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Sulfur amino acids and severe childhood undernutrition

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Abstract

In studies of glutathione (GSH) metabolism in children with severe childhood undernutrition (SCU), we found that slower erythrocyte GSH synthesis in those with edema was associated with lower concentrations of cysteine, the rate-limiting precursor of GSH synthesis. This finding suggested a shortage in cysteine availability for GSH synthesis in children with edematous SCU. The plasma concentration of methionine, the sulfur donor for cysteine synthesis, was also lower in children with edematous SCU, suggesting decreased methionine availability for cysteine synthesis. It is also possible that reduced methionine availability will result in decreased formation of S-adenosylmethionine, which could lead to an overall defect in methylation reactions. This review focuses on (i) the relationship between cysteine availability and GSH synthesis in children SCU (ii) whether there is an inadequate supply of cysteine in those with edematous SCU and, if so, (ii) whether this is due to a shortage of methionine or to decreased release from a protein breakdown. Finally, (iii) if there is a shortage of methionine, does this result in decreased synthesis of the universal methyl donor S-adenosylmethionine?

Introduction

The major clinical syndromes of severe childhood undernutrition (SCU) are marasmus (nonedematous SCU), kwashiorkor and marasmic-kwashiorkor (edematous SCU). Marasmus is relatively straightforward to treat and has a low mortality rate, but the edematous syndromes have a more complex cause, are difficult to treat, have a slower recovery and have high rates of morbidity and mortality (1–3). In addition to the wasting seen in marasmus, kwashiorkor and marasmic kwashiorkor are characterized by edema, altered Na/K ATPase activity, low plasma protein concentrations, dermatosis, hypopigmented hair, impaired immune and antioxidant capacities, neurologic abnormalities, and hepatomegaly caused by intense fatty infiltration (2). In addition there is evidence of more severe gut mucosal atrophy in children with edematous SCU compared to children with non-edematous SCU (4). Despite extensive research, the pathogenic factors that cause a child to develop the edematous instead of the nonedematous form of SCU in response to food deprivation are still not clear. Although an overall deficiency of dietary energy and protein plus clinical and/or subclinical deficiencies of most micronutrients underlie all syndromes, the etiology of edematous SCU is more complex and might involve the added physiological stresses of environmental toxins and/or infections (5). With respect to the pathogenesis of edematous SCU, the underlying mechanism(s) that cause these additional pathophysiological changes remain unclear. The observation that whole blood and erythrocyte concentrations of the ubiquitous antioxidant/detoxicant glutathione (GSH) are lower (6, 7) and plasma concentrations of biomarkers of oxidant-induced tissue damage are elevated in kwashiorkor and marasmic-kwashiorkor, but not in marasmus (6, 8, 9), led to the

proposal that oxidative stress, secondary to decreased antioxidant capacity, leads to free radical damage of cellular membranes and enzyme systems which contribute to the clinical (8) and pathophysiologic manifestations of the edematous syndromes of SCU (6). Because cysteine is the rate limiting amino acid for GSH synthesis, and methionine is required for initiation of protein synthesis and is a precursor for the synthesis of polyamines, SAME, the universal methyl donor, and the keratin of skin and hair, Roediger (10) has proposed that a profound shortage in the availability of sulfur amino acids during a time of greatest requirement, early childhood, may be responsible for some of the signs and symptoms of edematous SCU. This review will focus on (i) the relationship between cysteine availability and GSH synthesis in children with edematous and non-edematous SCU (ii) whether there is an inadequate supply of cysteine in children with edematous SCU and, if so, (ii) whether this is due to an overall shortage of its precursor methionine, hence decreased *de novo* synthesis, or to decreased release from a slower protein breakdown rate. Finally, (iii) if there is a shortage of methionine, does this result in decreased synthesis of the universal methyl donor S-adenosylmethionine (SAME) via the transmethylation pathway.

Relationship between Sulfur amino acid metabolism and Glutathione synthesis

Methionine plays an integral role in methyl group transfer via SAME and in the synthesis of cysteine via reactions of the transmethylation-remethylation and transsulfuration pathways respectively (Figure 1). As shown in the figure, methionine is first converted to SAME which then donates its methyl group through transmethylation (TM) reactions in which it is converted to S-adenosylhomocysteine, which in turn is converted to homocysteine. Homocysteine can follow either a remethylation (RM) or a transsulfuration (TS) pathway. In the RM pathway it receives a methyl group from methyltetrahydrofolate via vitamin B₁₂, to form methionine thereby completing the methionine cycle. Tetrahydrofolate is remethylated by either of the two primary methyl donors, serine and glycine, in reactions catalyzed by serine hydroxymethyltransferase and by the glycine cleavage system, respectively. In the TS pathway homocysteine first condenses with serine to form cystathionine which is then reduced to cysteine and α -ketobutyrate. The newly formed cysteine can react with glutamate and glycine to form glutathione in reactions catalyzed by γ -glutamylcysteine ligase and glutathione synthetase.

