The In Vivo Sparing of Methionine by Cysteine in Sulfur Amino Acid Requirements in Animal Models and Adult Humans\textsuperscript{1,2}

Ronald O. Ball,\textsuperscript{1,3} Glenda Courtney-Martin,\textsuperscript{1} and Paul B. Pencharz\textsuperscript{1}

\textsuperscript{1}The Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Canada, and \textsuperscript{2}the Department of Nutritional Science and Pediatrics, the University of Toronto and The Research Institute, The Hospital for Sick Children, Toronto, Canada

ABSTRACT Sulfur amino acid metabolism has been receiving increased attention because of the link to chronic diseases such as cardiovascular disease, Alzheimer's disease, and diabetes. In addition, the role of cysteine and optimal intakes for physiological substrates such as glutathione are currently of considerable interest in human health. Although the dietary indispensability of methionine is not in question, the ability of cysteine to substitute for a portion of its requirement has been the topic of much debate. Methionine is often the most limiting amino acid in the diets of the developing world's population because of its low concentration in cereal grains. Therefore, the ability of cysteine to substitute for methionine requirement is not just biologically interesting; it is also of considerable economic and social importance. The primary goal of this review is to discuss the available evidence on the effect of cysteine substitution for methionine to meet the total sulfur amino acid requirement in adult humans, including an assessment of the methodological features of experiments with conflicting results. Assessment of the requirement experiments for amino acids with complex metabolism such as methionine and cysteine must begin with a careful definition of requirements and what substitution means. As a result of these definitions, a set of criteria for the intakes of methionine that will allow demonstration of the substitution effect have been developed. Some recent publications are assessed using these definitions and criteria, and a possible reason for the conflicting results in the literature is proposed. An approach to estimating tolerable upper intakes is also proposed. Research on in vivo sulfur amino acid metabolism in humans is tremendously difficult, and therefore, we do not wish to be overly critical of the high-quality work of the ambitious and highly intelligent men and women who have conducted various studies. Our goal is to objectively review the data for the reader in a logical and comprehensive manner and propose methods that may avoid difficulties in future studies. J. Nutr. 136: 1682S–1693S, 2006.

KEY WORDS: methionine • cysteine • sulfur amino acids • requirements • human • pig

The sulfur amino acids, methionine and cysteine, have gained renewed interest in recent years largely because of their relation to homocysteine and glutathione (Fig. 1). Homocysteine is a central product in the metabolic pathway of methionine metabolism, and hyperhomocysteinemia has been identified as an independent risk factor for cardiovascular disease in adults (1), ischemic and hemorrhagic stroke in newborns and children (2,3), and Alzheimer's disease in adults (4,5). Glutathione, the most prevalent intracellular thiol (6) and an important endogenous antioxidant and scavenger (7), is synthesized de novo within all cells (8), and intracellular availability of cysteine is believed to be the most important rate-limiting factor for glutathione synthesis (9–11). More importantly, glutathione concentrations are reduced in several disease states including HIV (12), liver cirrhosis (13,14), diabetes (15), and Alzheimer's disease (16). Glutathione concentration is also found to be reduced in surgical trauma patients (17), septic patients (18), premature infants (19), and in children with severe protein–energy malnutrition (11,20). Comprehensive understanding of sulfur amino acid requirements and their complex metabolism clearly has many implications for human health.

Methionine is a dietary indispensable amino acid required for normal growth and development of humans (21–25), other mammals (26), and avian species. In addition to being a substrate for protein synthesis, it is an intermediate in transmethylation reactions, serving as the major methyl group donor in vivo (27,28), including the methyl groups for DNA and RNA intermediates. Methionine is a methyl acceptor for 5-methyltetrahydrofolate-homocysteine methyl transferase (methionine synthase), the only reaction that allows for the recycling of this form of folate, and is also a methyl acceptor for the catabolism of betaine. Methionine is also required for synthesis of cysteine.

Undisputed functions of cysteine include protein synthesis and the biosynthesis of taurine, sulfate (27), and glutathione.
However, the ability of cysteine to provide a portion of the total sulfur amino acid requirement in humans, thereby providing a sparing effect on the dietary methionine requirement, has been an area of considerable debate.

Methionine is accepted as the metabolic precursor for cysteine (29). Only the sulfur atom from methionine is transferred to cysteine; the carbon skeleton of cysteine is donated by serine (29). Cysteine is not a precursor for methionine because of the irreversibility of the cystathionase synthase reaction (21) (Fig. 1). Consequently, any substitution by cysteine for dietary methionine requirement can only be via inhibition of the sulfur amino acid pathway that leads to synthesis of the transsulfuration metabolites, including cysteine itself.

Womak and Rose (30) and Rose and Wixon, (31) were the first to demonstrate a substitution effect of cysteine for the methionine requirement, first in rats, then in humans. More recently, the substitution effect of cysteine on the methionine requirements in rats has been convincingly shown by the elegant work of Finkelstein et al. (26,32). In food and companion animals (e.g., poultry, pigs, cats, dogs), numerous experiments have clearly shown that cysteine can fulfill part of the sulfur amino acid requirement and thus reduce the amount of dietary methionine required (33). However, the studies examining whether cysteine can substitute for methionine in humans have produced opposing and often confounding results.

Based on our review of the literature on human sulfur amino acid metabolism, it appears that some authors are using different definitions of requirement for methionine, cysteine, and total sulfur amino acids. Cysteine sparing of the methionine requirement also appears to have been defined differently by different authors, judging from how they discuss it. In this review we propose a set of definitions for sulfur amino acid requirements in humans. We also propose a set of criteria for experiments with the purpose of measuring sulfur amino acid requirements. These criteria include a proposal that specific intakes of methionine are required before cysteine sparing can be demonstrated. Evidence for and against the sparing of methionine by cysteine, with special emphasis on recent research in adult humans and neonatal piglets, is discussed. These results are used to demonstrate our reasoning for proposing specific criteria for testing cysteine substitution for methionine.

We hope that this review will aid in resolution of the current controversy and clarify why a discrepancy exists in the literature regarding effect of substitution of cysteine for methionine in meeting the sulfur amino acid requirement in adult humans.

**Definitions of dietary requirements with respect to the sulfur amino acid**

Methionine is clearly accepted as a dietary indispensable amino acid, and cysteine as a dietary dispensable amino acid,
which can be entirely replaced with dietary methionine in human adults. This has been confirmed in all animal species examined to date. A complicating factor in determining sulfur amino acid requirements is that there is a cycle involved that links sulfur amino acid metabolism to several other functions. Because this cycle and these functions require multiple cofactors and metabolites, nutritionists must ensure that the intakes of these factors are more than adequate during the experiment. This caveat must be included in all the definitions because deficiencies, of the vitamin cofactors in particular, will affect the requirement that is measured. An additional issue to note is that the requirement definitions below assume that the dietary comparisons are made in moles of each amino acid, to ensure that the difference in molecular weight between methionine and cysteine does not complicate the comparisons.

