Effect of cystine intake on methionine kinetics and oxidation determined with oral tracers of methionine and cysteine in healthy adults\textsuperscript{1–3}

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ABSTRACT There is evidence based on nitrogen balance that dietary cystine spares, from ~16\% to 89\%, the total methionine requirement. In a previous study we did not detect, by tracer techniques, a sparing effect of cystine when the diet provided methionine at a limiting intake (requirement level: 13 mg · kg\(^{-1}·d\(^{-1}\)). One reason could be that we used an intravenous infusion of the tracer, which may not, therefore, have labeled the carbon dioxide derived from the splanchnic oxidation of dietary methionine. The aim of this study was to compare methionine metabolism and oxidation in eight healthy adults given 6 d each of three different diets: 13 mg (87.0 \(\mu\) mol) methionine · kg\(^{-1}·d\(^{-1}\)) and no cystine (diet A); 5 mg (33.5 \(\mu\) mol) methionine · kg\(^{-1}·d\(^{-1}\)) and no cystine (diet B), and 5 mg (33.5 \(\mu\) mol) methionine · kg\(^{-1}·d\(^{-1}\)) and 6.5 mg (52.4 \(\mu\) mol) cysteine · kg\(^{-1}·d\(^{-1}\)) (diet C). On day 7, tracers ([\(^{13}\)C]methyl-\(^{2}\)H\(_5\)methionine and [\(^{3}\)H\(_2\)]cysteine) were administered orally at 30-min intervals for 8 h. Blood and breath samples were obtained for analysis during 3-h fasting and consecutive 5-h feeding periods. During fasting, methionine oxidation and methionine methyl (\(Q_m\)) and carboxyl (\(Q_c\)) fluxes and plasma concentrations were not affected by the amount of sulfur amino acids in the three diets. In the fed state methionine oxidation was significantly lower during diets B (3.0 \(\pm\) 0.5 \(\mu\) mol · kg\(^{-1}·h\(^{-1}\)) and C (2.8 \(\pm\) 0.6 \(\mu\) mol · kg\(^{-1}·h\(^{-1}\)) than during diet A (4.1 \(\pm\) 0.9 \(\mu\) mol · kg\(^{-1}·h\(^{-1}\))); there were no significant differences between diets B and C. \(Q_m\) and \(Q_c\) decreased with decreased methionine intake but no effect was observed by adding cystine. Cysteine flux (\(Q_{cysteine}\)) was not affected by diet composition but it was lower during feeding than during fasting. In conclusion, replacing ~60\% of the total requirement for methionine with cystine over a short diet period did not result in a detectable sparing of methionine oxidation. Am J Clin Nutr 1997;66:283–92.

KEY WORDS Methionine balance, sulfur amino acid requirement, cysteine, cystine, carboxyl flux, methyl flux, humans

INTRODUCTION

The current requirement for total sulfur amino acids (methionine plus cystine) in healthy adults, as proposed by the FAO/WHO/UNU (1), is 13 mg · kg\(^{-1}·d\(^{-1}\). This amount, which was derived from nitrogen balance data, represents the upper range of the requirement for healthy adults (2, 3).

As part of our continuing investigations into the requirements for indispensable amino acids of healthy adults we previously carried out a stable-isotope-tracer study to estimate the sulfur amino acid requirement, which we define as the minimum intake required to maintain body amino acid balance (intake = oxidation). Our previous study (4) suggested that a methionine intake of 13 mg · kg\(^{-1}·d\(^{-1}\) would be close to a mean requirement value when there is no dietary cystine intake.

There is evidence from various species (5–7), including humans (8), that dietary cystine acts to spare the requirement for methionine. The estimates of the maximum methionine-sparing effect of dietary cystine in humans vary widely, ranging from ~16\% to 89\% of the methionine requirement, when compared with a cystine-free diet (9). The mechanism for this effect may be via an inhibition of the activity of cystathionine synthase, which would effectively reduce the rate of methionine loss or oxidation (transsulfuration) because of an irreversible conversion of homocysteine to cystathionine (10). In addition, if cysteine supplementation has a sparing effect on methionine oxidation, regardless of the mechanism, we would be able to detect it by using [\(^{13}\)C]methionine and by measuring \(^{13}\)CO\(_2\) in breath.

We investigated several kinetic aspects of methionine-cysteine metabolism previously in human subjects (8, 11). Thus, the rate of methionine oxidization with a diet supplying adequate methionine but without cystine was compared with that measured with a sulfur amino acid-devoid diet or a diet supplying only cystine (8). Methionine oxidation was significantly reduced with both methionine-devoid diets, and after cysteine supplementation it further declined by ~50\%. Also, the rate of remethylation of homocysteine relative to its conversion.

