Dietary cysteine reduces the methionine requirement in men[1–4]

Marco Di Buono, Linda J Wykes, Ronald O Ball, and Paul B Pencharz

ABSTRACT

Background: Despite early evidence suggesting that dietary cysteine has a sparing effect on methionine requirements, some recent reports question the existence of a measurable sparing capacity.

Objective: The goal of the present study was to determine whether dietary cysteine could reduce the requirement for methionine in men consuming diets with and without cysteine.

Design: Six men were randomly assigned to receive graded intakes of methionine while fed a diet containing either no exogenous cysteine or an excess of cysteine (21 $mg \cdot kg^{-1} \cdot d^{-1}$). The methionine requirement was determined by measuring the oxidation of L-[1-^{13}C]phenylalanine to $^{13}CO_2$ and estimated by using a linear regression crossover analysis.

Results: The mean and population-safe (upper limit of the 95% CI) methionine requirements in the absence of exogenous cysteine were found to be 12.6 and 21 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. The mean and population-safe methionine requirements in the presence of excess dietary cysteine were found to be 4.5 and 10.1 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively, representing a cysteine sparing effect of 64% in a comparison of mean methionine requirements and of 52% in a comparison of population-safe methionine intakes. Furthermore, the difference between population-safe intakes with and without dietary cysteine establishes a safe cysteine intake of 10.9 $mg \cdot kg^{-1} \cdot d^{-1}$ in the presence of adequate methionine intakes.

Conclusion: Our data suggest that dietary cysteine can reduce the exogenous requirement for methionine in men. These results strongly support the existence of a cysteine sparing effect in humans. *Am J Clin Nutr* 2001;74:761–6.

KEY WORDS Sulfur amino acid, indicator amino acid oxidation, amino acid requirement, stable isotopes, methionine, phenylalanine, cysteine sparing, men

INTRODUCTION

The current FAO/WHO/UNU population-safe intake of total sulfur amino acids (SAAs) in healthy adults, based on early nitrogen balance studies (1–3), is 13 $mg \cdot kg^{-1} \cdot d^{-1}$ (4). We previously reported that this value is 60% lower than the population-safe intake found in a study of men by indicator amino acid oxidation (IAAO) (5). Using L-[1-^{13}C]phenylalanine as an indicator, we found that the mean methionine requirement of 6 men in the absence of dietary cysteine was 12.6 $mg \cdot kg^{-1} \cdot d^{-1}$ and the upper limit of the 95% CI of this mean, which is an estimate of the population-safe intake, was 21 $mg \cdot kg^{-1} \cdot d^{-1}$ (5). Recent studies of

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SAA kinetics in humans confirmed, with the use of labeled methionine tracers (6, 7), that the current FAO/WHO/UNU recommendations for total SAA intake (4) are too low. In addition, using individual data provided in the early nitrogen balance study by Rose et al (1), we recalculated the mean and population-safe intake of total SAAs to be 13.2 and 18 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. Both of these recalculated values are similar to those found in our previous IAAO study (5) and further confirm that the population-safe intake of SAA is greater than the published FAO/WHO/UNU value of 13 $mg \cdot kg^{-1} \cdot d^{-1}$ (4).

The ability of cysteine to reduce the quantitative requirement for methionine in humans was reported in early studies (2, 8–10). In contrast, a more recent series of reports on methionine kinetics using methionine and cysteine tracers suggests that cysteine has no sparing effect on methionine requirements in humans (7, 11–15). However, the failure to detect a sparing effect in these recent experiments may have resulted from the investigators unknowingly supplying inadequate dietary SAA intakes. The test diets adopted in these kinetic studies were based on the FAO/WHO/UNU estimates, which we (5) and others (6, 7) maintain are too low. To detect a cysteine sparing effect on methionine requirements, cysteine must be present in amounts adequate to completely, or largely, arrest the flow of metabolites through the transsulfuration pathway, whereas methionine must be present in amounts adequate to meet all its other metabolic functions, includ-

[1] From the Departments of Nutritional Sciences and Paediatrics, the University of Toronto; The Research Institute, The Hospital for Sick Children, Toronto; the School of Dietetics & Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Canada; and the Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.

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[4] Address reprint requests to PB Pencharz, Division of Gastroenterology/Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. E-mail: paul.pencharz@sickkids.ca.

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TABLE 1
Subject characteristics and energy intakes

Subject	Age	Weight	BMI	Energy intake[1]
	y	*kg*	*kg/m²*	*MJ/d*
1	21	63.5	22.2	13.8
2	26	93.0	29.9	14.4
3	24	81.7	27.0	12.3
4	29	82.5	25.6	12.5
5	27	85.0	28.9	14.2
6	31	85.5	24.5	12.6
$\bar{x}$ ± SD	26.3 ± 3.6	81.9 ± 9.8	26.4 ± 2.9	13.3 ± 0.9

[1] Calculated from the resting metabolic rate, as determined by indirect calorimetry, multiplied by an activity factor of 1.7.

ing protein synthesis, transmethylation, and the provision of homocysteine for remethylation reactions necessary for folate and betaine metabolism. Unless the total SAA needs of all subjects are met, addition of cysteine to the diet will lead to an immeasurably small sparing effect on methionine requirements (13, 14).

We hypothesized that a sparing effect of cysteine on methionine requirements would be observed if the total SAA content of the diet was ≥21 mg·kg^{-1}·d^{-1}, our current estimated safe SAA intake (5). Thus, we used IAAO, with L-[1-^{13}C]phenylalanine in the presence of excess tyrosine as an indicator, to assess the methionine requirements of men fed a diet containing 21 mg cysteine·kg^{-1}·d^{-1}.

SUBJECTS AND METHODS

Subjects

Six healthy men participated in the study on an outpatient basis at the Clinical Investigation Unit at The Hospital for Sick Children, Toronto. Subject characteristics are described in **Table 1**. None of the subjects had a history of recent weight loss, unusual dietary practices, or endocrine disorders, and none were using medication at the time of entry in the study. The design and aims of the study and the potential risks involved were fully explained to each subject and informed, written consent was obtained. All procedures were approved by the Ethics Review Board of The Hospital for Sick Children. Subjects received financial compensation for their participation.

Experimental design

The study design was based on the noninvasive IAAO model of Bross et al (16). Each subject randomly received each of 6 dietary methionine intakes: 0, 2.5, 5.0, 7.5, 10.0, and 13.0 mg·kg^{-1}·d^{-1}. Dietary cysteine was held constant at an intake of 21 mg·kg^{-1}·d^{-1}. Each study consisted of a 2-d adaptation period to a prescribed diet (17). The diet provided 1.0 g protein·kg^{-1}·d^{-1} and was followed by a single study day on which phenylalanine kinetics were measured with the use of L-[1-^{13}C]phenylalanine at 1 of the 6 dietary methionine intakes and a protein intake of 1.0 g·kg^{-1}·d^{-1}. The study dietary periods were separated by ≥1 wk; all subjects completed all 6 studies within 3 mo.

Dietary and energy intakes

Dietary intakes during the 2-d adaptation period were provided in the form of milk shakes (Scandishake; Scandipharm, Birmingham, AL), which were weighed in daily portions for each subject and supplemented with additional protein (Promod; Ross Laboratories, Columbus, OH) and energy (Caloreen; Nestlé Clinical Nutrition, North York, Canada), depending on each subject's requirements. Subjects were instructed to add a predetermined volume of homogenized milk containing 3.25% fat to their daily portion and to drink the milk shake at regular meal times throughout the day. Energy intakes were based on each subject's resting metabolic rate, as determined by indirect calorimetry (2900 Energy Measurement System-Paramagnetic; Sensormedics, Yorba Linda, CA), multiplied by an activity factor of 1.7 (Table 1). Subjects also consumed a daily multivitamin supplement (Centrum; Whitehall-Robins Inc, Mississauga, Canada) containing 0.4 mg folic acid, 3 mg vitamin B-6, and 9 μg vitamin B-12 for the entire duration of the 6 studies. The daily choline content of the diet was ≈14 μg/kJ (60 μg/kcal; average intake: 215 ± 25 mg/d). No other food or beverages, including diet products containing artificial sweeteners, were consumed during the adaptation period.

On each of the 6 study days, the diet was provided as an experimental formula developed for amino acid kinetic studies (18). Briefly, a liquid formula (protein-free powder, product 80056; Mead Johnson, Evansville, IN) flavored with orange and fruit crystals (Tang and Kool-Aid, respectively; Kraft Foods Canada, Toronto) and protein-free cookies supplied the main source of energy in the diet. A crystalline amino acid mixture (1.0 g·kg^{-1}·d^{-1}), based on the amino acid composition of egg protein, provided the only source of amino nitrogen in the diet. The macronutrient composition of the experimental diet, expressed as a percentage of dietary energy, was 55% carbohydrate, 35% fat, and 10% protein. The diets were prepared and weighed in the metabolic kitchen and were portioned into isoenergetic, isonitrogenous meals. The diet was consumed as hourly meals; each meal represented one-twelfth of the subjects' total daily protein and energy requirements. Subjects had free access to water throughout the study day. The amount of L-[1-^{13}C] phenylalanine given during the study day was subtracted from the dietary provision of phenylalanine such that the total phenylalanine intake was 14 mg·kg^{-1}·d^{-1}; 40 mg tyrosine·kg^{-1}·d^{-1} was supplied to ensure an excess.

Tracer protocol

$NaH^{13}CO_3$ (99 atom%) and L-[1-^{13}C]phenylalanine (99 atom%) were used as tracers (Mass Trace, Woburn, MA). Isotope solutions were prepared in deionized water and stored at −20°C. Oral priming doses of $NaH^{13}CO_3$ (0.176 mg/kg) and L-[1-^{13}C] phenylalanine (0.664 mg/kg) were given with the fifth hourly meal. An hourly oral dosing protocol of L-[1-^{13}C]phenylalanine (1.2 mg·kg^{-1}·d^{-1}) was commenced simultaneously and continued throughout the remaining 6 h of the study.

Sample collection and analysis

We previously showed that plasma amino acid enrichments can be determined from urine (16, 19, 20). Baseline samples of breath carbon dioxide and urine were collected 60, 45, and 30 min before the isotope protocol began. A background isotopic steady state was achieved in all subjects within 4 h of the start of feeding. Breath carbon dioxide and urine samples were also collected at 30-min intervals during isotopic steady state, 120–320 min after isotope administration began, and were stored at −20°C. Breath samples were collected in disposable Haldane-Priestley tubes (Venoject; Terumo Medical Corp, Elkton, MD) with use of a collection mechanism that permits the

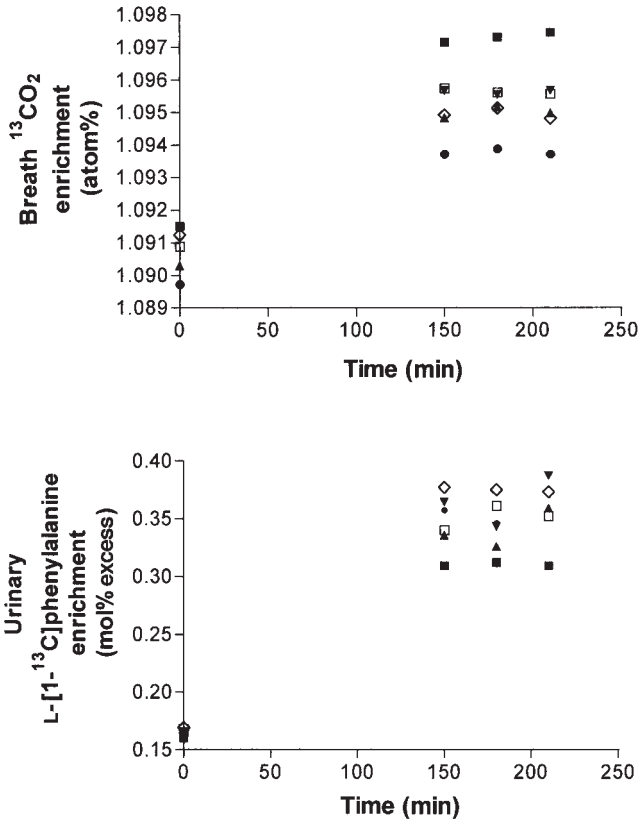

FIGURE 1. Effect of methionine intakes of 0 (■), 2.5 (▲), 5.0 (▼), 7.5 (◇), 10 (●), and 13 (□) mg·kg^{-1}·d^{-1} on breath $^{13}CO_2$ enrichment and urinary L-[1-^{13}C]phenylalanine enrichment in a typical subject. Establishment of a plateau in breath and urine samples, on the basis of no significant differences among timed samples, was confirmed with the use of repeated-measures ANOVA.

removal of dead-space air (21). Samples were stored at room temperature until analyzed. Carbon dioxide production was measured during each study day for 30 min with a variable-flow indirect calorimeter (2900 Energy Measurement System-Paramagnetic; Sensormedics).

The enrichment of ^{13}C in breath carbon dioxide was measured on a continuous-flow isotope ratio mass spectrometer (PDZ Europa Ltd, Cheshire, United Kingdom). Breath $^{13}CO_2$ enrichments were expressed as atom% over a reference standard of compressed carbon dioxide gas. Urinary L-[1-^{13}C]phenylalanine enrichment was measured by gas chromatography (selected ion monitoring-negative chemical ionization)–mass spectrometry (model 5890 gas chromatograph and model 5988A mass spectrometer; Hewlett-Packard, Mississauga, Canada). Urine samples (500 μL) were deproteinized and acidified with 250 μL of 400 g trichloroacetic acid/L and centrifuged at 7000 × *g* at room temperature for 5 min. Amino acids were separated from the supernatant fluid with a cation-exchange resin (Dowex 50W-X8, 100–200 mesh H^+; Bio-Rad Laboratories, Richmond, CA) and were derivatized according to the method of Patterson et al (22) to their *N*-heptafluorobutyramide *n*-propyl esters. Selected-ion chromatograms were obtained by monitoring mass-to-charge ratios of 383 and 384 for L-[1-^{13}C]phenylalanine corresponding to the unenriched (*m*) and enriched (*m*+1) peaks, respectively. The areas under the peaks were integrated with use of Hewlett-Packard G1034C MS-CHEM software (α Omega Technologies, Brielle, NJ). Isotopic enrichment was expressed as mol% excess.

Isotope kinetics

Phenylalanine kinetics were calculated according to the stochastic model of Matthews et al (23), as previously used by Zello et al (24). Isotopic steady state in the metabolic pool was represented by plateau in free L-[1-^{13}C]phenylalanine in urine and $^{13}CO_2$ in breath. The difference between mean breath $^{13}CO_2$ enrichments of the 3 baseline and 7 plateau samples was used to determine atoms percent excess above baseline at isotopic steady state.

Phenylalanine flux (μmol·kg^{-1}·h^{-1}) was measured during isotopic steady state from the dilution of the L-[1-^{13}C]phenylalanine infused into the urine metabolic pool. The rate of $^{13}CO_2$ release from L-[1-^{13}C]phenylalanine oxidation ($F^{13}CO_2$, in μmol $^{13}CO_2$·kg^{-1}·h^{-1}) was calculated according to the method of Matthews et al (23) from $^{13}CO_2$ expiration by using a factor of 0.82 to account for the $^{13}CO_2$ retained in the body as a result of bicarbonate fixation (25). The rate of phenylalanine oxidation (μmol·kg^{-1}·h^{-1}) was calculated from $F^{13}CO_2$ and urinary free phenylalanine enrichment (23).

Statistical analysis

Repeated-measures analysis of variance was performed on primary and derived variables to assess the effects of methionine intake, of the order in which the test intakes were administered, of subject, and of interactions (SAS, version 6.6; SAS Institute Inc, Cary, NC). The significance of differences between intakes was examined by using Tukey's post hoc test. In all cases, results were considered to be statistically significant at $P < 0.05$.

Estimates of the mean and population-safe methionine intakes for men were derived by breakpoint analysis with a 2-phase linear regression crossover model similar to that described previously (24). This model minimizes the residual SE in a stepwise partitioning of data points between 2 regression lines. The 95% CI for the mean total SAA requirement was calculated by using Fieller's theorem; the upper limit of the 95% CI was used to represent the population-safe intake of total SAAs. The requirement for individual subjects was determined by visual inspection.

RESULTS

Mean (±SD) baseline L-[1-^{13}C]phenylalanine enrichments were not significantly different between study days, illustrating no significant carryover of isotope between studies. As shown for a typical subject in **Figure 1**, a plateau in breath $^{13}CO_2$ and urinary L-[1-^{13}C]phenylalanine enrichment occurred within 120 min of isotope administration. Establishment of plateau in breath and urine samples, based on a lack of significant difference among timed samples, was confirmed with the use of repeated-measures analysis of variance. Mean L-[1-^{13}C]phenylalanine flux and oxidation data at the 6 dietary methionine intakes are shown in **Table 2**. The effect of dietary methionine intake on $F^{13}CO_2$ with dietary cysteine is shown in **Figure 2**.

Linear regression crossover analysis resulted in the identification of a mean methionine requirement, in the presence of exogenous cysteine, of 4.5 mg·kg^{-1}·d^{-1} and a population-safe

TABLE 2
Effect of methionine intake on phenylalanine flux and oxidation as measured by the rate of $^{13}CO_2$ release[1]

Methionine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)	Phenylalanine flux	Phenylalanine oxidation
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	
0	26.4 ± 6.9	2.3 ± 0.6[a]
2.5	27.0 ± 7.7	2.0 ± 0.5[b]
5.0	26.4 ± 7.0	1.9 ± 0.8[b]
7.5	24.7 ± 8.8	1.7 ± 0.6[b]
10.0	25.0 ± 5.8	1.9 ± 0.9[b]
13.0	24.1 ± 6.7	1.6 ± 0.8[b]

[1] Methionine had no significant effect on phenylalanine flux but did have a significant effect on oxidation, $P = 0.016$. Flux and oxidation rates were significantly different among individuals, $P = 0.01$. Means within columns with different superscript letters are significantly different, $P = 0.05$.

intake of 10.1 $mg \cdot kg^{-1} \cdot d^{-1}$. The range of breakpoint estimates determined by visual inspection of individual data was 2.5–10 $mg \cdot kg^{-1} \cdot d^{-1}$. The mean and population-safe methionine requirements in the absence of dietary cysteine were previously found to be 12.6 and 21 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively (5). The present results, therefore, represent a cysteine sparing effect of 64% in a comparison of mean requirements and of 52% in a comparison of population-safe intakes. The difference between the population-safe intake with and without dietary cysteine also establishes a population-safe cysteine intake of 10.9 $mg \cdot kg^{-1} \cdot d^{-1}$.

DISCUSSION

The evidence in support of cysteine having a sparing effect on methionine requirements in humans and animals is substantial. As early as 1941, Womack and Rose (8) showed a 17% sparing effect of cysteine on methionine requirements in rats when growth rates were used as an indicator. Shortly thereafter, a series of nitrogen balance studies in men and women showed a sparing effect ranging from 48% to 89% (2, 9, 10). In addition to these studies, subsequent studies showed that dietary cysteine suppresses transsulfuration in rats (26, 27) and humans (11), thus providing a metabolic basis for the sparing effect of cysteine on methionine requirements.

Finkelstein and Mudd (26) showed that cystine feeding in rats diminishes the liver content of cystathionine β-synthase (CBS). These results were confirmed in a later study in which the authors showed that, although the net flow of metabolites through the betaine–homocysteine *S*-methyltransferase (BHMT) enzyme did not change with cystine feeding, there was a significant increase in hepatic BHMT, resulting in increased utilization of homocysteine by BHMT (27). Finkelstein et al (27) concluded that BHMT may play an important role in the sparing effect of cysteine. Using L-[1-^{13}C; methyl-$^{2}H_3$]methionine, Storch et al (11) showed a 50% reduction in transsulfuration in 8 adult men fed a diet devoid of methionine and containing cysteine.

The available evidence indicates that the sparing effect of cysteine is based on a repartitioning of homocysteine between competing pathways. Although no change appears to occur in the rate of homocysteine remethylation to methionine by either of the available remethylation pathways (11, 26, 27), there is a clear reduction in the rate of transsulfuration. The net result is that the fractional remethylation of homocysteine increases while that portion metabolized by transsulfuration decreases. The most likely explanation for this response is a reduction in CBS synthesis by dietary cysteine. Finkelstein and Mudd (26) found a modest inhibition of CBS by cystine in vitro but found no dissociable inhibitor to account for an irreversible reaction between cystine and CBS. This finding suggested that a change in tissue CBS content underlies the effect of dietary cystine. Evidence from cell culture experiments of human CBS supports the notion of transcriptional regulation of CBS (28). The short half-life of the major CBS isoforms (29, 30) suggests that dietary cysteine could lead to changes in CBS messenger RNA transcription and, therefore, in activity, in the time frames of the experiments conducted by Finkelstein and Mudd (26), Finkelstein et al (27), and Storch et al (11).

In the current experiment we found a mean methionine requirement of 4.5 $mg \cdot kg^{-1} \cdot d^{-1}$ and a population-safe intake of 10.1 $mg \cdot kg^{-1} \cdot d^{-1}$ when cysteine was fed at an excess of 21 $mg \cdot kg^{-1} \cdot d^{-1}$. In a recent IAAO study (5), we determined that the mean requirement and population-safe intake of total SAAs in men fed a diet devoid of cysteine were 12.6 and 21 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. The mean methionine requirement in the presence of dietary cysteine in the present study is 64% lower than the mean methionine requirement in the absence of exogenous cysteine found in our earlier study (5). In addition, the population-safe methionine intake in the presence of dietary cysteine in the present study is 52% lower than that found in the absence of dietary cysteine in our earlier study (5). These reductions in the mean and population-safe intakes represent a sparing effect of cysteine on methionine requirements. Both values, 64% and 52%, fall well within the observed range of cysteine sparing in humans found previously (2, 8–10). We argue that the 64% sparing effect on the mean requirement is quantitatively more important because the mean requirement is more representative of a metabolic index than is the population-safe intake. The population-safe intake is a value derived on the basis of experimental variation, unlike the mean requirement, which is a measured value. Nonetheless, both sparing estimates are consistent with the extent of reduced transsulfuration described by Finkelstein and Mudd (26, 27). This further supports the extent of cysteine sparing observed in the current study.

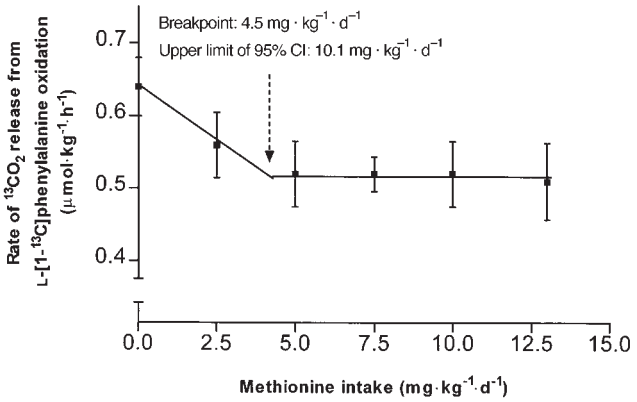

FIGURE 2. Mean (±SD) rate of $^{13}CO_2$ release from L-[1-^{13}C]phenylalanine oxidation after methionine intakes of 0, 2.5, 5.0, 7.5, 10, and 13 $mg \cdot kg^{-1} \cdot d^{-1}$ in 6 subjects ($n = 36$ observations). The dashed arrow indicates the mean total sulfur amino acid requirement in the presence of excess dietary cysteine (4.5 $mg \cdot kg^{-1} \cdot d^{-1}$).

TABLE 3
Summary of studies with no observed cysteine sparing effect

Reference	Subjects	Diet		Observation
		Methionine	Cysteine	
7	11 Adults	No methionine or cysteine		No cysteine sparing
11	8 Men	2.4%[1] 0% 0%	0% 0% 2%[2]	Addition of cysteine to a diet free of sulfur amino acids results in a nonsignificant 50% reduction in transsulfuration
12	8 Men	13 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1}	0 mg·kg^{-1}·d^{-1} 0 mg·kg^{-1}·d^{-1} 5.2 mg·kg^{-1}·d^{-1} 10.5 mg·kg^{-1}·d^{-1} 20.9 mg·kg^{-1}·d^{-1}	No observed sparing; no reduction in transsulfuration
13	6 Men, 2 women	13 mg·kg^{-1}·d^{-1} 5 mg·kg^{-1}·d^{-1} 5 mg·kg^{-1}·d^{-1}	0 mg·kg^{-1}·d^{-1} 0 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1}	No observed sparing; no reduction in transsulfuration
14	Elderly adults	13 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1}	0 mg·kg^{-1}·d^{-1} 5.2 mg·kg^{-1}·d^{-1} 20.9 mg·kg^{-1}·d^{-1}	Modest, nonsignificant reduction in transsulfuration
15	12 Adults	13 mg·kg^{-1}·d^{-1} 5 mg·kg^{-1}·d^{-1} 5 mg·kg^{-1}·d^{-1}	0 mg·kg^{-1}·d^{-1} 0 mg·kg^{-1}·d^{-1} 6.5 mg·kg^{-1}·d^{-1}	No observed sparing

[1] Methionine content (by wt) of an L-amino acid mixture.
[2] Cysteine content (by wt) of an L-amino acid mixture.

To observe a sparing effect of cysteine on methionine requirements, it is essential that subjects receive an adequate supply of total SAAs. More specifically, subjects must be receiving an adequate supply of methionine such that all functions of methionine, including protein synthesis and transmethylation, are satisfied, and subjects must be receiving an adequate amount of cysteine to meet the body's needs for cysteine and to reduce transsulfuration. To ensure adequate provision of cysteine while trying to identify a sparing effect in the present study, we fed an excess of 21 mg dietary cysteine·kg^{-1}·d^{-1} and subsequently defined a population-safe methionine intake of 10 mg·kg^{-1}·d^{-1}. Our earlier results suggest that the population-safe intake of total SAAs is 21 mg·kg^{-1}·d^{-1} (5). Therefore, the population-safe cysteine intake, defined as the difference between the 2 population-safe methionine intakes shown above, is 10.9 mg·kg^{-1}·d^{-1}. Thus, the inference is that a minimum of 10.9 mg dietary cysteine·kg^{-1}·d^{-1} is required to meet the body's needs for cysteine and to reduce transsulfuration.

Several recent reports suggest that cysteine cannot reduce the exogenous requirement for methionine in humans (7, 11–15). On closer examination of the test diets adopted in these studies (**Table 3**), it appears that subjects may not have consumed enough methionine or cysteine to observe a cysteine sparing effect on methionine requirements. These studies based the dietary SAA intakes they used on the FAO/WHO/UNU (4) population-safe intake, which was based on the only direct measurement of SAA requirements at the time but that we recently showed to be underestimated by 60% (5). A study by Hiramatsu et al (12) studied an amount of dietary cysteine similar to that used in the current study; however, methionine and cysteine tracers were administered intravenously. A major effect of route of administration on the fate of ingested amino acids is likely to be first-pass metabolism through the splanchnic region. Given that the enzymes of transsulfuration are found predominantly in the liver, kidney, small intestine, and pancreas (31–33), it is possible that the authors' failure to observe a cysteine sparing effect was due to the absence of first-pass metabolism of the tracer. A study similar to that by Hiramatsu et al (12) was undertaken by Raguso et al (13). In that study, subjects were fed orally; however, the subjects were fed methionine and cysteine, which is unlikely to result in any cysteine sparing effect on the basis of our recent data (5). In fact, both Hiramatsu et al and Raguso et al suggest that the sparing effect of cysteine could have been immeasurably small at low methionine intakes, which may have accounted for the lack of any observed effect. Of the remaining studies concerned with identifying a cysteine sparing effect in humans (7, 14, 15), only one provided dietary cysteine at an intake we now believe to be sufficient to observe any sparing effects (14). The authors observed a modest, nonsignificant reduction in transsulfuration in elderly adults fed 6.5 mg methionine·kg^{-1}·d^{-1} and 20.9 mg cysteine·kg^{-1}·d^{-1} (14).

In light of the evidence suggesting that the FAO/WHO/UNU population-safe intake of total SAAs was underestimated (5–7), future dietary protocols designed to investigate the ability of cysteine to spare methionine requirements should be revised to ensure adequate provision of both methionine and cysteine. On the basis of our results, cysteine reduces the mean methionine requirement by 64% when fed at an amount of 21 mg·kg^{-1}·d^{-1}. These data confirm the existence of a cysteine sparing effect on methionine requirements in humans and suggest that the dietary requirement of total SAAs can be met with both dietary methionine and cysteine.

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