**Total sulfur amino acid requirement.** Assuming that methionine can be converted to cysteine as required, and therefore can meet 100% of the metabolic requirement for cysteine, total sulfur amino acid requirement must be defined as the dietary methionine intake in the absence of cysteine that satisfies the response criteria of the requirement experiment (e.g., growth, nitrogen balance, indicator oxidation, transulfuration, methyl donation).

To test the hypothesis that cysteine can substitute for part of the dietary methionine required, we must assume that cysteine is capable of fulfilling some proportion of the total sulfur amino acid requirement. The corollary of this means that there must be some minimum obligatory requirement for methionine; this represents the proportion of the total sulfur amino acid (methionine) requirement that cannot be replaced by cysteine.

The minimum obligatory requirement for methionine can be defined as the dietary intake of methionine that cannot be replaced by cysteine and that will not be reduced by addition of any methyl donor, cofactor, or any other metabolite. The consequence of this definition is that the minimum obligatory requirement can be measured only when there is an excess dietary intake of cysteine, cofactors, and so on in the diet.

**Cysteine sparing.** This term often leads to misunderstanding. Cysteine sparing is derived from the definitions of total sulfur amino acid requirement and minimum obligatory methionine requirement as the proportion of the dietary requirement for total sulfur amino acid (as described above) that can be fulfilled by dietary cysteine.

Acceptance of these definitions means that a minimum of 3 experiments must be conducted to determine the 3 defined values: 1) total sulfur amino acid requirement, which is measured by feeding graded intakes of methionine and zero dietary cysteine; 2) minimum obligatory methionine requirement, which is measured by feeding an excess of dietary cysteine and graded intakes of methionine; and 3) cysteine substitution, which is measured by feeding the minimum obligatory methionine requirement and graded intakes of cysteine. This third experiment has often not been conducted except in food animals. The acceptable number of graded intakes required for these experiments is discussed below in the section on statistical estimates of requirement.

A consequence of these definitions is that the quantity, on a molar basis, of cysteine that can substitute for methionine should be similar to the difference between total sulfur amino acid requirement and the minimum obligatory requirement for methionine. Based on the assumption that cysteine is a dietary dispensable amino acid, the total of cysteine plus methionine cannot be higher than the total sulfur amino acid requirement. If this is observed, it means that cysteine was a dietary indispensable amino acid under the conditions of the experiment. Animal research (33) demonstrates that the intake (usually expressed on a weight basis) of methionine plus cysteine required to meet the total sulfur amino acid requirement is usually lower than the weight of total sulfur amino acids measured using methionine alone. This difference is partly caused by expression on a weight rather than molar basis. However, if the total of cysteine plus methionine on a molar basis is less than methionine alone, this can be taken to indicate that it is metabolically more efficient to feed the correct sulfur amino acid balance. These definitions and derived conclusions are important to bear in mind when interpreting the metabolic and requirement experiments described below.

**Dietary criteria for measuring sulfur amino acid requirements.** A set of criteria for experiments seeking to measure sulfur amino acid requirements can be developed by combining the definitions described above with knowledge of the regulation of the well-known and well-described sulfur amino acid pathways (4,26,32,34). These criteria, as described below, must be satisfied if the results of the experiments are to be accepted as valid estimates of requirement.

**Bioavailability of methionine and cysteine.** Bioavailability of dietary methionine and cysteine sources should be known and clearly stated in the method description. This point is important because foods vary widely in their amino acid bioavailability (35), and therefore comparison of the estimates among experiments is valid only if conducted on a bioavailable basis. For experiments using synthetic diets, the differences between humans and animal species in bioavailability of L-methionine must be recognized. L-Methionine is 100% bioavailable in animal models (e.g., chicks, dogs, pigs, rats), whereas it is only 30% bioavailable in humans, and the mixture of L- and DL-methionine is only 65% bioavailable (35). The reason for this difference in bioavailability is presently unknown. This should be investigated because it may have other implications for methionine requirements and metabolism in health and disease.

Methionine and cysteine are unstable during storage and are readily susceptible to oxidation to methionine sulfone, methionine sulfoxide, and cysteic acid. Reducing conditions can produce H2S. Although storage is not often reported or recognized as an issue, it must be considered, especially when diets may be subjected to heat, humidity, acidic conditions, or storage for periods longer than a few weeks. Research diets should always be stored in cool dark conditions, but this is even more critical for experiments involving methionine and cysteine metabolism.

**Molecular weight difference of methionine and cysteine.** The molecular weight difference of methionine and cysteine must be recognized and used in calculation of dietary sulfur amino acid concentrations. Many experiments in the published literature have not used dietary treatments that considered the 20% difference in molecular weight. This lack leads to difficulties in interpretation and sometimes to erroneous conclusions. For example, milligrams of cysteine and milligrams of methionine cannot be summed to calculate total sulfur amino acid in milligrams (e.g., 8 mg cysteine is the molar equivalent of 10 mg methionine, or 10 mg cysteine is equal to 12.5 mg methionine). When experiments use methionine and cysteine isotope tracers, the results are usually expressed in µmol/kg, but the dietary intakes are usually designed and reported as comparisons between equal milligrams of each amino acid. If the experimental design or the interpretation is based on the assumption that 1 mg of cysteine can replace 1 mg of methionine, then the conclusions will be incorrect.

**Cofactors and interacting metabolites.** Cofactors and metabolites interacting with the methionine cycle must be more than adequate and constant in all diet treatments (unless of
course the focus is on one of these factors). Therefore, the diet must be controlled for methyl donors, sulfate donors, antioxidants, cofactors, and so on. All recent experiments appear to have recognized the need for adequate B vitamins, but there are other metabolites that can have an equally large impact. For example, some experiments have used glycine and serine to balance the diets for nitrogen and amino acid intake. Glycine has been shown to cause a change in transmethylation activity (36). Serine, because it is the carbon donor for cysteine synthesis, may affect cysteine status (36). Adequate adaptation to a change in intake of the cofactors must also be considered. If the subjects may have been deficient in B vitamins for a period of time, B-vitamin supplementation may need to begin much in advance of the experiment.

Finally, there are a few minor issues that are often ignored or not reported but should be considered. For example, the antioxidant/oxidant capacity of the diets may affect cysteine demand for glutathione synthesis. Sulfate content of the diets may vary; if cysteine and methionine are not equimolar for all treatments, then sulfur status has probably changed (37). Acid-base balance and redox status of the subjects and diets also need to be considered because of their effects on urinary excretion of amino acids, nitrogen, and sulfur (38).

**Range in sulfur amino acid intakes and statistical estimates of requirement.** Experiments must include diets with both deficient and excess intake of sulfur amino acids; an experiment cannot be accepted as defining a requirement unless both deficient and excess levels of sulfur amino acids were included. Otherwise, the actual requirement could be either higher or lower than the diets that were studied. This criterion also means that the results must allow statistically valid tests within both the excess and deficient ranges of response. The number of excess and deficient diets used in human experiments is often too few to allow the robust statistical examination that is required in animal studies (39). For example, 3 treatments cannot reliably identify an unknown requirement, although this number of diets is not unusual in human experiments. At a minimum, there should be 4 dietary treatments, 2 above and 2 below the requirement, but this requires prior knowledge of the requirement unless you are very lucky. If the authors wish to demonstrate a linear or curvilinear response, then a minimum of 6 diets is required.

