Total (g/170 mL) 4.12 4.13 4.14 4.17 4.21
(Table 1) and, therefore, were not limiting for protein synthesis. Before the introduction of the study formula, almost all infants received their mothers’ (expressed) breast milk, and only a few infants received standard preterm formula (Nenatal; Nutricia, Zoetermeer, the Netherlands). The cyst(e)ine concentration of human milk varies widely, but the preterm formula provided 35 cyst(e)ine/100 mL.

The IAAO technique uses as indicator a labeled essential amino acid that is different than the test amino acid. The indicator is independent of the different intake amounts of the test amino acid. If the test amino acid is deficient in the diet, this will limit overall protein synthesis and all other essential amino acids will be oxidized. Increasing the dietary intake of the test amino acid will linearly decrease oxidation of the indicator until requirement of the test amino acid is met. We chose [1-13C]phenylalanine as the indicator (13).

After 24-h adaptation, subjects received a primed (10 μmol/kg) continuous (10 μmol·kg⁻¹·h⁻¹) enteral infusion of [13C]bicarbonate [sterile pyrogen free, 99 atom percent excess (APE; Cambridge Isotopes, Woburn, MA] for 2.5 h to quantify individual carbon dioxide production. We infused the tracer enteraly to minimize invasiveness, which was validated by our group (14). The labeled sodium bicarbonate infusion was directly followed by a primed (30 μmol/kg) continuous (30 μmol·kg⁻¹·h⁻¹) enteral infusion of [1-13C]phenylalanine (93 APE; Cambridge Isotopes) for 5 h. One hour before the start of the oxidation study, the feeding regimen was changed into continuous drip-feeding. Enteraly infused tracer was mixed with the study formula and infused continuously by an infusion pump through the nasogastric tube. All infants were breathing spontaneously, and 14 infants needed supplemental oxygen by a nasal prong.

Breath samples were obtained with the use of the direct sampling method described by van der Schoor et al (15). In brief, a 6-French gastric tube (6 Ch Argyle; Cherwood Medical, Tul-lamore, Ireland) was inserted 1–1.5 cm into the nasopharynx, and end-tidal breath was taken with a syringe connected to the end. Collected air was transferred into 10-mL sterile, non-silicon-coated evacuated glass tubes (Van Loenen Instruments, Zaandam, the Netherlands) and stored at room temperature until analysis. Baseline samples were obtained 15 and 5 min before starting tracer infusion. Duplicate 13C-enriched breath samples were collected every 30 min and every 15 min during the last 45 min of the tracer infusion.

Analytic methods and calculations

13CO2 isotopic enrichment in expired air was measured by isotope ratio mass spectrometry (ABCA; Europe Scientific, Van Loenen Instruments, Leiden, the Netherlands) and expressed as APE above baseline (15). APE was plotted relative to time.

It is necessary to determine the individual carbon dioxide production to determine the rate of substrate oxidation. In general, the rate of oxidation is calculated by multiplying the isotopic enrichment of carbon dioxide in breath by the total rate of carbon dioxide excreted; the latter needs to be determined by indirect calorimetry to estimate the rate of carbon dioxide production. Alternatively, body carbon dioxide production can be determined by quantifying the excretion of 13CO2 in expired air during [13C]bicarbonate infusion, which avoids the quantification of total expired air (15).

Estimated body carbon dioxide production (in mmol·kg⁻¹·h⁻¹) was calculated as described previously (14). The rate of fractional [1-13C]phenylalanine oxidation was calculated as follows:

\[
\text{Fractional phenylalanine oxidation (\%)} = \left[ \frac{\text{IE}_{B\text{PH}} \times i_B}{\text{IE}_{P\text{HE}} \times i_{P\text{HE}}} \right] \times 100 \quad (1)
\]

where IE_{BPH} is the 13C isotopic enrichment in expired air during [1-13C]phenylalanine infusion (in APE), i_B is the infusion rate of [13C]bicarbonate (in μmol·kg⁻¹·h⁻¹), IE_{PHE} is the infusion rate of [1-13C]phenylalanine (in μmol·kg⁻¹·h⁻¹), and IE_{PHE} is the 13C isotopic enrichment in expired air during [13C]bicarbonate infusion (16).

