Total Sulfur Amino Acid Requirements Are Not Altered in Children with Chronic Renal Insufficiency, but Minimum Methionine Needs Are Increased

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Abstract

Background: The total sulfur amino acid (TSAA) and minimum Met requirements have been previously determined in healthy children. TSAA metabolism is altered in kidney disease. Whether TSAA requirements are altered in children with chronic renal insufficiency (CRI) is unknown.

Objective: We sought to determine the TSAA (Met in the absence of Cys) requirements and minimum Met (in the presence of excess Cys) requirements in children with CRI.

Methods: Five children (4 boys, 1 girl) aged 10 ± 2.6 y with CRI were randomly assigned to receive graded intakes of Met (0, 5, 10, 15, 25, and 35 mg · kg⁻¹ · d⁻¹) with no Cys in the diet. Four of the children (3 boys, 1 girl) were then randomly assigned to receive graded dietary intakes of Met (0, 2.5, 5, 7.5, 10, and 15 mg · kg⁻¹ · d⁻¹) with 21 mg · kg⁻¹ · d⁻¹ Cys. The mean TSAA and minimum Met requirements were determined by measuring the oxidation of L-[1-¹³C]Phe to ¹³CO₂ (F¹³CO₂). A 2-phase linear-regression crossover analysis of the F¹³CO₂ data identified a breakpoint at minimal F¹³CO₂. Urine samples collected from all study days and from previous studies of healthy children were measured for sulfur metabolites.

Results: The mean and population-safe (upper 95% CI) intakes of TSAA and minimum Met in children with CRI were determined to be 12.6 and 15.9 mg · kg⁻¹ · d⁻¹ and 7.3 and 10.9 mg · kg⁻¹ · d⁻¹, respectively. In healthy school-aged children the mean and upper 95% CI intakes of TSAA and minimum Met were determined to be 12.9 and 17.2 mg · kg⁻¹ · d⁻¹ and 5.8 and 7.3 mg · kg⁻¹ · d⁻¹, respectively. A comparison of the minimum Met requirements between healthy children and children with CRI indicated significant (P < 0.05) differences.

Conclusion: These results suggest that children with CRI have a similar mean and population-safe TSAA to that of healthy children, suggesting adequate Cys synthesis via transsulfuration, but higher minimum Met requirement, suggesting reduced remethylation rates.

Keywords: methionine, cysteine sparing, indicator amino acid oxidation, amino acid requirement, renal insufficiency

Introduction

The kidney is involved in the metabolism of sulfur amino acids. A net release of taurine and Cys has been observed to occur in the renal vein (1, 2). Chronic renal disease resulting in chronic renal insufficiency (CRI) and end-stage renal disease (ESRD) affects sulfur amino acid metabolism. Abnormal patterns of plasma sulfur amino acids have been described in patients with chronic renal disease (3, 4). Cys, homocysteine, and Cys sulfenic acid concentrations have been reported to be high in the plasma of uremic patients, whereas Met concentrations have been found to be normal and taurine concentrations low (3, 5). Homocysteine is a key intermediate in the biosynthesis of Cys, which is required for protein synthesis and for the synthesis of the important antioxidant molecule glutathione. Cys is synthesized

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Abbreviations used: CRI, chronic renal insufficiency; ESRD, end-stage renal disease; GFR, glomerular filtration rate; REE, resting energy expenditure; TSAA, total sulfur amino acid.

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de novo, whereby the Cys sulfur is derived from Met via the transsulfuration of homocysteine to Cys and the carbon skeleton of Cys is donated by Ser (6). The transsulfuration pathway is found principally in the liver and kidney. In CRI and ESRD, renal call mass is reduced, and hence renal transsulfuration activity may be considerably reduced with the net effect of a reduced ability to make Cys in ESRD. Because both Met and Cys are required for protein synthesis, this may limit the proper utilization of dietary proteins for growth and maintenance. It is known that children with ESRD are stunted in their height (7), have high plasma homocysteine concentrations (8), and are at an increased risk of cardiovascular disease (9). It is therefore essential to determine the effects of CRI and ESRD on total sulfur amino acid (TSA) and minimum Met requirements to devise treatments to improve nutritional status (growth) and normalize plasma homocysteine concentrations to reduce cardiovascular disease risk.