Readers must recognize that human in vivo research is very expensive, very difficult, and time consuming; for this reason researchers sometimes use fewer diets than necessary to meet these strict criteria. These experiments should not be dismissed; the limited research in the area makes them valuable. However, more care must be applied during interpretation if they are used to estimate sulfur amino acid requirements. In addition, an exception to the number of required treatments may be possible with the use of isotopes to measure sulfur amino acid kinetics. For example, cysteine sparing may be measured by comparing methionine kinetics for 3 diets containing 1) methionine in excess of the total sulfur amino acid intake, 2) methionine and cysteine at equimolar intakes totaling the same total sulfur amino acid intake as diet 1, and 3) methionine at the minimum obligatory intake and cysteine in excess to the total in diet 2. The proportion of the total sulfur amino acid requirement that could be met by cysteine could then be determined by comparing the rates of transsulfuration (i.e., oxidation of excess methionine).

### Estimating the tolerable upper intake of sulfur amino acid

Pertinent to this discussion of the effect of sulfur amino acid intake on requirement estimates is the problem of determining a tolerable upper intake (i.e., probable safe intake) for sulfur amino acids. Very little data exist in this area in humans for most amino acids (40), mainly because toxicity studies in humans are not ethical. Requirement experiments, if they include diets with sufficiently high intake of amino acids, have the opportunity to contribute data to this current lack of safety information. Those conducting amino acid experiments in the future should acquire blood, urine, and tissue samples when possible and search for appropriate biomarkers of toxicity. These analyses would supply information about the potential safety of these amino acids to help guide further research.

An additional approach to potentially identifying tolerable upper intakes of amino acids is to use requirement experiments in a different way. There is a pattern of change in amino acid retention with increasing intake, which is typical of many amino acids. This generalized pattern is comprised of 3 phases: a phase with a positive slope, a plateau, and a second positive slope. The first phase results from increasing retention of the amino acid as a result of increasing utilization of the limiting amino acid for protein synthesis and other required metabolic functions. The second phase usually has no slope (i.e., a plateau) or some minimal slope, depending on the amino acid, and the intercept of phase 1 and 2 represents a measure of dietary requirement often called the breakpoint estimate. Phase 2 represents a range of intakes where additional increments of the test amino acid are primarily catabolized in proportion to the extra intake. This increasing catabolism in proportion to intake occurs because each additional increment in intake is in excess of the requirements for metabolism, and the amino acid is broken down and used for energy. The third phase is characterized by a positive slope, and the change in slope creates a second intercept or breakpoint. This change in slope results from increasing retention of the amino acid in body pools. This increase in retention is a result of dietary intake exceeding the metabolic capacity to catabolize the amino acid in direct proportion to intake. This third phase, and the second breakpoint, are usually also characterized by an increasing rate of excretion of the amino acid in urine. This second breakpoint may be regarded as one estimate of the tolerable upper intake because it represents the intake where the normal regulatory mechanisms are no longer sufficient to dispose of the excess. The second breakpoint does not necessarily mean that the amino acid or its metabolites are toxic at intakes above this level, nor that toxicity cannot occur at lower intakes—this will probably vary with each amino acid. However, the dietary intake represented by the second intercept could reasonably be described as an intake above which the risk of adverse events is increasing. As a result, we suggest that the intake at which this second intercept occurs would be a good starting point for assessing toxicity of certain amino acids.

Assessment of the tolerable upper intake must also consider the adaptation that occurs with excess amino acid intake. The catabolic mechanisms for most amino acids are up-regulated with chronic intake in excess of requirement (41). In addition there are overflow pathways for several amino acids that do not become evident until an excess intake is consumed for a period of time (41). The dietary intake at which the second intercept occurs may therefore shift to the right (i.e., greater intake) following adaptation. The up-regulated normal pathways or the new mechanisms that are recruited could either reduce or increase the toxic effects of the amino acid. Therefore, a solid understanding of the metabolism of the test amino acid is required to interpret these results.

### In vivo tracer studies of sulfur amino acid metabolism in humans

Mudd et al. (42,43) were the first to establish a method (methyl balance) to identify and quantify different aspects of
methionine metabolism in humans by measuring turnover of substrates. However, because these substrates turn over relatively slowly, acute changes could go undetected. Therefore, this approach was considered problematic because it may have been insensitive in terms of quantifying some methyl group excretion and oxidation (44).

In an effort to alleviate these problems, Storch et al. (44) developed a stable isotope tracer method for quantifying various aspects of methionine metabolism in humans. Various aspects of methionine metabolism were examined in both the fed and fasted states after a 5-d adaptation period to an adequate diet in which methionine and cysteine were provided at intakes of ~30 and 29 mg kg\(^{-1}\) d\(^{-1}\), respectively. The tracer was administered via a primed constant intravenous infusion of [methyl-\(2^\text{H}\)]methionine and [\(^{1-13}\)C]methionine.

This experiment showed that compared to the fasted state, feeding led to a decrease in breakdown and an increase in transmethylation, transsulfuration, and remethylation of methionine. Following feeding, there was an increase in methionine oxidation (transsulfuration) because more methionine was partitioned into the transmethylation pathway. This was accompanied by an increased rate of recycling of methionine via remethylation compared to disposal via transsulfuration. Therefore, during the fed state, methionine metabolism was directed toward anabolism, with the increased flux partly accounted for by enhanced flow of methionine into the pathways of transmethylation and remethylation relative to the fasted state. In the fasted state, methionine was primarily used for protein synthesis, which took precedence over transmethylation.

In a subsequent experiment, using a doubly labeled methionine tracer (L-[\(^{1-13}\)C; methyl-\(2^\text{H}\)]methionine) instead of 2 different tracers, Storch et al. (45) altered both methionine and cysteine intakes. Three diets provided either 25 mg/kg methionine without cysteine (adequate diet), a total sulfur amino acid–free diet, or zero methionine and 20 mg/kg cysteine. With the adequate diet, the rates of \(Q_c\) (flux of cysteine) and \(Q_m\) (flux of methionine) and the rates of remethylation and transsulfuration were similar to those observed previously (44) (Table 1). When the different patterns of the methionine cycle (Table 1) were compared with that observed previously, the results suggested that the adequate methionine diet was also adequate in total sulfur amino acids. The data may be taken as evidence confirming that methionine can provide the entire sulfur amino acid requirement and that cysteine is dispensable in humans. The diet free of sulfur amino acids caused a significant decrease in all aspects of the methionine methyl cycle (Table 1). With the addition of cysteine to the sulfur amino acid–free diet, the only significant change observed was a decline in the rate of transsulfuration. This experiment confirmed their other results (44), which showed that methionine was efficiently used for protein synthesis when sulfur amino acid intakes were low or absent. Additionally, the ratio of homocysteine remethylation to homocysteine oxidation (remethylation:transsulfuration) increased when the sulfur amino acid–free diet was consumed, resulting in a decrease in the proportion of methionine oxidized via transsulfuration (Table 1). Homocysteine formation (transmethylation) as well as homocysteine recycling (remethylation) were considerably reduced.