GSH synthesis and Cysteine availability

A consistent finding in earlier studies at the Tropical Metabolism Research Unit was that erythrocyte and whole blood concentrations of GSH were lower in children with edematous SCU compared to children with non-edematous SCU (6, 7). Whether this was due to increased utilization to neutralize oxidant species or to an underlying defect in GSH synthesis, or to a combination of both factors was not known. Based on the observation that intracellular erythrocyte GSH concentration increased when whole blood from children with edematous SCU was incubated without addition of exogenous cysteine, glycine, and glutamine, Golden and Ramdath (6) proposed that depletion of erythrocyte GSH in children with edematous SCU was due primarily to increased consumption rather than decreased synthesis. They argued that the observed increase in erythrocyte GSH concentration indicated that substrates were not limiting for GSH synthesis and that its biosynthetic pathway was not impaired in children with edematous SCU. On the other hand, the consistent finding of an overall reduction in total plasma free amino acid concentrations in children with edematous SCU, including reduced glutamine and cysteine concentrations (e.g. (11)) strongly suggested a reduction in GSH synthesis secondary to a shortage of the precursor amino acids cysteine and glutamate. In addition, the plasma concentration of methionine, the precursor for *de novo* cysteine synthesis, is 60% lower in children with

edematous SCU than in healthy children (10). For these reasons we measured erythrocyte GSH synthesis *in vivo* in children with edematous and non-edematous SCU three times during hospitalization; shortly after admission when the children were infected and malnourished (phase 1), 8 days post-admission, when they were no longer infected (phase 2) and when recovered (phase 3) (12). After correction of fluid and electrolyte imbalances, the subjects were started on a maintenance milk-based diet which provided 417 kJ. kg⁻¹.d⁻¹ and 1.2 g. kg⁻¹.d⁻¹ of protein with supplements of vitamins and trace elements. This amount of protein provided 156.5 µmol/kg/d of cysteine and 198.6 µmol/kg/d of methionine. This diet was continued until appetite returned (~ 9 days post-admission), when they were switched to an energy-dense, milk-based formula which provided 625–750 kJ. kg⁻¹.d⁻¹ and approximately 3 g protein. kg⁻¹.d⁻¹ during the period of rapid catch-up growth. This amount of protein provided 391.2 µmol/kg/d of cysteine and 496.5 µmol/kg/d of methionine. This diet was continued until the growth rate plateaued and weight for length was at least 90% of expected. The same maintenance diet (417 kJ. kg⁻¹.d⁻¹ and 1.2 g. kg⁻¹.d⁻¹ of protein) was fed during all studies. At study 1, they had received the diet for 1–2 days. At study 2, they had been on this diet for 7–10 days. The children were restarted on the maintenance diet 3 days prior to the final study based on the observation that adults re-establish whole body protein homeostasis within this time period when they are switched from an adequate to a marginal protein intake (13). Children with edematous SCU had significantly lower erythrocyte GSH concentration and slower absolute rate of synthesis than children with non-edematous SCU both shortly after admission and ~ 9 days later when their infections had resolved (Figure 2). At these times, the edematous group also had significantly lower erythrocyte GSH concentration and absolute rate of synthesis than at recovery. Plasma and erythrocyte-free cysteine concentrations of the edematous group were significantly lower at phase 1 and 2 than at recovery (Table 1). In contrast, erythrocyte GSH concentrations, rates of GSH synthesis and plasma as well as erythrocyte-free cysteine concentrations of the nonedematous group were similar at all three time points and greater at phase 1 and 2 than in the edematous group. These results confirmed that GSH deficiency was characteristic of edematous SCU, and suggested that it was due to a reduced rate of synthesis secondary to a shortage in cysteine supply. This study also showed that the children with non-edematous SCU, despite having similar infections and degree of severity of undernutrition, were still able to maintain normal concentrations and rates of synthesis of GSH, suggesting that GSH precursor supply was also adequate. Whereas the concentrations of methionine in plasma and cysteine in erythrocytes were markedly reduced in the children with edematous SCU (Table 1), they were not in the children with non-edematous SCU, suggesting that one factor contributing to an inadequate cysteine supply was decreased methionine availability to support an adequate rate of de novo cysteine synthesis.

Early dietary cysteine supplementation and GSH synthesis

If inadequate cysteine supply was mainly responsible for the slower rate of synthesis of GSH, then early nutritional resuscitation of children with edematous SCU should include cysteine to accelerate restoration and maintenance of GSH homeostasis. We therefore investigated the effect of dietary cysteine supplementation on GSH synthesis of children with edematous SCU (14). Two groups of children with edematous SCU were supplemented with either 0.5 mmol kg⁻¹.d⁻¹ of N-acetylcysteine or isonitrogenous alanine as control. We chose 0.5 mmol kg⁻¹.d⁻¹ because this the approximate amount of cysteine provided by the 3 g of protein/kg consumed during the period of rapid catch-up growth. This amount of N-acetylcysteine was well tolerated by the children and no adverse effects were observed. After 7 days, the cysteine supplement elicited a 514% increase in erythrocyte cysteine concentration which was associated with a 76% faster rate of synthesis of GSH and a 30% increase in GSH concentration. By contrast, in the alanine supplemented group, cysteine concentration increased by only 166 % and GSH concentration and synthesis rate increased

by only 15% and 27% respectively (Figures 3 and 4). These results confirmed that GSH synthesis rate and concentration could be restored during the early phase of treatment if patients were supplemented with cysteine. In addition to this early restoration of GSH synthesis rate and concentration the children had faster resolution of edema (~5 days less) compared to the control group suggesting early restoration of cell integrity and function.

