Effects of randomized supplementation of methionine or alanine on cysteine and glutathione production during the early phase of treatment of children with edematous malnutrition

Curtis O Green, Asha V Badaloo, Jean W Hsu, Carolyn Taylor-Bryan, Marvin Reid, Terrence Forrester, and Farook Jahoor

ABSTRACT
Background: We have shown that a low glutathione concentration and synthesis rate in erythrocytes are associated with a shortage of protein-derived cysteine in children with edematous severe acute malnutrition (SAM).

Objective: We tested the hypothesis that methionine supplementation may increase protein-derived cysteine and upregulate cysteine synthesis, thereby improving glutathione synthesis during the early treatment of edematous SAM.

Design: The cysteine flux, its de novo synthesis and release from protein breakdown, and erythrocyte glutathione synthesis rate were measured in 12 children with edematous SAM in the fed state by using stable isotope tracers at 3 clinical phases as follows: 3 ± 1 d (±SE) [clinical phase 1 (CP1)], 8 ± 1 d [clinical phase 2 (CP2)], and 14 ± 2 d (clinical phase 3) after admission. Subjects were randomly assigned to receive equimolar supplements (0.5 mmol · kg⁻¹ · d⁻¹) of methionine or alanine (control) immediately after CP1.

Results: In the methionine compared with the alanine group, cysteine flux derived from protein breakdown was faster at CP2 than CP1 (P < 0.05), and the change in plasma cysteine concentration from CP1 to CP2 was greater (P < 0.05). However, there was no evidence of a difference in cysteine de novo synthesis and its total flux or erythrocyte glutathione synthesis rate and concentration between groups.

Conclusions: Methionine supplementation increased cysteine flux from body protein but had no significant effect on glutathione synthesis rates. Although cysteine is made from methionine, increased dietary cysteine may be necessary to partially fulfill its demand in edematous SAM because glutathione synthesis rates and concentrations were less than previous values shown at full recovery. This study was registered at clinicaltrials.gov as NCT00473031.

INTRODUCTION
A low concentration of blood reduced glutathione is one contributor to increased morbidity in children with edematous severe acute malnutrition (SAM) than with nonedematous SAM (1–3). Glutathione has many important functions including a central role in the regulation of numerous enzymatic pathways and, most importantly, the body’s antioxidant and detoxification systems (2, 4, 5). We have shown that a reduced rate of red cell glutathione synthesis in children with edematous SAM was restored after ~11 d supplementation with N-acetylcysteine (NAC) (6, 7). This result suggested that a low glutathione synthesis rate and concentration are associated with a shortage of cysteine, which is the rate-limiting precursor for glutathione synthesis.

Cysteine in the body is derived from the diet, de novo synthesis from methionine and serine, and protein breakdown. Therefore, we further explored factors that underlie the shortage of cysteine by comparing cysteine and methionine kinetics in edematous and nonedematous SAM while children consumed the same standard treatment diet. Results showed that, in the acute, infected state, children with edematous SAM had a slower production of cysteine (8) and its precursor methionine (9) than did nonedematous children because of a slower rate of release from protein breakdown. However, there was no difference in methionine transsulfuration to cysteine between edematous and nonedematous children. Nevertheless, de novo cysteine synthesis in children with edematous SAM was faster when children were acutely ill than after recovery ~6 wk later. These results suggested an upregulation of methionine transsulfuration to cysteine possibly to meet an increased demand for cysteine during early nutritional rehabilitation. In addition, because methionine is a precursor for the synthesis of methionyl-transfer RNA, which is the cofactor for the initiation of synthesis of all body proteins, methionine deficiency may be related to the overall suppression of protein turnover in SAM (10). Therefore, we hypothesized that methionine supplementation during the early phase of treatment would increase the cysteine availability through enhanced protein turnover and de novo synthesis, which, in turn, would enhance glutathione synthesis, thereby quickly restoring the antioxidant capacity.

1 From the Tropical Metabolism Research Unit, Tropical Medicine Research Institute, University of the West Indies, Kingston, Jamaica (COG, AVB, CT-B, MR, and TF), and the USDA/Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX (JWH and FJ).

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3 Address correspondence to AV Badaloo, Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica. E-mail: asha.badaloo@uwimona.edu.jm.

4 Abbreviations used: CP1, clinical phase 1; CP2, clinical phase 2; CP3, clinical phase 3; NAC, N-acetylcysteine; SAM, severe acute malnutrition.