The Storch et al. studies (44,45) are important because they show that in the presence of a sulfur amino acid–free diet, methionine was highly directed to protein synthesis relative to transmethylation (the methionine locus). There was also an increase in the partitioning of homocysteine into remethylation relative to transsulfuration (the homocysteine locus), which served to conserve methionine by reducing oxidation.

With the addition of cysteine (20 mg kg\(^{-1}\) d\(^{-1}\)) to the sulfur amino acid–free diet (45), there was a significant decrease in transsulfuration by ~50% from 1.15 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\) to 0.65 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\). In addition, there was a trend toward an increase in protein synthesis relative to transmethylation (synthesis:transmethylation) and remethylation relative to transsulfuration (remethylation:transsulfuration) (Table 1). We suggest that these data indicate that the addition of cysteine increased protein synthesis and, therefore, that cysteine can provide a portion of the sulfur amino acid requirement in humans. Although we fully understand and appreciate the practical and technical difficulties inherent in the conduct of these studies, we suggest that the addition of a low, but not devoid, methionine diet with adequate or excess cysteine would have much more clearly shown the sparing effect of cysteine on methionine use for protein synthesis. We suggest that this would occur because zero methionine intake resulted in very low rates of protein synthesis and must result in methionine always being the limiting factor for protein synthesis. The only pathway by which cysteine can reduce the methionine requirement is by reducing the methionine used in transsulfuration. Transsulfuration rate in the experiment (45), in the complete absence of methionine in the diet, was extremely low (0.65 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\)). Therefore, the maximum possible cysteine-sparing effect that could be measured would be equal to no more than the rate of transsulfuration. The responses to cysteine addition were limited by the severe methionine deficiency and therefore were too small to be detected.

This view is supported by the work of Finkelstein et al. (26,32). Rats were fed a diet containing 1% methionine for 7 d (control group) or a diet with 1 g/100 g methionine to which 0.8 g cysteine/100 g diet was added (experimental group). The only statistically significant result from all of the parameters measured was a 30% lower level of S-adenosylhomocysteine in liver. However, when the same cysteine supplement of 0.8 g/100 g was added to a diet containing 0.25 g/100 g methionine and 0.5 g/100 g cysteine, there was a very significant decrease in the hepatic concentration of S-adenosylmethionine (28%) and serine (33%), and an 88% increase in cysteine. This was accompanied by a significant increase in hepatic cystathionine synthase activity. Using an in vitro system, Finkelstein et al. (32) subsequently demonstrated a 44% decrease in cystathionine synthesis, when 0.8 g cysteine/100 g diet was supplemented to the diet containing 0.25 g methionine/100 g and 0.5 g cysteine/100 g diet. However, there was no change in transsulfuration when 0.8 g cysteine/100 g was added to the diet containing 1 g methionine/100 g.

These results showed that cysteine supplementation to a diet marginal in total sulfur amino acid resulted in decreased hepatic cystathionine synthase, whereas there was no change in cystathionine synthase activity when a diet with excess methionine (1 g/100 g diet) was further supplemented with 0.8 g cysteine/100 g. A significant result was observed only when 0.8 g cysteine/100 g was added to a diet marginal but adequate in methionine and total sulfur amino acid intake. These data clearly show that the ability of cysteine to provide part of the sulfur amino acid requirement can be demonstrated only under specific dietary conditions that “allow for the efficient conservation of a limited methionine pool by means of augmenting homocysteine remethylation and or decreased cystathionine synthesis (transsulfuration)” (32).

As later stated by Finkelstein, “the dietary content of methionine is an important determinant of the effect of supplemental cysteine on hepatic methionine metabolism” (46).

In a subsequent study by Finkelstein et al. (26), the content of methionine and cysteine in the diets was varied to determine...
## TABLE 1

Summary of results from studies showing various components of the methionine cycle in response to different diets

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<th>Cys, mg/kg</th>
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<th>( Q_c )</th>
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1 Mean data were used for calculations where individual data for the parameter were not included in the paper. Calculations for S and B were according to the formulas: \( S = Q_c - TS \), \( B = Q_c - I \). SAA, sulfur amino acid; MET, methionine; CYS, cysteine; \( Q_m \), methionine flux; \( Q_c \), cysteine flux; RM, remethylation; TS, transsulfuration; TM, transmethylation; S, synthesis; B, breakdown; IV, intravenous; IG, intragastric.
the limit of adaptation of rats. The lowest dietary level of methionine, without cysteine and choline, that would support growth and prevent hepatic steatosis was 0.75 g methionine/100 g diet, the control group. However, 0.25 g methionine/100 g with 0.5 g cysteine/100 g and 0.3 g choline/100 g was the best diet, and all diets containing <0.25 g methionine/100 g were inadequate for growth regardless of the cysteine and choline supplementation. In other words, the cysteine-sparing effect (i.e., the ability of cysteine to provide a portion of the sulfur amino acid requirement) was clearly shown only when methionine intake was within a specific range. This range is less than the total sulfur amino acid requirement (i.e., 0.75 g/100 g) but more than the minimum methionine requirement (i.e., 0.25 g/100 g). We propose below that the failure to satisfy these criteria is one of the reasons why there is disagreement in the human literature regarding cysteine sparing.

Sulfur amino acid requirements and cysteine sparing by nitrogen balance

Rose et al. (47), using positive nitrogen balance as the criterion of adequacy, were the first to demonstrate that methionine was an indispensable amino acid in humans. Rose et al. (48) designated a minimal tentative requirement of 1.1 g/d and twice that (2.2 g/d) as the safe daily intake. With a calorie intake of 55 kcal·kg⁻¹·d⁻¹, a mean nitrogen intake of approximately 140 mg·kg⁻¹·d⁻¹ (mean weight 71.43 kg), and a protein intake of approximately 0.8 g/kg BW, the recalculated mean methionine requirement of the 6 male subjects is 13.25 mg·kg⁻¹·d⁻¹, and the proposed safe allowance is 26.5 mg·kg⁻¹·d⁻¹.

Rose and Wixom (31) subsequently published the report that led to much controversy in the ensuing years. They summarized the results of 3 experiments in which the methionine requirement was first determined in the absence of cysteine followed by the requirement determined in the presence of a fixed dietary excess of cysteine. Those results showed that L-cysteine was capable of replacing 80 to 89% of the methionine requirement of adult men; this provided evidence for a regulatory mechanism not only in rats (30) but also in humans (31).

