Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition\(^1\text{-}^3\)

Asha Badaloo, Marvin Reid, Terrence Forrester, William C Heird, and Farook Jahoor

**ABSTRACT**

**Background:** Children with severe edematous malnutrition have higher than normal oxidant damage and lower concentrations of the antioxidant reduced glutathione (GSH), which are associated with slower synthesis of GSH and with lower extracellular concentrations of the precursor amino acid cysteine.

**Objective:** We tested whether early dietary supplementation with cysteine could restore a normal GSH concentration and synthesis rate in these children.

**Design:** Erythrocyte cysteine and GSH concentrations and the fractional and absolute synthesis rates of GSH were measured in 2 groups of 16 edematous malnourished children, 10 boys and 6 girls aged 6–18 mo, at 3 times after hospital admission: at ≈2 d (period 1), when they were malnourished and infected; at ≈11 d (period 2), when they were malnourished but cleared of infection; and at ≈50 d (period 3), when they had recovered. Supplementation with either 0.5 mmol·kg\(^{-1}\)·d\(^{-1}\) N-acetylcysteine (NAC group) or alanine (control group) started immediately after period 1 and continued until recovery.

**Results:** From period 1 to period 2 the concentration and the absolute synthesis rate of GSH increased significantly (\(P < 0.05\)) in the NAC group but not in the control group. The increases in the GSH concentration and synthesis rate were ≈150% and 510% greater, respectively, in the NAC group than in the control group. The increases in the NAC group were associated with a significant effect of supplement (\(P < 0.03\)) on erythrocyte cysteine concentration.

**Conclusion:** These results suggest that the GSH synthesis rate and concentration can be restored during the early phase of treatment if patients are supplemented with cysteine.

Key Words: Glutathione, severe malnutrition, cysteine, edema, oxidant damage, children

**INTRODUCTION**

In children with severe malnutrition, it has consistently been shown that the concentration of reduced glutathione (\(\gamma\)-glutamylcysteinylglycine, or GSH) in plasma and whole blood is lower than normal in those with edema but not in those without edema (1–3). Because the GSH redox cycle is a major component of the body’s overall antioxidant defenses, a lower concentration of GSH is indicative of impaired antioxidant capacity. Indeed, children with edematous malnutrition have elevated levels of several biomarkers of oxidant-induced lipid peroxidation such as malondialdehyde, hexanal (4), and lipid hydroperoxide (5). On the basis of similar observations it was proposed that the impaired antioxidant defenses of kwashiorkor and marasmic kwashiorkor lead to free radical damage of cellular membranes and that this damage plays an important role in the pathogenesis of the disease (1, 2). This possibility is supported by a recent study by Fechner et al (6) showing a relation between restoration of erythrocyte GSH concentrations and the clinical outcome of children with kwashiorkor. If true, therapy aimed at restoring GSH availability during the early resuscitative phase of treatment should decrease overall morbidity and mortality. To implement such therapy, however, the mechanism or mechanisms responsible for the reduced availability of GSH must be identified.

The availability of any metabolite represents the balance between its rate of synthesis and the rate at which it is used. Although it has been proposed that GSH depletion in malnutrition results from infection-induced increases in oxidative stress and hence an accelerated rate of consumption of GSH (2), in a recent study of GSH metabolism in edematous and nonedematous malnourished children, we showed that GSH concentration was low only in the edematous group, despite the presence of concurrent infections in both groups (5). The lower GSH concentration in the edematous group appeared to be caused by impaired synthesis associated with lower concentrations of cysteine (5), the rate-limiting precursor of GSH. In fact, the erythrocyte cysteine concentration in the malnourished state was <2 mmol/L, a value much less than the apparent \(K_m\) value (0.1–0.3 mmol/L) of \(\gamma\)-glutamylcysteine synthetase (EC 6.3.2.2) (7). On the basis of these observations, we proposed that dietary supplementation with cysteine during the early resuscitative phase of treatment of children with severe edema might increase the availability of cysteine. Repeat studies, however, showed that the increase in cysteine concentration was small (8). These results suggest that additional mechanism(s) are involved in the reduced synthesis rate of GSH in severe malnutrition.