\textsuperscript{1} From the Laboratory of Human Nutrition, School of Science, Massachusetts Institute of Technology, Cambridge, MA, and the Shriners Burns Institute, Boston.

\textsuperscript{2} Supported by NIH grants DK 15856, GM 02700, RR 88 (CRC core grant), and P-30-DK-40561, and grants from the Shriners Hospitals for Crippled Children, Ajinomoto USA, Inc, Teaneck, NJ, donated the amino acids used in this study.

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Received August 14, 1996, Accepted for publication March 4, 1997.
to cystathionine increased from 0.6% to 3.4% when cystine was added to the methionine-free diet. These results suggest a sparing effect of dietary cystine on methionine metabolism when dietary methionine intake is either very low or absent. Note that in this previous study the methionine intake supplied by the adequate-methionine, cystine-devoid diet was 25 mg · kg⁻¹ · d⁻¹, which exceeds minimal physiologic needs. In a subsequent study (11) we compared methionine oxidation and balance after a 6-d diet period during which the amount of methionine given was approximately the amount recommended by the FAO/WHO/UNU (1) —13 mg · kg⁻¹ · d⁻¹— without cystine, or after a 6-d diet period during which 6.5 mg methionine · kg⁻¹ · d⁻¹ plus 0, 5.2, 10.5, or 20.9 mg cystine · kg⁻¹ · d⁻¹ was given. Methionine oxidation declined after the lower methionine intake, but we did not observe a sparing effect of dietary cystine on methionine oxidation. A possible reason for the lack of an observed effect of dietary cystine on methionine oxidation in that dietary study may have been the route of tracer administration. The tracer model in that case involved intravenous infusions of labeled methionine and cystine. However, it is to be expected that a fraction of both dietary cystine and methionine is metabolized during its initial passage within the splanchnic region. Therefore, if the sparing effect of dietary cystine on methionine oxidation is exerted principally during this phase of methionine utilization, then the intravenous tracer model might have failed to detect an actual sparing effect of cystine. The hypothesis, then, is that the route of tracer administration could have an important effect on the results and this is supported by the finding that labeled methionine appears to be oxidized to a greater extent when it is given orally than when it is given intravenously (12), at least when dietary intakes of methionine are well above requirement levels. In addition, when cystine was added to a low-methionine diet in rats, it increased the activity of only one of the two enzymes involved in homocysteine remethylation—betaine–homocysteine methyltransferase (13). This enzyme is located mainly in the liver and is apparently the most important enzyme in contributing to homocysteine remethylation, at least in rodents (14). Finally, we reported previously that effects of dietary betaine on methionine kinetics were only detected when the methionine tracer was given orally rather than intravenously (12). Therefore, we carried out a study in eight healthy adults using an experimental approach similar to that used in our previous methionine-cystine diet and kinetic study (11), except that the labeled methionine and cystine tracers were given orally. Three l-amino acid–based diets were studied.

The study and the consent form were approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Advisory Committee of the MIT CRC. Informed consent was obtained from each volunteer and the volunteers were paid for their participation in the study.

Experimental design and diets

The study consisted of three 7-d experimental diet periods.

1) Diet A: 13 mg (87.0 μmol) methionine · kg⁻¹ · d⁻¹, an amount that we believe approximates or is slightly lower than the mean physiologic requirement in adults, and no cystine (4);

2) Diet B: 5 mg (33.5 μmol) methionine · kg⁻¹ · d⁻¹, which is 40% of the recommended dietary allowance (RDA; 15), and no cystine; and

3) Diet C: 5 mg (33.5 μmol) methionine · kg⁻¹ · d⁻¹ and 6.5 mg (52.4 μmol) cystine · kg⁻¹ · d⁻¹. This amount of cystine was added to raise the total intake of sulfur amino acids to equal that for diet A. We subsequently refer to diet A as an “adequate diet.”

During the first 6 d of each period, subjects were adjusted to the varying dietary intakes of methionine and cystine. Each subject received the three diets in random order and there was a break period of ≥ 1 wk between each dietary period. On day 7 of each period, an 8-h tracer-infusion experiment (3-h fast followed immediately by 5-h fed) was performed.

Three isenergetic meals were provided during the 6 d preceding each 8-h tracer experiment. Subjects consumed their breakfast and lunch at the CRC and were allowed to take their dinner out. During the initial 6 d of each diet period the subjects attended classes and lived in their college residences. Their daily energy intake remained constant—between 171 and 188 kJ/kg. The energy from lipid and carbohydrate was provided in the form of protein-free, wheat-starch cookies and a sherbet-based drink. Nitrogen (160 mg kg⁻¹ · d⁻¹) was supplied as an l-amino acid mixture. The amino acid mixture (Table 1) was similar to that used previously (11), supplying dispensable amino acids in amounts that we suggested previously to be sufficient to meet the mean requirement in healthy young adults (16) but higher than the requirements proposed by the FAO/WHO/UNU (1). Dispensable (nonessential) amino acids were adjusted to maintain a constant total nitrogen content for the three 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 enrichments of the expired carbon dioxide between the fasted and fed states, particularly during the tracer studies.