With the use of the minimally invasive indicator amino acid oxidation technique, we previously determined the mean and population-safe TSA and minimum Met requirements of healthy school-aged children to be 12.9 and 17.2 mg·kg⁻¹·d⁻¹ (10) and 5.8 and 7.3 mg·kg⁻¹·d⁻¹ (11), respectively. To our knowledge, there are no specific isotope kinetics–based estimates of TSA and minimum Met requirements in children with CRI. Hence, the primary objective of this study was to determine the TSA (with no Cys intake) and minimum Met requirements (with excess Cys intake) in children with CRI and to compare the results obtained previously following the same experimental protocol in healthy children (10, 11) and adult studies (12, 13).

Methods

Participants. Five children with CRI (4 boys, 1 girl) were studied to determine TSA and minimum Met requirements at the Hospital for Sick Children between April 2005 and October 2006. Because of severe nausea, 1 boy could not proceed to the minimum Met requirement study. The children with CRI had a mean glomerular filtration rate (GFR) of 27.8 mL/min (range: 24.4–40 mL·min⁻¹·1.73 m²⁻¹). The Hospital for Sick Children Ethics Review Board approved all procedures. Informed consent was obtained from parents or guardians and assent from the participating child. The parent or guardian of each participating child received financial compensation for taking part in the studies.

Experimental design and tracer protocol. The study design has been used previously in healthy adults (12–15) and children (10, 11, 16). For both the TSA and minimum Met requirement studies, 2 d before the study day, children consumed a maintenance diet of 1.5 g·kg⁻¹·d⁻¹ protein and energy at 1.7× resting energy expenditure (REE). In the TSA requirement study, on the study day after a 12-h fast, 5 subjects were randomly assigned to receive 1 of 6 dietary concentrations of Met (0, 5, 10, 15, 25, and 35 mg·kg⁻¹·d⁻¹) along with an l-amino acid mixture to give a final protein intake of 1.5 g·kg⁻¹·d⁻¹ and energy intake of 1.7× REE. The protein intake was devoid of Cys. In the minimum Met study, on the study day after a 12-h fast, 4 of the same subjects were randomly assigned to receive 1 of 6 dietary concentrations of Met (0, 2.5, 5, 7.5, 10, and 15 mg·kg⁻¹·d⁻¹) along with an l-amino acid mixture (including 21 mg·kg⁻¹·d⁻¹ Cys) to give a protein intake of 1.5 g·kg⁻¹·d⁻¹ and energy intake of 1.7× REE. The study-day diet was consumed as 8 isonitrogenous and isocaloric hourly meals, in which each meal represented ~8% of the child’s total daily requirements. Apart from the study diet, children were only allowed water during the study day.

Phe kinetics were measured with the use of l-[1-¹³C]Phe (99 atom% excess) (Cambridge Isotope Laboratories). Oral priming doses of 0.176 mg/kg NaH¹³CO₃ (99 atom% excess) (Cambridge Isotope Laboratories) and 1.09 mg/kg l-[1-¹³C]Phe were given with the fifth hourly meal. In addition, an hourly oral dosing protocol of l-[1-¹³C]Phe (1.958 mg·kg⁻¹·h⁻¹) was commenced simultaneously (with the fifth meal) and continued for the remaining 3 h of the study. The amount of l-[1-¹³C]Phe given during the study day was subtracted from the dietary intake of Phe such that the total intake of Phe was 25 mg·kg⁻¹·d⁻¹ with a Tyr intake of 61 mg·kg⁻¹·d⁻¹ (to ensure an excess of Tyr). Study day periods were separated by ≥1 wk, and all children completed all 6 studies within a 3-mo period.

Study diets. The maintenance diet for the 2 d before the study day was prescribed by the study dietitian based on the participant’s 3-d food records. REE was measured by open-circuit indirect calorimetry (2900 Computerized Energy Measurement System; Sensormedics). For the entire duration of the 12 studies, children also consumed a daily supplement that contained 1.0 mg folic acid, 10 mg vitamin B-6, and 6 μg vitamin B-12 (Replavite; Landmark Medical Systems Inc.). This was to ensure adequate folate and vitamin B intake as cofactors for the metabolism of Met and homocysteine (12).