The recognition of methionine as a limiting amino acid in the diets of people of underdeveloped nations (49) subsequently stimulated additional research on sulfur amino acid requirements. Reynolds et al. (50) conducted the first studies on methionine and cysteine requirements in women. With intake of 500 mg of cysteine, a total of 240 to 290 mg of methionine was needed to maintain subjects in nitrogen equilibrium (50). This was the zone in which the difference between the intake and excretion did not exceed ±5% (i.e., the excretion is within 95 to 105% of intake). This represented a total sulfur amino acid requirement of 740 to 790 mg/d. Using the mean weight of 62.6 kg for the 15 subjects, this represents 11.8 to 12.62 mg·kg⁻¹·d⁻¹ mean total sulfur amino acid requirement.

When 10 mg of cysteine was fed with 290 mg of methionine, 9 of the 12 subjects were in positive nitrogen balance. When 260 mg of cysteine was combined with 290 mg of methionine (550 mg total sulfur amino acid), all 6 subjects studied were in positive nitrogen balance. The authors concluded that a total of 550 mg/d of total sulfur amino acid (260 mg cysteine and 290 mg methionine) was adequate for maintaining nitrogen balance. For an average weight of 55.98 kg, this represents a mean requirement of 9.8 mg·kg⁻¹·d⁻¹ for total sulfur amino acid (50).

An interesting point from this experiment (50) was that 1 woman who was 64 y of age (other women were 18–36 y old) was in negative nitrogen balance at all total sulfur amino acid intake levels ranging from 40 to 860 mg/d total sulfur amino acid intake. This led the authors (50) to suggest that sulfur amino acid requirement might be greater in older than in younger adults.

The authors (50) did not determine a methionine requirement in the absence of cysteine before varying the cysteine intake. Nevertheless, it should be noted that only when the methionine intake was ~5 mg·kg⁻¹·d⁻¹, in the presence of a cysteine intake that was similar or higher, was there a trend toward nitrogen equilibrium in most subjects. This supports the concept stated earlier that sparing is demonstrated only when the intakes are within the correct range.

Clark et al. (51) determined that the minimum methionine required ranged from 260 to 700 mg/d in the presence of a cysteine intake of 280 to 400 mg/d. Cysteine supplied between 36 and 52% of the sulfur amino acid requirement of these subjects. Subjects in a second experiment were fed graded intakes of methionine (310, 460, 610, 760, and 910 mg/d) with a fixed cysteine intake of 340 mg/d (for a MW-uncorrected total sulfur amino acid intake of 650, 800, 920, 1070, and 1220 mg/d). Only at the methionine intake of 910 mg/d was nitrogen balance significantly increased. Clark et al. (51) concluded that the lack of response occurred because when the minimal need for sulfur-containing amino acids was met, further increases in methionine did not induce a marked response. They also suggested that the lack of response regarding the sparing effect of cysteine was caused by a decreasing sparing effect of cysteine as methionine became adequate. This lack of response in nitrogen balance with increasing methionine intake has also been observed with the indicator amino acid oxidation technique, where increasing intake of the test amino acid intake above requirement produces no further increases in protein synthesis and thus no change in nitrogen excretion or oxidation of the indicator amino acid (52,53). In other words, when the sulfur amino acid requirement is already met by methionine without cysteine, adding cysteine will not show a sparing effect of cysteine on the methionine (sulfur amino acid) requirement.

This point of view is also supported by the study conducted by Albanese et al. (54). Five healthy infants were fed a diet in which the protein was provided by a casein hydrolysate to which supplemental tryptophan, methionine, and cysteine were added in graded amounts. In the absence of cysteine, normal weight gain and nitrogen balance were achieved with a methionine intake of 85 mg·kg⁻¹·d⁻¹. A deficient intake of 65 mg of methionine but with 50 mg of cysteine resulted in weight gain and nitrogen balance similar to that observed with intakes of 85 mg of methionine and 15 mg of cysteine. They concluded that 35 mg of cysteine reduced methionine requirement by 20 mg and that cysteine furnished 33% of the total sulfur (methionine) requirement. These authors were able to measure an effect of cysteine substitution because the methionine intakes used were suitable for this purpose.

Sulfur amino acid requirements by stable isotope tracer kinetics

Important recent contributions to our knowledge of the dietary requirement for sulfur amino acid and the effect of cysteine on the methionine requirement have resulted from experiments using stable isotope tracer kinetics. These experiments have been by the MIT group, headed by the late V. R. Young and his collaborators, and the combined Alberta/Toronto group directed by R. O. Ball and P. B. Pencharz. These 2 groups have employed the techniques of direct amino acid oxidation and indicator amino acid oxidation (IAAO), respectively, to determine the total sulfur amino acid requirement and the effect of cysteine on these requirements.
groups have obtained similar results with respect to the total sulfur amino acid requirement but have arrived at opposite conclusions regarding whether cysteine can provide a portion of the total sulfur amino acid requirement and thus spare methionine. There are much more data now than when these experiments were designed. With the benefit of hindsight we can now examine these experiments and analyze why some found that cysteine could provide a portion of the sulfur amino acid (methionine) requirement and some did not.

**Direct amino acid oxidation.** This series of investigations began with a study by Young et al. (55) designed to explore methionine metabolism and body methionine balance (Table 1) using L-[3H2-methyl-1-13C]methionine with the diet supplying methionine (without cysteine) at 13 mg·kg⁻¹·d⁻¹, the total sulfur amino acid RDA recommended by the 1985 FAO/WHO/UNU. Compared with Storch et al. (45), in which the methionine intake was 25 mg·kg⁻¹·d⁻¹, the methionine and cysteine flux, designated by Qm and Qc, were much lower in this study. Synthesis and breakdown rates were ~30% and 16% lower, with 13 mg·kg⁻¹·d⁻¹ methionine versus 25 mg·kg⁻¹·d⁻¹, respectively. The flow of methionine via the transmethylation and transsulfuration pathways was also lower with an intake of 13 mg·kg⁻¹·d⁻¹. Based on their estimation of methionine balance, which required a number of assumptions, Young et al. (55), concluded that: "a methionine intake of 13 mg·kg⁻¹·d⁻¹ in the absence of dietary cysteine approximates the mean requirement for the healthy young adult male, but the upper range of the requirement (RDA) is probably below ~25 mg·kg⁻¹·d⁻¹."

Clearly, the decreased flux and breakdown along with the reduced synthesis indicated that methionine was being more efficiently used at 13 mg·kg⁻¹·d⁻¹ and that the 25 mg·kg⁻¹·d⁻¹ used by Storch et al. (45) was probably a more than adequate methionine intake. However, the data of Young et al. (55) were insufficient to support their conclusion because they had tested only 1 intake of methionine. In addition, comparison with the data of Storch et al. (45) could also be taken to suggest that the intake of 13 mg·kg⁻¹·d⁻¹ was below the minimum requirement. With respect to the RDA, we agree with Irwin and Hegstead (56), who stated: "the average requirement is an inadequate base from which to establish a safe allowance or daily recommendation, since these must be established enough above average needs to provide for most subjects, and thus an estimate of the range of requirement (apart from the errors in the determinations of such requirement) is important."

The MIT group subsequently conducted another study using 5 diets varying in methionine and cysteine intake (57). Diet 1 contained 13 mg·kg⁻¹·d⁻¹ methionine and zero cysteine. Diets 2 to 5 contained 6.5 mg·kg⁻¹·d⁻¹ methionine and varying cysteine (0, 5.2, 10.5, and 20.9 mg·kg⁻¹·d⁻¹) intakes, respectively. The results of this study (Table 1), including our additional calculations, show that for diet 1 (13 mg·kg⁻¹·d⁻¹ methionine) the Qm was very similar to that previously observed at the same intake (55). However, the Qc seemed to be lower. This may be because different subjects were involved in this study or with intake being near the mean requirement, the variation (normal population distribution) among individuals in requirement may have had a substantial effect on kinetic measurements. Overall, the data for diet 1 were similar to those of the previous study (55).

When the methionine intake was reduced to 6.5 mg·kg⁻¹·d⁻¹ and the cysteine intakes were 0, 5.2 and 10.5 mg·kg⁻¹·d⁻¹ (57), the kinetic parameters were not different among diets (Table 1). However, when compared with diet 1, we observed what we believe to be an increase in synthesis relative to transmethylation and in remethylation relative to transsulfuration (Table 1). For diet 5, there was a possible trend toward a decrease in transsulfuration rates with an additional trend toward increased remethylation:transsulfuration rates. From diets 2 to 4 there was a 33% decrease in transsulfuration relative to remethylation; this means there was nearly a 90% increase in methionine being partitioned toward remethylation as opposed to transsulfuration.

Based on our review of the literature, we believe that at the intake of 6.5 mg·kg⁻¹·d⁻¹ of methionine and 20.9 mg·kg⁻¹·d⁻¹ cysteine a small sparing effect of cysteine should have been observed. Our additional calculations using their mean data indicate a trend toward a cysteine effect for diet 5, although we are unable to test this statistically. The authors suggested that their failure to measure a sparing effect might have been the result of bypassing the splanchnic region, a consequence of the intravenous infusion of the tracer, because in a previous study examining the effect of betaine on methionine metabolism (58), differences were observed only when the isotope was infused orally.

Another study (59) was conducted with the tracer infused enterally instead of intravenously, with 3 diets (2 of which were different from previous experiments): diet A 13 mg·kg⁻¹·d⁻¹ methionine without cysteine, diet B 5 mg·kg⁻¹·d⁻¹ methionine without cysteine, and diet C 5.0 mg·kg⁻¹·d⁻¹ methionine plus 6.5 mg·kg⁻¹·d⁻¹ cysteine. The ratio of remethylation:transsulfuration, (recycling:oxidation) was significantly lower for diets C and B compared with diet A, and the rate of transsulfuration was higher relative to conversion of methionine to homocysteine (transmethylation). Additional interpretations can be developed if we accept the authors’ previous assertion (55) that the methionine intake of 13 mg·kg⁻¹·d⁻¹ (diet A) just satisfied the total sulfur amino acid requirement and that when methionine was fed at 5 mg·kg⁻¹·d⁻¹ (diets B and C, Table 1) (59), intake was approximately the minimum obligatory dietary requirement for methionine. These data therefore mean that all, or nearly all, the methionine provided by diets B and C was necessarily utilized to satisfy the methionine specific metabolic demands. Only a small amount of methionine was used by the transsulfuration pathway; this quantity probably represents the minimum obligatory rate of methionine oxidation (~3 μmol·kg⁻¹·h⁻¹, Table 1) (59). The kinetic measures were not different between diets B and C (Table 1). The similar transsulfuration rates mean that the additional cysteine intake in diet C was only providing enough cysteine to meet the demands for cysteine specific metabolism (probably almost all was used for protein synthesis). It is important to note that the lack of an enzyme to convert cysteine into homocysteine means that methionine and cysteine are not in equilibration; cysteine cannot be used to synthesize methionine. Furthermore, cysteine intake within the range that we are interested in has a very small influence on the activity of these enzymes compared with methionine (26,32). Therefore, because all of the methionine in diet C was required for methionine specific metabolism, the small amount of cysteine provided was used in cysteine specific metabolism, and the metabolic uses of cysteine are external to influences on the methionine pathways, no changes in methionine kinetics would be detected in this experiment. Because there was no excess of methionine in diet C, very little or no effect of cysteine on methionine requirement would have been observed even if more cysteine had been fed; there was no extra methionine (i.e., no more than the obligatory minimum requirement) to be spared.

The conclusion made by the authors from the studies described above, that cysteine could not substitute for methionine in the total sulfur amino acid requirement (59), led them to design a study to follow up on the suggestion by Reynolds...
et al. (50) that sulfur amino acid requirement might be higher in older adults than in younger adults (60). The results (Table 1) show a trend toward a decrease in transsulfuration of methionine between diet 1 and diet 5. Evidence of efficient utilization of methionine was reflected by the increase in remethylation relative to transsulfuration from diet 1 to diet 5. These data suggest that the addition of cysteine reduced methionine oxidation; which is evidence for cysteine supplying part of the sulfur amino acid requirement.

**Indicator amino acid oxidation.** The IAAO technique, developed for use in humans by the Alberta/Toronto group, has contributed 6 reports (52,53,61–64) to current knowledge on sulfur amino acid metabolism; 3 in male adults and 3 in the neonatal piglet (62–64) as a model (65) for the human infant. These experiments attempted to follow the definitions and criteria described above for sulfur amino acid requirement experiments.

With l-[1-13C]phenylalanine used as an indicator amino acid, 6 men received each of 6 graded intakes of methionine, in the absence of cysteine, in random order after a 2-d adaptation to a prescribed diet (52) to determine the methionine (total sulfur amino acid) requirement without cysteine. Using F13CO2 recovery, a breakpoint in oxidation was identified by 2-phase linear regression analysis. The mean methionine (total sulfur amino acid) requirement of adult men was 12.6 mg·kg⁻¹·d⁻¹. The safe population estimate (RDA), based on individual variation, was 21 mg·kg⁻¹·d⁻¹ methionine (total sulfur amino acid) if provided by methionine only. Because the combination of methionine and cysteine necessary to meet the total sulfur amino acid requirement has typically been shown in animals to be less, when expressed on a weight basis, than that required when only methionine is provided (33), it is important to make the distinction shown above.

This mean population estimate (52) was very similar to that obtained by Rose et al. (48) using nitrogen balance (12.25 mg·kg⁻¹·d⁻¹) and Young et al. (55) using methionine kinetics (13 mg·kg⁻¹·d⁻¹). Taken together, these 3 experiments represent a robust estimate of the mean requirement. Therefore, the suggested RDA set by the 1985 FAO/WHO/UNO (66) of 13 mg·kg⁻¹·d⁻¹ was clearly much too low.