Statistical analysis

Descriptive data are expressed as mean ± SD. Steady state of 13CO2 in expired breath during the [1-13C]phenylalanine was achieved when the linear factor of the slope was found not to be significantly different from zero (P ≥ 0.05). The cyst(e)ine requirement was determined with the IAAO method. The indicator oxidation rate is plotted against the varying dietary intakes of cyst(e)ine. The inflection or breakpoint in the indicator oxidation rate represents the physiologic requirement of cyst(e)ine (17). Data were analyzed with the use of mixed model analysis of variance in SPSS SOFTWARE (version 14.0: SPSS Inc, Chicago, IL), while encoding the patients who participated twice with the same number. Repeated measures analysis of variance was performed on primary and derived variables to assess the effects of dietary intake and of subjects. Regression analysis was performed to analyze oxidation rates. Power calculation showed that, assuming 5 formula groups with a group variance of 16, an intergroup variance of 5.5, and a power of 80%, a breakpoint should be detected in 6 subjects per group. Statistical significance was assumed at the 5% level of significance (P ≤ 0.05).

RESULTS

We included 25 LBW infants (12 boys, 13 girls) born at mean (±SD) GA of 33 ± 1 wk (range: 32–34 wk). They were studied at mean postmenstrual age of 36 ± 1 wk (range: 35–38 wk), ie, approximately at postnatal age 1 mo. Subject characteristics are depicted in Table 2. Five infants participated twice and received 2 different study formulas. Aiming at 6 measurements per formula, we performed a total of 30 labeled phenylalanine oxidation rate measurements in these 25 infants.

GA, study age, birth weight, and study weight did not differ between the 5 formula groups as shown in Table 2. In addition, the total enteral intake did not differ between the 5 formulas (P = 0.07). Although the nitrogen content of study formula 5 was somewhat higher, the nitrogen intake did not differ significantly between the formula groups (P = 0.25).

To detect any differences between the 5 groups receiving the different formulas we corrected for sex, study age, and study weight. The baseline 13C enrichment in expired breath did not differ between the 5 formula groups (−17.24 ± 0.56, −16.77 ± 0.62, −17.95 ± 1.38, −17.87 ± 1.01, and −17.58 ± 1.55 Pee Dee Belemnite, respectively; P = 0.67) after correction for birth weight and study weight (Table 3). Each subject reached a plateau during both [13C]bicarbonate and [1-13C]phenylalanine tracer infusions. As an illustration, the 13C enrichments in
expired breath during the infusion of [1-13C]phenylalanine of 6 subjects receiving 54 mg cyst(e)ine \( \text{kg}^{-1} \cdot \text{d}^{-1} \) are shown in Figure 1.

The mean fractional [1-13C]phenylalanine oxidation did not differ between the groups \( (P = 0.73) \). Regression of the data did not show a linear decrease in indicator oxidation rate, and consequently a breakpoint is missing (Figure 2). A trend in the formula could not be detected \( (P = 0.90) \). This implies that the cyst(e)ine requirement under these circumstances is already met at an intake of 18 mg cyst(e)ine \( \text{kg}^{-1} \cdot \text{d}^{-1} \). At the intakes of 18–109 mg \( \text{kg}^{-1} \cdot \text{d}^{-1} \), cyst(e)ine is not the limiting amino acid for protein synthesis and is therefore deficient in the diet.