Vitamins and minerals were supplied as a daily supplement to meet or exceed the RDA or the safe and adequate intakes (15). Choline was added as a supplement to provide an intake of 500 mg/d.

Tracer studies

On the morning of day 7 of each diet period, subjects were admitted to the infusion room of the MIT CRC after having fasted overnight (12 h). An indwelling catheter was inserted in a retrograde direction into a dorsal hand or low forearm vein of the arm, which was placed in a heating box to sample arterialized venous blood. After blood and breath samples were

SUBJECTS AND METHODS

Subjects

Eight healthy volunteers, six males and two females (69.9 ± 9.0 kg body wt, 25.0 ± 3.6 y of age), participated in this study. The subjects were all from the Massachusetts Institute of Technology (MIT) student community and they gave a medical history and received a physical examination. In addition, blood and urine samples were collected for a biochemical and clinical screening conducted at the Clinical Research Center (CRC) laboratories.
TABLE 1
Composition of t-aminoc acid mixtures used to supply different methionine and cystine intakes

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>13.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.0</td>
<td>0.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>23.0</td>
<td>23.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>40.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>25.9</td>
<td>25.9</td>
<td>25.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Valine</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Histidine</td>
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<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>73.5</td>
<td>73.5</td>
<td>73.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>124.4</td>
<td>124.4</td>
<td>124.4</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>185.8</td>
<td>185.8</td>
<td>185.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>205.4</td>
<td>213.4</td>
<td>213.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>104.8</td>
<td>104.8</td>
<td>104.8</td>
</tr>
<tr>
<td>Proline</td>
<td>160.6</td>
<td>160.6</td>
<td>155.7</td>
</tr>
<tr>
<td>Serine</td>
<td>146.7</td>
<td>146.7</td>
<td>146.7</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>160.0</td>
<td>160.0</td>
<td>160.0</td>
</tr>
</tbody>
</table>

collected to measure background isotopic enrichments, oral priming doses of $^{13}$C bicarbonate (0.8 μmol/kg) (MassTracer, Woburn, MA), $^{1-13}$C methyl-3H$_2$ methionine (2 μmol/kg) (MassTracer), and L-$^{3}$3,3-2H$_2$ cysteine (1.5 μmol/kg) (CIL, Andover, MA) were given as a drink, with 30 mL water. Then, the labeled methionine (2 μmol⋅kg$^{-1}$⋅h$^{-1}$) and labeled cysteine (1.5 μmol⋅kg$^{-1}$⋅h$^{-1}$) were administered at 30-min intervals as oral boluses with 10–15 mL water throughout the 8-h experiment. During the first 3 h, subjects consumed no food (fasted phase); during the following 5 h they received, with the tracers, 10 small isonitrogenous meals supplying one-twelfth of daily intake per hour (fed phase).

Blood and expired air samples
Blood and breath samples were collected every 15 min during the last 90 min of the fasting phase and during the last 150 min of the feeding phase. Blood was collected in chilled, heparin-containing tubes and was centrifuged immediately at $1000 \times g$ for 10 min at 4°C; the plasma was stored at $-20°C$ until analyzed. Breath samples were collected as described previously (17) and stored at room temperature until analyzed by isotope ratio mass spectrometry (MAT Delta E; Finnigan, Bremen, Germany). Total carbon dioxide production and total carbon dioxide utilization were measured by indirect calorimetry (DeltaTrak; Datex, Yorba Linda, CA) twice during each phase over 30 min each.

Sample analysis
We described previously, in detail, treatment of blood and expired air samples for determination of isotopic enrichment, measurement of total $^{13}$CO$_2$ production, and analysis of plasma free methionine and cysteine (11). Briefly, N-methyl-N-(tert-butyl dimethylsilyl) trifluoroacetamide (Pierce Chemical Co, Rockford, IL) was used to form the tert-butyl dimethylsilyl derivative of these amino acids. Ethanethiol was also used in the derivatization mixture to convert cystine to cysteine and to serve as an antioxidant. Isotopic enrichments were measured by electron-impact ionization with gas chromatography–mass spectrometry (HP 5890 Series II and HP 5988A; Hewlett Packard, Palo Alto, CA). Methionine, $^{1-13}$C methionine, and $^{1-13}$C, methyl-2H$_3$ methionine were monitored at a mass-to-charge ratio ($m/z$) of 320, 321, and 324, respectively. Cysteine and [H$_2$] cysteine were monitored at $m/z$ 406 and 408, respectively. The isotopic enrichment of the experimental samples was determined by multivariate spectral deconvolution (18) by using the observed abundances of known tracer-tracer combinations, with molar ratios from 0 to 0.1 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-butyl dimethylsilyl derivatization approach afforded an average accuracy error and intersample precision of <7% each. All enrichment values reported here are expressed as a molar ratio (%) above baseline (18).

