



# Glycine production in severe childhood undernutrition<sup>1-3</sup>

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## ABSTRACT

**Background:** Although nutritionally dispensable amino acids are not essential in the diet, from a biochemical standpoint, dispensable amino acids such as glycine are essential for life. This is especially true under unique circumstances, such as when the availability of labile nitrogen for dispensable amino acid synthesis is reduced, as in severe childhood undernutrition.

**Objective:** We aimed to measure glycine production in children with edematous and nonedematous severe childhood undernutrition.

**Design:** Glycine flux and splanchnic glycine extraction were measured in 2 groups of children with edematous ( $n = 8$ ) and nonedematous ( $n = 9$ ) severe childhood undernutrition when they were infected and malnourished (clinical phase 1), when they were still severely malnourished but no longer infected (clinical phase 2), and when they were recovered (clinical phase 3).

**Results:** Total and endogenous glycine flux and splanchnic glycine uptake did not differ significantly between the edematous and nonedematous groups during any clinical phase. In both groups of subjects, none of the glycine kinetic parameters changed significantly from clinical phase 1 through phases 2 and 3. Compared with the value at clinical phase 3, plasma glycine concentrations were not significantly lower during clinical phase 1 or 2 in either group.

**Conclusions:** These findings suggest that children with severe childhood undernutrition can increase their de novo glycine synthesis to compensate for the reduced contribution from chronic food deprivation. The maintenance of the plasma glycine concentration suggests that the rate of glycine production was sufficient to satisfy metabolic demands in these children when they were acutely undernourished and infected. *Am J Clin Nutr* 2006;84:143-9.

**KEY WORDS** Glycine kinetics, edematous severe childhood undernutrition, nonedematous severe childhood undernutrition

## INTRODUCTION

In severe childhood undernutrition (SCU), fewer amino acids of dietary origin are available for metabolic purposes because of the reduced protein intake associated with chronic food deprivation. Because breakdown of body proteins is the major contributor to the overall flux of amino acids (1), our finding that the whole-body protein breakdown rate is slower in children with the edematous forms of SCU than in children with the nonedematous form of SCU (2) suggests that a more severe shortage in the availability of amino acids will exist in children with edematous SCU. Whereas such a reduction in availability will be especially true for the indispensable amino acids, supply of the dispensable amino acids will also be compromised unless there is an increase in de novo synthesis to compensate for the decreased supply from

the diet and the breakdown of body proteins. Although dispensable amino acids are considered to be nonessential in the diet, in reality they are critical for the maintenance of physiologic and metabolic homeostasis; hence, biochemically, they are needed in relatively large amounts to maintain good health. It is not known whether the supply of dispensable amino acids is maintained by increased de novo synthesis in children with SCU.

The de novo synthesis of the so-called dispensable amino acids is important for survival because these amino acids are precursors for the synthesis of numerous biochemical compounds and peptides that are necessary for the maintenance of good health. Glycine is a good example of a dispensable amino acid in high demand. Besides being a neurotransmitter and a one-carbon donor, it is a precursor for the formation of numerous essential biological compounds, such as the purines, porphyrins, creatine, glutathione, and, through its interconversion to serine, phospholipids and cysteine (3). Because glycine is unavailable for reutilization after its incorporation in most of these compounds, under certain circumstances, such as severely stressed states, the higher demand for this amino acid may exceed its rate of synthesis. This will be especially true during periods of chronic low dietary protein intakes, as in SCU, when the availability of labile nitrogen for dispensable amino acid synthesis is severely reduced (1). At present, it is not known whether de novo synthesis of glycine is increased to compensate for decreased supply from the diet and from protein breakdown in children with SCU and infections. The findings of Persaud et al (4) that the rate of 5-oxoproline excretion, an index of glycine insufficiency, was increased during the early phase of rapid tissue repletion in children with SCU strongly suggest that the supply of glycine may be insufficient to satisfy metabolic demands in children recovering from SCU. In this study, we aimed to determine whether glycine production was slower in the acutely malnourished and infected state (clinical phase 1) than in the malnourished but infection-free state (clinical phase 2) and the recovered state (clinical phase 3) in

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**TABLE 1**Age, physical characteristics, and albumin concentrations of the subjects<sup>1</sup>

