Sulfur amino acid metabolism in children with severe childhood undernutrition: cysteine kinetics

Farook Jahoor, Asha Badaloo, Marvin Reid, and Terrence Forrester

ABSTRACT

Background: Children with edematous but not nonedematous severe childhood undernutrition (SCU) have lower plasma and erythrocyte-free concentrations of cysteine, the rate-limiting precursor of glutathione synthesis. We propose that these lower cysteine concentrations are due to reduced production secondary to slower de novo synthesis plus decreased release from protein breakdown.

Objective: We aimed to measure cysteine production, de novo synthesis, and the rate of cysteine release from protein breakdown in children with SCU.

Design: Cysteine flux, de novo synthesis, and release from protein breakdown were measured in 2 groups of children with edematous (n = 11) and nonedematous (n = 11) SCU when they were infected and malnourished (clinical phase 1), when they were still severely malnourished but no longer infected (clinical phase 2), and when they had recovered (clinical phase 3).

Results: In clinical phase 1, cysteine production and its release from protein breakdown were slower in both groups of children than were the values in the recovered state. These kinetic variables were significantly slower, however, in the children with edematous SCU than in those with nonedematous SCU. De novo cysteine synthesis in clinical phase 1 was faster than the rate at recovery in the edematous SCU group, and there were no significant differences between the groups at any clinical phase.

Conclusion: These findings suggest that cysteine production is reduced in all children with SCU because of a decreased contribution from protein breakdown and not from decreased de novo synthesis. The magnitude of this reduction, however, is much greater in children with edematous SCU than in those with nonedematous SCU.

KEY WORDS Cysteine kinetics, edematous severe childhood undernutrition, nonedematous severe childhood undernutrition

INTRODUCTION

In 2 studies of glutathione (GSH) metabolism in children with edematous and nonedematous severe childhood undernutrition (SCU), we showed that the edematous group but not the nonedematous group had an erythrocyte GSH concentration that was lower than the value at recovery (1, 2). This lower erythrocyte GSH concentration was associated with a slower synthesis rate (1, 2). Inability to sustain the normal rate of synthesis of GSH can result from either a shortage in the supply of one or more of its precursor amino acids or a defect in the GSH biosynthetic pathway. Our observation that slower GSH synthesis was associated with lower concentrations of cysteine (1), the rate-limiting precursor of GSH synthesis, and that both the rate of synthesis and the concentration of erythrocyte GSH increased in response to supplements of cysteine during the immediate postadmission period (2) suggested that the slower GSH synthesis of children with edematous SCU was due to a shortage in the supply of cysteine. Whether such a shortage exists and its underlying cause or causes are not known.

The supply of a nonessential amino acid derives from the diet, de novo synthesis, and the breakdown of body proteins. We (3) and others (4) have reported that the rate of whole-body protein breakdown is slower in children with edematous SCU than in children with nonedematous SCU. Hence, in children with edematous SCU, a slower body protein breakdown rate plus the lack of an adequate dietary intake of protein will eventually result in an overall shortage of all amino acids, including cysteine and methionine, the latter being the sulfur donor for cysteine synthesis. Hence, we propose to test the hypothesis that at the time they are admitted to the hospital for treatment, children with edematous SCU will have a slower cysteine production rate than will children with nonedematous SCU because of decreased cysteine release from protein breakdown plus a slower rate of de novo synthesis. We also aimed to compare the response of cysteine kinetics to treatment by the 2 groups. Stable-isotope-tracer methods were used to determine the rate of production of cysteine, de novo cysteine synthesis, and its rate of release from protein breakdown in children with edematous and nonedematous SCU. Phylalanine flux, an index of the whole-body protein breakdown rate, was also measured. A secondary aim of the study was to compare values for cysteine derived from protein breakdown by the use of 2 different approaches, a direct approach based on cysteine kinetics and an indirect approach based on endogenous
phenylalanine flux and the molar ratio of cysteine to phenylalanine concentrations in mixed body proteins. This is the first of 2 articles reporting on cysteine and methionine metabolism in children with SCU.

SUBJECTS AND METHODS

Subjects

Twenty-two children who were admitted to the Tropical Metabolism Research Unit, University of the West Indies, for treatment of SCU participated in the study. During hospitalization, the children were managed according to a standard treatment protocol as previously described by us (1–3). As shown in Table 1, each subject had a deficit in body weight for age of >20%, indicating severe undernutrition. Type of SCU, ie, marasmus, kwashiorkor, or marasmic kwashiorkor, was diagnosed on the basis of the Wellcome Classification (5). Eleven children (6 boys, 5 girls) had nonedematous SCU, and 11 had edematous SCU (6 boys, 5 girls), 8 with kwashiorkor and 3 with marasmic kwashiorkor (Table 2).

