Minimum methionine requirement and cysteine sparing of methionine in healthy school-age children

Mohammad A Humayun, Justine M Turner, Rajavel Elango, Mahroukh Rafii, Veronika Langos, Ronald O Ball, and Paul B Pencharz

ABSTRACT

Background: Cysteine can provide a portion of the sulfur amino acid requirement in adults. Whether this is true in children—and, if so, to what extent—is not known.

Objectives: The objectives were to determine minimum methionine requirements in healthy, school-age children when excess cysteine is provided and to subsequently determine the cysteine-sparing effect by comparing these methionine requirements with those determined previously in the same children when no cysteine was provided.

Design: Six healthy, school-age children randomly received graded intakes of methionine (0, 2.5, 5, 7.5, 10, and 15 mg·kg⁻¹·d⁻¹) along with 21 mg cysteine·kg⁻¹·d⁻¹ in the diet. The mean methionine requirement was determined by using a biphasic linear regression crossover analysis of measurements of the rate of appearance of ¹³CO₂ in the breath (F¹³CO₂), which identified a breakpoint at the minimal F¹³CO₂ in response to graded levels of methionine intake.

Results: The mean and population-safe minimum methionine requirements, in the presence of excess dietary cysteine, were found to be 5.8 and 7.3 mg·kg⁻¹·d⁻¹, respectively. The mean and population-safe (upper 95% CI) methionine requirements, in the absence of dietary cysteine, were previously determined to be 12.9 and 17.2 mg·kg⁻¹·d⁻¹, respectively. These values represent a cysteine-sparing effect of 55% and 58% in comparison with mean and population-safe methionine requirements, respectively.

Conclusion: Excess intake of dietary cysteine results in the reduction in the requirements for methionine to a minimum obligatory requirement level.

KEY WORDS Sulfur amino acid, minimum methionine, indicator amino acid oxidation, amino acid requirement, cysteine sparing, children

INTRODUCTION

The sulfur amino acids (SAAs) methionine and cysteine are important in human nutrition. Methionine is a dietary indispensable amino acid required for normal growth and development of humans (1–5). In addition to being a substrate for protein synthesis, methionine is a precursor in transmethylation reactions and is the main methyl group donor to choline and creatine and to both DNA and RNA intermediates (6–9). Homocysteine (conversion to methionine) is a methyl acceptor for 5-methyltetrahydrofolate homocysteine methyl transferase (methionine synthase), which allows for the recycling of this form of folate, and is also a methyl acceptor for betaine, the oxidation product of choline. Functions of cysteine include protein synthesis and a role as a precursor for the synthesis of several metabolites, such as glutathione (8, 10–12), taurine, 3-phosphoadenosine-5′-phosphosulfate (ie, active sulfate), and coenzyme A.

Cysteine is synthesized de novo by mammals when the cysteine sulfur is derived from methionine by transsulfuration of homocysteine to cysteine and the carbon skeleton of cysteine is donated by serine (13). Dietary cysteine can satisfy a portion of the SA requirement, thereby providing a sparing effect on the dietary methionine requirement. The reduction in methionine requirements appears to be related to suppression of transsulfuration to cystathionine by dietary cysteine (14, 15). This phenomenon of cysteine sparing is well established in animals (16–22), but it has been an area of considerable debate in humans. Early nitrogen balance studies (23–25) and recent stable isotope studies that used indicator amino acid oxidation (IAAO) (26) and 24-h IAAO and indicator amino acid balance (IAAB) (27) techniques suggest a cysteine-sparing effect that ranges from 17% to 90%. In contrast, some of the studies that used the direct amino acid oxidation technique suggest that the cysteine-sparing phenomenon does not occur in humans (28–31). The lack of an observed cysteine-sparing effect in those studies appeared to result from the intake of methionine below the minimum obligatory requirement level.