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SUBJECTS AND METHODS

Subjects

Children, who were admitted to the ward at the Tropical Metabolism Research Unit of the University of the West Indies, Jamaica, for treatment of primary SAM were eligible for the study if they were diagnosed with edematous SAM (kwashiorkor or marasmic-kwashiorkor) according to the Wellcome Classification of malnutrition (11). Participants were studied as 2 groups supplemented with either methionine or isonitrogenous alanine as a control. A sample size of 7 children/group was based on the detection of a 1-SD difference between alanine and methionine groups in the main outcome, the glutathione synthesis rate, with the assumption of a moderate correlation between baseline and repeat measurements of 0.8 at 5% significance and 80% power. The treatment followed a standard protocol as described previously (12). This study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983 for human experimentation. The proposal 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.

Study design

With the use of a stable-isotope tracer methodology, rates of production of reduced glutathione (glutathione) and cysteine were measured before and at 2 time points after subjects received supplements of methionine or isonitrogenous alanine as a control. Subjects were randomly assigned in parallel to receive supplements for ~12 d starting immediately after the first experimental measurement. Subjects were allocated to receive supplements on a 1:1 ratio on the basis of a sequence generated by the rallog command in STATA 12 software (StataCorp LP). The randomization sequence was performed by MR who retained sole custody of the sequence. Eligible subjects were assigned to supplement by AVB after consultation with MR. Participants and care providers were blinded to the treatment allocation. Measurements were carried out in the following 3 phases: clinical phase 1 (CP1) took place at 3 ± 1 d (±SE) postadmission when subjects were both infected and malnourished but clinically stable as indicated by blood pressure, pulse, and respiration rates; clinical phase 2 (CP2) took place at 8 ± 1 d postadmission; and clinical phase 3 (CP3) took place at 14 ± 2 d postadmission when the children were still severely malnourished but after treatment of infection and loss of edema and had an improved effect and appetite. All children received an antibiotic treatment for 10 d.

Diet

All 3 measurements were conducted during the clinical stabilization period when a diet that provided maintenance quantities of energy and protein (417 kJ · kg⁻¹ · d⁻¹ and 1.2 g protein · kg⁻¹ · d⁻¹) was fed according to a standard treatment protocol as previously described (6–9). Thus, subjects had been receiving the therapeutic diet for ~2 d before CP1, ~7 d before CP2, and ~13 d before CP3 measurements. Each child received 2 mL vitamin solution/d, 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 nicotinamide (Tropicvit; Federated Pharmaceuticals) and a mineral mix of potassium, magnesium, and zinc salts [37.28 g KCl + 50.84 g MgCl₂.0.6H₂O + 3.36 g Zn(CH₂COO)₂.2H₂O per liter H₂O; Sigma).

To ensure that the same amount of energy and protein was 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 method provided 17.4 kJ · kg⁻¹ · h⁻¹ and 0.05 g protein · kg⁻¹ · h⁻¹. Weight and length were monitored throughout hospitalization, the former was measured daily with an electronic balance (model F150S; Sartorius), and the latter was measured weekly with a horizontally mounted stadiometer (Holten Ltd).

Supplement

Immediately after CP1, subjects began receiving either L-methionine (0.5 mmol · kg⁻¹ · d⁻¹) or an equimolar quantity of L-alanine as a control made up in sterile water. Supplements were of Sigma United State Pharmacopoeia grade (methionine M5308 and alanine A7469). The total daily supplement was given orally in equal aliquots just before each feed. Because 1 mol methionine yields 1 mol cysteine, the dose of methionine supplement was equimolar to the NAC supplement that had a beneficial effect in our previous study (4). An additional supporting reason was that the dose was equivalent to intake during catch-up growth when protein intake is ~3 g · kg⁻¹ · d⁻¹, which has 74.1 (ie, 24.7 × 3) mg methionine · kg⁻¹ · d⁻¹ or 0.5 mmol · kg⁻¹ · d⁻¹.

Isotope infusion protocol

Isotopic tracers were administered by a constant intravenous infusion. Total cysteine production was measured by using [²H₂]cysteine, and the conversion of [U-¹³C₃]-serine to cysteine was used to measure its de novo synthesis rate. The glutathione synthesis rate in erythrocytes was determined from the rate of incorporation of erythrocyte-free [²H₂]-glycine (precursor) into erythrocyte glutathione (product).