The study was approved by the Medical Ethics Committee of the University Hospital of the West Indies and the Baylor Affiliates Review Board for Human Subject Research of Baylor College of Medicine. Written informed consent was obtained from at least one parent of each child enrolled.

Treatment diets

As previously described by us (1–3), the children were fed a milk-based maintenance diet that aimed to provide ≈417 kJ · kg⁻¹ · d⁻¹ and ≈1.2 g · kg⁻¹ · d⁻¹ protein during the early resuscitative phase of treatment and an energy-dense, milk-based formula that provided ≈625–750 kJ · kg⁻¹ · d⁻¹ and ≈3 g · kg⁻¹ · d⁻¹ protein during the rapid catch-up growth phase. In addition, both diets were supplemented with vitamins (Tropivite; Federated Pharmaceuticals, Kingston, Jamaica) and a mineral mix prepared in the Tropical Metabolism Research Unit metabolic kitchen. Each child received 2 mL/d of the vitamin solution, which contained 6000 IU vitamin A (palmitate), 1600 IU vitamin D (calciferol), 2 mg thiamine HCL, 3.2 mg riboflavin, 120 mg vitamin C (ascorbic acid), 4 mg vitamin B-6 (vitamin B-6 HCL), and 28 mg niacinamide. They also received 5 mg folic acid/d and 2 mL of a mineral mix per kg daily. The mineral mix consisted of 37.28 g KCl · L⁻¹, 0.3 g HCl, 150 mg sodium-ascorbate, 1.6 mg copper-sulfate, 0.2 mg zinc-sulfate, 86.4 mg sodium-molybdate, 0.3 mg iron-sulfate, 0.2 mg manganese-sulfate, 0.2 mg cobalt-sulfate, 10.6 mg calcium-potassium-phosphate, 0.1 mg sodium-tungstate, 1.2 mg sodium-selenite, 0.2 mg sodium-iodate, and 0.2 mg sodium-chromate. All other vitamins and minerals were added at appropriate levels to meet the nutrient requirements of the children.

TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonedematous patients (n = 11)</th>
<th>Edematous patients (n = 11)</th>
<th>Nonedematous patients (n = 11)</th>
<th>Edematous patients (n = 11)</th>
<th>Nonedematous patients (n = 11)</th>
<th>Edematous patients (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>10.3 ± 2.4</td>
<td>9.9 ± 0.9</td>
<td>10.6 ± 2.4</td>
<td>10.3 ± 0.9</td>
<td>12.1 ± 1.9</td>
<td>11.3 ± 0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.5 ± 0.5</td>
<td>6.3 ± 0.6</td>
<td>4.5 ± 0.5</td>
<td>6.3 ± 0.6</td>
<td>6.8 ± 0.4</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>61.3 ± 2.6</td>
<td>64.7 ± 2.1</td>
<td>61.8 ± 2.5</td>
<td>65.1 ± 2.3</td>
<td>66.2 ± 2</td>
<td>67.9 ± 1.8</td>
</tr>
<tr>
<td>Weight-for-age (%)</td>
<td>50.2 ± 2.9</td>
<td>68.4 ± 4.9</td>
<td>50.5 ± 3.1</td>
<td>66.8 ± 4.9</td>
<td>72.4 ± 2.4</td>
<td>82.8 ± 3.5</td>
</tr>
<tr>
<td>Weight-for-length (%)</td>
<td>73.7 ± 1.9</td>
<td>90.1 ± 3.3</td>
<td>73.4 ± 2.0</td>
<td>87.7 ± 3.1</td>
<td>94.6 ± 3.1</td>
<td>102 ± 2.4</td>
</tr>
<tr>
<td>Length-for-age (%)</td>
<td>85.4 ± 1.5</td>
<td>89.2 ± 1.7</td>
<td>85.4 ± 1.3</td>
<td>89.1 ± 1.6</td>
<td>88.7 ± 0.8</td>
<td>91.3 ± 1.4</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>30.5 ± 1.7</td>
<td>23.6 ± 1.1.4</td>
<td>33.6 ± 2.9</td>
<td>33.2 ± 2.1</td>
<td>37.8 ± 1.3</td>
<td>37.25 ± 1.5</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. Clinical phase 1, ≈2 d after admission, when the subjects were infected and malnourished; clinical phase 2, ≈14 d after admission, when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, ≈57 d after admission, when the subjects had recovered. Cell means were compared by repeated-measures 2-factor ANOVA.

2 Main effect of clinical phase, P < 0.001.

3 Significantly different from corresponding clinical phase 3 value, P < 0.001 (post hoc comparison by Bonferroni method).