Recently, with the use of the IAAO technique, the mean and population-safe SAA requirements (in the absence of cysteine) of healthy school-age children were determined to be 12.9 and 17.2 mg·kg⁻¹·d⁻¹, respectively (32). The main objectives of the current study were to determine the minimum methionine requirements (with excess cysteine intake) for healthy school-age children and to examine whether a cysteine-sparing effect was present by following the same experimental protocol as was used in our studies in adults (26, 33). Another objective was to...
MINIMUM METHIONINE REQUIREMENT

TABLE 1
Characteristics of 6 preadolescent children (5 M and 1 F) and energy intakes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>9.4 ± 2.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>40 ± 13.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>140.7 ± 14.4</td>
</tr>
<tr>
<td>Percentage of IBW (%)</td>
<td>102.9 ± 7.4</td>
</tr>
<tr>
<td>LBM (kg)²</td>
<td>29.9 ± 8</td>
</tr>
<tr>
<td>RMR (MJ/d)³</td>
<td>5.8 ± 0.75</td>
</tr>
<tr>
<td>Energy intake (MJ/d)</td>
<td>9.9 ± 1.3</td>
</tr>
</tbody>
</table>

1 All values are ± SD. IBW, ideal body weight; LBM, lean body mass; RMR, resting metabolic rate.
2 Measured by using bioelectrical impedance analysis.
3 Measured by using open-circuit indirect calorimetry.

generate results for comparison with results from our similar, ongoing study in children with end-stage renal disease. Children with end-stage renal disease are stunted in their height (34), have high plasma homocysteine concentrations (35), and are at high risk of cardiovascular disease (36).

SUBJECTS AND METHODS

Subjects

Six healthy, school-age children (5 boys, 1 girl) were studied (on an outpatient basis) in the Clinical Investigation Unit at the Hospital for Sick Children (Toronto, Canada). Subject characteristics, body composition, and energy intakes are described in Table 1. None of the subjects had a history of recent weight loss or illness, and none was using any medication at the time of entry into the study.

Written informed consent was obtained from a parent or guardian, and assent was obtained from the participating child. The Research Ethics Board of the Hospital for Sick Children approved all procedures. The parent or guardian of each participating subject received financial compensation for inconvenience.

Experimental design and tracer protocol

The study design was based on the minimally invasive IAAO model (37) used in healthy adults (26, 33, 38) and children (32, 39). Two days before the study day, subjects consumed a maintenance diet that supplied 1.5 g protein · kg⁻¹ · d⁻¹ and 1.7 X resting energy expenditure (REE). On the study day after a 12-h fast, subjects randomly received 1 of 6 dietary levels of methionine and isovaline acid composition of egg protein), and protein-free cookies. The crystalline amino acid mixture (based on the amino acid composition of egg protein), and protein-free cookies. The study day intakes of energy and protein were the same as those of the maintenance diet. The macronutrient composition of the diet was 53% of energy from carbohydrate, 37% of energy from fat, and 10% of energy from protein (equivalent to 1.6% nitrogen).

Sample collection and analysis

Breath and urine samples were collected as described previously (33). Breath samples were stored at room temperature until they were analyzed. Urine samples were stored at −20 °C. During each study day, open-circuit indirect calorimetry was performed for the period of 20 min to measure the rate of carbon dioxide production.

Enrichment of 13C in breath was analyzed by continuous-flow isotope ratio mass spectrometer (20/20 isotope analyzer; PDZ Europa Ltd, Cheshire, United Kingdom). All analyses were performed in triplicate. Enrichments were expressed as APE compared with a reference standard of compressed CO₂ gas. The enrichment of l-[1-13C]phenylalanine in urine samples was analyzed by using a triple quadrupole mass analyzer (API 4000; Applied Biosystems/MDX SCIEX, Concord, Canada) coupled to an Agilent 1100 HPLC system (Agilent, Mississauga, Canada) as described previously (32). Isotopic enrichment was expressed as molar percent excess and was calculated from peak area ratios at isotopic steady state at plateau and baseline.