Intravenous access sites were established in opposite arms by the insertion of 22G or 24G catheters after preparation of access sites with a topical anesthetic (EMLA cream; Astra Pharmaceuticals Ltd). One intravenous catheter was used for the infusion of labeled serine, cysteine, and glycine, and the other intravenous catheter was used for blood sampling. A nasogastric tube was inserted into the child’s stomach, and a Flexifloto Magna-Port Y-Port connector (Ross Products Division, Abbott Laboratories) was attached to the proximal end. Approximately 33% of the child’s daily dietary intake was given over the next 8 h by a continuous intragastric infusion into one limb of the Y port by using an enteral infusion pump (Flexiflo companion enteral nutrition pump; Ross Laboratories). Sterile solutions of [²H₂]-cysteine, U-[¹³C₇]-serine, and [²H₂]-glycine (each 98% enriched; Cambridge Isotope Laboratories) were prepared in 9 g NaCl/L. Approximately a 2.2-mL blood sample was drawn after 2 h continuous intragastric feeding, which was followed immediately by 1 d before CP1, ~7 d before CP2, and ~13 d before CP3 measurements. Each child received 2 mL vitamin solution/d, 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 nicotinamide (Tropicvit; Federated Pharmaceuticals) and a mineral mix of potassium, magnesium, and zinc salts [37.28 g KCl + 50.84 g MgCl₂.0.6H₂O + 3.36 g Zn(CH₂COO)₂.2H₂O per liter H₂O; Sigma).

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by simultaneous primed-continuous intravenous infusions of
2H2-cysteine (prime: 1.5 μmol/kg; infusion rate: 1.5 μmol ·
kg⁻¹ · h⁻¹), 2H2-glycine (prime: 30 μmol/kg; infusion rate: 30
μmol · kg⁻¹ · h⁻¹), and U13C3-serine (prime: 8.0 μmol/kg;
infusion rate: 8.0 μmol · kg⁻¹ · h⁻¹). Four additional ~2.2-mL
blood samples were drawn every 0.5 h during the last 1.5 h of
infusions. Infusion rates and blood sampling protocols were the
same for the 2 subsequent experiments performed in subjects at
CP2 and CP3.

Sample analyses

Previously described analytic procedures were used in the
measurement of glutathione and cysteine kinetics (13). Immedi-
ately after each timed blood sample was taken, a 1-mL aliquot
was processed for subsequent measurements of the plasma free
cysteine concentrations and red cell glutathione concentration
and enrichment. The same 1-mL aliquot was processed for these
3 measurements. The 1-mL aliquot was quickly placed in a tube
that contained an equal volume of isotonic ice-cold mono-
bromobimane buffer (pH 7.4) solution (5 mmol monobromobimane/
L, 17.5 mmol Na2 EDTA/L, 50 mmol potassium phosphate/L, 50
mmol serine/L, 50 mmol boric acid/L) and for plasma free cyste-
ine derivatization. The whole blood monobromobimane buffer
mixture was centrifuged at 1000 × g for 10 min at 4°C. The plasma
monobromobimane supernatant fluid was removed and placed in
the dark at room temperature for 20 min for the development of
the plasma free cysteine-monobromobimane derivative. Erythrocytes
were immediately lysed by a rapid freeze and thaw after the addition of 1 mL of the 5-mmol monobromobimane/L buffer, and the mixture was left in the
dark at room temperature for 20 min for the development of the
erthrocyte reduced glutathione-monobromobimane derivative. Proteins
were precipitated from both the plasma and erythrocyte
mixtures with ice-cold 1-mol perchloric acid/L, and the super-
natant fluid was stored at −70°C for later analysis of glutathione
and cysteine concentrations and for isotopic enrichment of
glutathione.

With the use of the remaining blood, hematocrit was first
determined by using ~80 μL blood with a Micro Hematocrit
Centrifuge (Damon/IEC Division). A 1-mL aliquot of each of
the remaining timed blood samples was processed for later analysis
of the enrichment of red cell free glycine and of plasma
cysteine and serine. The same 1-mL aliquot of blood was pro-
cessed for enrichment analyses of all 3 amino acids. The 1-mL
aliquot of blood was centrifuged at 1000 × g for 10 min at 4°C,
and erythrocytes were removed and washed 3 times with an ice-
cold 9-g NaCl/L solution followed by the precipitation of pro-
teins with an ice-cold 1-mol perchloric acid/L solution. The
supernatant fluid from this mixture that contained erythrocyte
free amino acids was stored at −70°C for the later isolation of
free glycine (glutathione precursor) and measurement of its
isotopic enrichment. The plasma was stored −70°C for the later
isolation of serine and cysteine and measurement of their iso-
topic enrichments.