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edematous malnutrition would elicit an increase in GSH synthesis, thereby restoring the GSH concentration and availability faster.

The study reported here was performed to determine the effect of cysteine supplementation on GSH kinetics in children with edematous malnutrition receiving supplements of either N-acetylcysteine (NAC) or alanine (control).

**SUBJECTS AND METHODS**

**Subjects**

Sixteen children admitted to the Tropical Metabolism Research Unit, University of the West Indies, for treatment of severe edematous malnutrition participated in the study. The diagnosis of severe edematous malnutrition, kwashiorkor, or marasmic kwashiorkor was based on the Wellcome Classification (8). The clinical characteristics of the subjects are shown in Table 1. All were anemic with clinical evidence of infection (ie, the presence of one or more of the following: leukocyte count > 11 × 10^9 cells/L, temperature at admission > 37°C or < 35.5°C, an abnormal chest X-ray, a positive blood, urine, skin, or stool culture).

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, Baylor College of Medicine. Written, informed consent was obtained from at least one parent of each child enrolled.

**Treatment**

The children were managed according to a standard treatment protocol (9). During the resuscitative phase, they were started on broad-spectrum antibiotics, usually parenteral penicillin and gentamicin, plus oral metronidazole, as well as a milk-based diet that provided 417 kJ·kg⁻¹·d⁻¹ and 1.2 g protein·kg⁻¹·d⁻¹ supplemented with vitamins (Tropivite; Federated Pharmaceuticals, Jamaica) and a mineral mix. Each child received 2 mL Tropivite solution/d, which was composed of 6000 IU vitamin A (palmitate), 1600 IU vitamin D (calciferol), 2 mg thiamine HCL, 3.2 mg riboflavin 5’ phosphate Na, 120 mg vitamin C (ascorbic acid), 4 mg vitamin B-6 (pyridoxine HCL), and 28 mg nicotinamide. They also received 5 mg folic acid/d and 2 mL·kg⁻¹·d⁻¹ of a mineral mix of potassium, magnesium, and zinc salts (37.28 g KCl + 50.84 g MgCl₂·6H₂O + 3.36 g (CH₃COO)₂Zn·2H₂O/L H₂O) (BDH Chemicals, Poole, United Kingdom). This diet was continued until the infections had resolved, fluid and electrolyte imbalances were corrected, and appetites had improved (~11 d after admission). They were then switched gradually to an energy-dense, milk-based formula to facilitate rapid catch-up growth until nutritional recovery was achieved. This diet provided 625–750 kJ·kg⁻¹·d⁻¹ and 2.75–3.3 g protein·kg⁻¹·d⁻¹. During this catch-up growth phase, the children received vitamins and minerals as described above as well as 60 mg ferrous sulfate/d. At recovery the subjects had attained ≥90% of the appropriate weight in relation to their length or had experienced spontaneous reductions in dietary intake and in the rate of weight gain for 3 d. Weight was measured daily and length weekly throughout hospitalization, the former with an electronic balance (Sartorius model F150S; Göttingen, Germany) and the latter with a horizontally mounted stadiometer (Holttain Ltd, Crymych, United Kingdom). Each child was assessed for edema daily from admission until it was no longer present by a physician who was blinded to the identity of the supplement to which each patient was assigned. Edema was noted as present or absent and was detected by applying constant pressure with the thumb over the bony prominence of the tibia for 30 s. On removal, a depression indicated pitting edema.

**Study design**

Once enrolled in the study, the 16 subjects were randomly assigned to receive either NAC or control supplements starting immediately after the first experimental protocol (period 1). All subjects were studied on 3 occasions. The first experiment was performed within 1–3 d after admission, when the patients were both infected and malnourished but clinically stable, as indicated by blood pressure and pulse and respiration rates. The second experiment (period 2) was performed 7–13 d later, when they were
still severely malnourished but no longer infected; they also had lost edema, their affect and appetite had improved, and all clinical features of the infection (eg, diarrhea, chest crepitations, and fever) had resolved. The third experiment (period 3) was performed just before discharge after recovery, as defined above. At each experiment, the rate of synthesis of erythrocyte GSH was measured with the use of a constant infusion of $^{3}H_{2}$-glycine.