### DISCUSSION

To our knowledge this is the first study to determine the exact cyst(e)ine requirement in LBW infants. With the use of the IAAO method, we found it to be <18 mg \( \text{kg}^{-1} \cdot \text{d}^{-1} \) after birth, which suggests that cyst(e)ine is not a conditionally essential amino acid in these infants at this age who are receiving generous amounts of methionine. The IAAO method is based on the assumption that the partition of any essential amino acid between oxidation and protein synthesis is sensitive to the amount of the most limiting amino acid in the diet \( (17, 18) \). Thus, a limitation of this method is the necessity of providing all amino acids in excess, except for the one under study. Accordingly, insufficient amounts of essential amino acids could have resulted in another essential amino acid than cystine being limiting for protein synthesis. However, we do not believe this is the case in this study, seeing that the fractional [1-13C]phenylalanine oxidation did not differ between the 5 test diets and that essential amino acids were supplied in excess of the estimate dietary requirements for preterm infant formula \( (19) \). Although the dietary intake of formula 1 (170 mL \( \text{kg}^{-1} \cdot \text{d}^{-1} \)) was higher than formula 4 (163 mL \( \text{kg}^{-1} \cdot \text{d}^{-1} \)), it did not significantly differ. We do not believe that this had a major influence on the obtained results. The higher intake of formula 1 could have resulted in a lower phenylalanine oxidation rate because of a slightly higher cyst(e)ine intake compared with formula 2. However, we did not find a difference in the phenylalanine oxidation.

The current guidelines on requirements of individual amino acids for infants are based on the factorial method. To calculate the deposit of each amino acid, growth rate is assumed to be constant at 15 g \( \text{kg}^{-1} \cdot \text{d}^{-1} \) for a fetus from 900 to 2400 g and protein retention at 70% of total protein intake. Furthermore, the protein maintenance requirement in preterm infants ranges from 0.55 to 0.75 g \( \text{kg}^{-1} \cdot \text{d}^{-1} \) \( (20) \). Fetal amino acid accretion during normal intrauterine growth was determined by Widdowson \( (21) \). Cyst(e)ine accretion by the human fetus was not determined, however; thus, the requirement could not be estimated by the factorial approach.

### TABLE 2

Patient characteristics\(^1\)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Subjects</th>
<th>GA</th>
<th>SA</th>
<th>BW</th>
<th>SW</th>
<th>Enteral intake</th>
<th>Cyst(e)ine intake</th>
<th>SAA intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>( \text{wk} )</td>
<td>( \text{wk} )</td>
<td>( \text{kg} )</td>
<td>( \text{kg} )</td>
<td>( \text{mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} )</td>
<td>( \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} )</td>
<td>( \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} )</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>33 ± 2(^1)</td>
<td>36 ± 1</td>
<td>1.88 ± 0.28</td>
<td>2.26 ± 0.24</td>
<td>170 ± 6</td>
<td>18 ± 1</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>32 ± 1</td>
<td>35 ± 0</td>
<td>1.81 ± 0.36</td>
<td>2.10 ± 0.38</td>
<td>167 ± 1</td>
<td>36 ± 0</td>
<td>105 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>33 ± 1</td>
<td>36 ± 1</td>
<td>1.81 ± 0.38</td>
<td>2.17 ± 0.38</td>
<td>167 ± 2</td>
<td>54 ± 1</td>
<td>123 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>33 ± 1</td>
<td>36 ± 0</td>
<td>1.81 ± 0.24</td>
<td>2.17 ± 0.27</td>
<td>163 ± 5</td>
<td>70 ± 2</td>
<td>138 ± 4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>32 ± 1</td>
<td>36 ± 1</td>
<td>1.58 ± 0.38</td>
<td>2.04 ± 0.31</td>
<td>166 ± 3</td>
<td>108 ± 2</td>
<td>177 ± 4</td>
</tr>
<tr>
<td>All</td>
<td>30</td>
<td>33 ± 1</td>
<td>36 ± 1</td>
<td>1.78 ± 0.32</td>
<td>2.15 ± 0.31</td>
<td>167 ± 5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) GA, gestational age; SA, study age; BW, birth weight; SW, study weight; SAA, sulfur amino acid. No significant differences were detected in GA \( (P = 0.20) \), SA \( (P = 0.38) \), BW \( (P = 0.55) \), SW \( (P = 0.86) \), or enteral intake \( (P = 0.07) \) between the 5 formula groups.