4 Significantly different from corresponding clinical phase 2 value, P < 0.05 (post hoc comparison by Bonferroni method).

5 Diagnosis × clinical phase interaction, P < 0.05.

6 Main effect of diagnosis, P < 0.05.

7 Significantly different from nonedematous patients in the same clinical phase, P < 0.01 (post hoc comparison by Bonferroni method).

Study design

The study consisted of 11 children with edematous SCU and 11 with nonedematous SCU. Whole-body cysteine flux, de novo synthesis, and phenylalanine flux were measured 3 times during the maintenance phase but not in the maintenance phase, the children also received 60 mg FeSO₄.

TABLE 2

Clinical characteristics of the subjects at admission

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonedematous patients (n = 6 M, 5 F)</th>
<th>Edematous patients (n = 6 M, 5 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infections (no. of subjects)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.2 ± 0.5</td>
<td>7.8 ± 0.16</td>
</tr>
<tr>
<td>White blood cells (10⁹ cells/L)</td>
<td>10.9 ± 1.2</td>
<td>15.5 ± 1.9</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.25 ± 0.11</td>
<td>37.55 ± 0.23</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>31.3 ± 2.2</td>
<td>23.7 ± 1.3</td>
</tr>
</tbody>
</table>

1 x ± SE (all such values).

2 Significantly different from the nonedematous group, P < 0.05 (unpaired t test).
hospitalization by the use of constant intravenous infusions of stable isotopes of cysteine, serine, and phenylalanine: \( \approx 2 \) days after admission, when the subjects were both infected and malnourished but clinically stable as indicated by blood pressure, pulse, and respiration rates (clinical phase 1); \( \approx 14 \) d after admission, when the subjects were still severely malnourished (anthropometrically) but no longer infected, (ie, all clinical features of the infective episode had resolved), they had lost edema, and had improved affect and appetite (clinical phase 2); and \( \approx 57 \) d after admission, when the rate of catch-up growth had reached a plateau and weight-for-length was \( \geq 90\% \) of expected (clinical phase 3).

A diet providing maintenance quantities of energy and protein (417 kJ \( \cdot \) kg\(^{-1} \) \( \cdot \) day\(^{-1} \) and 1.2 g \( \cdot \) kg\(^{-1} \) \( \cdot \) day\(^{-1} \) protein) was fed during each isotope infusion protocol. The subjects had been receiving this therapeutic diet for \( \approx 2 \) d at the clinical phase 1 measurement and for \( \approx 13 \) d before the clinical phase 2 measurement. For the clinical phase 3 measurement, the subjects were taken off their regular high-energy diet (which provided \( \approx 625–750 \) kJ \( \cdot \) kg\(^{-1} \) \( \cdot \) day\(^{-1} \) and \( \approx 3 \) g \( \cdot \) kg\(^{-1} \) \( \cdot \) day\(^{-1} \) protein) and were placed on this maintenance diet for 3 d before the infusion protocol.

To ensure that the same amounts of energy and protein were given during the course of the isotope infusions, 33% of the child’s daily intake was given by continuous intragastric infusion over an 8-h period starting 2 h before the isotope infusions. This provided 17.4 kJ \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \) and 0.05 g \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \) protein.

Weight and length were monitored throughout hospitalization. The former daily with an electronic balance (model F150S; Sartorius, Göttingen, Germany), and the latter weekly with a horizontally mounted stadiometer ( Holtain Ltd, Crymych, United Kingdom).

Infusion protocol

Two intravenous access sites were established in opposite arms by the insertion of 22G or 24G catheters after preparation of the access sites with a topical anesthetic (EMLA cream; Astra Pharmaceuticals Ltd, Langley, United Kindom). One intravenous catheter was used for infusion of the labeled cysteine, serine, and phenylalanine and the other for blood sampling. A nasogastric tube was inserted into the child’s stomach, and a Flexiflo Magna-Port Y-Port connector (Ross Products Division, Abbott Laboratories, Columbus, OH) was attached to the proximal end. About 33% of the child’s daily dietary intake was then given over the next 8 h by continuous intragastric infusion into one limb of the Y-port by using an enteral infusion pump (Flexifo companion enteral nutrition pump; Ross Laboratories).