Concentration of erythrocyte glutathione

The glutathione concentration in the stored erythrocyte glu-
tagione-monobromobimane supernatant fluid was measured by
using reverse-phase HPLC as previously described (13) on
a Hewlett-Packard 1090 HPLC equipped with a Model HP 1046A
fluorescence detector (Hewlett-Packard). A fraction collector was
used to collect the erythrocyte glutathione-containing eluant for
hydrolysis of glutathione to its constituent amino acids and
isolation of glycine for isotopic enrichment.

Concentration of plasma cysteine

The cysteine concentration in the stored plasma cysteine-
monobromobimane supernatant fluid was measured by using
reverse-phase HPLC as previously described (13) on a Hewlett-
Packard 1090 HPLC equipped with a Model HP 1046A fluo-
rescence detector (Hewlett-Packard).

Isotopic-enrichment analysis

Enrichment of erythrocyte glutathione. Glutathione enrich-
ment was determined from the enrichment of glycine incor-
porated into the glutathione peptide. Glycine was isolated
from the erythrocyte glutathione fraction, which was collected
during the HPLC determination of the glutathione concentration.
The erythrocyte glutathione fraction was dried, and the peptide
was hydrolyzed for 4 h in 6 mol HC1/L at 110°C to yield its constituent
amino acids. The hydrolysate was dried, and released amino acids
were converted to their n-propyl ester heptafluorobutyramide
derivatives for mass spectrometric analysis. The tracer-to-tracer ratio
of the glycine derivative was measured by using negative chemical
ionization gas chromatography–mass spectrometry with the selec-
tive monitoring of ions at an m/z of 293–295.

Enrichment of erythrocyte-free glycine and plasma cysteine
and serine. The enrichment of glycine in the stored supernatant
fluid that contained erythrocyte-free amino acids and cysteine
and serine in plasma were also measured by using negative
chemical ionization gas chromatography–mass spectrometry.
Amino acids in each of these samples were first isolated by
using cation-exchange chromatography before conversion to
their n-propyl ester heptafluorobutyramide derivatives for mass
spectrometric analysis. For the plasma cysteine dithiothreitol,
0.075 mL of a 10-mmol/L solution was added to the de-
vitrification mixture to convert cystine to cysteine. The tracerto-tracer ratio of n-propyl ester heptafluorobutyramide deri-
vatives were monitored at a selective m/z of 293–295 for those
of erythrocyte-free glycine, 535–538 for plasma cysteine, and
519–522 for plasma serine.

Calculations

Total cysteine flux (Q) was calculated by using plasma plateau
tracer:tracer ratios in the following steady state equation:

\[ Q (\mu mol \cdot kg^{-1} \cdot h^{-1}) = I(E_{Inf} + E_{pl}) \]

where \( E_{Inf} \) is the tracer:tracer ratio of the infusate, \( E_{pl} \) is the
tracer:tracer ratio of plasma cysteine (M+2 isotopomer), and \( I \)
is the rate of infusion of the 2H2-cysteine tracer.

Because \( Q \) represents the sum of all inputs (and outputs) of an
amino acid through the body pool,

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

where \( B_{Cys} \) is cysteine released from body protein breakdown,
\( I_{Cys} \) is cysteine intake from the diet and tracer infusion, and \( S_{Cys} \)
is cysteine synthesized de novo. Hence, endogenous cysteine flux \([\text{IE}_{\text{Cys}} + \text{SC}_{\text{Cys}}]\) was calculated by subtracting \(L_{\text{Cys}}\) from \(Q_{\text{Cys}}\).

Similarly, cysteine derived from protein breakdown \(B\) can be calculated as

\[
B_{\text{Cys}} = Q_{\text{Cys}} - (L_{\text{Cys}} + S_{\text{Cys}}) \tag{3}
\]

Cysteine produced by de novo synthesis, which receives its carbon atoms from serine and its sulfur from methionine, can be obtained from the rate of conversion of serine to cysteine by using the equation

\[
\text{Cysteine synthesis} = Q_{\text{Cys}} \times (E_{\text{CYS}} + E_{\text{SER}}) \tag{4}
\]

where \(Q_{\text{Cys}}\) is cysteine flux obtained with the \(^{7}\text{H}_{2}\)-cysteine tracer, \(E_{\text{CYS}}\) is the tracer:tracee ratio of cysteine (M+3 isotopomer) derived from the U.\(^{15}\text{C}_{\text{Y}}\)-serine, and \(E_{\text{SER}}\) is the plasma tracer:tracee ratio of serine at an isotopic steady state.