The same diet (providing $\approx 417$ kJ · kg$^{-1}$ · d$^{-1}$ and $\approx 1.2$ g protein · kg$^{-1}$ · d$^{-1}$) was fed during all 3 experimental protocols. At period 1, the subjects had received this diet for 1–3 d and at period 2, they had received this diet for 9–15 d. They were restarted on this diet 3 d before the final experiment (period 3), ie, $\approx 47$ d after admission.

Isotope administration

At each experiment, $\approx 33\%$ of the subject’s daily formula intake was given by constant intragastric infusion starting 2 h before the isotope infusion was started and continuing throughout the 6-h infusion. A sterile solution of 18 mmol/L $^{2}H_{2}$-glycine (Cambridge Isotope Laboratories, Woburn, MA) was prepared in 9 g/L saline. After a 2-mL venous blood sample was drawn, a priming dose of $^{3}H_{2}$-glycine (40 μmol/kg) was administered by nasogastric tube and followed immediately by a continuous 6-h nasogastric infusion of $^{2}H_{2}$-glycine at a rate of 30 μmol · kg$^{-1}$ · h$^{-1}$. Additional 2-mL blood samples were drawn after 3, 4, 5, and 6 h of the infusion. The infusion and blood-sampling protocols for all 3 experiments were identical.

Supplement

Immediately after period 1, the subjects began receiving the assigned supplement, either 0.5 mmol cysteine · kg$^{-1}$ · d$^{-1}$ given as 81.6 mg NAC · kg$^{-1}$ · d$^{-1}$ or an equimolar quantity (44.5 mg · kg$^{-1}$ · d$^{-1}$) of alanine. The total daily supplement, which was adjusted for increases in body weights, was given orally in equal aliquots just before each feeding. The amounts of cysteine and alanine supplied by the milk-based diets were 0.26 and 0.45 mmol/g protein, respectively.

Sample analyses

A 1-mL aliquot of each blood sample was placed immediately in an equal volume of isotonic ice-cold monobromobimane (MBB) buffer (pH 7.4) solution (5 mmol monobromobimane/L, 17.5 mmol Na$_2$EDTA/L, 50 mmol potassium phosphate/L, 50 mmol serine/L, 50 mmol boric acid/L) for GSH derivatization and isolation. The whole-blood–MBB buffer mixture was centrifuged at 1000 g for 10 min at 4°C, and the plasma MBB supernatant fluid was removed. One milliliter MBB buffer and 0.045 mL 20 mmol D-penicillamine/L (internal standard) were added to the packed erythrocytes, which were immediately lysed by rapid freeze and thaw, and the lysed erythrocyte mixture was shaken and left in the dark at room temperature for 20 min for development of the erythrocyte GSH-MBB derivative. Proteins were precipitated from the mixture with ice-cold 1 mol perchloric acid/L, and the supernatant fluid was stored at $-70°C$ for later analysis. The hemocrit of each blood sample was determined with a Micro Hematocrit Centrifuge (Damon/IEC Division, Needham Heights, MA). Whole blood was centrifuged at 13 460 × g for 5 min at 25°C.

The remaining 1-mL aliquot of blood was centrifuged immediately at 1000 × g for 10 min at 4°C, and the plasma was removed and stored at $-70°C$ for later analysis. The packed erythrocytes of this aliquot were washed 3 times with an ice-cold 9 g sodium chloride/L solution, and the proteins were precipitated with an ice-cold 1 mol perchloric acid/L solution. The protein-free supernatant fluid, containing erythrocyte free amino acids, was stored at $-70°C$ for later determination of the isotopic enrichment of the erythrocyte free glycine.

Measurement of erythrocyte cysteine and erythrocyte GSH concentrations and isolation of erythrocyte GSH were done by reverse-phase HPLC as previously described (10) with a Hewlett-Packard 1090 HPLC equipped with a Model HP 1046A fluorescence detector (Hewlett-Packard, Avondale, PA). Standards included known concentrations of cysteine, glutathione, and D-penicillamine (Sigma, St Louis) prepared and diluted in the same manner as the samples. The GSH-containing fractions were collected on a fraction collector, and, after drying, the peptide was hydrolyzed for 4 h in 6 mol HCl/L at 110°C.