Sterile solutions of 3,3-\(^2\)H\(_2\)-cysteine, U-\(^{13}\)C\(_5\)-cysteine, U-\(^{13}\)C\(_5\)-serine, and ring-\(^{2}\)H\(_2\)-phenylalanine (98%, 98%, 99.9%, and 98% enriched, respectively; Cambridge Isotope Laboratories, Woburn, MA) were prepared in 9 g/L NaCl. After 2 h of continuous intragastric feeding, a 5-mL blood sample was drawn, which was followed immediately by simultaneous primed-continuous intravenous infusions of \(^2\)H\(_2\)-cysteine (prime = 1.5 \( \mu \)mol/kg, infusion rate = 1.5 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \)), U-\(^{13}\)C\(_5\)-cysteine (prime = 12 \( \mu \)mol/kg, infusion rate = 12 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \)), and \(^{2}\)H\(_2\)-phenylalanine (prime = 4 \( \mu \)mol/kg, infusion rate = 4 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \)) for 6 h. A priming dose of 1.0 \( \mu \)mol/kg U-\(^{13}\)C\(_5\)-cysteine was also administered intravenously at this time. Four additional 2-mL blood samples were drawn every 0.5 h during the last 1.5 h of the infusions. The infusion and blood sampling protocols were the same for the 2 subsequent experiments performed at clinical phases 2 and 3.

Sample analyses

The blood samples were centrifuged immediately at 1000 \( \times \) \( g \) for 15 min at 4 °C, and the plasma was removed and stored immediately at −70 °C for later analyses. Plasma amino acids were isolated from 0.2 mL plasma by ion-exchange (Dowex 200x) chromatography and were converted to the \( n \)-propyl ester, heptafluorobutyramide derivative. Dithiothreitol, 0.075 mL of a 10-mmol/L solution, was added to the derivatization mixture to convert cysteine to phenylalanine. The tracer-tracee ratios of plasma cysteine, phenylalanine, and serine were determined by negative chemical ionization gas chromatography–mass spectrometric analysis by selectively monitoring ions at mass-to-charge (m/z) ratios of 535 to 538 (cysteine), 383 to 388 (phenylalanine), and 519 to 522 (serine) with a Hewlett-Packard 5890 quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA).

Calculations

Total cysteine and phenylalanine fluxes \( (Q) \) were calculated by using their plasma plateau tracer-tracee ratios in the following steady state equation:

\[
Q (\mu\text{mol-kg}^{-1}\cdot\text{h}^{-1}) = I(\text{Tr/TrInf})/(\text{Tr/Trp}) \tag{1}
\]

where Tr/TrInf is the tracer-tracee ratio of the infusate, Tr/Trp is the tracer-tracee ratio of either cysteine (\( M + 2 \) isotopomer) or phenylalanine in plasma, and \( I \) is the tracer infusion rate.

Because cysteine receives its 3 carbons from serine during de novo synthesis, its rate of synthesis (\( S_{\text{Cys}} \)) can be calculated by using the equation

\[
S_{\text{Cys}} = Q_{\text{Cys}} \times (\text{Tr/Tr}_{\text{m} + 3\text{Cys}})/(\text{Tr/Tr}_{\text{m} + 3\text{Ser}}) \tag{2}
\]

where \( Q_{\text{Cys}} \) is cysteine flux obtained with the \(^2\)H\(_2\)-cysteine tracer, Tr/Tr\(_{\text{m} + 3\text{Cys}} \) is the steady state tracer-tracee ratio of the \( M + 3 \) isotopomer of cysteine derived from the U-\(^{13}\)C\(_5\)-serine tracer, and Tr/Tr\(_{\text{m} + 3\text{Ser}} \) is the steady state tracer-tracee ratio of the \( M + 3 \) isotopomer of serine in plasma.

Cysteine derived from protein breakdown was calculated as follows. Because \( Q \) represents the sum of all inputs (and outputs) of an amino acid through the body pool,

\[
Q_{\text{Cys}} = B_{\text{Cys}} + I_{\text{Cys}} + S_{\text{Cys}} \tag{3}
\]

where \( B_{\text{Cys}} \) is cysteine released from protein breakdown, \( I_{\text{Cys}} \) is cysteine intake from the diet and tracer infusion, and \( S_{\text{Cys}} \) is cysteine synthesized de novo. Hence, cysteine derived from protein breakdown \( B \) can be calculated as

\[
B_{\text{Cys}} = Q_{\text{Cys}} - (I_{\text{Cys}} + S_{\text{Cys}}) \tag{4}
\]

Endogenous cysteine flux, that is, cysteine derived from protein breakdown plus de novo synthesis (\( B_{\text{Cys}} + S_{\text{Cys}} \)), was also calculated by subtracting \( I_{\text{Cys}} \) from \( Q_{\text{Cys}} \).