The fractional synthesis rate (FSR) of erythrocyte-glutathione was calculated according to the precursor-product equation (13):

\[
\text{FSR}_{\text{GSH}}\left({\%}/d\right) = \left[\left(E_{t6} - E_{t4.5}\right) + E_{\text{inc}}\right] \times \left[2400 \div \left(t_{6} - t_{4.5}\right)\right] \tag{5}
\]

where \(E_{t6} - E_{t4.5}\) is the increase in the tracer:tracee ratio of erythrocyte glutathione-bound glycine between the 4.5th and 6th hours of infusion, when the tracer:tracee ratio of erythrocyte-free glycine \((E_{\text{inc}})\) had reached a steady state. The absolute synthesis rate of erythrocyte glutathione was calculated as the product of the erythrocyte glutathione concentration and the fractional synthesis rate. Units of the absolute synthesis rate are expressed as millimoles per liter of packed erythrocytes per day.

All kinetic data are expressed per kilograms of body weight. Body weight measured in the edematous state was corrected by subtracting the contribution of edema fluid. Edema fluid was estimated as the difference between body weights on the day of measurement and the lowest postexperiment weight indicating the loss of edema.

**Statistics**

Kinetics data were approximately normally distributed and analyzed by using 2-factor repeated-measures ANOVA with the supplement (methionine and alanine) as the between-group factor and the repeated factor as measurements over time at CP1, CP2, and CP3. Formal tests of sphericity such as Mauchly’s test often do not hold for small sample sizes as in this study. Consequently, we performed inferential tests by using the Huynh-Feldt–corrected \(P\) value, which is an example of a df correction test for all analyses. If the repeated-measures ANOVA was significant, post hoc pairwise comparisons were performed by using Tukey’s and Bonferroni’s methods. Stata Statistical Software (version 8 for Windows; StataCorp LP) was used for the analysis. Results were considered to be statistically significant at \(P < 0.05\).

**RESULTS**

Eight children in each group were randomly assigned to receive methionine or alanine, but measurements were completed and analyzed in 12 of these children. Two children recruited in the alanine group were dropped from the study because the hemoglobin concentration (4.9 g/L) in one patient was considered too low by the pediatrician in charge for taking experimental blood samples, and the other child was diagnosed with sickle cell disease. No measurements were carried out in 2 patients assigned to the methionine group because of difficulty in establishing intravenous lines. No adverse effect of supplements was observed during the experimental period or during the rehabilitation of participants. Age and physical characteristics of the 12 patients in whom measurements were completed are shown in Table 1. Ages, lengths, and weights were not significantly different between alanine- and methionine-supplemented groups. There was no significant gain in weight or in height over the period of measurements. Clinical characteristics of children at admission are shown in Table 2. Eleven of the 12 children studied had between 1 and 4 different infections at admission. Hemoglobin and albumin concentrations ranged between 5.5 and 10.4 g/L and between 15 and 28 g/L, respectively. The white blood cell count ranged between 6.4 and 27.6 \(\times 10^9\) cells/L, and body temperatures of the children were between 36°C and 38°C.

Total methionine intake from feeds and the supplement was 0.77 mmol \(\cdot\) kg\(^{-1}\) \(\cdot\) day\(^{-1}\). Cysteine and kinetics data are shown in Table 3. De novo cysteine synthesis at CP1 was higher than at CP3 \((P = 0.04)\), but there was no effect of the methionine

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Clinical phase 1</th>
<th></th>
<th>Clinical phase 2</th>
<th></th>
<th>Clinical phase 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine ((n = 6))</td>
<td>Methionine ((n = 6))</td>
<td>Alanine ((n = 6))</td>
<td>Methionine ((n = 6))</td>
<td>Alanine ((n = 6))</td>
<td>Methionine ((n = 6))</td>
</tr>
<tr>
<td>Age (mo)(^2)</td>
<td>11.5 ± 4.1</td>
<td>8.5 ± 1.8</td>
<td>11.7 ± 4.1</td>
<td>8.7 ± 1.8</td>
<td>12.0 ± 4.1</td>
<td>9.0 ± 1.8</td>
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<tr>
<td>Weight (kg)</td>
<td>5.8 ± 1.0</td>
<td>5.9 ± 0.6</td>
<td>5.7 ± 1.1</td>
<td>5.9 ± 0.6</td>
<td>5.8 ± 1.1</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>63.1 ± 4.7</td>
<td>64.3 ± 3.3</td>
<td>63.2 ± 4.7</td>
<td>64.4 ± 3.4</td>
<td>63.4 ± 4.7</td>
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<tr>
<td>Weight-for-age (%)(^2)</td>
<td>63.5 ± 6.2</td>
<td>70.5 ± 3.7</td>
<td>61.4 ± 5.8</td>
<td>69.6 ± 3.6</td>
<td>60.6 ± 8.2</td>
<td>65.3 ± 3.6</td>
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<tr>
<td>Weight-for-length (%)(^2)</td>
<td>91.5 ± 3.3</td>
<td>88.8 ± 5.8</td>
<td>88.8 ± 3.5</td>
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<tr>
<td>Length-for-age (%)(^2)</td>
<td>86.1 ± 3.2</td>
<td>91.8 ± 1.7</td>
<td>86.0 ± 3.1</td>
<td>91.5 ± 1.8</td>
<td>85.5 ± 3.9</td>
<td>90.7 ± 1.6</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SEMs. Clinical phase 1 took place at \(3 ± 1\) d \((\pm SE)\) after admission when subjects were both infected and malnourished but clinically stable; clinical phase 2 took place at \(8 ± 1\) d after admission; and clinical phase 3 took place at \(14 ± 2\) d after admission when subjects were still severely malnourished but no longer infected, had lost edema, and had improved affect and appetite. Weight-for-age, weight-for-length, and length-for-age are expressed as the percentage of the median for a reference child of the same age or height.