Variable	Clinical phase 1		Clinical phase 2		Clinical phase 3	
	Nonedematous (n = 9)	Edematous (n = 8)	Nonedematous (n = 9)	Edematous (n = 8)	Nonedematous (n = 9)	Edematous (n = 8)
Age (mo) <sup>2,3</sup>	14.9 ± 1.4 <sup>4</sup>	12.9 ± 3.3 <sup>4</sup>	15.3 ± 1.4 <sup>4</sup>	13.2 ± 3.3 <sup>4</sup>	17.4 ± 1.4	15.3 ± 3.5
Weight (kg) <sup>2</sup>	6.0 ± 0.3	6.0 ± 0.8	6.1 ± 0.3	6.1 ± 0.8	8.2 ± 0.2	7.8 ± 0.9
Length (cm) <sup>2</sup>	68.1 ± 1.4	64.7 ± 4.0	68 ± 1.5	65.3 ± 4.0	71.1 ± 1.2	67.4 ± 3.6
Weight-for-age (%) <sup>2</sup>	55.9 ± 2.2	59 ± 3.8	57 ± 2.2	60.3 ± 3.9	74 ± 1.3	73.7 ± 3.1
Weight-for-length (%) <sup>2,5</sup>	76.0 ± 1.5	87.4 ± 3.0	77.1 ± 2.2	86.5 ± 3.3	94 ± 2.6	103.5 ± 2.6
Fat-free mass (kg) <sup>2</sup>	5.5 ± 0.24	5.3 ± 0.9	5.48 ± 0.24	5.25 ± 0.8	7.16 ± 0.24	6.3 ± 0.7
Fat-free mass (% wt) <sup>2</sup>	89 ± 2	92 ± 3	88 ± 1	90 ± 2	84 ± 2	84 ± 1
Albumin (g/L) <sup>2,3,5</sup>	37 ± 1.7	27 ± 1.5 <sup>4,6,7</sup>	38.1 ± 1.3	31.3 ± 1.3 <sup>4,7</sup>	41 ± 0.5	41 ± 0.7

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ . Clinical phase 1,  $\approx 3$  d after admission when the subjects were infected and malnourished; clinical phase 2,  $\approx 14$  d after admission when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3,  $\approx 57$  d after admission when the subjects were recovered. Cell means were compared by repeated-measures ANOVA.

<sup>2</sup> Main effect of clinical phase,  $P < 0.001$ .

<sup>3</sup> Diagnosis  $\times$  clinical phase interaction,  $P < 0.05$ .

<sup>4</sup> Significantly different from corresponding clinical phase 3 value,  $P < 0.001$  (post hoc comparison by Bonferroni method).

<sup>5</sup> Main effect of diagnosis,  $P < 0.05$ .

<sup>6</sup> Significantly different from corresponding clinical phase 2 value,  $P < 0.05$  (post hoc comparison by Bonferroni method).

<sup>7</sup> Within clinical phase, values are significantly different from similar phase nonedematous value,  $P < 0.01$  (post hoc comparison by Bonferroni method).

children with SCU. We also aimed to determine whether children with edematous SCU had slower glycine production rates than did children with nonedematous SCU.

## SUBJECTS AND METHODS

### Subjects

Seventeen children who were admitted to the Tropical Metabolism Research Unit, University of the West Indies, for treatment of SCU participated in the study. During hospitalization, the children were managed according to a standard treatment protocol as previously described by us (2, 5). As shown in **Table 1**, each subject had a deficit in body weight-for-age of  $> 20\%$ , indicating severe undernutrition. The diagnosis of type of SCU, ie, marasmus, kwashiorkor, or marasmic kwashiorkor, was based on the Wellcome Classification (6). As shown in **Table 2**, 9 children (6 boys, 3 girls) had marasmus (ie, nonedematous SCU), and 8 had edematous SCU (5 boys, 3 girls; 5 with kwashiorkor and 3 with marasmic-kwashiorkor). Fourteen of the children had evidence of one or more infections at admission.

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

### Study design

The study consisted of a group of 8 children with edematous SCU and a group of 9 children with nonedematous SCU. Whole-body and splanchnic glycine kinetics were measured 3 times during hospitalization by using simultaneous constant intravenous and intragastric infusions of 2 different stable isotopes of glycine  $\approx 3$  d after admission when the subjects were both infected and malnourished but clinically stable as indicated by blood pressure, pulse, and respiration rates (clinical phase 1);  $\approx 14$  d after admission when the subjects were still severely

malnourished (anthropometrically) but no longer infected (ie, all clinical features of the infective episode had resolved) and when they had lost edema and had improved effect and appetite (clinical phase 2); and  $\approx 57$  d after admission when the rate of catch-up growth had reached a plateau and weight-for-length was  $\geq 90\%$  of expected (clinical phase 3). Total body water, from which fat-free mass was calculated, was also measured at each time point by the dilution of deuterium oxide.