The second study of the series was conducted to test whether cysteine could substitute for a portion of the methionine (53) in the total sulfur amino acid requirement. Graded intakes of methionine were fed with a fixed cysteine intake of 21 mg·kg⁻¹·d⁻¹, this cysteine intake was chosen because it represented our estimate of the RDA for the total sulfur amino acid requirement (52) and thus should result in maximum reduction in the quantity of methionine required. A breakpoint was identified in F13CO2 recovery in response to increasing methionine at 4.5 mg·kg⁻¹·d⁻¹. This was much less that the previous breakpoint (12.6 mg·kg⁻¹·d⁻¹) measured in the absence of dietary cysteine. This clearly showed that there was a decrease in the methionine requirement when excess cysteine was present in the diet (Table 2). We concluded that dietary cysteine was able to provide part of the total sulfur amino acid requirement, to the extent of ~64% of the methionine, expressed on a weight basis [100 – (4.5/12.6)].

The combined results of the above 2 studies (52,53) were criticized, and a number of limitations were suggested in an editorial written to the American Journal of Clinical Nutrition (67). These comments were as follows. 1) The rate of 13CO2 release from l-[1-13C]phenylalanine at the tracer methionine intake above the respective breakpoints differed between the 2 studies; it was ≥0.5 μmol·kg⁻¹·h⁻¹ in 1 study (53) and ≤0.4 μmol·kg⁻¹·h⁻¹ in the other (52) when methionine was given without cysteine. 2) There appeared to be no sparing effect of endogenous methionine utilization by cysteine at the zero to very low methionine intakes when rates of 13CO2 release in the 2 studies were compared. 3) Even if the assumption of a sparing of methionine was definitely shown, the practical limitations of this finding remains unclear because the cysteine intake (21 mg·kg⁻¹·d⁻¹) studied relative to that of methionine at the breakpoint of 4.5 mg·kg⁻¹·d⁻¹ was not characteristic of typical diets.

Regarding the first criticism (67), there are several critical points to be considered. 1) The F13CO2 is the rate of production of 13CO2 as a function of the V13CO2. Di Buono et al. (53) presented data (52) only to illustrate the effect of cysteine substitution on the methionine requirement. These 2 studies were not conducted in the same subjects or during the

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**TABLE 2**

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* dl-Methionine used in study.
same time period. The differences in $F^{13}\text{CO}_2$, not $^{14}\text{CO}_2$ as mentioned in the commentary (67), resulted from the interindividual variability in $V\text{CO}_2$ between different subjects from the 2 different studies, $V\text{CO}_2$ being affected by body size. In our piglet studies, where the animals studied were very homogeneous and differences in $V\text{CO}_2$ were not a factor, we observed no difference in the $^{14}\text{CO}_2$ oxidized (as a percentage of dose) in piglets fed diets with or without cysteine (62,63). Finally, examination of $F^{13}\text{CO}_2$ in published papers by the author of the commentary (67) clearly demonstrates differences in $F^{13}\text{CO}_2$ among experiments similar to that which we experienced in these experiments. 2) Second, it is important to note that in statistical analysis of indicator oxidation breakpoint, the absolute values of $F^{13}\text{CO}_2$ and $V\text{CO}_2$ are not critical factors. The requirement breakpoint depends on the slope of the change in oxidation with changing test amino acid intake, not the absolute values. This is one of the strengths of the indicator oxidation method over the direct oxidation method, where small variations in $F^{13}\text{CO}_2$ and $V\text{CO}_2$ can have a large impact on the conclusions. 3) Another very important factor to consider is the flux measurement. Within the 2 different studies, phenylalanine flux was not affected by changes in the methionine intake (52,53). This means the phenylalanine pool was constant in size and total movement. When pool size and flux are constant, changes in phenylalanine oxidation must be reflecting changes in the partitioning of phenylalanine between protein synthesis and oxidation resulting from changes in methionine intake.

With respect to the second comment, that there appeared to be no sparing effect of cysteine on methionine at low intakes of methionine, the regulation of methionine and cysteine metabolism that we have proposed suggests that this is the correct response. As described above, if methionine is at or below the minimum obligatory methionine requirement, we predict that the cysteine-sparing effect cannot be measured.

To address the third comment, Di Buono et al. (61) studied 3 ratios of methionine to cysteine, varied within our estimates of sulfur amino acid requirements and in such a way as to be representative of their ratios in common foods. With use of orally administered l-[1-13C]-methyl-2H3]methionine and the isotope approach previously used by Storch et al. (45) and Young et al. (55), 5 healthy men were fed 3 different diets in random order after 56-h adaptation periods. The 3 diets were diet A, 24 mg kg−1·d−1 methionine without cysteine; diet B, 13 mg kg−1·d−1 methionine plus 11 mg kg−1·d−1 cysteine; and diet C, 5 mg kg−1·d−1 methionine plus 19 mg kg−1·d−1 cysteine. These diets were chosen to represent A) methionine intake to exceed the RDA for total sulfur amino acid; B) methionine intake at the mean total sulfur amino acid requirement plus cysteine in approximately equimolar amounts to meet our estimate of the RDA; and C) methionine intake at the estimated minimum obligatory requirement, with additional cysteine to obtain similar total sulfur amino acid intake as diet B.

These data (61) (Table 1) showed a significant decrease in transsulfuration (oxidation) when the diets containing cysteine were fed (diets B and C) compared with diet A. There was also a significant decrease in transmethylation rates between diet A and diets B and C. In the case of diet C, when dietary methionine intake was at the estimated minimum obligatory requirement and cysteine intake was provided to meet our estimate of the RDA for total sulfur amino acid, there was an almost 40% decrease in transsulfuration rates relative to transmethylation. This resulted in a 2.5-fold increase in remethylation rate relative to transsulfuration (Table 1). These data mean that cysteine can fulfill ~60% of the total sulfur amino acid requirement when sufficient methionine is consumed to meet at least the methionine specific demands (i.e., at least the minimum obligatory requirement).

Our conclusions from this study were that 1) the ratio of cysteine to methionine regulates whole-body sulfur amino acid metabolism in adult humans, 2) when both methionine and total sulfur amino acid intake are adequate, replacement of methionine with cysteine results in increased remethylation at the expense of transsulfuration, and 3) at high methionine intake, the methionine pool size is regulated by a higher rate of transsulfuration. We believe that we have clearly established that cysteine can furnish part of the total sulfur amino acid requirement in humans, thus reducing the dietary need for methionine. To detect this effect of cysteine, the dietary intakes of methionine and cysteine must be within specified parameters.

Data from our piglet work have shown similar results (62,63) to that observed in our human studies; cysteine clearly reduced the dietary quantity of methionine necessary to satisfy the total sulfur amino acid requirement. However, the proportion of total sulfur (methionine) requirement provided by cysteine in piglets was smaller than that observed in adult men (40 vs. 64%). The methionine requirement in the enterally fed piglet was about 30% higher than that in the parenterally fed piglet, indicating that the intestine of the neonatal piglet has a substantial demand for methionine. This also means that using enteral data to decide on the methionine intake of parenterally fed individuals will result in overfeeding of methionine. Interestingly, despite the abnormal intestine that develops with parenteral feeding, the sparing effect of cysteine was the same, ~40%, by both routes of feeding (63). This indicates that there is sufficient transsulfuration occurring elsewhere in the body to meet the cysteine requirement by transsulfuration of methionine.