\(^2\) Main effect of clinical phase, \(P < 0.05\) (repeated-measures ANOVA).
supplementation. However, the total cysteine flux increased from CP1 to CP3 ($P = 0.02$). Also, cysteine derived from protein breakdown increased in both methionine and control groups from CP1 to CP3, but the rise from CP1 to CP2 was greater in the methionine group than alanine group (clinical phase by supplement interaction: $P = 0.05$). Similarly, the plasma cysteine concentration increased from CP1 to CP3 in both groups. When the change in cysteine concentration was analyzed, the change from CP1 to CP2 was greater with methionine supplementation ($P = 0.01$; unpaired $t$ test). The glutathione absolute synthesis rate and concentration were higher at CP3 than CP1 and CP2 ($P = 0.00$), but there was no significant effect of methionine supplementation (Table 4).

**DISCUSSION**

Data showed that supplementation with 0.5 mmol methionine/d did not elicit an improvement in the cysteine concentration and glutathione synthesis and concentration as reported for an equimolar amount of NAC supplementation in our previous study with the same treatment diet. Also, there was not a faster rate of loss of edema. Similar to our previous findings (8, 9), the de novo cysteine synthesis was upregulated at CP1 before supplementation but was not significantly different between the 2 groups. Although, after about 5 d of methionine supplementation, the plasma cysteine concentration increased in association with an increase in cysteine derived from protein, there was no significant effect on glutathione kinetics. Moreover, at the end of the supplementation period, the glutathione absolute rate of synthesis and concentration were less than values shown at full nutritional recovery in our previous study (7), which suggested that the total cysteine availability was not sufficient to have a significant effect on glutathione kinetics.

There is a strong possibility that the difference in outcome between the NAC and methionine supplements in our studies was because NAC is more potent at facilitating an increase in cysteine

### Table 2

Clinical characteristics of subjects at admission

<table>
<thead>
<tr>
<th>Child</th>
<th>Diagnosis</th>
<th>Supplement group</th>
<th>Infection</th>
<th>Hemoglobin</th>
<th>Leukocyte</th>
<th>Albumin</th>
<th>Temperature</th>
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<tr>
<td>1</td>
<td>k</td>
<td>Met</td>
<td>B, SC, URTI, OM</td>
<td>8.4</td>
<td>10.0</td>
<td>28</td>
<td>37.8</td>
</tr>
<tr>
<td>2</td>
<td>mk</td>
<td>Ala</td>
<td>URTI, D, SC</td>
<td>6.7</td>
<td>27.6</td>
<td>18</td>
<td>36.0</td>
</tr>
<tr>
<td>3</td>
<td>mk</td>
<td>Met</td>
<td>B, OM, SC</td>
<td>6.4</td>
<td>18.9</td>
<td>22</td>
<td>37.0</td>
</tr>
<tr>
<td>4</td>
<td>k</td>
<td>Ala</td>
<td>D</td>
<td>6.7</td>
<td>7.9</td>
<td>25</td>
<td>36.8</td>
</tr>
<tr>
<td>5</td>
<td>k</td>
<td>Met</td>
<td>None</td>
<td>6.4</td>
<td>12.7</td>
<td>27</td>
<td>36.6</td>
</tr>
<tr>
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<td>Ala</td>
<td>B, URTI, UTI, SC</td>
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<td>8.1</td>
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<td>7</td>
<td>k</td>
<td>Met</td>
<td>SC</td>
<td>9.2</td>
<td>17.3</td>
<td>24</td>
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<tr>
<td>8</td>
<td>mk</td>
<td>Ala</td>
<td>B, OM, SC</td>
<td>7.4</td>
<td>22.4</td>
<td>21</td>
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</tr>
<tr>
<td>9</td>
<td>k</td>
<td>Met</td>
<td>URTI, SC</td>
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<td>10.4</td>
<td>6.4</td>
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<td>37.5</td>
</tr>
<tr>
<td>12</td>
<td>mk</td>
<td>Ala</td>
<td>OM</td>
<td>8.1</td>
<td>8.6</td>
<td>22</td>
<td>38.0</td>
</tr>
</tbody>
</table>