A diet providing maintenance quantities of energy and protein ( $417 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  and  $1.2 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) was fed during each isotope infusion protocol. The subjects had been receiving this therapeutic diet for  $\geq 3$  d at the time of the clinical phase 1 measurement and for  $\approx 11$  d before the clinical phase 2 measurement. For the clinical phase 3 measurement, the subjects were taken off their regular diet and were placed on this maintenance diet for 3 d before the infusion protocol. 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 the 8-h period of the isotope infusion protocol.

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

### Infusion protocol

Two intravenous access sites were established in opposite arms by the insertion of 24G catheters after preparation of the access sites with a topical anesthetic (EMLA cream; Astra Pharmaceuticals Ltd, Langley, United Kingdom). One intravenous catheter was used for infusion of the labeled glycine and the other for blood sampling. A nasogastric tube was inserted into the child's stomach and a Flexiflo Magna-Port Y-Port connector (Ross Products Division, Abbott Laboratories, Columbus, OH) was attached to the proximal end. About 33% of the child's daily dietary intake was then given over the next 8 h by continuous

TABLE 2

Clinical characteristics of the subjects at admission<sup>1</sup>

Subject	Sex	Diagnosis	Infection	Hemoglobin	WBC count	Temperature
				g/L	$\times 10^9$ cells/L	$^{\circ}$ C
Edematous subjects						
1	M	Kw	B, URTI, D	108	12.4	37.2
2	F	Kw	URTI	83	7.3	38.4
3	F	Kw	D	90	9	37.5
4	M	Kw	UTI, Cand	90	9.7	37.5
5	M	Kw	B, Cand	95	25.2	37
6	M	MarKw	URTI	80	10.3	38.9
7	M	MarKw	B	89	14.3	37
8	F	MarKw	B, UTI	80	12.8	38.2
$\bar{x} \pm$ SE	—	—	—	$93.2 \pm 3.3$	$12.7 \pm 2.5$	$37.5 \pm 0.19$
Nonedematous subjects						
1	F	Mar	None	76	17.4	36.3
2	M	Mar	None	89	8.4	37.1
3	M	Mar	None	86	9.7	37.5
4	M	Mar	D	84	6.5	36.4
5	M	Mar	D	85	7.9	36.8
6	M	Mar	D	88	14.2	37.1
7	M	Mar	D, Cand	83	11.9	37
8	F	Mar	B, D	81	7.7	37.4
9	F	Mar	B,	91	22.1	37.2
$\bar{x} \pm$ SE				$84.8 \pm 1.5^2$	$11.8 \pm 1.8$	$37 \pm 1.8$

<sup>1</sup> Kw, kwashiorkor; MarKw, marasmic kwashiorkor; Mar, marasmus; WBC, white blood cell; B, bacteremia; Cand, candidiasis; D, infective diarrhea; URTI; upper respiratory tract infection; UTI, urinary tract infection.

<sup>2</sup> Significantly different from corresponding value of edematous group,  $P < 0.05$  (unpaired  $t$  test).

intra-gastric infusion into one limb of the Y-port by using an enteral infusion pump (Flexiflo companion enteral nutrition pump; Ross Laboratories, Columbus, OH).

Sterile solutions of [1,2-<sup>13</sup>C<sub>2</sub>]glycine and [1-<sup>13</sup>C]glycine (99.9%; Cambridge Isotope Laboratories, Woburn, MA) were prepared in 9 g NaCl/L. After 2 h of intra-gastric feeding, a 3-mL blood sample was drawn, and a bolus of deuterium oxide (100 mg/kg, 99.9%; Cambridge Isotope Laboratories) was given intra-gastrically through the other port of the nasogastric tube. This was followed by a 2-mL flush with 9 g NaCl/L. Immediately after, a primed, continuous, intravenous infusion of [1,2-<sup>13</sup>C<sub>2</sub>]glycine (prime = 22  $\mu$ mol/kg, infusion rate = 22  $\mu$ mol  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>) and a primed, continuous, intra-gastric infusion of [1-<sup>13</sup>C]glycine (prime = 6  $\mu$ mol/kg, infusion rate = 6  $\mu$ mol  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>) were started and maintained for 6 h. Additional 1.5-mL blood samples were drawn hourly from 3 to 6 h. The infusion and blood sampling protocols were the same for the 2 subsequent experiments performed during clinical phases 2 and 3.