As described previously (12), the study-day diet consisted of a protein-free liquid formula (flavored with soft drink crystals), corn oil, the crystalline amino acid mixture (based on the amino acid composition of egg protein), and protein-free cookies. The study-day energy and protein intakes were the same as in the maintenance diet. The macronutrient composition of the diet (expressed as a percentage of dietary energy) was 53% carbohydrates, 37% fat, and 10% protein.

Sample collection and analysis. Breath and spot urine samples were collected and stored as described previously (12). During each study day, the VCO₂ rate was measured for 20 min (2900 Computerized Energy Measurement System; SensorMedics).

The enrichment of ¹³C in the breath was measured with the use of isotope ratio mass spectrometry (20/20 isotope analyzer; PDZ Europa Ltd.) and expressed as atom percent excess compared with a reference standard of compressed CO₂ gas. l-[1-¹³C]Phe enrichment in urine samples was analyzed with the use of an API 4000 triple quadrupole mass analyzer (Applied Biosystems/MDS SCIEX) coupled to an Agilent 1100 HPLC system as described previously (10), and enrichment was expressed as molecule % excess.

Urinary Met, homocysteine, Cys, and creatinine concentration was determined with the use of LC-electrospray ionization-tandem MS (17). Urinary sulfate was determined with the use of a micromethod reported by Swaroop (18). Because we had previously studied the TSA and minimum Met requirements in healthy children with the use of the same experimental design and test amino acid intakes (10, 11), we reasoned that we could use the urine samples collected previously with samples from children with CRI to measure the urinary excretion of sulfur amino acids and metabolites and to compare the values obtained in this study. All samples were analyzed at the same time to minimize variation.

Tracer kinetics. Phe kinetics were calculated as described previously (19). Isotopic steady state in the tracer enrichment at baseline and

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**TABLE 1** Characteristics of school-aged children with CRI

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>10.0 ± 2.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>29.4 ± 5.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>135.6 ± 15.3</td>
</tr>
<tr>
<td>IBW, %</td>
<td>96.2 ± 13.8</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>25.7 ± 4.7</td>
</tr>
<tr>
<td>REE, MJ/d</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>Urine creatinine, μmol/L</td>
<td>271 ± 88.8</td>
</tr>
<tr>
<td>GFR, mL·min⁻¹·1.73 m²⁻¹</td>
<td>27.8 ± 8.8</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs [n = 5 (4 boys, 1 girl)]. CRI, chronic renal insufficiency; GFR, glomerular filtration rate; IBW, ideal body weight; LBM, lean body mass; REE, resting energy expenditure.
plateau was represented by <5% CV values of \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) in \( \text{^{13}CO}_2 \) in the breath. Atom percent excess was calculated from the differences in mean breath \( \text{^{13}CO}_2 \) enrichments between baseline and plateau samples. The rate of the appearance of \( \text{^{13}CO}_2 \) in the breath (\( \text{F}^{13}\text{CO}_2 \)) from the oxidation of \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) was calculated with a factor of 0.82 to account for the retention of \( \text{^{13}CO}_2 \) in the bicarbonate pool of the body in the fed state (14, 19, 20).

Statistical analysis. Results are expressed as means ± SDs. Data were analyzed with the use of SAS version 9.4 (SAS Institute). Repeated-measures ANOVA was performed on primary and derived variables to assess the effects of Met intake on urinary excretion of sulfur metabolites, with Tukey’s test used for post hoc analysis. \( P < 0.05 \) was considered to be statistically significant.

The TSAA and minimum Met requirements were determined by applying a 2-phase linear-regression crossover model to assess the \( \text{F}^{13}\text{CO}_2 \) data (18). The model selects for the minimum residual SE in a stepwise partitioning of data points between the sloping and nonsloping regression lines (21). The safe level of intake (upper 95% CI equivalent to the RDA) was calculated with the use of Fieller’s theorem (22). In addition, the minimum Met requirement breakpoint determined in this study was compared with the minimum Met requirement breakpoint determined previously in healthy children (11) with the use of the pooled 2-sample \( t \) test as described previously (23, 24). Briefly, \( t = [(\text{BP1} - \text{BP2})/\sqrt{(\text{Sp}^2/\text{nBP1}) + (\text{Sp}^2/\text{nBP2})}] \), with \( \text{df} = \text{nBP1} + \text{nBP2} - 2 \), where \( \text{BP1} \) = breakpoint, \( \text{Sp}^2 \) = pooled variance, and \( \text{nBP} \) = sample size of the breakpoint. The pooled variance is calculated by averaging each sample variance with weights equal to its df and is calculated by \( \text{Sp}^2 = [(\text{BP1} - 1) \text{SBP}^2_{\text{BP1}} + (\text{BP2} - 1) \text{SBP}^2_{\text{BP2}}]/(\text{BP1} + \text{BP2} - 2) \), where \( \text{SBP} \) = the SD of the combined regression lines in the 2-phase crossover analysis.