Erythrocyte free glycine was extracted from the protein-free supernatant fluid fraction by cation-exchange chromatography. Erythrocyte free glycine and erythrocyte GSH-derived glycine were converted to the $\alpha$-propyl ester heptafluorobutyramide derivatives, and the isotope ratio of each was measured by negative chemical ionization gas chromatography–mass spectrometry, monitoring ions at a mass-to-charge ratio of 293 to 295. Erythrocyte amino acid concentrations were determined with the use of reverse-phase high-performance liquid chromatography (Waters, Millipore Corporation, Milford, MA).

Calculations

The fractional synthesis rate (FSR) of erythrocyte-GSH was calculated according to the precursor-product equation (10):

$$\text{FSR}_{\text{GSH}} (\%) = \frac{[\text{IR}_G - \text{IR}_N]/\text{IR}_N} \times \left[\frac{2400/(t_6 - t_4)}{1}\right]$$

where $\text{IR}_G - \text{IR}_N$ is the increase in the isotopic enrichment of erythrocyte GSH-bound glycine between the fourth and sixth hours of infusion, when the enrichment of erythrocyte free glycine, $\text{IR}_N$, had reached a steady state. The absolute synthesis rate of erythrocyte GSH was calculated as the product of the erythrocyte GSH concentration and the FSR. The units of the absolute synthesis rate are expressed as mmol · L packed erythrocyte$^{-1}$ · d$^{-1}$.

Statistical analysis

Data are expressed as means ± SEMs. The data were analyzed by two-factor repeated-measures analysis of variance (RMANOVA) with the between-group factor being the supplementation group and the measurements over time (period 1 through period 3) being the repeated factor. If the RMANOVA was significant, then post hoc pairwise comparisons were performed by the Tukey method with the use of the error term from the RMANOVA analysis. The Friedman test was used to determine differences in plasma cysteine concentrations between periods. If the overall $P$ value obtained from the Friedman test was $<0.05$, then post hoc pairwise comparisons were done by Wilcoxon’s signed-rank test and the Mann-Whitney $U$ test. Data analysis was performed with STATA STATISTICAL SOFTWARE version 6 for WINDOWS (Stata Corporation, College Station, TX).

RESULTS

The age, weight, and length of the children at each period are shown in Table 2. There were no between-group differences in age, weight, weight for age, or weight for length. The children in the NAC group, however, were significantly longer than children
in the control group at each period ($P < 0.05$). As expected, the weight, length, weight for age, and weight for length of both groups combined increased significantly ($P < 0.01$) from period 1 to period 3.

Supplementation with NAC resulted in significantly higher GSH concentration in the NAC group than in the control group (Figure 1). This higher GSH concentration in the NAC group was associated with significantly higher absolute rates of synthesis of GSH ($P < 0.001$). There were also significant period effects because the GSH concentration, fractional rate of synthesis, and absolute rate of synthesis increased from period 1 to period 3.

When changes in the acute resuscitative phase were analyzed, that is, from period 1 to period 2, there were significant increases ($P < 0.05$) in the concentration as well as the absolute rates of synthesis of erythrocyte GSH in the NAC group but not in the control group (Figure 2). These increases in erythrocyte GSH concentration and absolute rate of synthesis from period 1 to period 2 were approximately 150% and 510% greater, respectively, in the NAC group than in the control group and were associated with a significant effect of supplement ($P < 0.03$) on erythrocyte cysteine concentration (Figure 3). In addition, the erythrocyte concentration of cysteine was significantly higher at period 3 than at period 1.

There were no significant between-group differences in the plasma concentrations of amino acids involved in glutathione synthesis (Table 3). However, there were significant period effects for concentrations of the amino acids glycine, serine, and methionine. Thus, the concentrations were significantly higher in period 2 than in period 1 for glycine, serine, and methionine; significantly higher in period 2 than in period 3 for glycine; and significantly higher in period 3 than in period 1 for methionine. Conversely, there were no significant effects of period on plasma glutamate plus glutamine concentration or cysteine concentration.

The time taken to lose edema was significantly less ($P < 0.05$) in the NAC group (9 ± 1 d) than in the control group (14 ± 2 d).