In the case of phenylalanine, because it is not synthesized endogenously, in the fed state its flux represents phenylalanine derived from whole-body protein breakdown plus intake from the diet and from the tracer infusion. Hence, \( B_{\text{Phe}} = Q_{\text{Phe}} - I_{\text{Phe}} \)

Cysteine derived from body protein breakdown, \( B_{\text{Cys}} \), was also estimated indirectly by multiplying \( B_{\text{Phe}} \) by the molar ratio concentrations of cysteine to phenylalanine in mixed body protein, based on the assumption that both amino acids are released from
protein breakdown in the same proportion as their content in whole body protein. At present, no published data are available on the cysteine content of mixed whole-body human tissue protein. On the basis of the very close agreement between the concentrations of nearly all other amino acids in human and pig tissues (6), we elected to use a cysteine-to-phenylalanine molar ratio of 0.38, which was based on the amino acid composition of whole-body pig tissue protein (7). In the children with edematous SCU, body weight measured in the malnourished edematous state (ie, the clinical phase 1 measurement) was corrected by subtracting the contribution of edema fluid. Edema fluid was estimated as the difference between body weight on the day of the clinical phase 1 experiment, ≈2 days after admission, and the lowest post-experiment weight observed before the clinical phase 2 measurement. All kinetic data are expressed per kg body wt.

Statistics

Data are expressed as means ± SEMs. Differences between the 2 groups during clinical phase 1 were determined by use of unpaired, 2-tailed t tests to test our primary hypothesis that at the time they are admitted to the hospital for treatment, children with edematous SCU have a slower cysteine production rate than do children with nonedematous SCU because of decreased cysteine release from protein breakdown plus a slower rate of de novo synthesis. The unpaired t test was also used to compare the clinical characteristics of the 2 groups when they were admitted to the hospital. To determine the response to treatment and whether differences due to effects of treatment depend on diagnosis, 2-factor repeated-measures analysis of variance (ANOVA) was used with diagnosis as the between factor and clinical phase as the repeated factor. Because of the nonnormal distribution of the cysteine kinetic data and the difference in variance between the 2 groups, the data were log transformed before repeated-measures ANOVA was performed. If the repeated-measures ANOVA was significant, pair-wise comparisons were made by the Bonferroni method. Inferential tests were considered statistically significant if P < 0.05. A Bland-Altman analysis was used to compare agreement between the 2 methods used to estimate protein-derived cysteine. All data analyses were performed with GRAPHPAD PRISM version 4 software (GraphPad Software, San Diego, CA).

RESULTS

At the time they participated in the first isotope infusion (clinical phase 1), all the children were severely wasted, with a mean weight-for-age of 50.2% of expected in the nonedematous group and 68.4% in the edematous group (Table 1). The edematous group's weight, weight-for-age, and weight-for-length were significantly greater than the corresponding values in the nonedematous group at clinical phases 1 and 2. When recovered at clinical phase 3, weight-for-age and weight-for-length remained significantly greater in the edematous group than in the nonedematous group. Within each group, age and all anthropometric measurements were significantly smaller at clinical phases 1 and 2 than at clinical phase 3 when the subjects had recovered from SCU. Similarly, plasma albumin concentrations were significantly lower in both groups at clinical phase 1 than at clinical phase 3. In the edematous group, plasma albumin concentrations at clinical phase 1 were also lower than the value at clinical phase 2.

The clinical characteristics of both groups at admission are shown in Table 2. Seven of the children in each group had evidence of one or more infections at admission. However, 3 of the 4 children with edematous SCU, who were diagnosed as not having an infection at admission, had a white blood cell count >10 000 cells/mL, which suggests the presence of an occult infection. All 22 children were anemic, and all those with edematous SCU and 7 with nonedematous SCU were hypoalbuminemic.

The tracer-tracee ratios of all 3 amino acid tracers reached a plateau in plasma during the final 1.5 h of the infusion. As shown in Figure 1, in the case of cysteine, both the M + 2 and M + 3 isotopomers) and serine (M + 3 isotopomer) reached a plateau during the final 1.5 h of the infusions, which permitted the use of the precursor-product steady state equation to calculate the fraction of cysteine synthesized de novo from serine. At clinical phase 1, both total phenylalanine flux and its flux from protein breakdown were significantly slower (P < 0.05) in the edematous group than the corresponding values of the nonedematous group (Table 3). Although the diagnosis × clinical phase interaction was not significant, both diagnosis and clinical phase had significant effects (P = 0.0049 and P = 0.0369, respectively) on total phenylalanine flux and its flux from protein breakdown. Furthermore, whereas phenylalanine flux from protein breakdown was ≈20% slower at clinical phase 1 than at clinical phase 3 in the edematous group, it was only ≈14% slower in the nonedematous group.

At clinical phase 1, total and endogenous cysteine fluxes and cysteine derived from protein breakdown were all significantly slower (P < 0.01) in the edematous group than in the nonedematous group (Table 4). There were significant diagnosis × clinical phase interactions in endogenous cysteine flux and in cysteine derived from protein breakdown. Total cysteine flux, however, just missed achieving statistical significance (P = 0.083). Within groups, at clinical phase 1, total and endogenous cysteine fluxes...
Phenylalanine kinetics in children with edematous and nonedematous severe undernutrition

<table>
<thead>
<tr>
<th>Phenylalanine kinetics</th>
<th>Clinical phase 1</th>
<th>Clinical phase 2</th>
<th>Clinical phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet + tracer inflow</td>
<td>(11.9 \pm 0.12)</td>
<td>(11.8 \pm 0.04)</td>
<td>(11.7 \pm 0.04)</td>
</tr>
<tr>
<td>Total flux (^2,3)</td>
<td>(89 \pm 5.3)</td>
<td>(74 \pm 2.9^d)</td>
<td>(86 \pm 3.5)</td>
</tr>
<tr>
<td>Protein-derived flux (^2,3)</td>
<td>(73 \pm 5.4)</td>
<td>(59 \pm 3^d)</td>
<td>(71 \pm 4.5)</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm SE\). Clinical phase 1, \(\approx 2\) d after admission, when the subjects were infected and malnourished; clinical phase 2, \(\approx 14\) d after admission, when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, \(\approx 57\) d after admission, when the subjects had recovered. Within clinical phase 1 (baseline), values were compared by unpaired \(t\) test; to determine differential response to treatment, a repeated-measures ANOVA was performed.

In both groups, at clinical phases 1 and 2, the ratio of protein-derived cysteine flux to phenylalanine flux was lower than the ratio of the molar concentrations of cysteine to phenylalanine (0.38) in mixed pig tissues. At clinical phase 3, the ratios increased to values that were close to 0.38 (Figure 2). When cysteine derived from protein breakdown was estimated indirectly from endogenous phenylalanine flux, the values obtained were different than the corresponding values calculated by using actual cysteine kinetics (Table 5). Comparison of data obtained with the 2 methods by Bland-Altman analysis indicated a lack of agreement between the methods. As shown in Table 5, the upper and lower 95% limits of agreement were large, indicating considerable discrepancy between the 2 methods. The bias, that is, the mean of the difference between the 2 methods, was large at clinical phases 1 and 2 and was not consistent from one clinical

<table>
<thead>
<tr>
<th>Cysteine kinetics</th>
<th>Clinical phase 1</th>
<th>Clinical phase 2</th>
<th>Clinical phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet + tracer inflow</td>
<td>(7.9 \pm 0.06)</td>
<td>(7.8 \pm 0.02)</td>
<td>(7.8 \pm 0.02)</td>
</tr>
<tr>
<td>Total flux (^2,3)</td>
<td>(37.2 \pm 2.5^d)</td>
<td>(27.9 \pm 2.4^d)</td>
<td>(37.7 \pm 2.8^d)</td>
</tr>
<tr>
<td>Endogenous flux (^2,5,6)</td>
<td>(29.3 \pm 2.6^d)</td>
<td>(20 \pm 2.4^d,7)</td>
<td>(30 \pm 2.8^d)</td>
</tr>
<tr>
<td>De novo synthesis (^2,5,6)</td>
<td>(8.6 \pm 0.4)</td>
<td>(9.4 \pm 0.8^d)</td>
<td>(8.7 \pm 0.2)</td>
</tr>
<tr>
<td>Protein-derived flux (^2,5,6)</td>
<td>(20.7 \pm 2.5^d)</td>
<td>(11.4 \pm 1.7^d,5,7)</td>
<td>(21.2 \pm 2.7^d)</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm SE\). Clinical phase 1, \(\approx 2\) d after admission, when the subjects were infected and malnourished; clinical phase 2, \(\approx 14\) d after admission, when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, \(\approx 57\) d after admission, when the subjects had recovered. Within clinical phase 1 (baseline), values were compared by unpaired \(t\) test; to determine differential response to treatment, a repeated-measures ANOVA was performed.

**Main effect of diagnosis, \(P < 0.05\).**

Significantly different from corresponding clinical phase 3 value, \(P < 0.001\) (post hoc comparison by Bonferroni method).

Significantly different from nonedematous patients in the same clinical phase, \(P < 0.05\) (unpaired \(t\) test).
Nonedematous patients (variable clinical phase 1 clinical phase 2 clinical phase 3 group, and there were no significant differences between the 2 state was faster than the rate at recovery in the edematous SCU. De novo cysteine synthesis in the infected malnourished children produced less cysteine than did the children with nonedematous SCU. This may explain why plasma and intracellular cysteine concentrations are much lower in children with edematous SCU than in those with nonedematous SCU (1–3). Together, our past (1–3) and present findings suggest that all children with edematous SCU have reduced cysteine production because of a decreased contribution from protein breakdown, not because of decreased de novo synthesis. However, the magnitude of this reduction is greater in children with edematous SCU because of a marked suppression of the protein breakdown rate. Finally, the lack of agreement between the values obtained for protein-derived cysteine flux with use of a direct approach based on cysteine kinetics and an indirect approach based on endogenous phenylalanine flux suggests that the indirect approach may not be valid.

The findings of the present study show slower endogenous cysteine production in both children with edematous and those with nonedematous SCU. This was surprising because in past studies we found that only children with edematous SCU, and not those with nonedematous SCU, had concentrations of plasma and erythrocyte-free cysteine that were lower than the values at recovery (1–3). This consistent finding plus our past (3) and present observation that the protein breakdown rate is slower in children with edematous SCU than in those with nonedematous SCU led to our proposal that children with edematous SCU will produce less cysteine than will those with nonedematous SCU because of a decreased contribution from protein breakdown. Actually, although both groups were producing less cysteine in the malnourished state (compared with recovery), the amount produced by the children with edematous SCU was 32% less than the amount produced by their nonedematous counterparts. This was because, whereas cysteine released from protein breakdown was 37% slower than the rate at recovery in the nonedematous SCU group, it was 61% slower in the edematous SCU group. Together, our past (1–3) and present findings suggest that although less cysteine is being produced by the nonedematous group, the amount is still sufficient to maintain plasma and intracellular concentrations and the GSH synthesis rate in the infected malnourished state. The same may not be true for the edematous SCU group, however, because the amount of cysteine produced is far less. This may explain why plasma and intracellular cysteine concentrations are much lower in children with edematous SCU than in those with nonedematous SCU (1–3). This may also explain why in the malnourished, infected state,

DISCUSSION

The results of the present study show that when infected and malnourished, all children with SCU produced cysteine at a rate slower than the rate at recovery because of decreased release from protein breakdown. The magnitude of this reduction, however, was much greater in those with edematous SCU. As a consequence, in the infected and malnourished state, these children produced less cysteine than did the children with nonedematous SCU because of a much slower release from protein breakdown. De novo cysteine synthesis in the infected malnourished state was faster than the rate at recovery in the edematous SCU group, and there were no significant differences between the 2

![FIGURE 2. Mean (±SEM) ratio of protein-derived cysteine flux to protein-derived phenylalanine flux (Cys-Phe flux ratio) in 11 children with nonedematous and in 11 children with edematous severe childhood undernutrition at clinical phase 1, = 2 d after admission, when the subjects were infected and malnourished; at clinical phase 2, = 14 d after admission, when the subjects were still severely malnourished but no longer infected and edematous; and at clinical phase 3, = 57 d after admission, when the subjects had recovered. The dotted line represents the molar ratio of cysteine to phenylalanine concentrations (0.38) of whole-body (WB) pig tissue protein.](image)

### TABLE 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinical phase 1</th>
<th>Clinical phase 2</th>
<th>Clinical phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonedematous patients (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD Cys flux from cysteine kinetics²</td>
<td>20.7 ± 2.5</td>
<td>21.2 ± 2.7</td>
<td>32.7 ± 3.3</td>
</tr>
<tr>
<td>PD Cys flux from phenylalanine flux¹</td>
<td>27.3 ± 2</td>
<td>26.5 ± 1.3</td>
<td>31.8 ± 3.1</td>
</tr>
<tr>
<td>Bias (mean of differences) ± SD¹</td>
<td>−6.53 ± 5.9</td>
<td>−5.33 ± 9.1</td>
<td>0.89 ± 14</td>
</tr>
<tr>
<td>95% Limit of agreement</td>
<td>−18.1 to 5.06</td>
<td>−23.2 to 12.5</td>
<td>−26.6 to 28.4</td>
</tr>
<tr>
<td><strong>Edematous patients (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD Cys flux from cysteine kinetics²</td>
<td>11.4 ± 1.6</td>
<td>19.2 ± 2.2</td>
<td>29.4 ± 2.8</td>
</tr>
<tr>
<td>PD Cys flux from phenylalanine flux¹</td>
<td>22.1 ± 1.1</td>
<td>23.6 ± 1.2</td>
<td>27.9 ± 1.9</td>
</tr>
<tr>
<td>Bias (mean of differences) ± SD¹</td>
<td>−10.6 ± 6.5</td>
<td>−4.4 ± 5.6</td>
<td>1.47 ± 7.3</td>
</tr>
<tr>
<td>95% Limit of agreement</td>
<td>−23.4 to 2.11</td>
<td>−15.4 to 6.5</td>
<td>−12.9 to 15.9</td>
</tr>
</tbody>
</table>

¹ Clinical phase 1, = 3 d after admission, when the subjects were infected and malnourished; clinical phase 2, = 14 d after admission, when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, = 57 d after admission, when the subjects had recovered.

² T ± SEM.

¹ Calculated by the Bland-Altman method.
children with edematous SCU cannot maintain the GSH synthesis rate but those with nonedematous SCU can (1, 2).

The present findings support our proposal that decreased cysteine release from protein breakdown is a primary contributor to the diminished cysteine production in children with edematous SCU. After 2 wk of treatment when the children were still severely malnourished (anthropometrically) but no longer infected and their appetite had returned, cysteine production and its release from protein breakdown increased significantly in the edematous group but remained slower than the values at recovery. Because there were no such changes in the nonedematous group, cysteine kinetics were similar in the 2 groups at this stage. This finding suggests that factors underlying the signs and symptoms of the edematous forms of SCU may be responsible for the greater suppression of protein breakdown and cysteine production. The 20% slower phenylalanine flux from protein breakdown in the malnourished state compared with the value at recovery in the edematous group corroborates our earlier findings with leucine kinetics that whole-body protein breakdown is slower in children with edematous SCU (3). Interestingly, in the nonedematous SCU group, cysteine production at clinical phase 1 was also slower because of decreased release from protein breakdown, because endogenous phenylalanine flux was ∼14% slower at clinical phase 1 than at recovery. A similar observation was made with leucine kinetics in a previous study (3).

It is difficult to reconcile this modest reduction in the rate of protein breakdown (∼14%) with the much greater reduction in protein-derived cysteine (∼36%) in the children with nonedematous SCU. A similar mismatch between protein-derived phenylalanine flux (∼20% reduction) and protein-derived cysteine flux (∼61% reduction) can be seen in the children with edematous SCU. A possible explanation is that the relative amount of cysteine released from the breakdown of proteins in the malnourished state is less than in the recovered state. Such a discord between the ratio of phenylalanine flux to cysteine flux from the malnourished to the recovered state is only possible if the types of proteins being broken down in the malnourished state have a lower cysteine content than the proteins being broken down in the recovered state. Indeed, in both groups in the malnourished states, when protein-derived cysteine flux was expressed as a ratio to protein-derived phenylalanine flux, it was ∼0.24, which indicates that for every 4 phenylalanine molecules released only 1 cysteine molecule is released. This ratio increased to 0.4 when the children had recovered, which suggests that for every 5 molecules of phenylalanine released, 2 cysteine molecules are released. In 3 different mammalian species, the rat, pig, and calf, it can be calculated that the molar ratio of cysteine to phenylalanine concentrations in liver tissue is always smaller than the value for the whole body (pig, 0.2 compared with 0.38; rat, 0.2 compared with 0.6; calf, 0.39 compared with 0.5; 7–9). Assuming that the liver is representative of other soft organs, it is possible that in the malnourished state, protein-derived cysteine is mostly from soft organs rather than muscle tissues. This is possible because children with SCU lose 60–70% of their muscle mass (10); hence, a decreased contribution from muscle protein breakdown may underlie the slower cysteine production in children with SCU.

The present findings do not support our proposal of a slower de novo cysteine synthesis in children with edematous SCU (1, 2). This proposal was based on our observations that both plasma and intracellular methionine concentrations were markedly lower in the children with edematous SCU but not in the children with nonedematous SCU (1, 2), which suggests a possible shortage in the availability of methionine for cysteine synthesis. To the contrary, the edematous group had a faster cysteine synthesis rate in the malnourished and infected state compared with the recovered state, which suggests that they were trying to compensate for the decreased cysteine production from protein breakdown by up-regulating de novo synthesis. Hence, impaired de novo cysteine synthesis was not a contributor to the slower cysteine production of children with SCU.

Finally, because of the difficulties associated with measuring the de novo synthesis of dispensable amino acids in vivo, researchers (11) have calculated it indirectly by estimating its protein-derived flux, which is then subtracted from its measured endogenous flux. Protein-derived flux is estimated from the measured flux of an indispensable amino acid (eg, phenylalanine) and the molar ratio concentrations of the dispensable amino acid to the indispensable amino acid in mixed body proteins. Our present finding that protein-derived cysteine flux estimated indirectly from phenylalanine flux is consistently different from the value calculated directly from cysteine kinetics suggests that the indirect approach of estimating the rate of release of an amino acid from protein breakdown may not be valid.

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