Cysteine kinetics and oxidation at different intakes of methionine and cystine in young adults¹⁻³

Comasia A Raguso, Meredith M Regan, and Vernon R Young

ABSTRACT

Background: We previously studied methionine kinetics and oxidation with the tracer L-[1-13C, methyl-2H₃]methionine.

Objectives: We sought to explore methionine-cysteine interrelations in adults by using L-[1-13C]cysteine under different dietary conditions.

Design: In experiment 1, 12 adults consumed a protein-free diet for 6 d. On day 7, methionine (n = 6) or cysteine (n = 6) oxidation rates were measured during an 8-h continuous infusion of L-[1-13C, methyl-2H₃]methionine or L-[1-13C]cysteine, respectively. In experiment 2, 6 young men consumed 3 diets for 6 d each before a tracer study on day 7 with L-[1-13C]cysteine. The amounts (in mg·kg⁻¹·d⁻¹) of methionine and cysteine, respectively, were: high-methionine (HM) diet, 13 and 0; low-methionine (LM) diet, 6.5 and 0; and methionine-plus-cysteine (MC) diet, 6.5 and 5.6. Cysteine flux and oxidation rates were determined and sulfur amino acid (SAA, methionine plus cysteine) balances were estimated.

Results: In experiment 1, rates of methionine and cysteine oxidation were similar to losses predicted from obligatory nitrogen losses. In experiment 2, SAA balance was less negative when subjects consumed the HM diet than the LM and MC diets (interaction, P = 0.034), largely because of a difference in fed-state balance (HM compared with LM, P < 0.01; HM compared with MC, P < 0.05). There was no evidence of a sparing effect of dietary cysteine on the methionine requirement.

Conclusion: These studies support use of [1-13C]cysteine for studying whole-body SAA oxidation and conclusions that maintenance of SAA balance is best achieved by supplying methionine as transsulfuration of 13 mg·kg⁻¹·d⁻¹ (5). This compares with the upper requirement of 13 mg·kg⁻¹·d⁻¹ for total sulfur amino acids (SAAs, methionine plus cysteine) proposed by FAO/WHO/UNU (6). Furthermore, we have not detected, with these tracer techniques, any major sparing effect of dietary cysteine on the methionine intake needed to balance methionine oxidation (1, 2).

To expand our investigations of SAA kinetics in healthy adults, we conducted studies to quantify whole-body cysteine turnover and oxidation. We believed that if the results for cysteine oxidation were found to be consistent with those predicted from the findings in young adults for methionine oxidation, this would provide additional support for the conclusions drawn previously (2, 3).

During the first study (experiment 1), 2 groups of healthy adult subjects consumed a protein-free but otherwise adequate diet for 6 d. We then determined the rate of either methionine or cysteine oxidation. In the second study (experiment 2), we explored the effects of 3 diets (providing adequate methionine without cysteine or low methionine with or without cysteine) on cysteine kinetics and oxidation.

SUBJECTS AND METHODS

Subjects

A total of 12 healthy volunteers (10 males and 2 females) participated in this investigation. In experiment 1, the subjects were divided into 2 groups. Group A included 4 males and 2 females (72.6 ± 12.7 kg body weight, 174.9 ± 11.1 cm height, 23.3 ± 3.5 y of age) and group B included 6 males (68.9 ± 6.9 kg body weight, 173.1 ± 5.4 cm height, 22.8 ± 2.2 y of age). Group B also participated in experiment 2. The subjects were all from the Massachusetts Institute of Technology (MIT) student community

INTRODUCTION

We have investigated the relations between dietary methionine and cysteine in young adults (1–3) and elderly individuals (4) by using L-[1-13C, methyl-2H₃]methionine and [3,3-2H₂]cysteine as tracers. From estimates of methionine transsulfuration (oxidation), we have concluded that the mean requirement for dietary methionine, in the absence of dietary cystine, is ≈13 mg·kg⁻¹·d⁻¹ (5). This compares with the upper requirement of 13 mg·kg⁻¹·d⁻¹ for total sulfur amino acids (SAAs, methionine plus cysteine) proposed by FAO/WHO/UNU (6). Furthermore, we have not detected, with these tracer techniques, any major sparing effect of dietary cysteine on the methionine intake needed to balance methionine oxidation (1, 2).

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TABLE 1
Composition of protein-free and of l-amino acid–based diets varying in methionine and cystine contents that were given to young adults for 6 d

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Protein-free diet</th>
<th>l-Amino acid–based diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid mixture (g · kg⁻¹ · d⁻¹)</td>
<td>0</td>
<td>1.17</td>
</tr>
<tr>
<td>Beet sugar (g · kg⁻¹ · d⁻¹)</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Wheat starch (g · kg⁻¹ · d⁻¹)</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Butter (g · kg⁻¹ · d⁻¹)</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Safflower oil (g · kg⁻¹ · d⁻¹)</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Sherbet (g · kg⁻¹ · d⁻¹)</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Flavored drink (g dry powder · kg⁻¹ · d⁻¹)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Supplements

| Multivitamin-multiminerals capsules² | 1/d | 1/d |
| Potassium tablets³ | 3/d | 3/d |
| Calcium tablets⁴ | 4/d | 4/d |
| Sodium chloride tablets⁴ | 2/d | 2/d |
| Choline⁵ | 2/d | 2/d |

¹Kool-Aid; Kraft General Foods Inc, White Plains, NY.
²One-A-Day; Miles Inc, Elkhart, IN.
³K-L YTE (978 mg or 25 mmol tablets); Bristol Laboratory, Evansville, IN.
⁴TUMS; SKB Corporation, Pittsburgh.
⁵One-gram tablets; Eli Lilly & Company, Indianapolis.
⁶250-mg capsules; Lee Nutrition Inc, Cambridge, MA.

and were screened for health by medical history and physical examination. In addition, blood and urine samples were collected for a biochemical and clinical screening that was carried out in the Clinical Research Center laboratories.

