Methionine-Adequate Cysteine-Free Diet Does Not Limit Erythrocyte Glutathione Synthesis in Young Healthy Adult Men

Glenda Courtney-Martin,3,4 Mahroukh Rafii,3 Linda J. Wykes,6 Ronald O. Ball,4,7 and Paul B. Pencharz3–5,7*

Abstract

Most methods of determining amino acid (AA) requirements are based on endpoints that determine adequacy for protein synthesis. However, the sulfur AA (SAA) cysteine is believed to be the rate-limiting substrate for synthesis of the most abundant intracellular antioxidant, glutathione (GSH). Our objectives were to determine whether supplementation of cysteine in a diet containing adequate SAA for protein synthesis, as methionine, increased GSH synthesis by measuring the fractional and absolute synthesis rates, and if concentration of GSH changed in response to feeding 5 graded intakes of cysteine (0, 10, 20, 30, and 40 mg·kg⁻¹·d⁻¹) in a random order with a fixed methionine intake of 14 mg·kg⁻¹·d⁻¹ and a protein intake of 1 g·kg⁻¹·d⁻¹. Each subject received a multivitamin and choline supplement during the study. Four healthy adult men each underwent 5 isotope infusion studies of 7-h duration after a 2-d adaptation to the level of cysteine intake being studied on the isotope infusion day. The isotope used was [U-¹³C₂ –¹⁵N]glycine. Analyses included erythrocyte GSH synthesis rates and concentration and urinary sulfate excretion. The GSH synthesis rates and concentration, measured at a methionine intake of 14 mg·kg⁻¹·d⁻¹, did not change with increasing intakes of cysteine. Urinary sulfate excretion showed a significant positive relationship with cysteine intake (r = 0.92; P < 0.01). In conclusion, this study provides preliminary evidence that consumption of SAA adequate to meet the requirement for protein synthesis does not limit GSH synthesis in healthy adult men receiving an otherwise adequate diet. J. Nutr. 138: 2172–2178, 2008.

Introduction

Methods for determining amino acid (AA) requirements, including nitrogen balance and carbon oxidation methods, use endpoints that reflect uptake of the AA for protein synthesis. In addition to their roles for protein synthesis, the sulfur AA (SAA), methionine and cysteine, are required for DNA and RNA methylation (1,2), creatine, epinephrine, and carnitine synthesis (methionine), and for maintenance of the body’s redox status, which is mediated by the body’s most abundant antioxidant and free radical scavenger, glutathione (GSH) (3) (cysteine).

GSH status has been shown to be very sensitive to changes in cysteine intake (4,5) and cysteine is considered the rate-limiting substrate for GSH synthesis (6–8). Cysteine deficiency, as measured by changes is GSH synthesis and concentration, has been observed in healthy adults consuming a SAA-free diet (6), as well as healthy adults consuming an energy-adequate, low-protein diet.

In addition, cysteine deficiency has been observed in stress and disease states, including children with edematous malnutrition (9), adults with HIV (10), and sickle cell disease (11). The cysteine deficiency, as reflected by decreased GSH synthesis and concentrations in those studies, was mainly attributed to an increase in the cysteine requirement due to its increased utilization for GSH synthesis. The short-term supplementation of N-acetyl-cysteine in subjects in those studies (8,10) led to significant improvement in GSH synthesis and concentration.

There is some evidence from animal and human data that GSH synthesis is not adequate at SAA intakes adequate for protein synthesis and maintenance of nitrogen balance (4,7,8,12). These data have been interpreted to mean that protein synthesis has a higher priority for cysteine than does GSH synthesis (13). The findings by Kurpad et al. (14), that undernourished Indian men have the same requirement for total

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3 Abbreviations used: AA, amino acid; ASR, absolute synthesis rate; FSR, fractional synthesis rate; FSRGSH, FSR of erythrocyte GSH; GSH, glutathione; REE, resting energy expenditure; SAA, sulphur amino acid; TSAA, total sulphur amino acid.

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SAA (as provided by methionine alone without cysteine) as their well-nourished Indian counterparts and well-nourished North American men, therefore begs the question as to whether the SAA requirements as determined using currently available methods, which reflect the needs for protein synthesis, underestimates the SAA intake necessary to maintain a normal GSH status.

The goal of this study was to determine whether GSH synthesis would increase following supplementation of the level of total SAA (TSAA) intake that supported maximum protein synthesis. This was determined by measuring erthrocyte GSH fractional and absolute synthesis rates (ASR) as well as erthrocyte GSH concentration in healthy adult men fed a diet providing 1 g/kg protein in the presence of the mean population requirement for TSAA (as methionine only) of 14 mg·kg⁻¹·d⁻¹ and a varying additional cysteine intake.

**Subjects and Methods**

**Subjects**

Ethical approval for the study was obtained from the Research Ethics Board at the Hospital for Sick Children. Written informed consent was obtained from each subject after the protocol was explained to them fully. Four young healthy adult men participated in this study. To participate in the study, each subject had to be in good health as determined by medical history and blood test, which included normal cell count and no evidence of anemia as determined by white and red cell count, hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin concentration within the normal range for age. Exclusion criteria were: presence of diseases known to affect GSH concentration (e.g. HIV and diabetes), anemia, medications known to affect protein and amino acid metabolism (e.g. steroids), substantial weight loss during the past month, consumption of weight-reducing diets, inability to tolerate the experimental diet, unwillingness to have blood drawn from a venous access during the study, substantial caffeine consumption (e.g. coffee), or significant alcohol consumption [equivalent to >2 cups (500 mL) of coffee per day], or significant alcohol consumption (>1 drink per day, e.g. 1 beer (355 mL, 4.2 g alcohol), one-half glass of wine (125 mL, 5 g alcohol)]. Before the commencement of the study, height, weight, and body composition were measured for each individual (Table 1).

**Study design and dietary intervention**

The 4 subjects each underwent 5 stable isotope infusion studies of 7-h duration, in a repeated-measures design, to measure GSH synthesis in response to 5 different intakes of dietary cysteine assigned in random order. The decision to use 4 subjects and 5 levels of cysteine intake per subject was carefully made. A repeated-measures design, in which each subject acts as their own control, is a powerful design to reduce the impact of subject variability, and this approach is also more sensitive to treatment differences than the alternative designs. In addition, we had predicted, based upon observations reported in the literature, that a curvilinear response was probable. We decided to use 5 cysteine intakes to ensure that we could adequately detect and prove a curvilinear response, if it occurred. Alternatively, with 5 increasing intakes of cysteine per subject, if there was a linear response in any of our measured variables, this would be clearly established.

Each individual study was carried out over a period of 3 d and individuals completed all their studies within a 3-mo period. The first 2 d were the adaptation days during which subjects were adapted to the level of cysteine administered for that study. Day 3 was the isotope infusion study day. A 2-d adaptation period was chosen because Lyons et al. (6) showed that the fractional GSH synthesis rate in blood was 65%, suggesting that the half-life of GSH in RBC was 18 h. In addition, data from Lee et al. (15) showed that the activity of the rate-limiting enzyme for GSH synthesis reached a new steady state in liver within 16 h of changing from a low- to a high-protein diet and that liver GSH and cysteine concentrations reached a new steady state within 12 h. In addition, Jackson et al. (7) found a significant decrease in GSH fractional synthesis rate (FSR) on d 3 after switching healthy participants from their habitual protein intake to the WHO-recommended intake of 0.75 g/d. In the same study, after a 9-d adaptation on the WHO 0.75-g/kg protein intake, the FSR was the same as that measured on d 3 of the diet. The suggestion is that the AA pool had already undergone an adequate adaptation after 2 d. Based on these data, we judged a 48-h adaptation period to be adequate to achieve equilibration of GSH in erthrocytes after each individual change in cysteine intake.

The diet was provided as an experimental formula and protein-free cookies developed for AA kinetic studies (16). Briefly, a liquid formula (protein-free powder, Product 80056, Mead Johnson) flavored with orange and fruit crystals (Tang and Kool-Aid, respectively, Kraft Foods) and protein-free cookies supplied the main source of energy in the diet (Table 2). The nitrogen content of the diet was provided as a crystalline AA mixture (1.0 g·kg⁻¹·d⁻¹) based on the AA pattern of egg protein

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<th>Nutrient, unit/100 g powder</th>
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**TABLE 1** Physical characteristics of the 4 adult men who participated in the study

| Age, y | 20.3 ± 1.5 |
| Weight, kg | 67.8 ± 5.6 |
| Height, m | 1.73 ± 0.1 |
| BMI, kg/m² | 22.7 ± 1.6 |
| Lean body mass, kg | 51.2 ± 5.5 |

1 Values are means ± SD, n = 4.
(17). The reason for using egg protein composition is that most studies on AA requirement have used this pattern. Certainly, all studies from which the total SAA requirement was based for this current study used egg protein. For consistency and to allow comparisons to be drawn, the same AA pattern was chosen (17). The adaptation diet for the 2 d before the tracer infusion study provided energy content for resting energy expenditure (REE) × 1.7, whereas the energy content of the diet on the isotope infusion day, provided as REE × 1.5. REE, was measured by open circuit indirect calorimetry (Deltatrac; SensorMedics, Yorba Linda). The macro-nutrient content of the experimental diet, expressed as a percentage of dietary energy, was 53% carbohydrate, 37% fat, and 10% protein. The diet was weighed (Sartorius Balance model BP110 S; Sartorius) and prepared in the research kitchen at the Hospital for Sick Children. Subjects also consumed a daily multivitamin supplement (Centrum Forte; Whitehall Robins) containing 0.6 mg folic acid, 5 mg vitamin B-6, and 20 μg vitamin B-12 beginning 2 wk before the start of the study and continuing for the entire duration of the 5 studies. In addition, a choline supplement of 500 mg was provided daily beginning 1 wk before the start of the first experiment and continuing for the entire 3-mo study duration. The multivitamin supplement was provided to ensure adequate supply of all cofactors involved in SAA metabolism. The choline supplement was provided because the experimental diet was low in choline (<150–200 mg/d choline), whereas the adequate intake for choline is 550 mg/d for adult male (18). It also provided consistency among studies on SAA metabolism, because previous studies had also given 500 mg/d choline to the subjects (19,20).

During the adaptation days, the diet was provided as 4 equal meals per day to be consumed at the same time each day. Subjects were allowed water in their desired quantity, but caffeinated beverage, alcohol, or any other drinks except that provided by the diet were not allowed. On the isotope infusion day, the diet was provided as 10 isoenergetic, isonitrogenous meals, each representing one-twelfth of the subjects’ total daily requirement. Subjects had free access to water on the study day.

Methionine was provided at an intake of 14 mg·kg⁻¹·d⁻¹, which is a mean of the 2 mean published estimates (using carbon oxidation techniques) for TSAA requirement in adult humans (19,21). We chose this level of methionine because it represented the mean estimated total SAA requirement for protein synthesis as estimated by nitrogen balance (22), indicator AA oxidation technique (21), and 24-h indicator oxidation-balance technique (19). The levels of cysteine studied were 0, 10, 20, 30, and 40 mg·kg⁻¹·d⁻¹. The 0 mg·kg⁻¹·d⁻¹ allowed for the estimation of GSH kinetics at the mean TSAA requirement of 14 mg·kg⁻¹·d⁻¹. The cysteine intake of 10 mg·kg⁻¹·d⁻¹ combined with the methionine intake of 14 mg·kg⁻¹·d⁻¹ provided the total SAA intake of 24 mg·kg⁻¹·d⁻¹, the estimated recommended daily allowance for total SAA requirement (19,21). The cysteine intakes of 20, 30, and 40 mg·kg⁻¹·d⁻¹ provided cysteine in an amount typically consumed in the western diet by individuals consuming a mixed protein diet providing >1.0 g·kg⁻¹·d⁻¹ protein (7,10). These intakes also represented 30, 50, and 65%, respectively, of the amount given in a supplementation study of children with malnutrition (8). The 40 mg·kg⁻¹·d⁻¹ of cysteine was chosen to represent the highest possible level beyond which increases in GSH synthesis would be unlikely in healthy subjects.

Glycine was provided at an intake of 69.5 mg·kg⁻¹·d⁻¹, which is twice that found in the high-quality egg protein at a protein intake of 1 g/kg. This was judged to be adequate to prevent it from being deficient for GSH synthesis. The amount of [¹⁵N–¹³C]glycine provided on the study day was subtracted from the dietary provision to maintain the intake at 69.5 mg·kg⁻¹·d⁻¹. The glycine content of the diet was increased to make glycine not limiting for GSH synthesis. The glycine content of egg protein is 3.8%, whereas the glycine content of human tissue protein is 7.2%. We chose to increase the glycine content to make it more comparable to the composition in human tissue (composition of the AA mixture presented in Table 3).

### Tracer protocol

[U-¹³C₂-¹⁵N]glycine (98% ¹³C₂, 98% ¹⁵N) was purchased from Cambridge Isotope Laboratories. Stock solutions were prepared in 0.9% sodium chloride (10 g/L) by the Research Pharmacy at the Hospital for Sick Children and were confirmed to be sterile and pyrogen free. We chose to use an M+3 glycine tracer as the GSH precursor to determine its incorporation into the GSH molecular ion by liquid chromatography tandem MS. In preliminary studies with M+2 glycine, we could not accurately detect the enrichment in the whole GSH molecule above background because of the high baseline M+2 GSH enrichment.

Each tracer infusion study was conducted on d 3, after completion of the 2-d adaptation on the liquid AA-based diet; subjects arrived at the Clinical Research Centre at the Hospital for Sick Children after a 12-h overnight fast. Ten hourly, isoenergetic, isonitrogenous meals were consumed beginning 3 h before the start of the i.v. isotope infusion. The cysteine content of each meal was dependent on the test level being studied. Because the amount of cysteine in the diet was manipulated, l-alanine was adjusted to keep the nitrogen content of the diet constant.

After consumption of the first 2 meals, i.v. catheters were inserted into superficial veins of both arms, 1 for continuous infusion of the tracer solution and the other for repeated blood sampling. Baseline blood (2.0 mL) was collected after the 3rd hourly meal. At the beginning of the 4th meal, a priming dose of [U-¹³C₂-¹⁵N]glycine (40 μmol/kg) was given over a 15-min period, followed immediately by a continuous infusion of [U-¹³C₂-¹⁵N]glycine (15 μmol·kg⁻¹·h⁻¹) for 7 h. Blood samples (1.5 mL) were taken hourly from the 3rd hour of the infusion until the 5th hour and then every 0.5 h until the end. To ensure arterialized blood, the hand was heated inside a thermostatic chamber maintained at 60°C for ≥15 min before the blood was sampled (23).

Urine samples were collected after each void for the 10 h of the study day. Samples were pooled and 2 × 2-mL aliquots were stored at −20°C for urinary sulfate analysis to relate changes in cysteine intake to sulfate excretion.

### Sample analysis

Blood (0.5 mL) for hematocrit was collect into tubes containing Na₂EDTA and immediately sent to the clinical biochemistry laboratory at the Hospital for Sick Children for analysis. Briefly, the red cells were analyzed using an Abbott CELL-DYN Sapphire Hematology analyzer. Hematocrit was then calculated using the formula HCT (L/L) = (RBC × MCV)/1000, where HCT is hematocrit and MCV is mean corpuscular volume.
Erythrocyte GSH concentration and enrichment

All chemicals were purchased from Sigma-Aldrich Canada. A 0.5-mL aliquot of each blood sample collected in Na2EDTA was centrifuged for 2 min at 13,000 x g (Beckman Microfuge-TM 11, Beckman Coulter Canada) within 5 min of collection. Each tube was weighed before and after blood collection to determine the amount of blood, because GSH concentration was normalized to hematocrit. After centrifugation, the plasma was immediately removed. Two hundred microliters of 100 mM ethylmalonate and 20 mL of 5 mM y-glutamyl-leucine (internal standard) were added to the separated RBC. The sample was then capped, mixed with a vortex mixer, and left for 10 min at room temperature. Cells were then lysed with 30 mL 0.4 mol/L ZnSO4 and the protein precipitated with 1 mL ice-cold methanol. The sample was then vortexed, centrifuged at 13,000 x g for 2 min, the supernatant removed and stored at -80°C until analysis.

GSH concentration and enrichment were analyzed using a triple quadrupole mass spectrometer API 4000 (Applied Biosystems/MDS SCIEX) operated in positive ionization mode with the Turbo Ion Spray ionization probe source (operated at 5.8 kV). This was coupled to an Agilent 1100 HPLC system. All aspects of system operation and data acquisition were controlled using The Analyst NT v 1.4.1 software. GSH concentration was measured using an external standard curve and the ratio of the analyte (GSH) to the internal standard (y-glutamyl-leucine). The parent-to-daughter transitions measured for GSH and the internal standard were 433.4 to 304.3 and 261.4 to 132.0, respectively. GSH enrichment was determined in the whole GSH molecule (as the tripeptide). There was no fractionation and hydrolysis step prior to measurement of enrichment. GSH enrichment was calculated as a ratio of the (enriched) M+3 to (unenriched) M peaks of the tripeptide molecule of GSH by measuring the transition of parent to daughter ions of 436.4 to 307.0 (M+3) and 433.4 to 304.3 (M+0) and was expressed as mole percent excess calculated from peak area ratios at the isotopic steady state of erythrocyte glycine enrichment in the last 2 h of the isotopic infusion. Interassay precision for GSH concentration was between 2.3 and 4.8%, whereas interassay precision for GSH enrichment was 3.7 ± 7.3%. The accuracy of the instrument for GSH concentration was measured by spiking samples with a known amount of GSH and comparing to the unspiked sample. Concentrations were determined using a standard curve. The accuracy of the GSH concentration was between 90 and 108% of that expected. Accuracy for GSH enrichment was measured using enrichment curves, which were linear within the ranges of expected sample enrichment. The results of the enrichment curve was y = 0.94x + 0.097; r² = 0.99.

Erythrocyte-free glycine enrichment

Each sample was collected and centrifuged as above. Plasma was quickly removed and the cells washed twice with 300 mL ice-cold saline on each occasion. Samples were vortexed between each wash. Cells were then lysed and deproteinated as above, vortexed, centrifuged for 2 min at 13,000 x g and the supernatant stored at -80°C until analysis.

Fifty microliters of each sample was then dried under nitrogen at 35°C. One hundred microliters of butanol-HCl (Sigma-Aldrich Canada) was then added and the sample vortexed, topped with nitrogen, and heated for 20 min at 55°C. The sample was again dried under nitrogen, reconstituted in 0.1% formic acid (Sigma-Aldrich Canada). Samples were then analyzed using a triple quadrupole mass analyzer as described above. Glycine enrichment was calculated as the ratio of the (enriched) M+3 to (unenriched) M peaks of glycine after derivatization with butanol-HCL. The masses of the parent to daughter transitions of butylated glycine monitored were 135.2 to 79.0 (M+3) and 132.2 to 76.0 (M).

The intra-assay precision of the triple quadrupole mass analyzer for measurements of erythrocyte-free glycine enrichment was between 3 and 5%. Accuracy for erythrocyte-free glycine enrichment was measured using enrichment curves. Enrichment curves were linear within the ranges of expected sample enrichment. The result of the enrichment curve was y = 0.732x + 0.042; r² = 0.99 for glycine enrichment.

Urinary sulfate

Urinary sulfate was measured using the method of Swaroop (24). Briefly, a standard curve was made using known concentrations of Ba2+ with sodium rhodizinate to form a red-colored complex that was measured at 520 nm against water. A known amount of sulfate was then added to form a BaSO4 precipitate, which resulted in a diminished color and absorbance. The standard curve was obtained by plotting the concentration of sulfate on the x-axis and differences in absorbance between blank (water) and the corresponding standard on the y-axis. One mL of each urine sample was then diluted to 200 mL with distilled water from which 0.05 mL was removed and 2.0 mL of ethanol added and vortexed. To each tube, BaCl2 and sodium rhodizate were added and the tubes vortexed. The tubes were then allowed to stand for 10 min in the dark, after which the red color produced was measured at 520 nm against water. The difference of absorbance between blank and sample was read on the graph and corrected for the dilution.

Calculations

Fractional synthesis rate of erythrocyte GSH. The FSR of erythrocyte GSH (FSR_GSH) was calculated using the precursor-product method of Jahoore et al. (25):

\[ \text{FSR}_{\text{GSH}} (\% / \text{d}) = \left( \frac{E_7 - E_5}{E_{\text{ERBC}}} \right) \times \left( \frac{24 \times 100}{(t_7 - t_5)} \right) \]

where \((E_7 - E_5)\) is the increase in the isotopic enrichment of erythrocyte GSH between the 5th and 7th h of infusion as a result of the incorporation of the labeled glycine; \(E_{\text{ERBC}}\) was the intracellular glycine enrichment at isotopic steady state; and \((t_7 - t_5)\) is the time interval between the 5th and 7th h, when the incorporation of glycine into GSH was measured:

\[
\text{ASR} = \text{GSH}_{\text{mass}} \times \text{FSR}_{\text{GSH}}
\]

where \(\text{GSH}_{\text{mass}}\) is the amount of the product cell (or cell number or cell protein) and the concentration of GSH in the cell. Hematocrit was calculated using the formula HCT (L/L) = (RBC x MCV)/1000.

Statistical analysis

The data were analyzed by repeated-measures ANOVA with the PROC MIXED procedure to assess the effects of cysteine intake on GSH FSR, ASR, and concentration. Other independent variables tested were cysteine intake, subject and order of cysteine intake, as well as the interaction between cysteine intake and order.

Repeated-measures ANOVA with the PROC MIXED and PROC general linear model procedures was also used to assess the effect of cysteine intake on urinary sulfate excretion.

When significant differences were identified, individual differences were assessed by post hoc analysis with Bonferroni correction for multiple comparisons. Differences were considered significant at \(P < 0.05\). Data were analyzed using SAS version 9.1 for Windows (SAS Institute).

Results

The age and physical characteristics of the 4 healthy, male subjects who participated in the study are presented in Table 1. Isotopic steady state was achieved in the erythrocyte-free intracellular glycine pool by 5 h after the start of the isotope infusion. This was determined by the absence of a significant slope between data points from 5 to 7 h using ANOVA (Fig. 1). Therefore, FSR was calculated based on the linear incorporation of glycine into GSH during the last 2 h of the infusion. Cysteine intake did not affect erythrocyte GSH concentration, FSR, or ASR (Fig. 2) (Table 4).

Urinary sulfate excretion normalized to creatinine excretion (Fig. 3) increased with increasing cysteine intake (\(P < 0.01\); \(r^2 = 0.92\)). Order of cysteine intake did not affect urinary sulfate excretion. Also, absolute urinary sulfate excretion (data not shown) increased with increasing cysteine intake (\(P < 0.01\); \(r^2 = 0.95\)). Order of cysteine intake did not affect absolute urinary sulfate excretion.
Discussion

This is the first study, to our knowledge, to report on the erythrocyte GSH kinetics in healthy adult male in response to varying cysteine intake levels in the presence of adequate protein and energy intakes and the mean methionine requirement of 14 mg·kg⁻¹·d⁻¹. These results suggest that in the presence of an adequate protein intake of 1 g·kg⁻¹·d⁻¹ and the mean methionine requirement of 14 mg·kg⁻¹·d⁻¹ (19,21), further increases in the SAA intake in the form of cysteine did not affect erythrocyte GSH metabolism (Fig. 2). The GSH synthesis rates and concentrations in the current study were similar to those in previous studies of GSH kinetics in healthy adults (6,7).

The inability to measure GSH kinetics in liver or muscle due to practical and ethical considerations is a potential limitation of in vivo studies with healthy subjects. However, erythrocyte GSH kinetics has been shown to respond to dietary changes in disease (10,11), malnutrition (8), and even to small decreases in protein intake (7), demonstrating that erythrocyte GSH is a sensitive pool from which to detect changes in GSH metabolism. At a protein intake of 0.75 g·kg⁻¹·d⁻¹, set by WHO as the safe intake (26), Jackson et al. (7) showed decreased erythrocyte GHS synthesis when compared with a habitual protein intake of 1.13 g·kg⁻¹·d⁻¹. In fact, this higher protein requirement was recently confirmed by our group, showing that a safe intake of protein is closer to 1 g·kg⁻¹·d⁻¹ (27). In addition, cysteine supplementation at only 15 mg·kg⁻¹·d⁻¹ produced significantly increased GSH synthesis in symptom-free HIV individuals (10), suggesting that at the cysteine intakes used in the current study, significant changes in GSH metabolism should have been observed had they occurred.

Although we did not observe changes in GSH metabolism in response to feeding graded intakes of cysteine to healthy adults receiving the previously derived mean methionine (TSA) requirement (Fig. 2), we observed a significant linear increase in urinary sulfate production in response to graded cysteine intakes (Fig. 3). The precise mechanisms governing all aspects of SAA metabolism are not yet completely understood. However, the increases in urinary sulfate excretion in the current study can be partly explained by work conducted by Stipanuk et al (13). Because excess cysteine is considered toxic, the liver regulates cysteine concentration within a small range and maintains a plasma concentration within a 2.5-fold range (13). Cysteine concentration has been found to be the key regulator of its own metabolism (4,5,15,28–30). When protein and/or SAA intake is low, γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis is upregulated, resulting in a greater partitioning of SAA toward GSH synthesis. On the other hand, when protein and/or SAA intake is increased, cysteine dioxygenase the enzyme that catalyzes cysteine to sulfate and taurine, is upregulated, resulting in greater partitioning of cysteine toward sulfate production. Thus, increasing urinary sulfate observed in the

![FIGURE 1](https://academic.oup.com/jn/article/138/11/2172/4670065)
present study appears to be due to increased partitioning of dietary cysteine toward catabolism in response to graded intakes of cysteine.

However, a closer look at the pattern of the isotope results reveals a similar rate of erythrocyte GSH synthesis at cysteine intakes of 0, 20, 30, and 40 mg kg\(^{-1}\) d\(^{-1}\), with an almost 45% increase from 0 at a cysteine intake of 10 mg kg\(^{-1}\) d\(^{-1}\). This increase in GSH synthesis, although not significant (P = 0.49), may be of biological importance, especially because other investigators have shown significant results at lower changes in synthesis rates (6,7). The observed similar GSH synthesis rates at cysteine intakes of 20 mg kg\(^{-1}\) d\(^{-1}\) and above to that observed at a 0 cysteine intake suggest a return to baseline at the higher cysteine intakes. This is supported by previous data that show that increasing levels of protein (soy and casein), as well as the SAA are increased, in the diets of rats or the addition of SAA to the culture medium of primary rat hepatocyte results in increased cysteine dioxygenase and decreased SAA activity (5,28,29). In healthy humans, a deficient protein and or SAA intake has been shown to significantly decrease GSH synthesis (5–15 mg kg\(^{-1}\) d\(^{-1}\)) in addition to a methionine intake of 14 mg kg\(^{-1}\) d\(^{-1}\).

This is the first study, to our knowledge, to report on GSH kinetics in response to varying cysteine intakes in healthy adult men. These data provide a point of focus for the design of future experiments on adequate SAA requirement for the maintenance of whole-body antioxidant status. These data also provide preliminary evidence that the currently derived TSAA requirement for healthy adults, when provided in the presence of an adequate protein intake (27), does not limit GSH synthesis in that population. Nevertheless, the need for TSAA may be higher in disease states where there is increased oxidative stress. Studies in rats have suggested an increase in cysteine requirement in septic rats compared with controls as evidenced by an increase in methionine transsulfuration and an increase in both methionine and cysteine flux compared with controls (31). A later study showing increased GSH synthesis in septic compared with control rats was used as a possible explanation for the increased cysteine requirement in sepsis (32). In humans with HIV and malnutrition, decreased GSH metabolism was ameliorated by cysteine supplementation, which served to increase GSH synthesis and concentration to that of control subjects. However, our current results show that typical cysteine intake in not rate limiting for GSH synthesis in healthy adult men, but rather that GSH synthesis is maximized at protein and TSAA intakes equivalent to those required for adequate protein synthesis in healthy adult subjects.

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**Literature Cited**