**DISCUSSION**

The primary aim of the present study was to determine whether dietary NAC supplementation would stimulate GSH synthesis, thereby restoring GSH concentration during the early phase of nutritional rehabilitation of children with edematous malnutrition. The results show a significantly greater increase in the erythrocyte GSH concentration by 9 d after hospital admission in the group that received NAC supplementation compared with the increase in the control group, which received alanine. This early

**TABLE 2**

Physical characteristics of the children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Period 1 Control group</th>
<th>Period 2 Control group</th>
<th>Period 3 Control group</th>
<th>Period 1 NAC group</th>
<th>Period 2 NAC group</th>
<th>Period 3 NAC group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>10.5 ± 1.4</td>
<td>10.9 ± 1.4</td>
<td>12.1 ± 1.0</td>
<td>11.2 ± 1.2</td>
<td>11.7 ± 1.2</td>
<td>12.1 ± 1.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>5.7 ± 0.6</td>
<td>5.7 ± 0.6</td>
<td>7.6 ± 0.8</td>
<td>6.6 ± 0.5</td>
<td>6.4 ± 0.4</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>Length (cm$^2$)</td>
<td>63.4 ± 3.0</td>
<td>64.2 ± 3.0</td>
<td>66.5 ± 2.7</td>
<td>67.0 ± 1.8</td>
<td>67.3 ± 1.9</td>
<td>67.4 ± 1.7</td>
</tr>
<tr>
<td>Weight for age (%) expected</td>
<td>62.3 ± 5.2</td>
<td>61.1 ± 5.4</td>
<td>77.8 ± 5.7</td>
<td>68.6 ± 4.3</td>
<td>65.2 ± 3.4</td>
<td>80.2 ± 4.3</td>
</tr>
<tr>
<td>Weight for length (%) expected</td>
<td>89.7 ± 5.0</td>
<td>86.0 ± 5.0</td>
<td>103.0 ± 2.2</td>
<td>87.2 ± 2.6</td>
<td>83 ± 1.8</td>
<td>103 ± 3.2</td>
</tr>
</tbody>
</table>

$^1$ ± SEM; $n = 8$ per group. The NAC group received N-acetylcysteine supplementation; the control group received alanine supplementation; % expected is the percentage of the median value for a child of the same age or the same length (11).

$^2$ Period term, $P < 0.0001$ (period 1 compared with period 3; period 2 compared with period 3, $P < 0.05$).

$^3$ Supplement-by-period interaction, $P < 0.0001$.

$^4$ Significantly different from control group, $P < 0.05$.

**FIGURE 1.** Mean (± SEM) erythrocyte glutathione (GSH) concentrations and absolute (ASR) and fractional (FSR) synthesis rates in children with severe edematous malnutrition supplemented with N-acetylcysteine ($\bigcirc; n = 8$) or alanine ($\square; n = 8$) when they were malnourished and infected (period 1), when infections were cleared and edema was lost (period 2), and when they had recovered (period 3). Repeated-measures ANOVA for GSH concentration: supplement term, $P < 0.01$; period term, $P < 0.001$ (period 1 compared with period 3, $P < 0.05$; period 2 compared with period 3, $P < 0.05$); supplement-by-period interaction, $P < 0.05$; repeated-measures ANOVA for GSH FSR: supplement term, $P < 0.01$; period term, $P < 0.001$ (period 1 compared with period 3, $P < 0.05$; period 2 compared with period 3, $P < 0.05$); supplement-by-period interaction, $P < 0.05$; repeated-measures ANOVA for GSH ASR: supplement term, $P < 0.001$; period term, $P < 0.001$ (period 1 compared with period 3, $P < 0.05$; period 2 compared with period 3, $P < 0.05$); supplement-by-period interaction, $P = NS$. Post hoc comparisons were done by using the Tukey test.
FIGURE 2. Mean (± SEM) changes in erythrocyte glutathione (GSH) concentration and absolute (ASR) and fractional (FSR) synthesis rates in children with severe edematous malnutrition after 9 d of supplementation with N-acetylcysteine (□; n = 8) or alanine (●; n = 8). *Significantly different from alanine, P < 0.01.

increase in the erythrocyte GSH concentration was accompanied by marked increases in the erythrocyte cysteine concentration and in the rate of synthesis of GSH. These findings suggest that a shortage of intracellular cysteine is the underlying cause of the slower rate of GSH synthesis and hence the low intracellular GSH concentration. Moreover, they show that supplementation with NAC rapidly corrects the GSH deficiency.