Our observation that the slower GSH synthesis was associated with lower plasma and erythrocyte-free concentrations of cysteine (12), the rate limiting precursor of GSH synthesis, and that both the rate of synthesis and concentration of erythrocyte GSH increased in response to supplements of cysteine during the immediate post admission period (14), strongly suggested that the slower GSH synthesis of children with edematous SCU was due to a shortage in the supply of cysteine. Why such a shortage existed and its underlying cause(s) were not known. However, the markedly lower plasma concentrations of methionine strongly suggested a shortage of this amino acid to support de novo synthesis of cysteine.

Methionine Production in Edematous versus Nonedematous SCU

The lower plasma concentration of methionine in children with edematous SCU (12,14,15) suggested decreased availability not only for cysteine synthesis but also for formation of SAME potentially leading to an overall defect in methylation reactions. SAME is the universal methyl-group donor and its deficiency has been associated with demyelination of the spinal cord and brain and vacuolar myelopathy in HIV-infected patients (16–18). Some of these neurological changes are similar to those seen in kwashiorkor (19), supporting the argument that methionine deficiency may be a contributing factor in this disorder (10).

Methionine is an indispensable amino acid whose production consists of intake from the diet, release from protein breakdown and methionine re-synthesized from the methylation of homocysteine. Since there does not seem to be a difference between the diets of children with edematous and non-edematous SCU (20), it is unlikely that dietary intake is a significant contributor to any difference in methionine supply between children with edematous and non-edematous SCU. On the other hand our finding that whole body protein breakdown rate was slower in children with the edematous SCU compared to those with non-edematous SCU (15) suggested that a more severe shortage of methionine existed in children with edematous SCU. The possible involvement of impaired homocysteine remethylation is not known, but a slower protein breakdown rate could in theory result in decreased serine production, hence a shortage of methyl group donors to facilitate homocysteine re-methylation to methionine. We therefore tested the hypothesis that at admission to hospital for treatment, methionine and serine kinetics will be slower in children with edematous SCU compared to those with non-edematous SCU.

Methionine, cysteine, serine and phenylalanine kinetics were measured by primed-continuous intravenous infusions of different tracers in children with edematous and non-edematous SCU at different times during hospitalization as previously described by us (21, 22). Briefly, whole body methionine flux, its rate of release from protein breakdown, its transmethylation and its synthesis from homocysteine remethylation were measured by a primed-continuous intravenous tracer infusions. Measurements were made three times during hospitalization, at ~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), at ~14 days after admission when the subjects were still severely malnourished (anthropometrically) but no longer infected, (*i.e.*, all clinical features of the infective episode had resolved), they had lost edema and had improved affect and appetite (Clinical phase 2), and at ~57 days after admission when the rate of catch-up growth had

reached a plateau and weight for length was at least 90% of expected (Clinical phase 3). A diet providing maintenance quantities of energy and protein ($417 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ and $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of protein) was fed during each isotope infusion protocol as described above.

At clinical phase 1, when the children were severely malnourished and infected, total methionine flux, flux from protein breakdown and flux to protein synthesis were slower ($P < 0.01$) in the edematous group compared to the rates of the non-edematous group (Table 2). Within the non-edematous group, total methionine flux, flux from protein breakdown and flux to protein synthesis were faster at clinical phase 1 compared to the values at clinical phase 2 and 3 ($P < 0.05$). On the other hand, in the edematous group there were no differences in total methionine flux and flux from protein breakdown among the different clinical phases. However, methionine flux to protein synthesis was significantly slower at clinical phase 1 compared to the value at clinical phase 3 ($P < 0.05$). Also, whereas methionine balance was 54% lower at clinical phase 1 compared to the value at clinical phase 3 in the edematous group it was only 26% lower in the non-edematous group.

At clinical phase 1 both methyl methionine (i.e. Q_m) and carboxyl methionine (i.e. Q_c) fluxes were slower ($P < 0.05$) in the edematous group compared to the rates of the non-edematous group (Table 3). There were, however, no differences in homocysteine remethylation and methionine transsulfuration and transmethylation between the groups. With respect to Q_m and Q_c there were also significant diagnosis by clinical phase interactions between the groups ($P < 0.05$). Within the non-edematous group, Q_c was faster at clinical phase 1 compared to the values at clinical phase 2 and 3 ($P < 0.05$). On the other hand in the edematous group, Q_m was slower at clinical phase 1 compared to the values at clinical phase 2 and 3 ($P < 0.05$). There were no between group and within group differences in serine flux at any of the 3 clinical phases (Table 4). These results suggested that in the acutely malnourished and infected state children with edematous SCU have slower methionine production compared to children with non-edematous SCU because of a slower rate of release from protein breakdown. This slower methionine production was not however, associated with slower rates of methionine transsulfuration and transmethylation and homocysteine remethylation compared to the rates of the nonedematous children.