The studies and their respective consent forms were approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Advisory Committee of the MIT Clinical Research Center. Informed consent was obtained from the volunteers and they were paid for their participation in the study.

Experimental designs

Experiment 1

Twelve healthy subjects (10 male, 2 female) were given a protein-free diet for 6 d. On the morning of day 7, the subjects underwent an 8-h tracer study consisting of an initial 3-h fast followed immediately thereafter by a 5-h fed period. The tracers used were either l-[1-¹³C], methyl-²H₃,methionine and l-[3,3-²H₂]cysteine (n = 6, group A) or l-[1-¹³C]cysteine (n = 6, group B).

Experiment 2

Six subjects (all males) participated in this experiment, which consisted of 3 separate 7-d diet periods. During the first 6 d of each period, subjects adjusted to the different dietary intakes of methionine and cystine. The 3 diets were: 1) high methionine (HM), which provided methionine (13 mg · kg⁻¹ · d⁻¹) with no cystine; 2) low methionine (LM), which provided methionine (5 mg · kg⁻¹ · d⁻¹) with no cystine; and 3) methionine plus cystine (MC), which provided methionine (5 mg · kg⁻¹ · d⁻¹) with cystine (6.5 mg · kg⁻¹ · d⁻¹). On day 7 of each period, an 8-h tracer study similar to the one performed in experiment 1 was conducted. Each subject received the 3 diets in random order. Between the different diet periods there were intervals of 10–20 d, during which subjects consumed free-choice diets. One subject dropped out of the study for personal reasons after he had completed one of the diet periods (diet HM).

Diets

Subjects received 3 isoenergetic, isonitrogenous meals on each of the 6 d preceding every 8-h tracer experiment. Daily energy intake was constant; the diet provided between 170 and 190 kJ/kg (41–45 kcal/kg) for these subjects. The dietary energy was mainly derived from lipid and carbohydrate sources provided in the form of protein-free wheat-starch and butter cookies and a sherbet-based drink (Table 1). Nitrogen (160 mg · kg⁻¹ · d⁻¹) was supplied as an l-amino acid mixture (amino acids were obtained from Ajinomoto USA, Inc, Teaneck, NJ) (Table 2). The amino acid mixture was similar to that used previously (2); it supplied dispensable amino acids in amounts that we have suggested are sufficient to meet the mean requirement in healthy young adults (7) but that are considerably higher than the requirements proposed by FAO/WHO/UNU (6). Dispensable (nonessential) amino acids were adjusted to maintain a constant total nitrogen content for the 3 diets. Beet sucrose and flavoring agents (Vivonex flavor packets; Norwich Eaton Pharmaceuticals, Norwich, NY) were added to improve the taste of the amino acid mixture. Beet sucrose was used to avoid changes in the background ¹³C-isotope enrichment of the expired carbon dioxide between the fasting and fed states.

Vitamins and minerals were supplied in daily supplements designed to meet or exceed recommended allowances or the safe and adequate intakes, as described previously (8). Choline supplements providing 500 mg/d were also given to subjects.

Tracer studies

Experiment 1

On the morning of day 7 of the protein-free-diet period, subjects were admitted to the infusion room of the MIT Clinical Research Center after they had fasted for 12 h overnight. As
described in greater detail previously (2), an indwelling catheter was inserted in retrograde direction into a dorsal hand or low forearm vein and a second catheter was inserted into the antecubital vein of the same arm. The hand was then placed in a heating box for purposes of sampling arterialized venous blood. After blood and breath samples were collected from subjects to measure background isotopic enrichments, subjects received intravenous priming doses of \([^{13}C]\)bicarbonate (0.8 μmol/kg; MassTrace, Woburn, MA) and either \(t-[^{13}C]\), methyl-[\(^3\)H]methionine (2.0 μmol/kg; MassTrace) and \(t-[3,3-\text{H}_2]\)cysteine (1.5 μmol/kg; CIL, Andover, MA) in group A or \(t-[^{13}C]\)cysteine (1.5 μmol/kg; MassTrace) in group B. Then the labeled methionine (2.0 μmol·kg\(^{-1}\)·h\(^{-1}\)) and [\(^3\)H\(_2\)]cysteine or [\(^1\)C]cysteine (both 1.5 μmol·kg\(^{-1}\)·h\(^{-1}\)) were infused continuously throughout the 8-h experiment in groups A and B, respectively.

During the first 3 h of each tracer study, the subjects continued to fast. Throughout the next 5 h (fed phase), every 30 min they received small, isonenergetic meals, each of which supplied one–twenty-fourth of the daily intake.