### Sample analyses

The blood samples were centrifuged immediately at 1000  $\times$  g for 15 min at 4  $^{\circ}$ C, and the plasma was removed and stored immediately at -70  $^{\circ}$ C for later analyses. Plasma amino acid concentrations were determined by reversed-phase HPLC on a Hewlett-Packard 1090 chromatograph equipped with a model HP 1046A fluorescence detector (Hewlett-Packard, Avondale, PA). The <sup>2</sup>H<sub>2</sub> content of plasma water was determined by reducing water extracted from 10  $\mu$ L of plasma with zinc in quartz vessels and determining the <sup>2</sup>H<sub>2</sub> abundance of the resulting hydrogen gas by gas-isotope-ratio mass spectrometry ( $\delta$ -E; Finnigan MAT, San Jose, CA). Plasma glycine was isolated by ion exchange (Dowex 200x) chromatography and was converted to

the  $n$ -propyl ester, heptafluorobutyramide derivative. The tracer-to-tracee ratio of plasma glycine was determined by negative chemical ionization gas chromatography-mass spectrometric analysis by selectively monitoring ions at mass-to-charge ratios of 293 to 295 with a Hewlett-Packard 5890 quadrupole mass spectrometer (Palo Alto, CA).

### Calculations

The percentage of enteral glycine (diet plus intra-gastric tracer) extracted by the splanchnic tissues (GLY<sub>splan</sub>) was obtained as previously described (2, 5):

$$\% \text{GLY}_{\text{splan}} = (1 - \{E_{\text{pIG}}/E_{\text{pIV}} \times i_{\text{IV}}/i_{\text{IG}}\}) \times 100 \quad (1)$$

where  $E_{\text{pIG}}$  and  $E_{\text{pIV}}$  are the plateau tracer-to-tracee ratios of the intra-gastric (IG) and intravenous (IV) tracers, and  $i_{\text{IG}}$  and  $i_{\text{IV}}$  are the rates of infusion of the IG and IV tracers.

Splanchnic glycine utilization (GLY<sub>splan</sub>) was obtained as the product of %GLY<sub>splan</sub> and enteral glycine intake.

Total glycine flux ( $Q$ ) was calculated as

$$Q(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = i_{\text{IV}}/E_{\text{pIV}} \quad (2)$$

Endogenous glycine flux (GLY<sub>endo</sub>), that is, glycine derived from protein breakdown plus de novo synthesis, was calculated as the difference between total glycine flux and all sources of exogenous glycine:

$$\text{GLY}_{\text{endo}}(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = Q - (\text{Diet GLY} + \text{IG GLY} + \text{IV GLY}) \quad (3)$$

Total body water (TBW) was calculated as follows:

$$\text{TBW}(\text{mL}) = (E_{\text{D}_2\text{O}} \times \text{dose})/(E_{\text{pD}_2\text{O}} \times 1.04) \quad (4)$$

TABLE 3

Whole-body and splanchnic glycine kinetics in children with edematous and nonedematous severe undernutrition<sup>1</sup>

Glycine kinetics	Clinical phase 1		Clinical phase 2		Clinical phase 3	
	Nonedematous (n = 9)	Edematous (n = 8)	Nonedematous (n = 9)	Edematous (n = 8)	Nonedematous (n = 9)	Edematous (n = 8)
	$\mu\text{mol} \cdot \text{kg fat free wt}^{-1} \cdot \text{h}^{-1}$		$\mu\text{mol} \cdot \text{kg fat free wt}^{-1} \cdot \text{h}^{-1}$		$\mu\text{mol} \cdot \text{kg fat free wt}^{-1} \cdot \text{h}^{-1}$	
Dietary inflow	12.8 ± 0.2	12.9 ± 0.4	13.1 ± 0.2	12.9 ± 0.3	13.8 ± 0.3	13.8 ± 0.1
Intragastric tracer	5.6 ± 0.1	5.6 ± 0.2	5.7 ± 0.1	5.6 ± 0.1	6 ± 0.1	6 ± 0.1
Intravenous tracer	22.2 ± 0.3	22.4 ± 0.7	22.7 ± 0.3	22.4 ± 0.5	23.8 ± 0.5	24 ± 0.2
Exogenous inflow	40.6 ± 0.6	41 ± 1.2	41.6 ± 0.6	41 ± 0.9	43.6 ± 0.9	43.8 ± 0.4
Total flux	332 ± 16	300 ± 18	332 ± 16	276 ± 23	331 ± 16	293 ± 24
Endogenous flux	291 ± 16	259 ± 19	291 ± 16	235 ± 23	288 ± 15	249 ± 24
Splanchnic uptake	3 ± 0.6	3.6 ± 0.6	4.5 ± 0.5	3.1 ± 0.6	4.4 ± 0.7	3.5 ± 0.7
Splanchnic uptake (% of enteral)	16 ± 3	20 ± 4	24 ± 2.6	16.6 ± 3	22 ± 3.6	17.6 ± 3.8