In one of our piglet studies (64), we measured plasma homocysteine concentration in response to feeding graded levels of methionine either parenterally or enterally in the presence or absence of cysteine. These results confirmed the previous observation by Storch et al. (58) that route of feeding affected sulfur amino acid metabolism. Homocysteine concentration was highest in the group fed methionine without cysteine enterally, and plasma homocysteine concentration was directly proportional to methionine intake. These data suggest that during normal enteral feeding in the neonate, the addition of cysteine decreases the partitioning of methionine toward homocysteine (transmethylation). This concept is supported by the study by Di Buono et al. (61) where we found a significant decrease in transmethylation rates when cysteine was added to the diet, to provide a total of ~24 mg·kg−1·d−1 total sulfur amino acid.

Therefore, the presence, and perhaps even quantity, of cysteine in the diet could be of benefit in the treatment or prevention of illness related to elevated homocysteine. Further in vivo research should determine whether there are specific intakes of methionine and cysteine that would optimize homocysteine metabolism.

**Twenty-four-hour indicator amino acid oxidation and balance.** The MIT group, with their collaborators in India, adapted our IAAO technique by infusing the isotope over a 24-h period, instead of 8 h, and measuring amino acid balance. Using the 24-h IAAO and balance technique, Kurpad et al. (68,69) studied 21 healthy Indian men, randomly assigned to 3 periods of 7 d in which methionine intakes (without cysteine) were varied (68). Twenty-four-hour IAAO studies were conducted on day 7 using an intravenous administration of l-[1-13C]leucine (as the indicator amino acid).
They identified a breakpoint in the F\(^{13}\)CO\(_2\) response at 14 mg·kg\(^{-1}\)·d\(^{-1}\), and the 24-h indicator amino acid balance suggested that the mean methionine (total sulfur amino acid) requirement in the absence of cysteine was 15 mg·kg\(^{-1}\)·d\(^{-1}\). These are similar to the requirement estimates derived by Di Buono et al. (52), using a short-term (8 h) IAAO technique, and others, using nitrogen balance (48) and methionine oxidation (55).

A possible sparing effect of cysteine on methionine use for total sulfur amino acid requirement was studied with 2 intakes of cysteine (5 and 12 mg·kg\(^{-1}\)·d\(^{-1}\)) (68). With a cysteine intake of 5 mg·kg\(^{-1}\)·d\(^{-1}\), the breakpoint was identified at a methionine intake of 20 mg·kg\(^{-1}\)·d\(^{-1}\), whereas at a cysteine intake of 12 mg·kg\(^{-1}\)·d\(^{-1}\), the methionine breakpoint was 10 mg·kg\(^{-1}\)·d\(^{-1}\). In their previous study (68) the methionine requirement without cysteine was 15 mg·kg\(^{-1}\)·d\(^{-1}\), for a total sulfur amino acid requirement (on a weight basis) of 15 mg·kg\(^{-1}\)·d\(^{-1}\). The authors concluded that cysteine may provide part of the methionine (total sulfur amino acid) requirement in healthy men but that the amount was difficult to quantify.

Based on the definitions and criteria stated above and the literature published to date, we propose that the intake of 5 mg·kg\(^{-1}\)·d\(^{-1}\) cysteine was less than that required to arrest the flow of methionine through the transsulfuration pathway, and therefore, the sparing effect observed was too small to measure, relative to the total potential effect of cysteine to replace methionine in providing the total sulfur amino acid requirement. When a diet with 12 mg·kg\(^{-1}\)·d\(^{-1}\) cysteine was fed, the estimated methionine requirement (10 mg·kg\(^{-1}\)·d\(^{-1}\)) was lower than both their previous estimates (15 mg·kg\(^{-1}\)·d\(^{-1}\)) and the estimates of others (~13 mg·kg\(^{-1}\)·d\(^{-1}\)). This shows that cysteine sparing of methionine was occurring and was equivalent to about 3 to 5 mg of methionine. However, some of the other data are rather surprising. For example, the total sulfur amino acid requirement, measured using the diet of methionine (15 mg·kg\(^{-1}\)·d\(^{-1}\)) without cysteine, represents total sulfur amino acid requirement because methionine is assumed to be converted to cysteine as required. However, the author's estimate of total sulfur amino acid requirement (corrected for MW difference and expressed in milligrams of methionine) was higher, at ~26.3 mg·kg\(^{-1}\)·d\(^{-1}\), when 5 mg·kg\(^{-1}\)·d\(^{-1}\) cysteine was fed and ~25 mg·kg\(^{-1}\)·d\(^{-1}\) when 12 mg·kg\(^{-1}\)·d\(^{-1}\) cysteine was fed. Based on our current understanding, the addition of cysteine to the diet should not result in a higher estimate of total sulfur amino acid requirement compared with methionine alone. There are 4 possible explanations for this unusual result. 1) Cysteine was a dietary indispensable amino acid in this population under the conditions of this experiment. 2) Glycine and serine were used in this experiment to balance nitrogen and amino acid intake. Glycine and serine interact with methionine as part of the methyl cycle, and serine provides the carbon skeleton for synthesis of cysteine. Differences in the dietary intake of these amino acids among treatments may have affected the rates of transsulfuration and/or remethylation of methionine, thus leading to incorrect estimates. 3) The statistical analyses may not have been optimal. 4) The bioavailabilities of the amino acids in the diets were lower than expected. Explanation 1, although not impossible, is highly unlikely. The other explanations are possible and need to be considered, but we suggest that 2 and 3 are the most likely explanations.

Conclusions

The mechanisms at play in the utilization, conservation, and regulation of the sulfur amino acids in animals (4,26,32) appear to be very similar to those in humans (46). There is no doubt that dietary cysteine can furnish a portion of the sulfur amino acid requirement in rats (30,32), pigs (62,63), and many other species (33).

Although the data on the ability of cysteine to substitute for methionine in the sulfur amino acid requirement in humans are varied, there can be little, if any, doubt that it occurs and that the mechanisms are similar in animals and humans. There appear to be a number of methodological reasons why some of the results in humans have been at variance to these conclusions. In the adult human studies reviewed, some have shown a definite ability of cysteine to substitute for a portion of the methionine in the sulfur amino acid requirement, whereas the results of others have not been as consistent. These in vivo experiments in humans are recognized as very difficult, time consuming, and expensive to conduct. The focus of this review was not to be critical of this valuable and outstanding work but to propose reasons why these differences were observed.

When no effect of cysteine was observed, it appears to have been because 2 important conditions were not met: 1) the methionine and/or total sulfur amino acid intake was too low (at or below the minimum obligatory methionine requirement), or 2) the ratio of the methionine to cysteine intake was not optimal. We conclude, based on the available evidence to date, that when these conditions were satisfied, cysteine can substitute for methionine in the sulfur amino acid requirement of adult humans.

LITERATURE CITED


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