**Experiment 2**

The tracer protocol followed on day 7 of each of the 3 diet periods was essentially the same as for experiment 1, except that we gave only [\(^{13}\)C]cysteine in place of the specific methionine and cysteine tracers used in experiment 1 for groups A and B. After an intravenous priming dose (1.5 μmol/kg), it was infused at a constant rate of 1.5 μmol·kg\(^{-1}\)·h\(^{-1}\).

**Blood and expired air samples**

Blood and breath samples were collected every 15 min during the last hour of each metabolic phase (fasting and fed states). Blood was collected in chilled tubes with heparin and was then immediately centrifuged (15 min at 1200 \(\times\) g at 4 °C); the plasma was stored at \(-20^\circ\)C until analyzed. Breath samples were collected as described previously (9) and were stored at room temperature until analyzed by isotope ratio mass spectrometry (MAT Delta E; Finnigan, Bremen, Germany). Total carbon dioxide production and total oxygen utilization were measured by indirect calorimetry (DeltaTrak; Datex, Yorba Linda, CA) twice during each phase over a period of 30 min for each.

**Sample analysis**

We have previously described in detail the treatment of blood and expired air samples for determination of isotopic enrichment, measurement of total \(^{13}\)C\(_2\) production, and analysis of plasma free methionine and cysteine (1–3, 10). Briefly, \(N\)-methyl-\(N\)-(\(\text{tert}-\text{butyl-dimethylsilyl}\)) trifluoracetamide (Pierce Chemical Co, Rockford, IL) was used to form the \(\text{tert}-\text{butyl-dimethylsilyl}\) derivative of these amino acids. Ethanethiol was also used in the derivatization mixture to convert cysteine to cystine and to serve as an antioxidant. Also, note that the cysteine bound to protein and dipeptides would not be recovered in this assay because the ethanethiol was added after the free amino acids had been extracted from the plasma. Therefore, the cysteine isotope enrichments reflect the combined free cysteine and cystine in plasma (i.e., total free plasma cysteine). This point has been discussed in greater detail in our previous papers on cysteine kinetics (1, 4).

Isotopic enrichments were measured by using a gas chromatograph and mass spectrometer (HP 5890 Series II and Hewlett Packard 5988A, respectively; Hewlett Packard, Palo Alto, CA). Methionine, \([^{13}\text{C}]\)methionine, and \([^{13}\text{C}]\), methyl-[\(^3\)H]methionine were monitored at m/z 320, 321, and 324, respectively. Cysteine, \([^{13}\text{C}]\)cysteine, and [\(^3\)H\(_2\)]cysteine were monitored at m/z 406, 407, and 408, respectively. The isotopic enrichment of the experimental samples was determined by multivariate spectral deconvolution (11) by using the observed abundances of known tracer and tracee combinations from 0 to 0.1 mol ratio as standards. The validation standards were analyzed before and after each set of unknowns to adjust for variations in instrument response. In this study, the tert-butyldimethylsilyl derivatization approach afforded an average accuracy error and intersample precision of <7% for each. All plasma enrichment values reported here are expressed as a molar ratio (%) above baseline (11).

**Whole-body kinetics**

**Methionine**

Methionine carboxyl (\(Q_c\)) and methyl (\(Q_m\)) flux rates, when specifically referring to measurements with the \([^{13}\text{C}]\)carboxyl and [\(^3\)H\(_2\)]methyl tracers, respectively, were calculated as described previously (10). Briefly, \(Q_c\) and \(Q_m\) were calculated as follows:

\[ Q_c = I_c \times [E_c \times 1/(E_c + E_s \times 0.8) - 1] \]  
\[ Q_m = I_m \times [E_m \times 1/(E_c + E_s \times 0.8) - 1] \]

where \(I_c\) and \(I_m\) are the infusion rate (μmol·kg\(^{-1}\)·h\(^{-1}\)) and the enrichment of the tracer \([^{13}\text{C}]\), methyl-[\(^3\)H]methionine. The term \((E_c + E_s \times 0.8)\) is the total enrichment of methionine when assuming that the intracellular enrichment of the parent tracer is 80% of the plasma enrichment (see below). \(E_c\) and \(E_s\) are the plateau plasma enrichments of methionine at \(m+1\) ([\(^{13}\text{C}]\)methionine) and \(m+4\) ([\(^{13}\text{C}]\), methyl-[\(^3\)H]methionine), respectively, where \(m\) is the nominal integer mass of the tracer ion. The enrichment of the infused tracer provides an estimate of the flux of the methyl moiety of methionine (\(Q_m\)) and the combined enrichment of the \(m+1\) and \(m+4\) species allows an estimate of the methionine-carboxyl flux and of the rate of transsulfuration.

We have argued previously (1) on the basis of the available evidence (12–14) that the alternative or transaminative pathway of methionine oxidation is not quantitatively significant in healthy volunteers. Therefore, the transsulfuration rate (methionine oxidation) was calculated as follows:

\[ \text{Transsulfuration rate} = \left( \frac{\Delta V_{\text{CO}_2} \times E_{^{13}\text{C}}} {V_{\text{CO}_2}} \times 1/R \right) \times \left( \frac{1}{E_q + (E_s \times 0.8)} \right) \]

where \(\Delta V_{\text{CO}_2}\) is the rate of carbon dioxide production in μmol·kg\(^{-1}\)·h\(^{-1}\), \(E_{^{13}\text{C}}\) is the enrichment of \([^{13}\text{C}]\)bicarbonate in expired air, and \(R\) is the bicarbonate recovery factor. Some of the \([^{13}\text{C}]\)bicarbonate was estimated to be 70% for the postabsorptive state and 82% for the fed state on the basis of our short-term bicarbonate infusion studies (15). Hence, the factor \(R\) used to correct our \([^{13}\text{C}]\)bicarbonate enrichment data in breath samples for the calculations of methionine and cysteine oxidation was 0.7 and 0.82 for fasting and fed conditions, respectively.