Our finding that methionine released from protein breakdown was slower in the edematous group compared to the nonedematous group in the malnourished and infected state corroborated our earlier findings with leucine kinetics (15) that the rate of whole body protein breakdown was slower in children with edematous SCU. Despite this slower methionine production by the children with edematous SCU, however, methionine transsulfuration to cysteine was maintained at a rate that was not different from that of the non-edematous group. As a consequence less methionine was available for protein synthesis. These findings indicated that the rate of methionine released from whole body protein breakdown and available for protein synthesis were slower in children with edematous SCU compared to children with non-edematous SCU in the infected and acutely malnourished state. This finding indirectly supported the argument of Roediger (10) that a profound shortage of methionine, during a time of greatest requirement, early childhood, may be a primary factor responsible for the overall decrease in protein synthesis and for the skin lesions and hair changes seen in kwashiorkor.

Methionine Transmethylation and Homocysteine Remethylation

Although in the acutely malnourished and infected state methionine transmethylation and homocysteine remethylation tended to be slower in the edematous group compared to the nonedematous group, these differences were not statistically significant. Hence, despite slower production rates of both methyl- and carboxy- methionine in children with

edematous SCU, flux through the transmethylation-remethylation cycle of methionine metabolism was not impaired. This may be due to the fact that production of glycine (23) and serine, two primary donors of methyl groups in the body, are not diminished in children with SCU. Further, this finding suggested that production of SAME, the universal methyl-group donor, was not impaired in children with edematous SCU. Hence, impaired methyl-group transfer was probably not involved in the pathogenesis of edematous SCU as suggested by Roediger (10). Interestingly, at clinical phase 2 both transmethylation and remethylation were faster, indicating that methylation reactions were proceeding at a faster rate compared to the clinical phase 1 values. At clinical phase 2, the edematous children had lost edema and their appetite had returned, indicating that their metabolic capacity had been sufficiently re-established to permit initiation of rapid catch-up growth. Hence more methyl groups were being utilized for new RNA and DNA syntheses and methylation to facilitate a net increase in protein synthesis. Methyl groups were also being consumed in the methylation of new proteins and peptides and in the synthesis of several important biomolecules such as epinephrine, serotonin, phosphatidylcholine, and creatine as their pools were being replenished.

Methionine Transsulfuration

An interesting observation was that in both groups of children, at all three clinical phases, methionine transsulfuration to cysteine was slightly more than the $\sim 8 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ dietary intake of methionine. That is, at an intake of $\sim 1.2 \text{ g protein/kg/d}$ (80% of RDA) methionine was being converted to cysteine at a rate equivalent to dietary intake. As a matter of fact the small positive methionine balance at each clinical phase was only possible because of the $2.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ of labeled methionine that was being infused. This finding suggested that the requirement for cysteine was not met by the protein provided by the maintenance diet ($1.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ of protein) normally fed during early nutritional rehabilitation, that is, from admission to the end of maintenance period (clinical phase 2). This may not be the case during the rapid catch-up growth phase, however, when the children were consuming about 3 g protein/kg/d . It also suggested that cysteine requirement, hence its synthesis from methionine, has a higher priority than methionine requirement for the early re-establishment of metabolic capacity. It is likely that a high priority for cysteine is necessary to re-establish gut structure and function to facilitate increased digestion of food and absorption of nutrients for rapid catch-up growth.

Cysteine Production in Edematous versus Nonedematous SCU

In severe undernutrition the gut is marked by reduced secretory mucins (24), mucosal atrophy (4) and reduced activity of the digestive enzymes (25, 26). Mucins are rich in cysteine (27), and gut mucosal and mucins protein turnover at extremely fast rates, $\sim 100 - 140\%/d$ (28, 29) suggesting a high requirement for amino acids, especially cysteine, to facilitate repair and re-establishment of function. Not surprisingly, it has been shown that in the piglet there is an unusually high uptake of dietary methionine, 30–40%, and of cysteine, $\sim 80\%$, by the portal drained viscera (30). Hence, during the early phase of treatment of the child with SCU, the demand for cysteine and methionine by the gut will be unusually high to facilitate repair and restoration of function. Unfortunately, providing more sulfur amino acids by feeding extra protein is not an option during the early phase of nutritional rehabilitation (2).