Tracer kinetics

Kinetics were calculated according to the stochastic model of Matthews et al (40), as previously used by Zello et al (41). Isotopic steady state in the tracer enrichment at plateau and baseline was represented by unchanged values of [1-13C]phenylalanine in urine and 13CO₂ in breath. At plateau, the APE was calculated by subtracting the mean breath 13CO₂ enrichments of the 3 baseline samples from the 4 plateau samples.
Phenylalanine flux (in μmol · kg\(^{-1} \cdot h^{-1}\)) was calculated from the dilution of orally administered L-[1-\(^{13}\)C]phenylalanine into the metabolic pool (at steady state) by using enrichments of L-[1-\(^{13}\)C]phenylalanine in urine (40, 41). The rate of appearance of \(^{13}\)CO\(_2\) in breath (F\(^{13}\)CO\(_2\); in μmol · kg\(^{-1} \cdot h^{-1}\)) after the oxidation of ingested L-[1-\(^{13}\)C]phenylalanine was calculated according to the model of Matthews et al (40), with the use of a factor of 0.82 to account for the retention of \(^{13}\)CO\(_2\) in the bicarbonate pool of the body in the fed state (42). The rate of phenylalanine oxidation (in μmol · kg\(^{-1} \cdot h^{-1}\)) was calculated from the F\(^{13}\)CO\(_2\) data and the urinary L-[1-\(^{13}\)C]phenylalanine enrichment (40, 41).

**Statistical analysis**

Data were analyzed with the use of PROC MIXED in SAS software (version 8.2; SAS Institute Inc, Cary, NC). Repeated-measures analysis of variance was performed on primary and derived variables to assess the effects of methionine intake, of subject, and of interactions. Tukey’s test was used for post hoc analysis of the results from the analysis of variance. Results are expressed as means ± SDs. Statistical significance was assumed at 5% level of significance (\(P < 0.05\)).

The minimum methionine requirement (breakpoint) was determined by applying a biphasic linear regression crossover model on the F\(^{13}\)CO\(_2\) data (41). This model selects for the minimum residual SE in a stepwise partitioning of data points between 2 regression lines. The safe level of intake (upper 95% CI; equivalent to recommended dietary allowance) was calculated by using Fieller’s theorem (43).

The study design included repeated graded levels within a subject (6 levels/subject). The 6 subjects, providing a total of 36 data points, were predicted to be a sample of adequate size from which to estimate the mean and population-safe requirements of minimum methionine in children by applying a 2-phase linear regression crossover analysis on the data, as determined previously in children and adults (32, 33).

**RESULTS**

**Subject characteristics**

Six healthy, school-age children (9.4 ± 2.3 y old) completed the study. Subject anthropometry (Table 1) was within the normal range for age (44). Similarly, energy and protein intakes of the subjects were adequate. According to self- and parent-rated Tanner staging, all subjects were in early puberty except 1 male subject, who was in midpuberty (45).

**Phenylalanine flux and oxidation**

Phenylalanine flux was not affected by methionine intake (Table 2), which provides evidence that the precursor pool for indicator oxidation did not change in size in response to the test amino acid (ie, methionine). Phenylalanine oxidation declined in response to increases in methionine up to an intake of 7.5 mg · kg\(^{-1} \cdot d^{-1}\). However, the changes in phenylalanine oxidation at a methionine intake of 0 mg · kg\(^{-1} \cdot d^{-1}\) differed significantly from those at all other intake levels, and the changes at a methionine intake of 2.5 mg · kg\(^{-1} \cdot d^{-1}\) differed significantly from those at intakes of 7.5, 10, and 15 mg · kg\(^{-1} \cdot d^{-1}\) (\(P < 0.05\) for all). Methionine intakes between 5.0 and 15 mg · kg\(^{-1} \cdot d^{-1}\) did not result in differences (\(P > 0.05\)) in phenylalanine oxidation.

**TABLE 2**

Phenylalanine flux and oxidation at 6 levels of methionine intake (n = 6 per mean) in 6 school-aged children

<table>
<thead>
<tr>
<th>Methionine intake</th>
<th>Phenylalanine flux</th>
<th>Phenylalanine oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg · kg(^{-1} \cdot d^{-1}))</td>
<td>(μmol · kg(^{-1} \cdot h^{-1}))</td>
<td>(μmol · kg(^{-1} \cdot h^{-1}))</td>
</tr>
<tr>
<td>0</td>
<td>51.2 ± 5.2</td>
<td>6.5 ± 1.7</td>
</tr>
<tr>
<td>2.5</td>
<td>50.8 ± 11.0</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>58.0 ± 8.2</td>
<td>3.5 ± 1.0</td>
</tr>
<tr>
<td>7.5</td>
<td>53.3 ± 11.3</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>47.7 ± 11.3</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>15</td>
<td>46.2 ± 10.4</td>
<td>2.1 ± 0.7</td>
</tr>
</tbody>
</table>

\(^{1}\) All values are \(\bar{x} \pm S.D\).