**Cysteine**

Cysteine flux rate (\(Q_{\text{cys}}\)) was calculated as for methionine flux:

\[ Q_{\text{cys}} = I_u \times [(E_s/E_{\text{cys}}) - 1] \]
the plateau plasma enrichment of $[13\text{C}]$- or $[2\text{H}_2]$cysteine tracer. The rate of cysteine oxidation was calculated as for methionine transsulfuration:

$$\text{Ox}_\text{cys} = (\delta CO_2 \times E\text{cys} \times 1/R) \times 1/E\text{cys} \quad (5)$$

As in previous methionine studies, a correction factor was used above to account for a likely plasma intracellular gradient in the methionine tracer enrichment. In the past, we have assumed that the intracellular enrichment of tracer methionine is 80% of the measured plasma enrichment of the relevant labeled species (1, 10). When we used this correction factor in our earlier studies on methionine kinetics (10), we found that it permitted a determination of methionine oxidation that was consistent with the rate anticipated for a generous methionine intake, where an equilibrium could be expected for body methionine balance.

For these initial cysteine oxidation studies, we have not made any assumptions about a possible difference between the $13\text{C}$ enrichment of the cysteine in the plasma compartment and that of the pool that is undergoing oxidation. The liberation of the $13\text{C}$ label from cysteine may or may not involve the intermediate formation of cysteinsulfinic acid (16) that, in turn, may be decarboxylated or transaminated with formation of pyruvate. As discussed below, the approach we have taken here appears appropriate for studies of cysteine oxidation.

**Estimates of sulfur amino acid balance**

Daily body sulfur amino acid kinetic balance (SAAB) in experiment 2 was determined to be the sum of the 12-h fasting SAAB (FaB) and the 12-h fed SAAB (FeB):

$$\text{SAAB} = \text{FaB} + \text{FeB} \quad (6)$$

FaB and FeB were calculated as follows:

$$\text{FaB} = (i - \text{Ox}) \times 12 + \text{tracer prime} \quad (7)$$

$$\text{FeB} = (i + \text{diet} - \text{Ox}) \times 12 \quad (8)$$

where $i$ is the constant infusion rate of cysteine (µmol·kg$^{-1}$·h$^{-1}$), Ox is the oxidation rate of cysteine, and diet is the methionine and cystine intake (µmol·kg$^{-1}$·h$^{-1}$).

In arriving at this estimate of balance, the assumption is made that the measurement of cysteine oxidation reflects the mean rate for each 12-h period (fasting or fed). From studies with L-leucine (17) and perhaps also phenylalanine (18, 19), this may be a reasonable assumption, although it should be recognized that the overall temporal 24-h pattern of amino acid oxidation (presumably including that for cysteine) may depend on the adequacy of dietary SAA intake. In their recent study of leucine oxidation in young adult and elderly subjects, Fereday et al (20) made similar assumptions to arrive at estimates of daily leucine balance and, by extrapolation, estimates of protein requirements. In addition, for the feeding conditions used here we have assumed that there was complete absorption of the methionine and cystine. However, if this was not the case or if the absorbed cystine did not mix with the intravenously administered labeled cysteine tracer, then it is possible that the actual rate of cysteine oxidation might have been somewhat higher than we calculated and that the intake of SAA has been overestimated. If this is the case, the values reported below for SAAB may be somewhat more positive or somewhat less negative than they actually should be. To further interpret the $13\text{C}$-derived estimates of whole-body cysteine oxidation, we predicted rates of cysteine oxidation with the assumption that the concentrations of methionine and cysteine in the mixed proteins are 120 and 206 mol/g protein, respectively (21).

**Statistical methods**

Data are presented as means ± SDs. For experiment 1, parameters were compared between the fasting and fed states by using a paired $t$ test. Plasma cysteine enrichment, $VCO_2$, and cystine flux were analyzed by using mixed models analysis of variance (ANOVA) with group as a between-subjects factor and metabolic condition as a within-subjects factor. For experiment 2, each parameter was analyzed by using mixed-models ANOVA with both diet and metabolic condition as within-subjects factors. For ANOVA results, interactions were reported if they were significant and were followed up with contrasts for the relevant pairwise comparisons; otherwise, significant main effects were reported and contrasts for differences between diets were examined as appropriate. All analyses were performed with SAS version 6.12 (SAS Institute, Inc, Cary, NC).

**RESULTS**

The isotopic enrichments of plasma methionine, cysteine, or both and of $13\text{C}$ in expired air during the fasting and fed periods reached a relatively steady state, as shown in Figure 1 for experiment 1 and in Figure 2 for experiment 2. Group mean values for the fasting and fed periods in experiments 1 and 2 are summarized in Tables 3 and 4, respectively.

**Experiment 1: protein-free diet**

On the basis of the enrichments of plasma methionine and cysteine and the $13\text{CO}_2$ output (Table 3), we estimated the fluxes of methionine and cysteine as well as methionine and cysteine oxidation. These results are summarized in Table 5.

For group A, the carboxyl methionine flux (Qc) in the fasting state was $14 \mu$mol·kg$^{-1}$·h$^{-1}$ and was significantly higher than that in the fed state ($P < 0.05$, paired $t$ test). Cysteine flux was $37\mu$mol·kg$^{-1}$·h$^{-1}$ in the fasting and fed states, respectively ($P < 0.01$, ANOVA). In group B, the cysteine flux ($Qc[13\text{C}^1]$) was $48\mu$mol·kg$^{-1}$·h$^{-1}$ for the fasting and fed states, respectively ($P < 0.01$, ANOVA). These values were higher ($P < 0.01$, ANOVA) than those obtained in group A with the deuterium tracer. The fasting-state methionine flux, under this condition of a protein-free diet, reflects the breakdown of body protein. Hence, we can calculate that this is equivalent to $3\text{ g protein}·\text{kg}^{-1}·\text{d}^{-1}$. The cysteine flux reflects cysteine entering the pool from protein turnover, its formation from methionine (a relatively small fraction, 4–5%, of the cysteine flux), and the turnover of circulating glutathione, as shown previously (22). Therefore, we cannot use these cysteine fluxes to compute protein turnover.

For the protein-free diet condition, methionine oxidation (extrapolated to 24 h) was $41 \mu$mol·kg$^{-1}$·d$^{-1}$ and cysteine oxidation was $125 \mu$mol·kg$^{-1}$·d$^{-1}$. These values compare well with predicted oxidation rates of $40\mu$mol·kg$^{-1}$·d$^{-1}$, respectively.

**Experiment 2: methionine and cystine intakes**

A summary of the main data used to assess the kinetic status of plasma $[13\text{C}]$cysteine metabolism is given in Table 6 for the 3 experimental diets. The output of expired carbon dioxide was not different among the 3 diet groups and increased ($P < 0.001$, ANOV A) with the deuterium tracer. The fasting-state methionine flux, $VCO_2$, and cystine flux were analyzed by using mixed-models ANOVA with both diet and metabolic condition as within-subjects factors. For ANOVA results, interactions were reported if they were significant and were followed up with contrasts for the relevant pairwise comparisons; otherwise, significant main effects were reported and contrasts for differences between diets were examined as appropriate. All analyses were performed with SAS version 6.12 (SAS Institute, Inc, Cary, NC).
ANNOVA) with the feeding of small meals. The enrichment of plasma cysteine was also higher in the fed than in the fasting state (\(P < 0.01, \text{ANOVA}\)) across all diet groups.

Plasma cysteine flux was consistently reduced with feeding (\(P < 0.01\)) across all diets and was higher for the high-cystine (MC) diet (\(P < 0.05\); Table 5). In comparison with the diets adequate in methionine (HM) and high in cysteine (MC), the daily rate of cysteine oxidation was lowest when the diet low in methionine and free of cystine (LM) was given (\(P < 0.001\) and \(P < 0.05\), respectively) (Tables 5 and 6).

We calculated the daily total SAAB by using cysteine oxidation as an index of the combined methionine and cysteine sulfur loss from the SAA pool. The input was the sum of total SAA intake via diet and tracer and the output was the cysteine oxidation. These balance values are summarized in Table 7.

For diet HM, the calculated daily balance was less negative than for diets LM and MC (interaction, \(P = 0.034\)). This was essentially due to the higher positive balance achieved in the fed state for diet HM (42.2 \(\mu\)mol·kg\(^{-1}\)·12 h\(^{-1}\)) than for diet LM (9.0 \(\mu\)mol·kg\(^{-1}\)·12 h\(^{-1}\), \(P < 0.01\)) and diet MC (13.9 \(\mu\)mol·kg\(^{-1}\)·12 h\(^{-1}\), \(P < 0.05\)). There were no significant differences in balance between diet HM and diets LM and MC for the fasting state (-43.6 compared with -34.5 and -61.6 \(\mu\)mol·kg\(^{-1}\)·12 h\(^{-1}\), respectively). Finally, there was no indication of a sparing effect of dietary cystine on overall SAAB for the amount of dietary methionine intake evaluated in this experiment.

**DISCUSSION**

The main objective of this investigation was to explore the whole-body kinetics of cysteine by using [1-\(^{13}\)C]cysteine as a tracer, with particular reference to our earlier conclusions that dietary cystine has little detectable sparing effect on the methionine requirement in healthy young adults (1, 2); in contrast, there may be a small sparing effect of cystine in elderly subjects (4). Furthermore, we wished to confirm our earlier theory (5) that a daily intake of methionine that was about half of the total SAA requirement proposed in the 1985 FAO/WHO/UNU report, when either consumed as the sole source of SAAs or with a significant cystine intake, would not be sufficient to maintain body methionine balance, as estimated by the \(^{13}\)C-tracer balance approach (1, 2).

Because there are no published \(^{13}\)C-tracer estimates of whole-body cysteine oxidation in healthy adults, we conducted experiment 2 to assess whether our approach for estimating whole-body cysteine oxidation (at various SAA intakes) would yield reasonable values. For experiment 1, our objectives were to estimate the rate of cysteine oxidation while subjects consumed a protein-free diet and to compare this with the predicted total loss...
(oxidation) of this SAA that could be derived from the amount of 
obligatory nitrogen loss (54 mg N·kg\(^{-2}\)·d\(^{-1}\)) and an assumed 
composition of mixed body proteins (6, 21).

Thus, we predict that while consuming a protein-free diet, 
the population mean total endogenous SAA loss would be 
\(< 110 \mu\text{mol}·\text{kg}^{-1}·\text{d}^{-1}\). By using the rate of cysteine oxidation 
that we measured during the 8-h tracer study and extrapolating it 
to a 24-h day, we estimated a mean value of 125 \(\mu\text{mol}·\text{kg}^{-1}·\text{d}^{-1}\) 
for whole-body cysteine oxidation. Hence, there is relatively 
good agreement between these different estimates. On this basis, 
it appears that our approach for determination of whole-body 
cysteine oxidation is satisfactory. Had we included an 80% 
correction factor, as for methionine, then the rate of cysteine 
oxidation would have been \(< 50% \) higher than the prediction.

Considering this, taken together with our calculations of body 
balance in experiment 2 (Table 7), it seems that a correction of 
this magnitude is not appropriate for estimating whole-body cysteine oxidation.

We can also conclude from Table 4 that the measured rate of 
methionine oxidation was quite close to that predicted from 
obligatory nitrogen losses. The extrapolated 24-h rate of 
methionine oxidation was 41 \(\mu\text{mol}·\text{kg}^{-1}·\text{d}^{-1}\), whereas the predicted loss is 40 \(\mu\text{mol}·\text{kg}^{-1}·\text{d}^{-1}\).

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasting</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma methionine enrichment (mol ratio %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([1-\text{13}C, \text{methyl}-\text{2H}_3]\text{methionine} ]</td>
<td>12.4 ± 2.2</td>
<td>13.3 ± 1.4</td>
</tr>
<tr>
<td>([1-\text{13}C]\text{methionine} ]</td>
<td>3.4 ± 0.8</td>
<td>5.7 ± 0.9(^2)</td>
</tr>
<tr>
<td>Plasma ([3,3-\text{2H}_2]\text{cysteine enrichment (mol ratio %)})</td>
<td>3.8 ± 0.3</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Breath (^{13}\text{CO}_2) enrichment (APE × 10(^3))(^4)</td>
<td>2.1 ± 0.4</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>(\text{VCO}_2 (\mu\text{mol}·\text{kg}^{-1}·\text{h}^{-1}))</td>
<td>7.2 ± 0.8</td>
<td>9.3 ± 0.7(^5)</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma ([\text{13}C]\text{cysteine enrichment (mol ratio %)})</td>
<td>3.1 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>Breath (^{13}\text{CO}_2) enrichment (APE × 10(^3))(^6)</td>
<td>1.5 ± 0.5</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>(\text{VCO}_2 (\mu\text{mol}·\text{kg}^{-1}·\text{h}^{-1}))</td>
<td>7.0 ± 0.6</td>
<td>9.5 ± 1.1(^5)</td>
</tr>
</tbody>
</table>

\(^1\)± SD; \(n = 6.\) APE, atom percent excess.

\(^2\)Significantly different from fasting, \(P < 0.01\) (paired \(t\) test).

\(^3\)Significantly different between groups, \(P < 0.05\), and between fasting and fed states, \(P < 0.01\) (both by ANOVA).

\(^4\)Significantly different between groups and between fasting and fed states, both \(P < 0.01\) (ANOVA).

\(^5\)Significantly different from fasting, \(P < 0.001\) (ANOVA).
Cysteine kinetics in young adults with different intakes of methionine and cystine (experiment 2)\(^1\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HM (n = 6)</th>
<th>Fasting</th>
<th>Fed</th>
<th>LM (n = 5)</th>
<th>Fasting</th>
<th>Fed</th>
<th>MC (n = 5)</th>
<th>Fasting</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{VCO}_2) ((\mu\text{mol·kg}^{-1}·\text{h}^{-1}))</td>
<td>7.7 ± 0.7</td>
<td>9.9 ± 0.8</td>
<td></td>
<td>7.0 ± 0.6</td>
<td>9.4 ± 0.9</td>
<td></td>
<td>7.7 ± 1.5</td>
<td>9.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Breath (^{13}\text{C}) enrichment ((\text{APE}×10^3))</td>
<td>1.5 ± 0.4</td>
<td>1.9 ± 0.2</td>
<td></td>
<td>1.5 ± 0.4</td>
<td>1.3 ± 0.6</td>
<td></td>
<td>1.5 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Plasma ([^{13}\text{C}]\text{cysteine enrichment (mol ratio %)})</td>
<td>3.2 ± 0.6</td>
<td>4.5 ± 0.3</td>
<td></td>
<td>3.3 ± 0.3</td>
<td>4.7 ± 0.6</td>
<td></td>
<td>3.1 ± 1.9</td>
<td>3.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Cysteine flux ((\mu\text{mol·kg}^{-1}·\text{h}^{-1}))</td>
<td>47.2 ± 11.0</td>
<td>31.9 ± 2.1</td>
<td></td>
<td>44.2 ± 4.4</td>
<td>30.6 ± 3.8</td>
<td></td>
<td>59.8 ± 30.5</td>
<td>46.2 ± 19.3</td>
<td></td>
</tr>
<tr>
<td>Cysteine oxidation ((\mu\text{mol·kg}^{-1}·\text{h}^{-1}))</td>
<td>5.3 ± 1.4</td>
<td>5.2 ± 0.74</td>
<td></td>
<td>4.5 ± 1.2</td>
<td>3.5 ± 1.9</td>
<td></td>
<td>6.8 ± 3.4</td>
<td>6.4 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\bar{x} ± \text{SD}. \text{Data were analyzed by using mixed-models ANOVA. For all diets, rate of [\text{\(^{13}\text{C}\)}cysteine infusion was 1.5 \(\mu\text{mol·kg}^{-1}·\text{h}^{-1}\). APE, atom percent excess; HM, high methionine; LM, low methionine; MC, methionine plus cysteine; V\text{CO}_2, \text{carbon dioxide production.}\)