The supply of cysteine derives from the diet, synthesis *de novo* and from the breakdown of body proteins. Hence, in the child with edematous SCU, a slower body protein breakdown rate (15) plus the lack of an adequate dietary intake of protein will eventually result in an overall shortage of not only cysteine, but also of methionine, the sulfur donor for cysteine

synthesis. In agreement, our observations that both plasma and intracellular methionine concentrations were markedly lower in the children with edematous SCU but not in children with non-edematous SCU (12, 14), suggested a possible shortage in methionine's availability for cysteine synthesis. We therefore reasoned that the markedly depleted intra and extracellular cysteine pools of children with edematous SCU (12) was probably due to its decreased release from protein breakdown rate plus decreased *de novo* synthesis from methionine. Hence, we tested the hypothesis that at the time they are admitted to hospital for treatment children with edematous SCU will have a slower cysteine production rate compared to children with non-edematous SCU because of decreased cysteine release from protein breakdown plus a slower rate of *de novo* synthesis. We therefore measured the rate of production of cysteine, cysteine synthesis *de novo* and its rate of release from protein breakdown using the tracer infusion protocols described in the previous section. Phenylalanine flux was also measured as an estimate of whole body protein breakdown rate.

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 compared to the rates of the non-edematous group (Table 5). There were significant diagnosis by clinical phase interactions in endogenous cysteine flux and 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 and cysteine derived from protein breakdown were all significantly slower ($P<0.01$) compared to the values at clinical phase 2 and 3 in the edematous group. Although these values increased by ~30%, 38% and 68% respectively from clinical phase 1 to clinical phase 2, they were still slower compared to the values at clinical phase 3. In the non-edematous group total and endogenous cysteine fluxes and cysteine derived from protein breakdown were slower at clinical phase 1 compared to the values at clinical phase 3. These parameters remained unchanged at clinical phase 2. Hence, at clinical phase 2, they remained significantly different ($P<0.05$) compared to the values at clinical phase 3. There were no differences in *de novo* cysteine synthesis between the two groups at any of the 3 clinical phases. However, within the edematous group at clinical phase 1, *de novo* cysteine synthesis was faster compared to the value at clinical phase 3. (Table 5).

At clinical phase 1 both total phenylalanine flux and its flux from protein breakdown were significantly slower ($P<0.05$) in the edematous group compared to the corresponding values of the non-edematous group (Table 6). Further, whereas phenylalanine flux from protein breakdown was ~20% slower at clinical phase 1 compared to the value at clinical phase 3 in the edematous group, it was only ~14% slower in the non-edematous group. In both groups, at clinical phase 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 5).

These results showed that when infected and undernourished all children with SCU produced cysteine at a slower rate compared to 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 the children with non-edematous SCU because of a much slower release from protein breakdown. *De novo* cysteine synthesis in the infected undernourished state was faster compared to the rate at recovery in the edematous SCU group and there were no differences between the two groups at any of the 3 clinical phases. These results suggested that all children with SCU have reduced cysteine production because of decreased contribution from protein breakdown, not because of decreased *de*

novo synthesis. However, the magnitude of this reduction was greater in the children with edematous SCU because of a marked suppression of protein breakdown rate.

The finding of a slower endogenous cysteine production in both children with edematous and non-edematous SCU was surprising because in past studies we found that only children with edematous SCU, not those with non-edematous SCU, had lower concentrations of plasma and erythrocyte-free cysteine concentrations compared to the values at recovery (12,14,15). This consistent finding plus our observation that protein breakdown rate was slower in children with edematous SCU compared to those with non-edematous SCU confirmed that children with edematous SCU produced less cysteine compared to those with non-edematous SCU because of decreased contribution from protein breakdown. Actually, although both groups were producing less cysteine in the malnourished state (compared to recovery), the amount produced by the children with edematous SCU was 32% less than the amount produced by their non-edematous counterparts. This was due to the fact that whereas cysteine released from protein breakdown was 37% slower compared to the rate at recovery in the non-edematous SCU group, it was 61% slower in the edematous SCU group. Together our findings suggested that although children with non-edematous SCU were producing less cysteine, the amount was still sufficient to maintain plasma and erythrocyte concentrations and GSH synthesis rate in the infected undernourished state (12,14,15). The same was not true for children with edematous SCU, however, because the amount of cysteine produced was far less. This explained why plasma and erythrocyte cysteine concentrations were much lower in children with edematous SCU than in those with non-edematous SCU (12,14,15). This also explained why in the undernourished infected state children with edematous SCU were unable to maintain GSH synthesis rate but those with non-edematous SCU could (12, 14).

Relationship between decreased body protein breakdown and cysteine production

These findings strongly suggested that decreased cysteine release from protein breakdown was the primary contributor to the diminished cysteine production in children with edematous SCU. After two weeks 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 non-edematous group, cysteine kinetics was similar in the two groups at this stage. This finding suggested that factors underlying the signs and symptoms of the edematous forms of SCU were responsible for the greater suppression of protein breakdown and hence, cysteine production. The 20% slower phenylalanine flux from protein breakdown in the undernourished state compared to the value at recovery in the edematous group, corroborated our earlier findings with leucine kinetics that whole body protein breakdown was slower in children with edematous SCU (15). Interestingly, in the non-edematous SCU group, cysteine production at clinical phase 1 was also slower because of decreased release from protein breakdown as endogenous phenylalanine flux was ~14% slower at clinical phase 1 compared to the rate at recovery, similar to our earlier observation with leucine kinetics (15). It is difficult to reconcile this modest reduction in rate of protein breakdown (~14%) with the much greater reduction in protein-derived cysteine (~36%) in the children with non-edematous SCU. A similar mismatch between protein-derived phenylalanine flux (~20% reduction) and protein-derived cysteine flux (~61% reduction) was seen in the children with edematous SCU. A possible explanation is that the relative amount of cysteine released from breakdown of proteins in the severely undernourished state was less than in the recovered state. Such a discord between the ratio of phenylalanine flux to cysteine flux from the undernourished to the recovered state is only possible if the types of proteins being