Whole-body kinetics
Flux (Q) rates of methionine carboxyl (Qc) and methyl (Qm) when specifically referring to measurements with the $^{13}$C [carboxyl and $^{3}$H$_2$] methyl tracers, respectively, were calculated as described previously (11). Briefly, however, Qc and Qm were calculated as follows:

\[
Q_m = I_m \times [(E_d/E_s) - 1] \tag{1}
\]

\[
Q_c = I_c \times [(E_d/E_s + E_d) - 1] \tag{2}
\]

where $I_m$ and $E_s$ are the infusion rate and the enrichment of the tracer $^{1-13}$C methyl-2H$_3$ methionine, respectively, and $E_d$ and $E_s$ are the plateau plasma enrichments of $^{1-13}$C methyl-2H$_3$ methionine and $^{1-13}$C, methyl-2H$_3$ methionine, respectively, from plasma samples obtained during the last hour of each metabolic phase.

Equations 3 and 4 below relate Qm and Qc rates to their individual components. These calculations and the assumptions involved were discussed previously (19). In steady state conditions, flux (Q) = the sum of inputs = the sum of outputs:

\[
Q_m = I + B + RM = S + TM \tag{3}
\]

\[
Q_c = I + B = S + TS \tag{4}
\]

where I is dietary intake, B is plasma methionine appearance via tissue protein breakdown, RM is methionine appearance from remethylation of homocysteine, S is methionine plasma disappearance via nonoxidative catabolism (assumed to be protein synthesis), TM is transmethylation (rate of conversion of methionine to homocysteine), and TS is transsulfuration, which we assume to be equivalent to methionine oxidation.

Thus, the following two equations can be derived from equations 3 and 4 as follows:

\[
RM = Q_m - Q_c \tag{5}
\]

\[
TM = RM + TS \tag{6}
\]

The TS rate was calculated as follows:

\[
TS = \dot{V}^{13}CO_2 \times [(11^{13}C]methionine pool enrichment) - (11^{13}C]methionine tracer enrichment)] \tag{7}
\]

where $\dot{V}^{13}CO_2$ is the rate of $^{13}$C output in expired air.
\[ Q_{\text{syn}} = I_t \times [(E_d/E_s) - 1] \]

where \( E_s \) is the plateau plasma enrichment of cysteine.

As in previous studies, a correction factor was used for the plasma intracellular gradient in the methionine and cysteine tracer enrichment. Hence, we assumed that the intracellular enrichment of tracer methionine and cysteine is 80% of the measured plasma enrichment of the relevant labeled species (19). We applied this correction in our earlier studies on sulfur amino acid kinetics (11, 19) and found that it permitted a determination of cysteine turnover and methionine oxidation that was consistent with the anticipated rate for a methionine intake that was generous and an equilibrium could be expected for methionine balance.

Some of the \( ^{13}\text{C} \) label of methionine that is liberated during the oxidative decarboxylation of \( \alpha\text{-ketobutyric acid as } ^{13}\text{CO}_2 \) is retained by the body and it is necessary to correct for this retention. The recovery of \( ^{13}\text{C} \) in breath after an intragastric infusion of \( [^{13}\text{C}]\text{bicarbonate was taken to be 74% for the postabsorptive state and 79% for the fed state on the basis of our previous short-term bicarbonate-infusion studies (20).} \)

These correction factors (ie, 74% and 79%) have been used to correct our \( ^{13}\text{C} \) enrichment data in breath samples for the calculations of methionine oxidation under fasted and fed conditions. Daily methionine balance was calculated as the difference between total 24-h methionine intake and total 24-h predicted oxidation as follows:

\[
\text{Balance} = \text{methionine intake (diet + tracer)} - [\text{oxidation during the third fasting hour} \times 12] - [\text{oxidation during the fifth feeding hour} \times 12]
\]

**Statistical methods**

Data were analyzed by two-way repeated-measures analysis of variance with a subject \( \times \) diet \( \times \) metabolic condition factorial design. Dependent variables were methionine and cysteine kinetics and independent variables (repeated measures) were diet and metabolic state (fasted and fed). Significant differences between mean values for methionine and cysteine kinetics (flux and oxidation) among the diets within fasted and fed states were determined from one-way repeated-measures analysis of variance followed by pair-wise comparison among means by using the Neuman-Keuls test. Data are presented as means \( \pm \) SDs. An \( \alpha \) level \(<0.05 \) was considered statistically significant. All statistical analyses were run by using SAS software (SAS Institute Inc. Cary, NC).