\(^2\)Significant main effect: fasting, \(P < 0.001\).

\(^3\)Significant main effect of diet for pairwise comparisons of HM and MC, and LM and MC, \(P < 0.05\).

\(^4\)Significant main effect of diet, \(P < 0.01\). Because no differences were detected between fasting and fed rates of oxidation, pairwise comparison was done regardless of metabolic phase: MC compared with LM, \(P > 0.001\); HM compared with LM, \(P < 0.05\).

The agreement between the measured and predicted methionine or total SAA (methionine plus cystine) losses implies that we have not substantially overestimated their rates of oxidation by using the \(^{13}\text{C}\)-tracer technique. However, we may have underestimated the rates of endogenous methionine and cysteine oxidation because these comparisons were made under conditions of a significant input of tracer during the 8-h infusion period. In the case of cysteine, this amounted to an input of 13 \(\mu\text{mol/kg}\) over the tracer period, whereas for methionine the input was 16 \(\mu\text{mol/kg}\). However, whether these amounts should be used to correct the daily estimates of methionine and cysteine oxidation is unclear. It seems likely that there was retention of tracer methionine and cysteine and possibly even a sparing effect of cystine on methionine oxidation during this protein-free, SAA-free diet condition. This situation is reminiscent of the response in rats, in terms of growth or nitrogen balance, when a protein-free diet was supplemented with methionine (23).

In experiment 1, we obtained different estimates for cysteine flux in the 2 groups; results based on the deuterium tracer were lower than those obtained with the \(^{13}\text{C}\)-tracer. The reason for this finding is unclear, because both estimates fall within the range of values that we obtained previously with the deuterium tracer (1, 4). We do not believe that our findings were due to an analytic problem or necessarily to an isotope effect of the kind we reported for deuterated phenylalanine (24) and that others noted for various \(^2\text{H}\)-labeled compounds (25–27). However, to rule out or accept this latter possibility, it would be desirable to explore simultaneously the effects of bolus doses of the 2 cysteine tracers in healthy adults under conditions similar to our investigations reported here. Our current conclusions, however, are not influenced by this particular issue.

The results obtained in experiment 2 are in accordance with our previous tracer experiments (1, 2) in healthy adults, which failed to reveal a sparing effect of dietary cysteine on the methionine requirement under the experimental conditions tested. Furthermore, our findings indicate that at a methionine intake substantially below the 1985 FAO/WHO/UNU (6) requirement for methionine plus cystine, body SAA cannot be achieved. As shown in Table 6, the addition of an extra \(\approx 40\) \(\mu\text{mol}\) dietary cysteine (given as cystine) per day to the LM diet increased cysteine oxidation by a somewhat greater extent than expected; the mean difference in daily oxidation between diets LM and MC amounted to 62 \(\mu\text{mol}\). Although all subjects had higher rates of cysteine oxidation, there was wide interindividual variation in response to cysteine supplementation. However, it is evident from this experiment that we failed to observe a significant sparing of the methionine requirement. This is consistent with our previous methionine-tracer studies (1, 2, 4).

Nevertheless, this observation does not refute the elegant biochemical findings reported by Finkelstein (28, 29) on the extent and mechanism of methionine sparing by cystine in rats, and, as has been pointed out in a recent editorial by Finkelstein (30) in reference to our study in elderly subjects (4). It merely serves to emphasize, again in agreement with Finkelstein (30), the complexity of tissue and interorgan methionine metabolism as well as the interactions between numerous dietary variables and the integrated response of the whole body.