GSH is synthesized de novo from glycine, cysteine, and glutamate in reactions catalyzed by γ-glutamylcysteine synthetase and GSH synthetase (EC 6.3.2.3). It can be regenerated from oxidized glutathione in a reaction catalyzed by GSH reductase (EC 1.6.4.2). The inability to sustain an adequate rate of synthesis of this tripeptide can result from a limitation in substrate supply or a defect in the GSH synthetic pathway or both. However, previous studies in rats showing that both the activity (12, 13) and amount (13) of hepatic γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis, were not decreased by consumption of a low-protein diet, suggest that the GSH synthetic pathway may not be impaired by protein malnutrition. The present results showing significant increases in the rate of synthesis of GSH in response to NAC supplementation indirectly support this finding and suggest that delayed restoration of cellular GSH levels in children with edematous malnutrition may be secondary to a shortage in the availability of cysteine.

Our argument that a shortage of intracellular cysteine is responsible for the impaired synthesis of GSH is based on our previous (5) and current finding that the intracellular concentration of cysteine is lower in children with edematous malnutrition but that concentrations of the other 2 GSH precursors, glutamate and glycine, are not. Cysteine is synthesized endogenously from serine and methionine and is derived from the diet and from whole-body protein breakdown. Because plasma methionine is lower in the malnourished children at admission than at recovery but plasma serine is not (Table 3), the shortage of cysteine may reflect decreased availability of methionine, an essential amino acid that must be supplied in the diet. Because diets associated with severe malnutrition are believed to be low in sulfur amino acid content (14), chronically restricted dietary intake is likely to contribute to a shortage of methionine and hence of cysteine in severely malnourished children. Alternatively, because severely malnourished children frequently have diarrhea, Jackson (1) has proposed that decreased cysteine availability can result from its increased use for taurine synthesis secondary to bile salt malabsorption.

Another factor that may contribute to a shortage of sulfur amino acids for GSH synthesis in children with edematous malnutrition is a slower rate of endogenous protein breakdown, as reported by Manary et al (15) in children with kwashiorkor and marasmic kwashiorkor than in those with marasmus. We have also shown that GSH homeostasis and intracellular cysteine concentration are not altered in marasmic children (5). On the other hand, there does not seem to be a quantitative or qualitative difference between the diets of children with edematous or nonedematous malnutrition (16). Together, these findings suggest that marasmic children can compensate for a restricted dietary supply of sulfur amino acids with those released from the breakdown of body proteins, whereas children with kwashiorkor and marasmic kwashiorkor cannot. In the present study, we supplemented with the sulfur amino acid cysteine instead of methionine because cysteine is the direct precursor of GSH and hence might be used more effectively to main-
tain its synthesis (17). Whether methionine supplementation is equally effective remains to be determined.

The established approach for dietary management of severely malnourished children during the acute resuscitative phase of rehabilitation is to restrict the energy and protein intakes to near maintenance amounts. The aim of this strategy is to keep dietary intake within the functional metabolic capacity of the child while infections are treated, fluid and electrolyte balance is reestablished, and specific micronutrient deficiencies are corrected (9). Adherence to this treatment protocol precludes increasing the intake of cysteine during this phase of treatment by simply feeding more protein. However, the cysteine content of the resuscitative diet of the edematous child can be increased easily with cysteine supplementation, thereby stimulating GSH synthesis and improving antioxidant capacity earlier.