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ . Clinical phase 1,  $\approx 3$  d after admission when the subjects were infected and malnourished. Clinical phase 2,  $\approx 14$  d after admission when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3,  $\approx 57$  d after admission when the subjects were recovered. Cell means were compared by repeated-measures ANOVA. There were no significant main effects of clinical phase or diagnosis or significant diagnosis  $\times$  clinical phase interactions.

where  $E_{\text{D}_2\text{O}}$  is the enrichment of the deuterium oxide dose,  $E_{\text{pD}_2\text{O}}$  is the plasma water plateau enrichment, and 1.04 is the factor that converts deuterium dilution space to total water (7).

Fat-free mass (FFM) was calculated as follows:

$$\text{FFM(kg)} = \text{TBW}/K \quad (5)$$

where  $K$  is the age- and sex-specific hydration constant for FFM as reported by Fomon et al (8). In the children with edematous SCU, total body water measured in the malnourished edematous state (ie, clinical phase 1 measurement) was corrected by subtracting the contribution from edema fluid. Edema fluid was estimated as the difference between body weight on the day of the clinical phase 1 experiment,  $\approx 3$  days after admission, and the lowest postexperiment weight observed before the clinical phase 2 measurement,  $\approx 14$  d after admission.

All kinetic data are expressed per kg fat free mass.

### Statistics

Data are expressed as means  $\pm$  SEMs. Two-factor repeated-measures analysis of variance (ANOVA) was used to determine differences between the 2 groups with diagnosis as the between factor and clinical phase as the repeated factor. If the repeated-measures ANOVA was significant, pairwise comparisons were made by Tukey's method. Unpaired  $t$  tests were used to compare the clinical characteristics of the 2 groups at the time the groups were admitted to the hospital. Inferential tests were considered statistically significant when  $P$  values were  $< 0.05$  (2-tailed). Data analysis was performed with GraphPad PRISM version 4 software (GraphPad Software, San Diego, CA).

### RESULTS

At the time they participated in the first isotope infusion (clinical phase 1), all the children were severely wasted with a mean weight-for-age of 55.9% of expected in the nonedematous group and 59% of expected in the edematous group (Table 1). There were no significant differences between the 2 groups in age, in any of the anthropometric variables, or in fat-free mass. Plasma albumin concentrations, however, were significantly lower in the edematous group than in the nonedematous group at clinical

phases 1 and 2. Within each group, when the subjects were recovered from SCU (clinical phase 3), age, all anthropometric measurements, and fat-free mass had increased significantly compared with the values at clinical phases 1 and 2. In the nonedematous group, albumin concentrations did not change significantly from clinical phase 1 to 3, but in the edematous group, albumin concentrations were significantly lower at clinical phases 1 and 2 than at clinical phase 3. In both groups, the percentage of body weight composed of fat-free mass decreased significantly from clinical phase 1 to clinical phase 3 ( $P < 0.05$ ).

The clinical characteristics of both groups at admission are shown in Table 2. All the subjects were anemic, and hemoglobin concentrations were significantly lower ( $P < 0.05$ ) in the nonedematous group than in the edematous group. Although 3 of the 17 subjects were diagnosed as not having an infection at admission, one of these subjects had a markedly elevated white blood cell count, which suggests the presence of an occult infection. The white blood cell count was elevated in only 8 of the 17 subjects.