\(^{2}\) Methionine had no statistically significant effect on phenylalanine flux, \(P > 0.05\).

\(^{3}\) Significantly different from methionine intakes of 2.5, 5, 7.5, 10, and 15 mg · kg\(^{-1} \cdot d^{-1}\), \(P < 0.05\).

\(^{4}\) Significantly different from methionine intakes of 7.5, 10, and 15 mg · kg\(^{-1} \cdot d^{-1}\), \(P < 0.05\).

**L-[1-\(^{13}\)C]phenylalanine label oxidation**

Dietary methionine intake reduced the oxidation of L-[1-\(^{13}\)C]phenylalanine measured as F\(^{13}\)CO\(_2\) in response to graded intakes of methionine (Figure 1). As was described above for phenylalanine oxidation, the F\(^{13}\)CO\(_2\) responses at a methionine intake of 0 mg · kg\(^{-1} \cdot d^{-1}\) differed significantly from those at all other intake levels, and the changes at a methionine intake of 2.5 mg · kg\(^{-1} \cdot d^{-1}\) differed significantly from those at intakes of 7.5, 10, and 15 mg · kg\(^{-1} \cdot d^{-1}\) (\(P < 0.05\) for all). As the methionine intake increased, the F\(^{13}\)CO\(_2\) decreased steadily (which represented increasing incorporation of label into protein) until the methionine intake reached 5–7.5 mg methionine/kg; no further decrease in F\(^{13}\)CO\(_2\) was observed with increases in methionine intake.

**FIGURE 1.** Relation between the rate of breath \(^{13}\)CO\(_2\) excretion and \(^{13}\)C\(_{\text{phenylalanine}}\) oxidation at 6 levels of methionine intake (n = 6 per mean). The female subject. Breath \(^{13}\)CO\(_2\) responses at a methionine intake of 0 mg · kg\(^{-1} \cdot d^{-1}\) were significantly different from those at all other intake levels, and those at a methionine level of 2.5 mg · kg\(^{-1} \cdot d^{-1}\) were significantly different from those at intakes of 7.5, 10, and 15 mg · kg\(^{-1} \cdot d^{-1}\) (\(P < 0.05\) for all).
methionine intake (ie, no further change in the incorporation of label into protein occurred). A biphasic linear regression crossover analysis of the 

methionine intake (ie, no further change in the incorporation of label into protein occurred). A biphasic linear regression crossover analysis of the $^{13}$CO$_2$ data in the presence of excess dietary cysteine in 6 school-age children (n = 6 per mean). The intersection of sloping and horizontal line represents a breakpoint (or the mean minimum methionine requirement) after biphasic linear regression crossover analysis of the breath $^{13}$CO$_2$ data.

**FIGURE 2.** Relation between various methionine intakes and the mean (±SD) rate of appearance of orally administered L-[1-$^{13}$C]phenylalanine as breath $^{13}$CO$_2$ in the presence of excess dietary cysteine in 6 school-age children (n = 6 per mean). The intersection of sloping and horizontal line represents a breakpoint (or the mean minimum methionine requirement) after biphasic linear regression crossover analysis of the breath $^{13}$CO$_2$ data.

DISCUSSION

This is the first report of determination of the minimum methionine requirements and cysteine sparing of methionine requirements in healthy, school-age children by using the IAAO technique. In the current study, the mean methionine requirement and the population-safe (95% CI) intake were determined to be 5.8 and 7.3 mg · kg$^{-1}$ · d$^{-1}$, respectively, when the cysteine intake was 21 mg · kg$^{-1}$ · d$^{-1}$. The mean methionine requirement of 5.8 mg · kg$^{-1}$ · d$^{-1}$ found in the current study represents the minimum obligatory methionine requirement at a cysteine intake of 21 mg · kg$^{-1}$ · d$^{-1}$. The minimum obligatory methionine requirement is defined as the intake of methionine that cannot be replaced by cysteine (22). The mean minimum and population-safe obligatory methionine requirements are 55% and 58% lower than the mean (12.9 mg · kg$^{-1}$ · d$^{-1}$) and population-safe (17.2 mg · kg$^{-1}$ · d$^{-1}$) methionine requirements of the same group of children when the diet was devoid of cysteine (32).