broken down in the malnourished state have a lower cysteine content compared to the proteins being broken down in the recovered state. Indeed in both groups in the undernourished states, when the protein-derived cysteine flux was expressed as a ratio to protein-derived phenylalanine flux it was ~0.24 indicating that for every 4 phenylalanine molecules released only 1 cysteine molecule was released (Figure 5). This ratio increased to 0.4 when the children were recovered suggesting that for every 5 molecules of phenylalanine released 2 cysteine molecules were released. In three 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 vs. 0.38; rat, 0.2 vs. 0.6; calf 0.39 vs. 0.5; refs. (31–33)). Assuming that liver is representative of other soft organs, it is possible that in the undernourished 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 (34), hence, decreased contribution from muscle protein breakdown may underlie the slower cysteine production in children with SCU.

These findings did not support our proposal of a slower *de novo* cysteine synthesis in children with edematous SCU (12, 14). To the contrary the edematous group had a faster cysteine synthesis rate in the undernourished and infected state compared to the recovered state suggesting that they were trying to compensate for the decreased cysteine production from protein breakdown by upregulating *de novo* synthesis. Hence, impaired *de novo* cysteine synthesis was not a contributor to the slower cysteine production of children with SCU.

Conclusion

The findings of these studies strongly suggest that the requirement for cysteine is not met by the protein provided by the maintenance diet (1.2 g·kg⁻¹·d⁻¹ of protein) normally fed during early nutritional rehabilitation of children with severe undernutrition. Further, our finding that edema was lost at a faster rate by the children whose GSH pools were restored early by supplemental cysteine indicates accelerated recovery of cell membrane integrity and function possibly because of decreased oxidant damage secondary to improved antioxidant capacity. This beneficial effect represents a reduction in morbidity that may extend to a reduction in mortality. Hence, supplemental cysteine should be added to the maintenance diet fed during early nutritional rehabilitation of children with severe undernutrition. In the past we have provided an extra 0.5 mmol N-acetylcysteine/kg/d, equivalent to the amount consumed during the rapid catch up growth phase, with no adverse effects. In current and future studies we plan to look at the effect of this supplement on the rate of recovery of gut integrity and function and on the rate of synthesis of gut mucosal protein and of skin protein.

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Figure 1. Methionine metabolism and its relationship to glutathione synthesis. The main enzymes are: 1= methionine adenosyltransferase; 2= X-methyltransferase; 3= S-adenosylhomocysteine hydrolyase; 4=methionine synthase; 5=cystathionine synthetase; 6= cystathionine γ -lyase; 7= γ -glutamylcysteine ligase; 8=glutathione synthetase. Abbreviations are: TM, transmethylation; RM, remethylation; TS, transsulfuration; THF, tetrahydrofolate.

Figure 2.

Erythrocyte glutathione concentration, fractional and absolute (FSR; ASR) synthesis rates in children with non-edematous (n=7) and edematous (n=7) SCU when they were malnourished and infected (Phase 1), when infections were cleared and edema was lost (Phase 2), and when they were fully recovered (Phase 3). Values are means $\pm$ SEM. a, significantly different from Phase 3 value, $P < 0.05$; b, significantly different from Phase 2 value, $p < 0.05$; c, significantly different from the same phase value of non-oedematous group, $P < 0.05$. Data taken from ref. #12, Reid et al (2000)

Figure 3.

Changes in erythrocyte glutathione concentration, fractional and absolute (FSR; ASR) synthesis rates in children with edematous SCU after ~9 days of supplementation with N-acetylcysteine (n=8) and alanine (n=8). Values are means $\pm$ SEM. a, indicates alanine vs cysteine group, $p < 0.01$. Data taken from ref. #14, Badaloo et al (2002)

Figure 4.

Erythrocyte cysteine concentration in children with edematous SCU supplemented with N-acetylcysteine (n=8) and alanine (n=8) when they are malnourished and infected (Phase 1), when infections were cleared and edema was lost (Phase 2), and when they were fully recovered (Phase 3). Values are means $\pm$ SEM. a, indicates vs Phase 1, $p < 0.05$; b, indicates vs Phase 2, $p < 0.01$; c, indicates vs cysteine, $p < 0.03$. Data taken from ref. #14, Badaloo et al (2002)

Figure 5.