### TABLE 4

Methionine and cysteine kinetics in young adults consuming a protein-free diet (experiment 1)\(^1\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasting</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine flux ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>19.2 ± 5.4</td>
<td>17.1 ± 2.6</td>
</tr>
<tr>
<td>Cysteine flux ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>13.5 ± 3.4</td>
<td>10.3 ± 1.5</td>
</tr>
<tr>
<td>Methionine oxidation ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>36.5 ± 3.2</td>
<td>27.7 ± 3.2</td>
</tr>
<tr>
<td>Cysteine oxidation ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>1.7 ± 0.5</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine flux ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>47.8 ± 6.1</td>
<td>34.4 ± 6.7</td>
</tr>
<tr>
<td>Cysteine flux ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>5.0 ± 1.8</td>
<td>5.4 ± 2.1</td>
</tr>
<tr>
<td>Cysteine oxidation ((\mu\text{mol·kg}^{-1}·\text{d}^{-1}))</td>
<td>125 ± 31</td>
<td>110</td>
</tr>
</tbody>
</table>

\(^1\bar{x} ± \text{SD}. \text{Infusion rates (\(\mu\text{mol·kg}^{-1}·\text{h}^{-1}\)) for group A were 2.0 for [\text{\(^{13}\text{C}·\text{methyl}\)}·\text{\(^2\text{H}\)}]methionine and 1.5 for [3,3-\text{\(^2\text{H}\)}]cysteine; for group B: 1.5 for [\text{\(^{13}\text{C}\)}]cysteine. n = 6.\)

\(^2\)Significantly different from fasting, \(P < 0.05\) (paired t test).

\(^3\)Cysteine fluxes were compared by using ANOVA (interaction, \(P < 0.01\)); pairwise differences were found between groups and between fasting and fed (\(P < 0.01\) for all).

\(^4\)Values in parentheses are the predicted daily oxidation rates based on nitrogen excretion data.
Table 6
Rates of cysteine oxidation for fasting and fed periods and daily for 6 subjects receiving different intakes of methionine and cysteine (experiment 2)¹

| Subject no. | HM (n = 6) | | | LM (n = 5) | | | MC (n = 5) | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|
|            | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           |
| 1          | 48.6       | 56.0       | 104.5      | 31.7       | 13.7       | 45.4       | 32.0       | 69.5       | 101.5      |
| 2          | 76.4       | 56.0       | 132.5      | 56.5       | 24.7       | 81.3       | 66.1       | 37.5       | 103.6      |
| 3          | 71.9       | 58.0       | 129.9      | 49.3       | 44.8       | 94.1       | 63.6       | 53.8       | 117.3      |
| 4          | 81.0       | 77.7       | 158.7      | 69.0       | 63.9       | 132.9      | 110.2      | 95.8       | 260.6      |
| 5          | 63.1       | 68.9       | 132.0      | 63.3       | 65.3       | 128.6      | 133.5      | 130.3      | 263.8      |
| 6          | 37.6       | 60.4       | 98.0       | 14.5       | 23.1       | 36.1       | 40.4       | 36.6       | 73.0       |
| x          | 63.1       | 62.8       | 125.9²     | 54.0       | 42.5       | 96.4²      | 81.1       | 77.4       | 158.4²     |
| SD         | 16.9       | 8.7        | 21.9       | 14.5       | 23.1       | 36.1       | 40.4       | 36.6       | 73.0       |

¹HM, high methionine; LM, low methionine; MC, methionine plus cystine.
²Significant interaction between diet and fasting compared with fed state, P = 0.034 (ANOVA). The fed balance was higher for the HM diet when compared with the LM diet (P < 0.01) and MC diet (P < 0.05). In the fasting state, the HM diet was not different from either the LM diet or MC diet.

Table 7
Sulfur amino acid balance for fasting and fed periods and daily for 6 subjects receiving different intakes of methionine and cysteine (experiment 2)²

| Subject no. | HM (n = 6) | | | LM (n = 5) | | | MC (n = 5) | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|
|            | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           | µmol·kg⁻¹·12 h⁻¹ | µmol·kg⁻¹·d⁻¹ |           |
| 1          | −29.1      | 49.0       | 20.0       | −12.2      | 37.8       | 25.6       | −12.5      | 21.8       | 9.3        |
| 2          | −56.9      | 49.0       | −8.0       | −37.0      | 26.8       | −10.3      | −46.6      | 53.8       | 7.2        |
| 3          | −52.4      | 47.0       | −5.4       | −29.8      | 6.7        | −23.1      | −44.1      | 37.5       | −6.5       |
| 4          | −61.5      | 27.3       | −34.2      | −49.5      | −12.4      | −61.9      | −90.7      | −4.5       | −95.2      |
| 5          | −43.6      | 36.1       | −7.5       | −43.8      | −13.8      | −57.6      | −114.0     | −39.0      | −153.0     |
| 6          | −18.1      | 44.6       | 26.5       | −34.5      | 9.0        | −25.4²     | −61.6      | 13.9       | −47.6²     |
| x          | −43.6      | 42.2       | −1.4²      | −34.5      | 9.0        | −25.4²     | −61.6      | 13.9       | −47.6²     |
| SD         | 16.9       | 8.7        | 21.9       | 14.5       | 23.1       | 36.1       | 40.4       | 36.6       | 73.0       |

²Significant main effect of diet, P < 0.01. Because no differences were detected between fasting and fed rates of oxidation, pairwise comparison was done regardless of metabolic phase: MC compared with LM, P < 0.001; HM compared with LM, P < 0.05.
intake of methionine that is supplemented with a generous amount of cystine. Thus, our experiments failed to expose a major sparing effect of dietary cystine on the minimum requirement for methionine. Our data suggest that, to meet the SAA requirements of healthy adults, it would be prudent to 1) supply methionine at an intake that approaches, if not equals, the FAO/WHO/UNU requirement for total SAAs and 2) simultaneously supply a reasonable, although as yet undefined, amount of cystine because it might be used more effectively than methionine to maintain cysteine and glutathione homeostasis (33).

REFERENCES