As shown in Table 3, there was no significant difference in total or endogenous glycine flux or splanchnic glycine uptake between the edematous and nonedematous groups during any of the 3 treatment phases. There was a trend, however, whereby glycine flux was consistently slower in the edematous group than in the nonedematous group by 11% at phase 1, by 19% at phase 2, and by 14% at phase 3. In both groups of subjects, there were no significant changes in any of the glycine kinetic variables from clinical phase 1 through clinical phases 2 and 3. Although not statistically significant, splanchnic glycine uptake in the nonedematous group was 50% greater at clinical phases 2 and 3 than at clinical phase 1.

Plasma amino acid concentrations are shown in Table 4. At clinical phase 1, 8 amino acids were significantly lower than recovered values: 6 essential amino acids (leucine, isoleucine, valine, methionine, phenylalanine, and lysine) and 2 nonessential amino acids (tyrosine and arginine). Similarly, at clinical phase 2, with the exception of lysine, the plasma concentrations of these amino acids remained lower than recovered values. The plasma concentrations of 6 essential amino acids (leucine, valine,



TABLE 4

Plasma amino acids in children with edematous and nonedematous severe childhood undernutrition<sup>1</sup>

Amino acid	Clinical phase 1	Clinical phase 2	Clinical phase 3	All subjects
Essential amino acids by diagnosis				
Leucine <sup>2</sup>				
Nonedematous	61 ± 7	75 ± 7	120 ± 17	85 ± 8 <sup>3</sup>
Edematous	30 ± 7	44 ± 10	116 ± 17	64 ± 10
All subjects	44 ± 6 <sup>a</sup>	58 ± 8 <sup>a</sup>	118 ± 10 <sup>b</sup>	73 ± 7
Isoleucine <sup>2,4</sup>				
Nonedematous	31 ± 2.6 <sup>5</sup>	39 ± 4 <sup>5</sup>	45 ± 4 <sup>5</sup>	38 ± 2
Edematous	17 ± 1.5	23 ± 4	68 ± 10	36 ± 6
All subjects	23 ± 3 <sup>a</sup>	30 ± 4 <sup>a</sup>	57 ± 6 <sup>b</sup>	37 ± 3
Valine <sup>2</sup>				
Nonedematous	75 ± 10	87 ± 16	185 ± 23	116 ± 15 <sup>3</sup>
Edematous	51 ± 3.7	51 ± 7.3	182 ± 41	95 ± 14
All subjects	62 ± 7 <sup>a</sup>	68 ± 10 <sup>a</sup>	184 ± 7 <sup>b</sup>	105 ± 10
Histidine				
Nonedematous	46 ± 5	78 ± 25	60 ± 4	59 ± 8
Edematous	52 ± 9	63 ± 23	52 ± 1	58 ± 4
All subjects	49 ± 6	70 ± 9	55 ± 3	58 ± 4
Methionine <sup>2</sup>				
Nonedematous	21 ± 1.8	22 ± 1.2	26 ± 1.5	23 ± 1 <sup>3</sup>
Edematous	13 ± 2.2	14 ± 2.2	22 ± 2	17 ± 2
All subjects	17 ± 2 <sup>a</sup>	18 ± 2 <sup>a</sup>	24 ± 1 <sup>b</sup>	20 ± 1
Lysine <sup>2</sup>				
Nonedematous	100 ± 8	112 ± 7	110 ± 12	108 ± 5 <sup>3</sup>
Edematous	70 ± 9	72 ± 10	93 ± 7	79 ± 3
All subjects	85 ± 8 <sup>a</sup>	91 ± 6 <sup>a,b</sup>	101 ± 3 <sup>b,c</sup>	92 ± 4
Phenylalanine <sup>2</sup>				
Nonedematous	33 ± 3.7	31 ± 3.6	40 ± 5	35 ± 2 <sup>3</sup>
Edematous	17 ± 1.8	18 ± 2.4	34 ± 5	23 ± 3
All subjects	24 ± 3 <sup>a</sup>	24 ± 3 <sup>a</sup>	37 ± 4 <sup>b</sup>	28 ± 2
Threonine				
Nonedematous	76 ± 8	74 ± 12	85 ± 16	78 ± 7 <sup>3</sup>
Edematous	39 ± 7	41 ± 9	75 ± 9	48 ± 6
All subjects	56 ± 7	59 ± 8	72 ± 11	62 ± 5
Nonessential amino acids				
Alanine				
Nonedematous	343 ± 24	382 ± 58	321 ± 30	347 ± 25
Edematous	240 ± 40	294 ± 33	293 ± 24	268 ± 22
All subjects	287 ± 29	335 ± 36	291 ± 24	304 ± 17
Glycine				
Nonedematous	272 ± 31	279 ± 15	239 ± 23	287 ± 17
Edematous	269 ± 35	220 ± 21	259 ± 33	240 ± 16
All subjects	254 ± 21	261 ± 21	268 ± 21	261 ± 12
Serine				
Nonedematous	121 ± 9	130 ± 3.7	129 ± 13	126 ± 6
Edematous	119 ± 22	140 ± 9	138 ± 10	156 ± 23
All subjects	120 ± 10	148 ± 30	158 ± 24	141 ± 12
GLX <sup>6</sup>				
Nonedematous	500 ± 58	559 ± 75	530 ± 36	530 ± 56
Edematous	418 ± 55	621 ± 123	486 ± 27	508 ± 68
All subjects	463 ± 56	595 ± 48	510 ± 30	520 ± 45
Tyrosine <sup>2</sup>				
Nonedematous	29 ± 5	35 ± 6	54 ± 8	39 ± 4 <sup>3</sup>
Edematous	11.5 ± 1.4	17 ± 3	43 ± 7	23 ± 4
All subjects	20 ± 4 <sup>a</sup>	25 ± 4 <sup>a</sup>	46 ± 5 <sup>b</sup>	30 ± 3
Arginine <sup>2</sup>				
Nonedematous	70 ± 15	77 ± 14	85 ± 16	86 ± 8 <sup>3</sup>
Edematous	32 ± 6	52 ± 8	79 ± 17	54 ± 8
All subjects	50 ± 10 <sup>a</sup>	69 ± 8 <sup>a</sup>	88 ± 12 <sup>b</sup>	69 ± 6