The ratio of protein-derived cysteine flux to protein-derived phenylalanine flux (Cys/Phe flux ratio) in 11 children with non-edematous and 11 children with edematous SCU at clinical phase 1, ~2 days post-admission when the subjects were infected and malnourished, at clinical phase 2, ~14 days post-admission when the subjects were still severely malnourished but no longer infected and edematous and at clinical phase 3, ~57 days post-admission when the subjects were recovered. The dotted line represents the molar ratio of cysteine to phenylalanine concentrations (0.38) of whole body pig tissue protein (WB Cys/Phe Molar Ratio). Values are means $\pm$ SEM. Data taken from ref. #21, Jahoor et al (2006)

Table 1

Concentrations of the glutathione precursor amino acids in plasma and red blood cells of children with severe childhood undernutrition¹

Amino Acid	Phase 1		Phase 2		Phase 3	
	Edema (n=7)	No-edema (n=7)	Edema (n=7)	No-edema (n=7)	Edema (n=7)	No-edema (n=7)
Plasma-free μmol/L						
Glycine	260±33	190±22	335±41	276±54	249±29	205±18
Glx	379±61 ^{3,4}	489±58	647±83 ⁵	559±79	526±34	536±37
Cysteine plus 2×Cystine	6.9±2.8 ^{2,3,4}	61±19	16±11 ^{2,5}	55±11	38±8	56±9
Methionine	5.3±0.5 ^{2,3,4}	9.1±1.0	6.7±0.7 ^{2,5}	11±1.7	10.5±0.5	11.3±0.9
Serine	150±20	148±19	192±21 ⁵	180±28	138±10	129±13
RBC-free						
Glycine	721±95 ⁴	565±75	1000±103 ^{2,5}	490±70	688±118 ²	490±11
Glutamate	565±75	779±74	695±61	666±130	538±49	602±48
Glutamine	183±37 ^{2,3,4}	386±68	271±26	392±93	263±32	373±69
Cysteine [*]	<1.0 ^{2,3} (<1,11.3)	17.5 (3.4,44)	1.7 ^{2,5} (<1,11.0)	16.2 (8.2,40)	22.1 (9.8,35)	30.9 (5.8,49)

Data taken from ref. #12, Reid et al (2000).

Abbreviations : Glx: Glutamate plus glutamine; RBC, red blood cell.

¹ All values are means ± SEM, except * values which are medians with range.

² edema vs no-edema, p<0.05;

³ phase 1 vs phase 3, p <0.001;

⁴ phase 1 vs phase 2, p<0.05;

⁵ phase 2 vs phase 3, p<0.05 by RMANOVA

Table 2

Whole body methionine kinetics in children with edematous and non-edematous severe undernutrition¹.

Methionine Kinetics $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$	Clinical Phase 1		Clinical Phase 2		Clinical Phase 3	
	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)
Total flux ²⁻⁴	63±3.6 ^{5,6}	50±0.96 ^a	53±1.8	52±2.4	56±2	53±1.7
Diet + Tracer inflow	10.6±0.1	10.6±0.03	10.5±0.03	10.5±0.02	10.6±0.02	10.5±0.03
Methionine TS ⁴	8.6±0.4	9.4±0.6 ⁶	8.7±0.2	8.4±0.4	7.9±0.4	7.6±0.3
Flux from PB ²⁻⁴	52±3.6 ^{5,6}	39±1.8 ^a	42±1.8	41±2.4	46±2.1	43±1.7
Flux to PS ²⁻⁴	54±3.4 ⁵	41±1.8 ^{a,6}	44±1.6	43±2.1	49±2.1	47±1.8
Balance ⁴	2±0.4	1.3±0.8 ⁶	1.8±0.2	1.7±0.5	2.7±0.4	2.8±0.3

Data taken from ref. #22, Jahoor et al (2006).

Abbreviations: TS, transsulfuration; PB, protein breakdown; PS, protein synthesis.

¹ Mean± SEM; Clinical Phase 1 (baseline) values were compared by non-paired t-test; to determine differential response to treatment, a RMANOVA was performed.

² Diagnosis by Clinical Phase Interaction, p<0.05.

³ Main effect of Diagnosis p<0.05

⁴ Main effect of Clinical Phase, p <0.05

⁵ Significantly different from corresponding Clinical Phase 2 value, P<0.05 (post hoc comparison by Bonferroni method)

⁶ Significantly different from corresponding Clinical Phase 3 value, P<0.001 (post hoc comparison by Bonferroni method)

^a Values are significantly different from non-edematous value p<0.05. (non-paired t-test).

Total methionine methyl and carboxy fluxes, transsulfuration (TS) and transmethylation (TM), and homocysteine remethylation (RM) in children with edematous and non-edematous severe undernutrition¹.

Table 3

Kinetic Parameter $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$	Clinical Phase 1		Clinical Phase 2		Clinical Phase 3	
	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)
Methionine flux (Q_m) ^{2,3}	84±5.6	66±1.9 ^{4,5,6}	76±2.7	76±3.3	82±3.1	74±3.3
Methionine flux(Q_c) ²⁻⁴	63±3.6 ^{5,6}	50±0.96 ^a	53±1.8	52±2.4	56±2	53±1.7
Homocysteine RM ⁴	21.6±3.2	15.5±1.3 ⁵	23±2.3	23±1.7	25.4±1.8	20±2.1
Methionine TS ⁴	8.6±0.4	9.4±0.8 ⁶	8.7±0.2	8.4±0.4	7.9±0.4	7.6±0.3
Methionine TM ⁴	30.4±3.2	25±1.3 ⁵	32±2.3	31.4±1.8	33.8 ±2	27.7±2.4

Data taken from ref. #22, Jahoor et al (2006).