**RESULTS**

Plasma methionine concentrations were not significantly different in the fasted state among the three diet groups (23.4 \( \pm \) 3.0, 21.6 \( \pm \) 3.3, and 22.3 \( \pm \) 1.5 \( \mu\text{mol/L} \) with diets A, B, and C, respectively; NS). During feeding, the plasma methionine concentration was significantly different from that after fasting: it increased by feeding with diet A (26.1 \( \pm \) 2.0 \( \mu\text{mol/L}; \) \( P < 0.05 \) compared with fasting) and decreased with diets B (19.2 \( \pm \) 3.4 \( \mu\text{mol/L} \) and C (18.7 \( \pm \) 2.7 \( \mu\text{mol/L} \) (\( P < 0.05 \) compared with fasting).

**Methionine and cysteine kinetics**

Plasma enrichment of \([^{13}\text{C} \text{ methionine and } [^{13}\text{C}]\text{cysteine are shown in Figure 1 and that of } [^{13}\text{H}]\text{cysteine in Figure 2.} ^{13}\text{CO}_2 \text{ enrichment in breath is shown in Figure 3.} \)

For the fasted state, plasma \( Q_s \) and \( Q_c \) were not significantly different during the three diet periods (Table 2). In the fed state, \( Q_m \) and \( Q_c \) decreased with the reduction in methionine intake (Table 2). However, we did not find any further change after supplementation of the low-methionine diet with cystine.

The effects of reducing the methionine content of the diet, with and without cystine supplementation, on methionine oxidation are also summarized in Table 2. Methionine oxidation during the fasted state was similar across all diets and it decreased during the fed state when the low-methionine diets were given. For the adequate-methionine diet, the mean difference in methionine oxidation between the fasted and fed states was not different from zero. For both low-methionine diets the rate of methionine oxidation declined significantly in all eight subjects when meal feeding began (\( P < 0.05 \)).

An estimate of the daily methionine balance in these subjects was calculated as described in SUBJECTS AND METHODS. For the adequate-methionine diet, five subjects were estimated to be in negative methionine balance and two in low positive balance; the group mean value was \(-11.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) or \(-2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (not significantly different from zero). Hence, this was considered to be neutral body methionine balance and this value was anticipated because of our previous experience (4, 11). Methionine balances were estimated to be distinctively negative, amounting to \(-8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) for both low-methionine diets. These balance values are taken to be predictions of the entire 24-h period. However, note that although the approach used here to determine methionine balance is supported by 24-h tracer studies at grossly inadequate leucine intakes (21) but not at low phenylalanine intakes (22). Hence, it is possible that these estimates of daily methionine balance may not be entirely accurate for the two low-methionine diets but for near- and above-maintenance amino acid intakes this kind of extrapolation appears to be quite reasonable (17, 23).

The methionine transmethylation and homocysteine remethylation rates (Table 2) were estimated from the relative level of enrichments of the methionine tracer \([^{13}\text{C} \text{ methionine and } [^{13}\text{C}]\text{methionine metabolites in the peripheral plasma during the plateau periods for the fasted and fed states.} \)

The absolute remethylation rates were not significantly different between the fasted and fed states or among the three diet groups. This finding also applied to the absolute rate of transmethylation, except that it was significantly lower (\( P < 0.05 \) during the fed state for the low-methionine, cystine-supplemented di -

The relations among remethylation, transmethylation, and transsulfuration (oxidation) rates and methionine fluxes are summarized in Table 3. In the fed state, the ratios of remethylation to transsulfuration and transsulfuration to the rate of conversion of methionine to homocysteine (homocysteine lo-
DISCUSSION

The aim of the present study was to investigate the effect of dietary cystine on methionine kinetics and oxidation when a portion of the sulfur amino “minimum requirement” intake was provided by cystine. It is generally accepted that dietary cystine spares the irreversible loss of methionine and from nitrogen balance studies this sparing effect has been variably estimated to range from 16% to 90% of the requirement for methionine when determined in the absence of cystine. Rose and Wixom (24), from their nitrogen balance studies in three adult subjects, concluded that “l-cystine was capable of replacing 80 to 89 percent of the minimal methionine needs of adult man, as measured by the maintenance of nitrogen equilibrium.” However, interpretation of these experiments is complicated, as we discussed previously (25), because of the questionable nitrogen balance criterion used to estimate nutritional adequacy and also the experimental design followed by these investigators (24).

Similarly, from the nitrogen balance studies carried out by Reynolds et al. (2) in adult women it is difficult to judge the extent to which cystine actually spared the requirement for methionine, again because of the wide range of responses, problems in experimental design, and the criterion of nitrogen equilibrium chosen to assess adequacy of intake.