\(^2\) \( \bar{x} \) ± SD (all such values).

### TABLE 3

Whole-body carbon dioxide production and fractional [1-13C]phenylalanine (phe) oxidation rates of 5 different cyst(e)ine intakes\(^1\)

<table>
<thead>
<tr>
<th>Cyst(e)ine intake</th>
<th>Carbon dioxide production</th>
<th>Fractional oxidation of [1-13C]phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 18 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} ) (( n = 6 ))</td>
<td>30.78 ± 5.31</td>
<td>20.32 ± 5.26</td>
</tr>
<tr>
<td>( 36 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} ) (( n = 6 ))</td>
<td>28.19 ± 2.61</td>
<td>18.73 ± 5.03</td>
</tr>
<tr>
<td>( 54 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} ) (( n = 6 ))</td>
<td>29.83 ± 3.08</td>
<td>20.33 ± 4.15</td>
</tr>
<tr>
<td>( 72 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} ) (( n = 6 ))</td>
<td>30.20 ± 1.97</td>
<td>21.48 ± 5.70</td>
</tr>
<tr>
<td>( 109 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} ) (( n = 6 ))</td>
<td>29.24 ± 2.22</td>
<td>19.07 ± 4.93</td>
</tr>
</tbody>
</table>

\(^1\) All values are \( \bar{x} \) ± SD. \( {13C} \)O2 enrichment of \( {13C} \) in expired air; PDB, Pee Dee Belemnite; bicarb, sodium bicarbonate; APE, atom percent excess. No significant differences were detected in baseline \( {13C} \)O2 \( (P = 0.67) \), carbon dioxide production \( (P = 0.61) \), or fractional oxidation of labeled phenylalanine \( (P = 0.73) \) between the 5 formula groups.
cysteine concentrations, with or without cysteine supplementation (24–26). Pohlandt (26) observed no differences in plasma cysteine concentrations between preterm infants receiving only glucose intravenously and preterm infants fed a mixture of synthetic amino acids free from cysteine. In both groups cysteine plasma concentrations were low, <5 μmol/L, indicating limited endogenous synthesis from methionine. Furthermore, Viňa et al (25) and Miller et al (27) reported impaired cysteine metabolism in premature infants on total parenteral nutrition. All those studies indicate that limited cystathionase activity makes cyst(e)ine a conditionally essential amino acid in preterm infants. Zlotkin et al (28), nevertheless, reported that cysteine supplementation did not affect growth rate and nitrogen balance in parenterally fed term and preterm infants. It did slightly increase urinary 3-methylhistidine excretion, however, suggesting that either muscle protein catabolism or muscle mass had increased. Also Malloy et al (29) did not find an improved nitrogen balance with cysteine supplementation, although plasma-free cyst(e)ine concentration increased. Contradictorily, findings from a recent study suggest that the transsulfuration pathway produces sufficient amounts of cysteine in the parenterally fed preterm infant (30).

Our study was performed when neonates received full enteral feeding supplied with a generous amount of methionine and shows that the cyst(e)ine synthesis pathway is active and sufficient at this time. We studied the indicator oxidation during 5 h when infants were continuously fed. The standard feeding regimen of these infants in our neonatal intensive care unit dictates feeding every hour or every 2 h, depending on clinical considerations. Thus, we studied neonates under physiologic circumstances. In our view, therefore, the absence of a postabsorptive state in these preterm infants does not necessitate the study of oxidation during 24 h.

In conclusion, our findings reject our hypothesis that cyst(e)ine is a conditionally essential amino acid in 1-mo-old fully enterally fed LBW preterm infants with a mean GA of 33 wk. These findings show indirectly that activity of the enzyme cystathionase is sufficient to synthesize adequate amounts of cyst(e)ine in these infants supplied with a generous amount of dietary methionine.

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