Abbreviations: Q_m methyl methionine flux; Q_c carboxyl methionine flux; RM, remethylation; TS, transsulfuration; TM, transmethylation

¹ Mean± SEM; Clinical Phase 1 (baseline) values were compared by non-paired t-test; to determine differential response to treatment, a RMANOVA was performed.

² Diagnosis by Clinical Phase Interaction, $p<0.05$.

³ Main effect of Diagnosis $p<0.05$

⁴ Main effect of Clinical Phase, $p <0.05$

⁵ Significantly different from corresponding Clinical Phase 2 value, $P<0.05$ (post hoc comparison by Bonferroni method)

⁶ Significantly different from corresponding Clinical Phase 3 value, $P<0.001$ (post hoc comparison by Bonferroni method)

^a Values are significantly different from non-edematous value $p<0.05$. (non-paired t-test).

Table 4

Whole body serine kinetics in children with edematous and non-edematous severe undernutrition¹.

Serine Kinetics	Clinical Phase 1		Clinical Phase 2		Clinical Phase 3	
	Non-edematous	Edematous	Non-edematous	Edematous	Non-edematous	Edematous
$\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$	(N=11)	(N=11)	(N=11)	(N=11)	(N=11)	(N=11)
Total flux	227±9.2	244±17	232±8	217±8.6	224±11	210±7.2
Diet + Tracer inflow	36±0.3	35.8±0.1	35.6±0.1	35.6±0.1	36±0.1	35.7±0.1
Endogenous flux	191±9	202±15	197±7.9	181±8.6	188±11	174±7.2

Data taken from ref. #22, Jahoor et al (2006)

¹Mean± SEM; Clinical Phase 1 (baseline) values were compared by non-paired t-test; to determine differential response to treatment, a RMANOVA was performed.

Table 5

Cysteine kinetics in children with edematous and non-edematous severe undernutrition¹.

Cysteine Kinetics $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$	Clinical Phase 1		Clinical Phase 2		Clinical Phase 3	
	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)
Diet + Tracer inflow	7.9±0.06	7.8±0.02	7.8±0.02	7.8±0.02	7.9±0.01	7.8±0.02
Total flux ^{2,3}	37.2±2.5 ⁶	27.9±2.4 ⁶	37.7±2.8 ⁶	35.3±2.4 ⁶	48.4±3.4	44.8±2.9
Endogenous flux ²⁻⁴	29.3±2.6 ⁶	20±2.4 ^{5,6}	30±2.8 ⁶	27.6±2.4 ⁶	40.6±3.4	37±2.9
De Novo Synthesis ²⁻⁴	8.6±0.4	9.4±0.8 ⁶	8.7±0.2	8.4±0.4	7.9±0.4	7.6±0.3
Protein derived flux ²⁻⁴	20.7±2.5 ⁶	11.4±1.7 ^{4,5,6}	21.2±2.7 ⁶	19.2±2.3 ⁶	32.7±3.3	29.4±2.4

Data taken from ref. #21, Jahoor et al (2006)

¹ Mean± SEM; Within Clinical Phase 1 (baseline) values were compared by non-paired t-test; to determine differential response to treatment, a RMANOVA was performed

² Main effect of Clinical Phase, p <0.001

³ Main effect of Diagnosis p<0.05

⁴ Diagnosis by Clinical Phase Interaction, p<0.05.

⁵ Significantly different from corresponding Clinical Phase 2 value, P<0.05 (post hoc comparison by Bonferroni method)

⁶ Significantly different from corresponding Clinical Phase 3 value, P<0.001 (post hoc comparison by Bonferroni method)

^a Significantly different from non-edematous value p<0.05. (non-paired t-test).

Table 6
Phenylalanine kinetics in children with edematous and non-edematous severe undernutrition¹.

Phenylalanine Kinetics	Clinical Phase 1		Clinical Phase 2		Clinical Phase 3	
	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)	Non-edematous (N=11)	Edematous (N=11)
$\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$						
Diet + Tracer inflow	11.9±0.12	11.8±0.04	11.7±0.04	11.7±0.03	11.9±0.02	11.8±0.03
Total flux ^{2,3}	89±5.3	74±2.9 ^a	86±3.5	79±3.9	101±8.3	90±5.2
Protein derived flux ^{2,3}	73±5.4	59±3 ^a	71±4.5	63±3.4	85±8	74±5

Data taken from ref. #21, Jahoor et al (2006)

¹ Mean± SEM; Within Clinical Phase 1 (baseline) values were compared by non-paired t-test; to determine differential response to treatment, a RMANOVA was performed

² Main effect of Clinical Phase, p <0.01

³ Main effect of Diagnosis p<0.05

^a Significantly different from non-edematous value p<0.05. (non-paired t-test)