These reductions in methionine requirements resulting from intake of excess cysteine represent the cysteine-sparing effect. Cysteine sparing is defined as the proportion of dietary requirement for SAAs (above the minimum obligatory methionine requirement) that can be fulfilled by dietary cysteine (22).

In the current study, the minimum mean obligatory methionine requirement was found to be 5.8 mg · kg$^{-1}$ · d$^{-1}$ with the intake of 21 mg cysteine · kg$^{-1}$ · d$^{-1}$. We propose that, if cysteine intake were reduced to <=15 mg · kg$^{-1}$ · d$^{-1}$ (21 - 5.8 mg · kg$^{-1}$ · d$^{-1}$), the mean methionine requirement would proportionally increase from the minimum mean obligatory methionine requirement of 5.8 mg · kg$^{-1}$ · d$^{-1}$ to the maximum mean methionine requirement when dietary intake of cysteine became zero. The evidence of an increase in mean methionine requirement is provided in the report of Kurpad et al (27), in which the mean methionine requirements were found to be 10 and 15 mg · kg$^{-1}$ · d$^{-1}$ when graded levels of methionine were fed in the presence of 12 mg cysteine · kg$^{-1}$ · d$^{-1}$ (approximately half of the amount in the current study) and in the absence of cysteine in the diet, respectively. From the data in that study, we calculated a cysteine-sparing effect of 33%, which is approximately half that found in the current study in children and in our previous study in adults (26). The lower cysteine-sparing effect on methionine requirement in the study of Kurpad et al (27) may be related to an insufficient intake of cysteine. We predict that a comparable cysteine-sparing effect of 55–66% would have been observed in the study of Kurpad et al (27) if the dietary cysteine intake were high enough to be comparable to that in the current study or our previous study in adults (26). Similarly, the evidence of a maximum mean methionine requirement is provided by studies in which the mean methionine requirement was determined to be between 12.6 and 15 mg · kg$^{-1}$ · d$^{-1}$ (33, 32, 46) when graded levels of methionine were fed with no cysteine in the diet. The maximum mean methionine requirement represents the mean SAA requirement in the absence of dietary cysteine intake. Further studies are needed to determine the estimated average requirement and population-safe intake levels for cysteine by feeding graded levels of cysteine with a methionine intake at a population-safe minimum obligatory requirement level of 7.2 mg · kg$^{-1}$ · d$^{-1}$ in children and 10.1 mg · kg$^{-1}$ · d$^{-1}$ in adults.