The protective functions of GSH are especially relevant to problems associated with the edematous forms of malnutrition. Golden and Ramdath (2) proposed that the peculiar clinical characteristics of the kwashiorkor syndrome are related to oxidant damage because of an imbalance between the generation of oxidants and antioxidant capacity. As a consequence, free radical damage occurs and contributes to the development of suboptimal liver and immune functions as well as edema. This hypothesis is supported by data showing marked improvements in natural killer cell activity and T cell proliferative responses to phytohemagglutinin and tetanus toxoid in HIV-infected patients treated with supplements of NAC (18), which was shown to stimulate GSH synthesis in vivo (19). The NAC treatment also has an anti-inflammatory effect, because it lowers plasma interleukin 6 concentration, a mediator of the acute phase response (18). The hypothesis is also supported by data showing increased peroxidative damage in various organs and tissues in conditions associated with impaired antioxidant defenses (20). Furthermore, there is now direct evidence that both oxidant stress and biomarkers of oxidant-induced cell membrane damage (21, 22) are higher in children with edematous malnutrition than in children with marasmus (4, 5). More recently, Fechner et al (6) reported markedly higher plasma concentrations of nitrite plus nitrate in children with edematous malnutrition, indicating increased production of the prooxidant nitric oxide. Together, the results from these studies strongly suggest that early restoration of GSH homeostasis in children with edematous malnutrition should improve immune function and reduce oxidant damage, thereby accelerating the recovery of cell membrane integrity and function. This is supported by our finding in the present study that edema was lost at a faster rate by the children whose GSH pools were restored early. This beneficial effect represents a reduction in morbidity that may extend to reduction in mortality. This possibility is supported by a recent study showing that children with kwashiorkor whose low GSH concentrations improved recovered from severe malnutrition, whereas those in whom GSH concentrations remained low did not (6).

Overall, much evidence suggests that the early restoration of antioxidant capacity may lead to earlier reestablishment of immunologic and metabolic capacities—and hence earlier resolution of clinical signs and symptoms of edematous malnutrition—thereby reducing morbidity and perhaps mortality. Other important positive effects of earlier restoration of antioxidant capacity may include a reduction in hospitalization and hence in the cost of treatment. These therapeutic and economic benefits are important because malnutrition is more prevalent in poor and developing countries that have few centers in which children can be housed and treated until full recovery.

We are grateful to the physicians and nursing staff of the Tropical Metabolism Research Unit for their care of the children and to Hyacinth Gallimore, Bentley Chambers, Sharon Howell, Margaret Frazer, and Melanie Del Rosario for their excellent work and support in the conduct of the studies and analysis of the samples.

REFERENCES


TABLE 3
Plasma concentrations of the glutathione precursor amino acids in children with severe edematous malnutrition

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control group</th>
<th>NAC group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Period 1</td>
<td></td>
<td></td>
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<tr>
<td>Glycine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>274 ± 64&lt;sup&gt;1&lt;/sup&gt;</td>
<td>222 ± 24</td>
</tr>
<tr>
<td>Serine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>143 ± 16</td>
<td>111 ± 10</td>
</tr>
<tr>
<td>Glutamate + glutamine</td>
<td>418 ± 81</td>
<td>375 ± 38</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9 ± 1</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Cystine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1&lt;sup&gt;4&lt;/sup&gt;(&lt;1–2)</td>
<td>1(&lt;1–5)</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>395 ± 88</td>
<td>423 ± 73</td>
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<tr>
<td>Serine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>195 ± 19</td>
<td>167 ± 18</td>
</tr>
<tr>
<td>Glutamate + glutamine</td>
<td>621 ± 123</td>
<td>506 ± 48</td>
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<tr>
<td>Methionine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>17 ± 1</td>
<td>14 ± 1</td>
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<td>Cystine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2(&lt;1–4)</td>
<td>4(&lt;1–9)</td>
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<td>Period 3</td>
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</tr>
<tr>
<td>Glycine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24 ± 395</td>
<td>24 ± 395</td>
</tr>
<tr>
<td>Serine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>11 ± 4</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>Glutamate + glutamine</td>
<td>19 ± 10</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2 ± 19</td>
<td>2 ± 17</td>
</tr>
<tr>
<td>Cystine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2(1–3)</td>
<td>2(1–3)</td>
</tr>
</tbody>
</table>

<sup>1</sup>NAC, N-acetylcysteine.
<sup>2</sup>Period term, P < 0.01 (glycine: period 1 compared with period 2; P < 0.05; period 2 compared with period 3, P < 0.05; serine: period 1 compared with period 2, P < 0.05; methionine: period 1 compared with period 3, P < 0.05).
<sup>3</sup>Median SEM.
<sup>4</sup>Mean ± SEM; range in parentheses.