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ . Clinical phase 1,  $\approx 3$  d after admission when the subjects were infected and malnourished; clinical phase 2,  $\approx 14$  d after admission when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3,  $\approx 57$  d after admission when the subjects were recovered. Cell means were compared by repeated-measures ANOVA.

<sup>2</sup> Main effect of clinical phase was significant,  $P < 0.05$ . Within rows, mean values of subjects at each clinical phase with different superscript letters are significantly different,  $P < 0.05$ . Post hoc comparisons were by Tukey's procedure.

<sup>3</sup> Main effect of diagnosis was significant,  $P < 0.05$ . Marginal mean values for nonedematous diagnosis were significantly different from marginal mean value of edematous diagnosis.

<sup>4</sup> Diagnosis  $\times$  clinical phase interaction was significant,  $P < 0.005$ .

<sup>5</sup> Within clinical phase, nonedematous diagnosis was significantly different from the edematous diagnosis on the basis of the multiple comparison procedure of Tukey.

<sup>6</sup> Glutamate plus glutamine.

phenylalanine, lysine, threonine, and methionine) and 2 nonessential amino acids (tyrosine and arginine) were greater in the nonedematous group than in the edematous group. However, for isoleucine, there was a significant clinical phase by diagnosis interaction evidenced by the nonedematous group having a significantly greater concentration at clinical phases 1 and 2 but a lower concentration on recovery (clinical phase 3).

## DISCUSSION

In this study, we aimed to determine whether glycine production is slower in the malnourished and infected state than in the malnourished but infection-free state and the recovered state in children with SCU. We also aimed to determine whether there are differences in whole-body glycine flux and splanchnic glycine extraction between children with edematous SCU and those with nonedematous SCU. Our results showed that there was no significant difference in glycine flux and splanchnic glycine extraction between the malnourished states (clinical phases 1 and 2) and the recovered state in both groups of children with SCU. There was also no significant difference between children with edematous SCU and those with nonedematous SCU in any of these kinetic variables at any phase of treatment. Plasma glycine concentrations were not lower in the malnourished state than in the recovered state in either group of children and there was no significant difference in glycine concentration at any clinical phase in the children with edematous SCU compared with those with nonedematous SCU. These results suggest that children with edematous and nonedematous SCU are capable of maintaining glycine production in the severely undernourished state, both when infections are present and when infections are cleared.