$^1$ Ala, alanine; B, bacteremia; D, infective diarrhea; k, kwashiorkor; mk, marasmic kwashiorkor; Met, methionine; OM, otitis media; URTI, upper respiratory tract infection; UTI, urinary tract infection; SC, skin candidiasis.

### Table 3

Cysteine kinetics in children with edematous severe acute malnutrition

<table>
<thead>
<tr>
<th>n</th>
<th>Total flux</th>
<th>Dietary inflow</th>
<th>Endogenous flux</th>
<th>De novo synthesis</th>
<th>Protein-derived flux</th>
<th>Plasma cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu mol \cdot kg^{-1} \cdot h^{-1}$</td>
<td>$\mu mol \cdot kg^{-1} \cdot h^{-1}$</td>
<td>$\mu mol \cdot kg^{-1} \cdot h^{-1}$</td>
<td>$\mu mol \cdot kg^{-1} \cdot h^{-1}$</td>
<td>$\mu mol \cdot kg^{-1} \cdot h^{-1}$</td>
<td>$\mu mol \cdot L^{-1}$</td>
</tr>
<tr>
<td>Clinical phase 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>6</td>
<td>41.3 ± 5.8$^2$</td>
<td>6.9 ± 0.2</td>
<td>32.9 ± 5.7</td>
<td>11.8 ± 2.5</td>
<td>21.1 ± 3.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>6</td>
<td>41.1 ± 7.9</td>
<td>7.3 ± 0.2</td>
<td>32.3 ± 7.8</td>
<td>12.5 ± 2.1</td>
<td>19.8 ± 6.8</td>
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<tr>
<td>Clinical phase 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Alanine</td>
<td>6</td>
<td>44.9 ± 3.9</td>
<td>6.3 ± 0.02</td>
<td>37.1 ± 3.9</td>
<td>10.0 ± 0.9</td>
<td>27.1 ± 3.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>6</td>
<td>54.6 ± 4.6</td>
<td>6.4 ± 0.02</td>
<td>46.8 ± 4.6</td>
<td>8.6 ± 1.4</td>
<td>38.15 ± 4.2$^3$</td>
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<tr>
<td>Clinical phase 3</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Alanine</td>
<td>6</td>
<td>49.7 ± 3.5</td>
<td>6.4 ± 0.02</td>
<td>41.9 ± 4.5</td>
<td>8.5 ± 1.1</td>
<td>33.4 ± 2.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>6</td>
<td>50.7 ± 3.6</td>
<td>6.3 ± 0.04</td>
<td>42.4 ± 2.4</td>
<td>8.4 ± 1.6</td>
<td>34.5 ± 2.5</td>
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<tr>
<td>$P$</td>
<td>Supplement × clinical phase</td>
<td>0.21</td>
<td>0.13</td>
<td>0.18</td>
<td>0.74</td>
<td>0.05</td>
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<tr>
<td>Supplement</td>
<td></td>
<td>0.60</td>
<td>0.19</td>
<td>0.61</td>
<td>0.88</td>
<td>0.48</td>
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<tr>
<td>Clinical phase</td>
<td></td>
<td>0.02</td>
<td>0.001</td>
<td>0.01</td>
<td>0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^1$ Clinical phase 1 took place at 3 ± 1 d (±SE) after admission when subjects were both infected and malnourished but clinically stable; clinical phase 2 took place at 8 ± 1 d after admission; and clinical phase 3 took place at 14 ± 2 d after admission when subjects were still severely malnourished but no longer infected, had lost edema, and had improved affect and appetite. Analyses were conducted by using repeated-measures ANOVA.

$^2$ Mean ± SEM (all such values).