In the current study, the rate of label baseline oxidation ($^{13}$CO$_2$) was found to be 0.55 μmol · kg$^{-1}$ · h$^{-1}$ at the mean methionine requirement level of 5.8 mg · kg$^{-1}$ · d$^{-1}$, when the diet provided 21 mg cysteine · kg$^{-1}$ · d$^{-1}$. This $^{13}$CO$_2$ value was similar to that found in the same group of children when we determined their mean maximum methionine requirement (diet was devoid of cysteine) as being 12.9 mg · kg$^{-1}$ · d$^{-1}$ (32). The same level of baseline label oxidation between the 2 studies suggests that the level of protein synthesis was similar between the 2 studies. Therefore, less methionine was necessary (which spared the methionine requirement) to maintain a similar level of protein synthesis in the presence of excess cysteine in the diet than in the context of the diet devoid of cysteine. This point is also supported by the study by Albanese et al (23), in which similar rates of weight gains and nitrogen balances were maintained in 5 healthy infants in the absence (85 mg methionine · kg$^{-1}$ · d$^{-1}$) and presence (65 mg methionine with 50 mg cysteine) of excess cysteine in the diet. They concluded that 35 mg cysteine spared 20 mg methionine and that cysteine spared 23% of the methionine requirement.
Cysteine sparing was shown in adults by nitrogen balance studies (23–25) and recent stable-isotope studies that used IAAO (26) and 24-h IAAO and IAAB (27) techniques in which graded levels of methionine were fed in the absence and presence of excess cysteine in the diet. In contrast, a lack of sparing effect of cysteine was suggested by several studies that used the direct amino acid oxidation technique in which a single level of methionine was tested in the presence of single (29) or graded (28, 30, 31) levels of cysteine intakes to determine a decrease in transsulfuration (a marker of cysteine sparing). At the time of those studies, a minimum obligatory methionine requirement level had not been defined, and in several cases methionine intake levels were below the minimum obligatory methionine level; hence, a methionine-sparing effect would not be expected. Furthermore, had there been a range of methionine intakes in the presence of cysteine, a cysteine-sparing effect would have been expected because of changes in transsulfuration. Hence, the lack of observation of a cysteine-sparing effect in those studies (28–31) appears to be related to the fact that the intake of methionine was below the minimum obligatory requirement level.

Briefly, in studies that lacked a cysteine-sparing effect (28–31), the level of SAA (methionine + cysteine) intake was based on the FAO/WHO/UNU population-safe recommendation of 13 mg · kg⁻¹ · d⁻¹, which recent IAAO (33) and 24-h IAAO and IAAB (46) techniques have shown to be inadequate because it represents only the mean SAA requirement rather than the population-safe intake level (ie, 21 mg · kg⁻¹ · d⁻¹). In those studies (28–31), when dietary intake of methionine was ≤6.5 mg · kg⁻¹ · d⁻¹ [representing a methionine intake below the population-safe minimum obligatory methionine requirement of 10.1 mg · kg⁻¹ · d⁻¹ (26)] and the cysteine intake was ≥5.0 mg · kg⁻¹ · d⁻¹ (range: 5.0–20.9 mg · kg⁻¹ · d⁻¹), cysteine sparing was not observed because of the lack of significant changes in transsulfuration. Lack of cysteine sparing appears to be related to methionine intake below the minimum obligatory requirement level, which cannot be replaced by cysteine because of the reversibility of the cystathionine synthase reaction (1). Inadequacy of methionine intake was, in fact, confirmed in those studies that reported a negative daily methionine balance at methionine intakes of ≤6.5 mg · kg⁻¹ · d⁻¹ (30, 31). We believe that the minimum obligatory methionine requirement and cysteine-sparing effect should be determined by conducting 2 experiments. First, SAA requirements should be determined by feeding graded intakes of methionine and zero dietary cysteine; second, the minimum obligatory methionine requirement should be determined by feeding graded intakes of methionine and excess cysteine. In human nutrition, it is important to feed graded levels of a substrate to objectively determine the nutrient requirements.

In conclusion, the results of the current study suggest that the intake of excess dietary cysteine (equal to population-safe SAA intake of 21 mg · kg⁻¹ · d⁻¹) can reduce methionine requirements by 55% with the identification of a minimum mean obligatory methionine requirement of 5.8 mg · kg⁻¹ · d⁻¹ in school-age children. These results confirm the sparing effect of cysteine on methionine requirements that was previously observed in adults (23–27). Further research is needed to ascertain whether reduced methionine requirements with excess cysteine supplementation could lead to better management of diseases or conditions in which toxic homocysteine concentrations are elevated in blood partly because of low conversion of homocysteine to cysteine.

We thank the subjects who participated in the study and Linda Chow in the Department of Nutrition and Food Services (Hospital for Sick Children) for preparing the protein-free cookies.

MAH, JMT, RE, ROB, and PBP were involved in the study design; VL was responsible for subject recruitment and management; MAH, JMT, and RE were responsible for data collection; MAH, JMT, RE, and MR were responsible for sample analysis; MAH, JMT, RE, MR, ROB, and PBP were responsible for data analysis; and MAH, JMT, RE, ROB, and PBP were responsible for writing the manuscript. None of the authors had a personal or financial conflict of interest.

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