In humans, glycine is regarded as a dispensable amino acid, meaning that it can be synthesized in sufficient quantities to meet the usual needs for normal growth and maintenance of good health. It is, however, a dispensable amino acid in very high demand because it is a precursor for the formation of numerous essential biological compounds (3). Because glycine is unavailable for reutilization after its incorporation in most of these compounds, it is not known whether de novo synthesis of glycine will be sufficiently increased to compensate for the decreased supply from the diet in children who have SCU and concurrent infections. Hence, whereas a healthy child consuming an adequate diet may be able to synthesize adequate quantities of glycine, this may not be true in a child with SCU and concurrent infection, because the metabolic and immunologic response to infection will increase the demand for this amino acid in the face of reduced availability of labile nitrogen to support its synthesis. The results of the present study in children with edematous and nonedematous SCU show that glycine production is maintained in the severely undernourished state, both when infections are present and when infections are cleared, compared with the recovered state. Hence, these children can up-regulate de novo glycine synthesis to compensate for the reduced contributions from body protein breakdown and chronic food deprivation.


Because the labile amino nitrogen pool available for de novo synthesis of dispensable amino acids is markedly reduced in children with SCU (2, 9), the finding that endogenous glycine flux was not slower in the malnourished state (clinical phases 1 and 2) than during recovery (clinical phase 3) was surprising. This was especially true for the children with edematous SCU in light of our recent finding that protein breakdown was  $\approx 50\%$

slower in these children when they were malnourished than when they had recovered (2). In the fed state, the flux of a dispensable amino acid represents the sum of its inflows into the free pool from protein breakdown, de novo synthesis, and the diet. We have shown in studies in adults that glycine derived from whole-body protein breakdown is a major contributor to its overall flux (1). Therefore, the finding of no change in glycine flux despite a marked reduction in the protein breakdown rate and, hence, of protein-derived glycine, indicates that the flux of glycine was maintained by increased de novo synthesis. This finding suggests that glycine synthesis has a high metabolic priority to ensure that the metabolic requirement is not severely compromised in children with edematous SCU. The maintenance of plasma glycine concentrations in these children further suggests that the metabolic demand for glycine was met by its rate of production.

We are not aware of any other study of glycine kinetics in severely undernourished children. In a study of children with SCU, Persaud et al (4) reported a significant increase in urinary 5-oxoproline excretion during the rapid catch-up growth phase, which suggests that the availability of glycine was insufficient to meet total metabolic requirements, specifically for glutathione synthesis. 5-Oxoproline excretion has been proposed as an index of glycine sufficiency because it indicates that there is an overproduction of  $\gamma$ -glutamyl-cysteine secondary to a shortage of glycine for glutathione synthesis (10). When the authors added glycine to the diets of children with SCU during the rapid catch-up growth phase, they reported a 50% reduction in urinary 5-oxoproline excretion and a doubling of blood glutathione concentrations, which strongly suggests that the supply of glycine was insufficient to satisfy both the accelerated protein and glutathione synthesis rates necessary for rapid tissue and glutathione repletion during this phase of rehabilitation. Hence, although we found that glycine production was more or less the same during the early phases of treatment and during recovery (when the children were not growing rapidly), this does not rule out the possibility that the amount of glycine produced was not enough to satisfy the rapid rate of protein synthesis plus maintenance of the numerous pools of other specialized products made from glycine, such as heme and glutathione, during the rapid catch-up growth phase of rehabilitation.

Another aim of the present study was to determine whether glycine flux was slower in the children with edematous SCU than in those with nonedematous SCU in the malnourished state. In a recent study, we reported that the protein breakdown rate in children with edematous SCU is  $\approx 40\%$  slower than that in children with nonedematous SCU (2). This finding suggests that children with edematous SCU will also have a slower glycine flux, because whole-body protein breakdown is a major contributor to overall glycine flux. Indeed, there was a trend whereby glycine flux was consistently slower in the edematous children than in the nonedematous children. However, these differences were not significant, which indicates that de novo glycine synthesis was sufficiently increased in the edematous children to compensate for the slower release from protein breakdown. On the other hand, one cannot rule out a type II error because of the relatively small sample size used in our study. For example, on the basis of the differences between groups and their SDs, we can calculate that at clinical phase 1, 40 subjects per group would have given an 80% power to detect a difference between means at a significance level of 0.05. At clinical phase 2, however, just 20 subjects per group would have given an 80% power to detect



a difference between means with a significance level of 0.05. Hence, there is a distinct possibility that at clinical phase 2, just as the children are entering the catch-up growth phase, an additional 10 subjects per group would have yielded a significant difference in glycine production. This would have supported the conclusion of Persaud et al (4) that the supply of glycine was insufficient to satisfy both the accelerated protein and glutathione synthesis rates necessary for rapid tissue and glutathione repletion during the catch-up growth phase of nutritional rehabilitation. 

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