$^3$ Compared with alanine at the same stage [95% CIs: 7.4, 29.3 (Tukey) and 6.7, 30.0 (Bonferroni)].
availability to enhance glutathione synthesis. Iminocyclase 1, which is the enzyme that catalyzes the deacetylation of NAC, has a low affinity for NAC and low activity in red blood cells (15). Therefore, a limited intracellular deacetylation of NAC to provide cysteine does not account for the full effect of NAC in supporting erythrocyte glutathione production such as after administration of paracetamol (14). As a substrate for cysteine, an NAC extracellular concentration 20 times that of cysteine can readily enter red blood cells to sustain glutathione synthesis. Despite an early increase in the amount of cysteine derived from protein breakdown in the methionine-supplemented group, the inability to meet the total cysteine requirement to meet the body’s demands was higher in edematous SAM than non-edematous SAM (18). This effect may be associated with a higher demand to synthesize cysteine-rich proteins such as acute phase proteins as a consequence of infection and to make gut mucosal proteins and cysteine-rich mucins for repair of the markedly atrophic gut mucosa that accompanies edematous SAM (18). Moreover, production rates of glycine (24) and serine (9) of methionine to cysteine in the face of a shortage of cysteine was not supported by the lack of an increase in the conversion of methionine to cysteine (6, 7) and the increased demand for cysteine in edematous SAM inferred that cysteine might be conditionally essential in these children. It is also possible that de novo cysteine cannot significantly contribute to satisfying the increased demand for cysteine in edematous SAM because there is a limit in the conversion of methionine to cysteine. For example, transmethylation reactions accounted for 23% and 35% of the methionine flux in healthy, adult men in the postabsorptive and fed state, respectively (22).

A limitation of this study was that methionine supplementation was not tested at different amounts. If the supplementation was in excess of the methionine requirement in SAM, there could have been competition for glycine between its use in the metabolism of excess methionine and its role as a precursor for glutathione. In the transmethylation of methionine, glycine is a methyl-group acceptor that forms sarcosine (N-methylglycine) and may contribute substantially to the transmethylation flux (23). In addition, serine which is used in the transsulfuration pathway, can be synthesized in part from glycine. However, methionine intake in excess of the demand was not supported by the lack of an increase in the conversion of methionine to cysteine in the face of a shortage of cysteine (6, 7) and the increased demand for cysteine in edematous SAM (18). Moreover, production rates of glycine (24) and serine (9) are not reduced in SAM.

In conclusion, methionine supplementation in children with edematous SAM increases the plasma cysteine concentration and cysteine derived from protein breakdown but not de novo cysteine or glutathione synthesis. Dietary methionine might not be able to satisfy the total demand for cysteine in these children. Therefore, cysteine might be conditionally essential in edematous SAM, and consequently, dietary cysteine might be needed to partially fulfill the total requirement for cysteine.

### Table 4

Reduced glutathione kinetics in erythrocytes of children with edematous severe acute malnutrition

<table>
<thead>
<tr>
<th>Sample</th>
<th>ASR mmol·L⁻¹·d⁻¹</th>
<th>FSR %/d</th>
<th>Concentration mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical phase 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.3 ± 0.1²</td>
<td>38.9 ± 6.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2 ± 0.04</td>
<td>58.5 ± 9.3</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td><strong>Clinical phase 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.6 ± 0.08</td>
<td>57.2 ± 5.4</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.6 ± 0.2</td>
<td>76.1 ± 17.4</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td><strong>Clinical phase 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.9 ± 0.6</td>
<td>61.0 ± 5.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9 ± 0.1</td>
<td>52.2 ± 6.3</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

¹Clinical phase 1 took place at 3 ± 1 d (± SE) after admission when subjects were both infected and malnourished but clinically stable; clinical phase 2 took place at 8 ± 1 d after admission; and clinical phase 3 took place at 14 ± 2 d after admission when subjects were still severely malnourished but no longer infected, had lost edema, and had improved appetite. Analyses were conducted by using repeated-measures ANOVA. ASR, absolute synthesis rate; FSR, fractional synthesis rate.

²Mean ± SEM (all such values).
We are very grateful to the physicians and nursing staff of the Tropical Metabolism Research Unit for their care of children and Lorraine Wilson, Bentley Chambers, and Grace Tang for their excellent work and support in the conduct of the studies and analysis of samples.

The authors’ responsibilities were as follows—FJ, TF, AVB, and MR: designed the research; CT-B: was the study pediatrician; AVB, CT-B, and COG: conducted the research; JWH, AVB, and MR: analyzed data or performed the statistical analysis; COG, AVB, TF, and FJ: wrote the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES