In vivo rates of erythrocyte glutathione synthesis in adults with sickle cell disease

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Submitted 24 June 2005; accepted in final form 21 January 2006

The sickle cell anemias are genetic diseases resulting from nucleotide changes in the gene for the β-chain of the adult hemoglobin (HbA) (19). This results in the production of distinct functional hemoglobin variants that aggregate or polymerize when deoxygenated, resulting in decreased cellular flexibility and decreased red cell survival (29). One of the commonest sickle hemoglobinopathies is homozygous β⁺ (HbSS) disease (44) with an estimated incidence of 0.31% in Jamaica (36).

The extent to which a cell is able to withstand the potentially damaging effects of oxidative stress is determined by the balance between the rate at which oxidant species are generated and the capacity of metabolic processes to produce antioxidants. The antioxidant defense systems include a complex of interrelated functions, each of which tends to buffer the effects of the others. The glutathione (GSH) system is a major component of overall antioxidant defenses (33). GSH is synthesized de novo within all cells from glutamate, cysteine, and glycine in a series of reactions catalyzed by glutamate cysteine ligase (also known as γ-glutamylcysteine synthetase, EC 6.3.2.2) and γ-L-glutamyl-L-cysteine:glycine ligase (also known as glutathione synthetase, EC 6.3.2.3). Glutamate cysteine ligase catalyzes the synthesis of the intermediate γ-L-glutamyl-L-cysteine from glutamate and cysteine, the rate-limiting step in GSH synthesis. Alternately, GSH is regenerated from glutathione disulfide (GSSG) by glutathione: NADP⁺ oxidoreductase (also known as glutathione reductase, EC 1.8.1.7).

There is abundant evidence that the GSH concentration in erythrocytes of individuals with sickle cell disease (SCD) is low and that they also have increased oxidative stress (11, 16–18, 46). It has been proposed that oxidative damage of the membrane and ionic channels of the SCD erythrocyte alters its polymerization and depolymerization kinetics, resulting in the formation of irreversibly sickled cells and microvascular occlusion (13, 14, 39–41). Furthermore, Gibson et al. (14) have reported that increasing the intracellular concentrations of GSH inhibits the formation of dense cells and irreversibly sickled cells in vitro by maintaining the sulfhydryl groups of β-actin, a membrane structural protein in the reduced form.

Despite several reports of lower GSH concentration in SCD (7, 33), the in vivo kinetic mechanism(s) responsible for GSH deficiency has not been determined, as the majority of the work in this area to date has relied on in vitro measurements. Notwithstanding, there are two obvious general kinetic mechanisms for the decreased intracellular GSH concentration, namely suppressed synthesis and/or increased consumption relative to synthetic capacity. In SCD there is indirect evidence for protein and energy deficiencies relative to metabolic demand (5, 42, 43). Additionally, in vivo kinetic experiments in normal adults have shown that GSH turnover is suppressed by diets deficient in sulfur amino acids (28) and by diets with marginal amounts of protein (21). Similarly, in animal models, dietary deficiencies of the precursor amino acids, especially cysteine, resulted in decreased GSH concentrations (4, 10, 15, 24). Therefore, it is plausible that substrate availability may constrain the in vivo synthesis of GSH in SCD, resulting in low concentrations.
GSH concentrations. Alternatively, the reported increased oxidative stress in SCD may consume GSH at a much faster rate than the rate of synthesis. To determine whether increased GSH consumption or suppressed synthesis was responsible for the lower erythrocyte GSH concentration, we used a stable-isotope tracer method (34) to measure erythrocyte GSH synthesis in vivo in individuals with SCD and in healthy controls. We also measured erythrocyte cysteine concentration, the rate-limiting precursor for GSH synthesis, and plasma markers of oxidant damage.

METHODS

Subjects

The sample comprised 23 adult patients with HbSS recruited from the Jamaica Sickle Cell Cohort and eight control HbAA subjects. Exclusion criteria were history or evidence of cardiovascular, hepatic, renal, or gastrointestinal disease; presence of chronic infectious disease (including HIV infection), and blood transfusion within 3 mo of the study. The Jamaican Sickle Cell Cohort includes patients with sickle cell disease detected using standard criteria during screening of 100,000 consecutive nonoperative deliveries at a large maternity hospital (the Victoria Jubilee Hospital) between 1973 and 1981 (37). For subjects with SCD, the study was performed in the steady state, i.e., free of acute clinical illness. Written informed consent was obtained from all participants enrolled in the study. The study was approved by University Hospital/University of the West Indies Faculty of Medical Sciences Ethics Committee and the Institutional Review Board, Baylor College of Medicine.

The subjects were admitted to the metabolic ward of the Tropical Metabolism Research Unit on the night before the study. After an overnight fast from 10 PM, the subjects were asked to void, and an aliquot of the urine was collected and stored at −20°C for subsequent analyses. The subjects were then weighed using an electronic scale (Seca 770, Hamburg, Germany) and had height measured using a Harpenden stadiometer. Anthropometric measurements were obtained with the subjects dressed in light clothing and with shoes and socks removed. Weight measurements were in kilograms to the nearest 100 g and height measurements in centimeters to the nearest millimeter. The same observer performed all measurements.

Tracer Infusion Protocol

A sterile solution of [2H2]glycine (Cambridge Isotope Laboratories, Woburn, MA) was prepared in 9 g/l saline. A stock solution of deuterium oxide was prepared by diluting deuterium oxide (100 g, 99.9% enriched in deuterium, Cambridge Isotope Laboratories, Woburn, MA) with deionized water to yield a concentration of ~5 g/100 ml. On the morning of the infusion, intravenous catheters were inserted into superficial veins of both arms, one for continuous monitoring of vital signs and the other for repeated blood sampling. After a 20-ml blood sample was drawn, a bolus dose of ~2 g/kg stock deuterium oxide (~100 mg/kg of 99.9% deuterium) was given orally. The exact amount given was determined by weighing. Immediately thereafter, a bolus intravenous infusion of [2H2]glycine (20 μmol/kg) was given to prime the glycine pool followed by a constant infusion of the same isotope at the rate of 15 μmol·kg⁻¹·h⁻¹. The duration of the constant infusion was 8 h. Additional 5-ml blood samples were taken at 4, 5, 6, 7, and 8 h for measurement of erythrocyte GSH-derived glycine isotopic enrichments.

Sample Analyses

Blood chemistries. The baseline blood sample was distributed into tubes containing Na2EDTA (1 mg/ml blood) for the measurement of hematological indices inclusive of hematocrit, plain tubes for the measurement of clinical chemistry, and heparinized tubes for the measurement of derivatives of reactive oxygen metabolites (D-ROM) test. Hemoglobin and white blood cell counts were measured with a hematology analyzer (Beckman Coulter Gen System 2, Fullerton, CA), serum albumin by Abbott Alcycon 300i, (Abbott Park), and serum urea, creatinine, total bilirubin, and serum aspartate aminotransferase with an Abbott Spectrum Clinical Chemistry Analyzer (Abbott Park). The hematocrit of each blood sample was determined by centrifugation at 13,460 g for 5 min at 25°C on a Micro Hematocrit Centrifuge (Damon/IEC Division, Needham Heights, MA). Blood samples for the determination of isotopic enrichments were drawn in prechilled tubes (containing Na2EDTA and a cocktail of sodium azide, merthiolate, and soybean trypsin inhibitor) and immediately centrifuged at 4°C, and the plasma was removed and stored at −70°C for later analysis.

Erythrocyte GSH analyses. To measure erythrocyte GSH concentration and isotopic enrichment, a 1-ml aliquot of each blood sample was mixed immediately in a cryotube with 0.5 ml of chilled, isotonic monobromobimane (MBB) buffer solution (pH 7.5) containing the following (in mmol/l): 5 MBB, 17.5 Na2EDTA, 50 potassium phosphate, 50 serine, and 50 boric acid. The whole blood-MBB mixture was centrifuged at 1,000 g for 10 min at 4°C, and then the supernatant fluid was incubated in the dark for 20 min for development of the plasma GSH-MBB derivative. Another 1.0 ml of MBB buffer was added to the packed erythrocytes, which were immediately lysed by rapid freeze and thaw with liquid nitrogen, and the lysed erythrocyte-MBB buffer 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 by using 0.5 ml of 2 mol/l perchloric acid, and the supernatant fluids were stored at −70°C until further analysis. To determine GSSG concentration, the reducing agent diithiothreitol (5 mM) was added first to a blood sample to convert GSSG to GSH and the sample was processed as described above. The GSSG concentrations were obtained by subtracting the GSH value from the GSH + GSSG value.

Isolation of erythrocyte GSH and measurement of its concentration were performed using a Hewlett-Packard 1100 HPLC system (Hewlett-Packard, Avondale, PA) equipped with a reverse-phase ODS Hypersil column (5 μm, 0.46 × 200 mm; Agilent Technologies, Wilmington, DE) and fluorescence detector (model HP 1046A; Hewlett-Packard) as previously described by us (3, 20, 26, 28). Elution of the GSH was accomplished with a 3–13.5% acetonitrile linear gradient in 1% acetic acid (pH 4.25) at a flow rate of 1.1 ml/min. The GSH elution was collected using a fraction collector, dried, and hydrolyzed for 4 h in 4 mol/l HCl at 110°C (34).

Erythrocyte free glycine was extracted from the protein-free supernatant by cation exchange chromatography. Erythrocyte free glycine and erythrocyte GSH-derived glycine were converted to the n-propyl ester heptafluorobutyramide derivatives, and the isotope ratio of each was measured by negative chemical ionization gas chromatography mass spectrometry, monitoring ions at mass to charge ratio (m/z) 293 to 295 (30).

The 2H2 content of plasma water was determined by reducing water extracted from 10 μl of plasma with zinc in quartz vessels and determining the 2H2 abundance of the resulting hydrogen gas by gas isotope-ratio mass spectrometry (Delta-E; Finnigan MAT, San Jose, CA) (47).

Oxidant markers. The D-ROMS test was used to measure plasma hydroperoxide concentration as an index of free radical formation (9). This test is based on the concept that the amount of organic hydroperoxides present in plasma is related to the free radicals from which they are formed. Briefly, plasma is reacted with an acidic acetate buffer (pH 4.8), which liberates transition metal ions that catalyze the decomposition of the hydroperoxides to alkoxy and peroxyl radicals. These newly formed radicals in turn oxidize the spectrophotometric marker (N,N-diethyl-p-phenylenediamine), which is detectable at an
Table 1. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 8)</th>
<th>SCD (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>5/3</td>
<td>11/12</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
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<td>25.4±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170.8±12.8</td>
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<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
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<td>54.4±6.7</td>
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<tr>
<td>BMI, kg/m²</td>
<td>22.1±2.1</td>
<td>19.1±2.8</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>Total body water, liters</td>
<td>39.2±7.2</td>
<td>32.3±5.1</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>53.2±9.9</td>
<td>44.2±7.0</td>
<td>&lt;0.007</td>
</tr>
</tbody>
</table>

Values are means ± SD. SCD, homozygous β⁺ sickle cell disease; NS, not significant.

absorption at 505 nm as U.CARR. (carratelli units). One U.CARR. is equal to 0.8 mg/l, hydrogen peroxide.

Plasma nitrotyrosine was measured as an index of protein oxidative damage by enzyme-linked immunoassay using a BIOXYTECH Nitrotyrosine-EIA kit, (OXIS International, Portland, Oregon). Additionally, urinary concentrations of isoprostane (marker of lipid membrane peroxidative damage) were measured using an Oxford Biomedical Research EIA kit (Oxford, MI).

Dietary Assessment

Subjects were issued with a 3-day food diary during the week prior to the infusion experiment with verbal and written instructions to add to their diary every time they ate or drank, describing the food/drink as accurately as possible and giving estimates of amounts. The diary consisted of two weekdays and one weekend day. All of the diaries were completed and collected when the subjects were admitted for the infusion experiments. The diaries were analyzed using Nutritionist Five (San Bruno, CA) software. The data were entered by a trained nutritionist.

Calculations

The fractional synthesis rate (FSR) of erythrocyte GSH per day was calculated according to the precursor-product equation (34)

\[
\text{FSR}_\text{GSH} \text{(%/day)} = \frac{\text{IR}_7 - \text{IR}_5}{\text{IR}_{bc}} \times \frac{1200}{t_7 - t_5} \tag{1}
\]

where \(\text{IR}_7 - \text{IR}_5\) is the increase in the isotope ratio of erythrocyte GSH-bound glycine between the fifth and seventh hours of infusion, when the isotope ratio of erythrocyte free glycine, \(\text{IR}_{bc}\), had reached a steady state. The units of FSR are percent per day (%/day). The absolute synthesis rate (ASR) of erythrocyte GSH per day was calculated as the product of the erythrocyte GSH concentration and the FSR. The units of ASR are expressed as millimoles per liter per day of packed erythrocyte.

Total body water (TBW) in moles is calculated from the dilution of deuterium in plasma. Briefly, prior to analysis on the isotope ratio mass spectrometer, an aliquot of the deuterium dose administered to each subject was diluted with deionized water. The amounts of dose diluted and water used were recorded. The enrichments of deuterium were measured in a diluted aliquot of the dose administered, \(E_{2H2O}\), in deionized water, \(E_{D2O}\), and in plasma sample, \(E_{2H2O}\). TBW was then calculated using the equation (47)

\[
\text{TBW(kg)} = \frac{E_{H2O} - E_{D2O}}{E_{H2O}} \times \frac{W_{D2O} \times W_{Dose}}{1000 \times W_{H2O} \times 1.04} \tag{2}
\]

where \(W_{D2O}\) is the weight of deionized water in grams used to dilute aliquot of dose, \(W_{Dose}\) is the weight in grams of deuterium stock given to subject, \(W_{H2O}\) is the weight in grams of the aliquot of dose, and 1.04 is the factor that converts deuterium dilution space to total water (47).

Fat free mass (FFM) was calculated as follows:

\[
\text{FFM(kg)} = \frac{\text{TBW}}{0.73} \tag{3}
\]

where 0.73 was taken as the hydration constant for FFM (45).

Statistics

Data are expressed as means ± SD. Differences in means between the SCD group and the control group were determined using an independent t-test with the Satterthwaite adjustment for unequal variances where appropriate. For variables where the distributions were skewed, the two-sample Wilcoxon test was used. Differences in net tracer-to-tracee ratio between groups at plateau were determined by repeated-measures analysis of variance. Data analysis was performed with the Stata statistical software, version 8.2, for Windows (Stata, College Station, TX). Results were considered to be statistically significant if \(P < 0.05\).

RESULTS

Clinical Characteristics

By design, the ages and sex distribution of the subjects with SCD and controls were not different. Subjects with SCD were lighter and had lower body mass indexes, TBW, and FFM compared with control subjects (Table 1).

The differences observed in hematology indexes were as expected, with subjects with SCD having lower hemoglobin concentrations and erythrocyte counts but higher nucleated cell concentrations and erythrocyte GSH kinetics (Table 2).

Erythrocyte GSH Kinetics

The tracer/tracee ratio of glycine reached steady state after 5 h of the isotope infusion in both SCD group and controls, and
there was a linear increase in the amount of labeled glycine incorporated into erythrocyte GSH (Fig. 1). The average net tracer/tracer ratio at plateau was significantly greater in controls compared with subjects with SCD (mean ± SD, 1.96 ± 0.13 vs. 1.3 ± 0.08 mol%, P < 0.001).

Compared with values of controls, subjects with SCD had significantly lower erythrocyte GSH concentrations (mean ± SD, 2.0 ± 0.7 vs. 2.6 ± 0.2 mmol/l, P < 0.04) but significantly faster GSH FSR (mean ± SD, 149 ± vs. 74 ± 15, %day⁻¹, P < 0.02 ; Figs. 2 and 3). The mean value of absolute rates of synthesis in subjects with SCD compared with control subjects was greater by ~57%, but this difference did not achieve conventional thresholds for statistical significance (mean difference with 95% CI 1.1, −0.1 to 2.3, P = 0.062; Fig. 3).

To determine whether there was impairment in the regeneration of GSH from GSSG, erythrocyte GSSG concentration and the ratio of GSH to GSSG concentrations were measured in a subset of subjects, (5 controls and 5 subjects with SCD). There were no differences between the SCD group and controls in GSSG concentrations (mean ± SD, 0.12 ± 0.16 vs. 0.12 ± 0.14 mmol/l) and GSH/GSSG ratios (mean ± SD, 40.4 ± 40.2 vs. 40.6 ± 29.4; Fig. 2).

The faster rates of synthesis of GSH in subjects with SCD were accompanied by lower concentrations of erythrocyte cysteine (P < 0.004). On the other hand, the concentrations of markers of oxidative damage (plasma D-ROMs, plasma nitrotyrosine, and urinary isoprostane/creatinine ratio; Table 3), as well as dietary intakes of energy, protein, and GSH precursor amino acids cysteine, glycine, and glutamate, were not different between subjects with SCD and controls (Table 4).

DISCUSSION

In this study, we used an in vivo stable-isotope method to measure rates of synthesis of GSH in vivo in the erythrocytes...
of subjects with SCD and in healthy controls. The data reported here demonstrate that erythrocyte GSH and the GSH precursor cysteine concentrations were lower in subjects with SCD compared with controls. On the other hand, the fractional and absolute rates of GSH synthesis were greater by 101 and 57% respectively. This was associated with no difference in the GSH/GSSG ratio and the concentrations of markers of lipid and protein oxidant damage between SCD subjects and controls. These findings suggest that the depleted erythrocyte GSH of this cohort of SCD patients is not due to suppressed synthesis or impaired regeneration but rather to increased consumption. Increased GSH consumption, a major intracellular antioxidant/detoxificant, may explain why these patients do not have evidence of oxidative stress in their plasma.

We have discussed previously (23, 34) the assumptions and technical limitations of the stable-isotope approach for measuring GSH synthesis using the precursor-product model. Briefly, the requirements for the calculation of the rate of GSH synthesis using the precursor-product model is the measurement of isotopic enrichment of GSH-bound tracer amino acid at a minimum of two time points during the quasi-linear portion of the exponential rise in GSH-bound amino acid labeling. In addition, it is essential to obtain an estimate of the isotopic enrichment of the amino acid tracer at the site of protein synthesis, i.e., the erythrocyte protein synthetic precursor pool. Comparing with measurements in whole blood, we measured the isotopic enrichment of the glycine tracer within the erythrocyte, our tissue of interest, thereby mitigating errors associated with the dilution of the tracer with glycine released from the free amino acid pools of other types of cell in whole blood. Nevertheless, the observed lower plateau enrichments of glycine in the erythrocytes of the SCD group compared with controls represent the confluence of differences in glycine transport (26) and glycine kinetics (rate of appearance from proteolysis, endogenous synthesis, nonoxidative disposal, and oxidation) in the erythrocytes of the SCD group compared with controls. We used labeled glycine in this study, as we have reported previously that both glycine and cysteine tracers yielded similar results (24). Indeed, we are heartened by the fact that our estimate of GSH synthesis rates in controls is similar in magnitude to the GSH synthesis rates reported by Lyons et al. (28) using a cysteine tracer in adult men.

There are two obvious general kinetic mechanisms for the decreased intracellular GSH concentration, namely suppressed synthesis and/or increased consumption relative to synthetic capacity. Our finding that GSH synthesis in SCD is not impaired is supported by the work of Kiessling et al. (26), who based their arguments on in vitro measurements of GSH precursor concentrations and cellular amino acid transport kinetics. In fact, the faster turnover and decreased erythrocyte concentration of GSH suggest that there is an increased but unsatisfied demand for GSH in SCD. The demand for GSH is driven by the need to maintain redox homeostasis, transport of amino acids via the γ-glutamyl cycle, and the production of inflammatory mediators.

GSH maintains redox homeostasis by two mechanisms, namely by reacting with hydroperoxides and by conjugation reactions (8). GSH is converted to its oxidized form GSSG when it reduces hydroperoxides, a reaction catalyzed by glutathione:hydrogen-peroxide oxidoreductase (also known as glutathione peroxidase, EC 1.11.1.9). Reduced GSH in turn is

Table 3. Concentration of oxidants in subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>SCD (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>D-ROMs U Carr</td>
<td>566.0</td>
<td>382.0</td>
<td>700.0</td>
</tr>
<tr>
<td>Nitrotyrosine, nmol/l</td>
<td>2.9</td>
<td>1.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Isoprostane:creatinine ratio, ng/mmol</td>
<td>688.3</td>
<td>138.5</td>
<td>1796.5</td>
</tr>
</tbody>
</table>

D-ROMs U Carr, Distribution of values was skewed. Comparisons of distributions performed by the two-sample Wilcoxon test.

Fig. 3. GSH fractional and absolute synthesis rates in SCD subjects (n = 23) and controls (n = 8). Values are means ± SE. *P < 0.02.
regenerated by glutathione:NADP+ oxidoreductase with NADPH as cofactors. Thus the ratio of GSH to GSSG is an important determinant of the redox state of the cells (12), and under physiological conditions variations in this ratio are small. In this study, although subjects with SCD had markedly lower erythrocyte concentrations of GSH compared with controls, the GSH/GSSG ratio was not different, suggesting no difference in the redox state of erythrocytes. Indeed, the maintenance of the GSH/GSSG ratio despite decreased GSH concentrations has been reported in animal studies (25, 27) and highlights the physiological importance of appropriate redox state for optimal cell functioning (27). Mechanistically, the maintenance of GSH/GSSG in SCD suggests that the regeneration of GSH from GSSG is not impaired. Additionally, the observation that there was no difference in urinary excretion of isoprostane, a marker of lipid peroxidation, between controls and subjects with SCD indicates that the level of oxidative stress by hydroperoxides was not different. However, the finding that GSH concentration was lower despite a faster rate of synthesis in SCD strongly intimates increased irreversible consumption of GSH in detoxification reactions. Furthermore, the depletion of the GSH pool in SCD implied that the upregulation of GSH synthesis was insufficient to simultaneously quench the oxidative stress and maintain GSH concentrations.

There is irreversible loss of GSH when it acts as a detoxicant forming mercapturic acids. GSH may be conjugated with electrophilic substances by nonenzymatic or by enzymatic reactions catalyzed by RX:glutathione R-transferase (glutathione S-transferase, EC 2.5.1.18) (8) or, as in the case of cysteiny1-leukotrienes, synthesis by leukotriene-C4 (LTC4) synthetase (EC 4.4.1.20) (1). Thus the decreased GSH concentration in SCD may be due to increased conjugation reactions to form GSH adducts. For example, net loss of GSH may arise because of increased excretion of GSH adducts as mercapto products (8) or via increased biliary excretion (6). Additionally, urinary excretion of cysteiny1-leukotrienes is reported to be increased in SCD (20). Leukotrienes are biologically potent eicosanoids that are derived from arachidonic acid and have significant roles in the modulation of the inflammatory process, cell adhesiveness, and vascular tone in SCD (38).

There is an abundance of evidence from our own work (3, 21, 22, 32) and others’ (28) that cysteine is a limiting precursor for in vivo synthesis of GSH in humans. Decreased cysteine intracellular concentrations can result from decreased transport of cysteine into cells, increased incorporation into GSH and body protein, increased oxidation, or decreased cysteine synthesis. On the basis of the measurements of Kiessling et al. (26), it is unlikely that decreased transport of cysteine into cells is the cause of low erythrocyte cysteine concentrations. On the other hand, the lower erythrocyte cysteine concentration plus the faster rate of GSH synthesis in the present study strongly suggest that the endogenous cysteine supply is not sufficient to meet all anabolic demands plus an upregulated GSH synthesis. As a consequence, the intracellular pool becomes reduced. We have previously reported (2) that in individuals with SCD whose whole body protein synthesis is increased by ∼100%, and in the present study GSH synthesis rates were increased by 57% in SCD subjects compared with controls. This metabolic profile would impose an increased demand for cysteine that would have to be met by increased synthesis or from the diet.

Cysteine is classified as a dispensable amino acid, which implies that the body can synthesize enough to satisfy demand given that an appropriate amount of its precursors, methionine and serine, are available. Methionine is an indispensable amino acid that is supplied by the diet (31). Cysteine is formed principally in the liver from methionine and serine in the transmethylolation-transsulfuration pathway. In this pathway, methionine is converted to homocysteine. Homocysteine in turn combines with serine to form cystathionine. The latter is then converted into cysteine and 2-ketobutyrate by cystathionine t-homocysteine lyase (cystathionase, EC 4.4.1.18). Therefore, decreased availability of methionine and serine, impairment of the transmethylolation-transsulfuration pathway, and/or deficient dietary intake of cysteine or gastrointestinal malabsorption may lead to cysteine deficiency. In this study, we did not measure cysteine absorption from gastrointestinal tract. However, tests of D-xylose absorption have been reported to be abnormal in children and adults with SCD (35), suggesting impairment of jejunal function. Thus, if the body is unable to synthesize enough cysteine to satisfy metabolic demands, then these reported observations would suggest an increased dietary requirement for cysteine. Indeed, the lower erythrocyte cysteine concentration in subjects with SCD and the similar dietary intakes of methionine, serine, and cysteine between controls and SCD subjects suggested that the increased demand for cysteine in SCD was unmet. In other words, the evidence here intimates that cysteine is a conditionally essential amino acid in SCD.

In conclusion, the findings of this study suggest that the depleted erythrocyte GSH of this cohort of SCD patients is not due to suppressed synthesis or impaired regeneration but rather to increased consumption. Increased GSH consumption may explain why these patients do not have evidence of oxidative stress in their plasma. In addition, the lower erythrocyte cysteine concentration plus the faster rate of GSH synthesis strongly suggest that the endogenous cysteine supply is not sufficient to meet all anabolic demands. Hence, cysteine may be a conditionally essential amino acid in individuals with SCD.

ACKNOWLEDGMENTS

We gratefully acknowledge the participation of the subjects in this study, as well as Sharon Howell, Bentley Chambers, Lorraine Wilson, Norma Lewis, O’Neil Brown, Margaret Frazer, and Melanie Del Rosario for their excellent work and support in the conduct of the studies.

GRANTS

This research was supported by National Institutes of Health Grant R03 TWO-5493 and with federal funds from the US Department of Agriculture/Agricultural Research Service under Cooperative Agreement No. 58-6250-6001.

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