Therefore, we considered it valuable to further explore dietary methionine-cystine relations using a tracer approach to expand on the limited and variable nitrogen balance response data that currently serve as the basis for sulfur (methionine plus cystine) amino acid requirements in healthy adult humans (1). Hence, in a previous study (8) we showed that by adding a generous amount of cystine to a methionine-devoid diet, methionine oxidation during the fed state decreased significantly from \(-1.2 to -0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). This methionine-sparing effect of cystine, under conditions of a methionine-free intake, is reminiscent of the improvement in nitrogen balance that was observed by Rose and Wixom (24) when cystine was added to a sulfur amino acid–free diet given to one of the three subjects who participated in their study. Furthermore, this effect of cystine in the absence of methionine in the diet parallels the
sparing effect on body nitrogen loss when methionine is added to a protein-free diet in rats (26).

An important question is whether this sparing effect of cystine is exerted equally over the entire submaintenance-to-near maintenance methionine (or total sulfur amino acid) intake. Thus, in a later study we did not detect any sparing effect on methionine oxidation by replacing half of the minimum daily requirement for total methionine (13 mg·kg⁻¹·d⁻¹ in the absence of cystine) with different amounts of cystine (0–21 mg·kg⁻¹·d⁻¹) (11). Our interpretation of the differences found between our two tracer-methionine studies (8, 11) was that at a low intake of methionine, rather than at a methionine-devoid intake, the sparing effect of cystine is probably small. Moreover, the intravenous route of methionine tracer administration used in that study (11) might not have been the most appropriate technique to detect a limited effect of dietary cystine on whole-body methionine metabolism. This would be true, especially, if the nutritionally relevant interactions between methionine and cystine occurred in the splanchnic area during the first pass uptake of these amino acids. A further rationale for this hypothesis is that dietary cystine is almost quantitatively removed by the intestinal tissues, or within the splanchnic bed, in pigs (27) and it has also been shown to stimulate the activity of the enzyme betaine–homocysteine methyltransferase (13), which is one of two major homocysteine remethylation enzymes located in the liver (14). Consequently, in the present study we chose to administer the tracers orally either by adding them to the amino acid mixture during feeding or to small drinks of water during the fasting period.

Our findings did not indicate any major effect of cystine on the irreversible loss of methionine when methionine intake is ~40% of the recommended amount (determined in the absence of dietary cystine). As in our previous study (11), we cannot rule out the possibility that at the low methionine intake the rate of cysteine tracer administration (1.5 μmol·kg⁻¹·h⁻¹)—equivalent to ~1 mg/kg for the 5-h fed period—resulted in a sufficient intake to trigger a cystine-sparing effect, limiting detection of any possible but small differences that might have been due to the dietary cystine supplement per se.

In contrast with our previous findings with leucine (17, 21) and lysine (28), the postabsorptive (fasting) rate of methionine oxidation was not affected by the prior dietary methionine intake. Thus, in the present study methionine oxidation was ~4 μmol·kg⁻¹·h⁻¹ for daily methionine intakes of ~87 μmol·kg⁻¹·d⁻¹ and ~34 μmol·kg⁻¹·d⁻¹. This oxidation rate is not significantly different from that found with gener-

**FIGURE 2.** Mean (± SE) cysteine enrichment in plasma during diets A, B, and C. Panel A: [³H₇]cysteine during the last 90 min of the fasting phase; panel B: [³H₇]cysteine during the last 150 min of the feeding phase.
ous, or supramaintenance, methionine intakes (8, 29) or in studies (11) of methionine intakes comparable with intakes supplied by diet A. Similarly, for the fasted state, $Q_m$ and $Q_c$ were not significantly different among the three diet groups and were similar to values reported for subjects receiving a generous methionine intake (8). A reasonable interpretation of these observations is that in the fasting state the dominant determinant of plasma methionine appearance is via tissue and organ protein breakdown rather than via remethylation of homocysteine or from methionine that is absorbed from the intestine. Additionally, plasma methionine concentrations did not differ significantly among the diet groups, suggesting that cellular methionine availability during this state was relatively constant and consequently the oxidation rate was maintained.

The fact that there was little response in methionine oxidation during the fasted state to a low methionine intake, compared with the response seen for leucine (17, 21), might help to explain the observations by Said and Hegsted (30). These investigators found in rats that the relation between the decrease in body water (or body nitrogen) and the decrease in specific amino acid intakes was described by a linear regression intersecting at $-40$ g water lost when there was no intake of methionine or cysteine (effect not different from that of a protein-free diet). However, the loss was only $-10$ g with other test amino acids such as leucine and phenylalanine. This suggests that in the fasting state methionine may be less efficiently retained by the body, especially at markedly restricted dietary methionine intakes compared with other indispensable amino acids.

During the fed state of each dietary period, methionine oxidation rates were generally similar to those reported previously by us at dietary methionine intakes comparable with those in the present study (11). Because the methionine tracer in the present study was given orally, and not intravenously as in previous experiments (4, 8, 11), it appears that the route of tracer administration did not affect the estimate of methionine oxidation. This contrasts with our previous findings (8) but they were obtained at a more generous methionine intake.

As also observed in our previous study (11), cysteine turnover was not affected by the composition of the diet. Additionally, the decline in $Q_\text{cysteine}$ with feeding also agreed with our finding in an earlier study in which an intravenous cysteine tracer was used (11). Recent studies in mice deficient in $\gamma$-glutamyl transpeptidase have shown that the catabolism of glutathione by $\gamma$-glutamyl transpeptidase is a major source of circulating cysteine (31), and we showed in humans that $\sim 50\%$ of
TABLE 2
Plasma and $^{13}$CO$_2$ enrichment, total carbon dioxide production, and methionine and cysteine kinetics in response to diets with different methionine and cystine contents in young adults.

<table>
<thead>
<tr>
<th></th>
<th>Diet A (87.0 μmol Met, no Cys)</th>
<th>Diet B (33.5 μmol Met, no Cys)</th>
<th>Diet C (33.5 μmol Met, 52.4 μmol Cys)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>Plasma methionine enrichment (% i)$^1$</td>
<td>9.2 ± 0.8</td>
<td>9.5 ± 0.5</td>
<td>9.1 ± 1.7</td>
</tr>
<tr>
<td>[1-[13]C, methyl-$^{2}$H$_4$Methionine]</td>
<td>4.1 ± 0.9</td>
<td>5.7 ± 1.6</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Plasma cysteine enrichment (% i)$^1$</td>
<td>2.4 ± 0.5</td>
<td>3.0 ± 0.5$^3$</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Breath $^{13}$CO$_2$ enrichment (APE × 10$^4$)</td>
<td>3.8 ± 1.0</td>
<td>4.2 ± 0.8</td>
<td>4.3 ± 1.2</td>
</tr>
<tr>
<td>VCO$_2$ (μmol·kg$^{-1}$·30 min$^{-1}$)</td>
<td>3.8 ± 0.4</td>
<td>4.8 ± 0.6$^3$</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Methionine flux</td>
<td>25.0 ± 2.8</td>
<td>24.1 ± 1.8</td>
<td>27.9 ± 6.2</td>
</tr>
<tr>
<td>Q$_m$ (μmol·kg$^{-1}$·h$^{-1}$)</td>
<td>16.7 ± 1.3</td>
<td>14.5 ± 2.4</td>
<td>18.6 ± 3.9</td>
</tr>
<tr>
<td>Methionine oxidation (μmol·kg$^{-1}$·h$^{-1}$)</td>
<td>4.2 ± 1.4</td>
<td>4.2 ± 0.9</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>Remethylation (μmol·kg$^{-1}$·h$^{-1}$)</td>
<td>8.4 ± 2.8</td>
<td>9.5 ± 1.8</td>
<td>9.2 ± 3.1</td>
</tr>
<tr>
<td>Transmethylation (μmol·kg$^{-1}$·h$^{-1}$)</td>
<td>12.6 ± 2.9</td>
<td>13.7 ± 2.1</td>
<td>13.5 ± 4.4</td>
</tr>
<tr>
<td>Cysteine flux (μmol·kg$^{-1}$·h$^{-1}$)</td>
<td>80.4 ± 18.2</td>
<td>61.8 ± 10.7$^3$</td>
<td>87.1 ± 46.7</td>
</tr>
</tbody>
</table>

$^1$ i ± SD; n = 8. Amounts of methionine and cystine in diets are per kg/d. VCO$_2$, carbon dioxide consumption; Q$_m$, methionine methyl flux; Q$_m$, methionine carboxyl flux; Cys, cystine; Met, methionine.

$^2$ Molar ratios.
$^3$ Significantly different from fed.
$^4$ P < 0.05.
$^5$ P < 0.01.
$^6$ Significantly different from diet A; P < 0.05.

plasma Q$_m$ appears to be determined by its release from the turnover of glutathione, with the remaining fraction being derived from protein breakdown and in the fed state from the diet (29). The suppressive effect of meals on Q$_m$ might be the result of either the decreased rate of protein breakdown that occurs in the fed state (32,33), the reduced rate of glutathione turnover, or both. This latter possibility requires investigation because glutathione synthesis is an important factor of absorbed cystine (34,35) as well as serving in turn as a significant source of plasma cysteine (31,36).

Finally, some comments are worth making about the approximations that might be made for the splanchic first-pass disappearance of methionine and cystine during the fasted and fed periods. Hence, using previous Q$_m$ data obtained with an intravenous administration of labeled cysteine (11) and calculated as described previously (37), we estimated that the first-pass disappearance of labeled cysteine during the fasted state was approximately 46% of the tracer intake and 44% during the fed state. This relatively high first-pass splanchic disappearance of cysteine is consistent with the findings made by Rerat et al (27) in pigs, who showed that dietary cystine was essentially, quantitatively removed from the lumen of the gut.

We can also make comparable approximations of the first-pass disappearance of tracer methionine, which we take to reflect the immediate metabolic fate of dietary methionine during the fed state. Thus, from our previous flux data, obtained at an equivalent dietary methionine intake (11), we calculated that the mean uptake of tracer methionine during the fast period was 15% for the adequate-methionine diet and 25% and 33% for the low-methionine, cystine-supplemented and nonsupplemented diets, respectively. We were not able to determine whether these values were significantly different because they were derived from comparisons of group mean data. It seems likely, however, that they represent reasonable mean approximations of the extent to which the tracer disappears between intestinal lumen and peripheral blood circulation during the fasted state. For the fed state, on the other hand, the mean estimates of the first-pass disappearance were close to zero: 1.4% for the adequate-methionine diet and 5% for the low-methionine, cystine-supplemented diet. Hence, it appears that there is little immediate net total loss of methionine within and by the splanchic region during, at the least, the initial part of the 12-h fed period of the 24-h d at a low to submaintenance methionine intake.

TABLE 3
Relations among methionine metabolic indexes in young adults given diets with different methionine contents.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>TS:TM</td>
<td>0.34 ± 0.11</td>
<td>0.31 ± 0.05</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>RM:TS</td>
<td>2.19 ± 1.06</td>
<td>2.37 ± 0.67</td>
<td>2.16 ± 0.39</td>
</tr>
<tr>
<td>TM:Q$_m$</td>
<td>0.48 ± 0.08</td>
<td>0.58 ± 0.05$^3$</td>
<td>0.50 ± 0.08</td>
</tr>
</tbody>
</table>

$^1$ i ± SD; n = 8. TS, transsulfuration rate (assumed to be equal to the methionine oxidation rate); RM, methionine appearance from remethylation of homocysteine; TM, rate of conversion of methionine to homocysteine; Q$_m$, methionine methyl flux.

$^2$ Significantly different from diet A; P < 0.05.

$^3$ Significantly different from fasted; P < 0.05.
The foregoing estimates of methionine first-pass uptake were based solely on measurement and comparison of the primary \( [1^{-13}C, \text{methyl}-^3H] \) methionine isotopomer. However, an intriguing finding worth mentioning concerns the appearance of the \( [1^{-13}C] \) methionine metabolite, when compared with that seen previously after intravenous administration of the tracer \( [1^{-13}C, \text{methyl}-^3H] \) methionine. Thus, although the present enrichment of \( [1^{-13}C, \text{methyl}-^3H] \) methionine in plasma was similar to that found by us previously (11) using an intravenous tracer administered at a similar rate, the enrichment of the derived methionine species \( ([1^{-13}C] \text{methionine}) \) was considerably higher in the present study during both the fasted and fed states. The remethylation and transmethylation rates were all computed to be higher in the present than in the previous study when an intravenous tracer was used (11). Thus, at a sub-to maintenance intake of methionine, it appears that the free methionine pool undergoes significant methyl group transfer and remethylation during passage of dietary methionine through the splanchnic region, with an equivalent of the fraction of dietary methionine involved in this metabolic activity being released from the liver into the circulation. The regulatory nature of the splanchnic metabolism and fate of limiting intakes of dietary methionine deserve further investigation.

In conclusion, a clear and significant sparing effect of dietary cystine on methionine metabolism was not revealed in this tracer study. Our findings point to a possibly quite small effect on methionine sparing by dietary cystine, in agreement with some earlier nitrogen balance studies (9). Methionine turnover and oxidation in the fasted state were not affected by dietary intake, whereas, in the fed state methionine turnover and oxidation decreased with restrictions in methionine intake. \( Q_{\text{cr}} \) was not affected by the change in dietary intake and this may have been due in part to a possible buffering effect of the glutathione pool on cysteine status and metabolism. For healthy subjects, a longer period of consumption of low sulfur amino acid diets or a pharmacologic depletion of glutathione (and therefore cysteine storage) might be needed to observe quantitatively significant interactions between dietary methionine and cystine intakes on the kinetics of methionine metabolism. Further, the desirability of developing tracer protocols to probe, in depth, cysteine metabolism, especially in relation to glutathione synthesis and turnover, is underscored by these observations.

We thank the MIT CRC nursing, dietary, and laboratory staff for their help in conducting these studies. The willingness and dedication of the subjects who volunteered for these studies is gratefully acknowledged.

REFERENCES


25. Young VR, Marchini JS. Mechanisms and nutritional significance of...