Our study design was based on a repeated-measures graded testing of Met intakes (6 test intakes/child in each experiment). With the use of a 2-phase linear-regression crossover analysis of the data, we reasoned that a total of 54 data points would be adequate to estimate the mean and population-safe TSAA and minimum Met requirements. In our previous studies of healthy school-aged children, we used a similar study design, in which 5–6 children were studied at 6–7 test intakes each to determine the requirements for branched-chain amino acids (16), Lys (25), TSAA (10), minimum Met (11), and branched amino acid needs in children with liver disease (26).

Results

Subject characteristics. Five children (4 boys, 1 girl) aged 10 ± 2.6 y with CRI completed the study. As expected, anthropometry (Table 1) indicated malnutrition compared with healthy children of the same age (27) and with our earlier studies (10, 11). There were 6 children (5 boys, 1 girl) aged 9.1 ± 2.2 y with an ideal body weight of 99% ± 8.2% in the TSAA study of healthy children and 6 children (5 boys, 1 girl) aged 9.4 ± 2.3 y with an ideal body weight of 102.9% ± 7.4% in the minimum Met requirement study. During the study, energy and protein intakes of the children were adequate. According to self- and parent-rated Tanner staging, children were in early- to midpuberty (28).

\( \text{l-}[1-\text{^{13}C}]\text{Phe} \) oxidation. Figure 1 shows that the intakes of dietary Met (in the absence of Cys) reduced the oxidation of \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) measured as the rate of label appearance in \( \text{F}^{13}\text{CO}_2 \). With increasing Met intakes, the oxidation of \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) decreased steadily (representing increased incorporation for protein synthesis) until a point was reached (10–15 mg/kg) after which there was no further decrease in \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) oxidation (representing no further increased incorporation of the label into protein synthesis) (Figure 1). A 2-phase linear-regression crossover analysis of the \( \text{F}^{13}\text{CO}_2 \) data identified a breakpoint (mean TSAA requirement) at 12.6 mg · kg⁻¹ · d⁻¹ (\( R^2 = 0.668 \)) and safe level of intake [the upper 95% CI equivalent to RDA (29)] at 15.9 mg · kg⁻¹ · d⁻¹.

Figure 2 shows that the intakes of dietary Met (in the presence of excess dietary Cys) reduced the oxidation of \( \text{l-}[1-\text{^{13}C}]\text{Phe} \) measured as the rate of label appearance in \( \text{F}^{13}\text{CO}_2 \) in response to graded intakes of Met. A 2-phase linear-regression crossover analysis of the \( \text{F}^{13}\text{CO}_2 \) data identified a breakpoint (estimate of mean minimum Met requirement equivalent to the Estimated Average Requirement) at 7.3 mg · kg⁻¹ · d⁻¹ (\( R^2 = 0.651 \)) and safe level of intake [the upper 95% CI equivalent to the RDA (29)] at 10.9 mg · kg⁻¹ · d⁻¹ (Figure 2). A comparison of the breakpoints obtained for the minimum Met requirement between children with CRI and healthy children (11) with the use of our previously described method (23, 24) indicated a significant difference (\( P < 0.001 \)).
Urinary excretion of sulfur metabolites. Figure 3 shows the urinary concentration of the metabolites of sulfur metabolism from children with CRI with increasing Met intakes and compares it with samples from healthy children (10, 11). All values are expressed as per-mole creatinine in urine because we used spot urine samples from each study day. When the subjects who received no Cys (TSAA study) were studied, urinary homocysteine and Cys were consistently elevated compared with healthy children, although it was significant (P < 0.05) only at baseline Met intake for homocysteine (Figure 3B) and at 10 mg · kg⁻¹ · d⁻¹ Met (Figure 3C) for Cys. In the absence of dietary Cys, urinary Met and sulfate excretions did not differ from those of healthy children (P > 0.05). Conversely, in the studies with excess dietary Cys in the diet, all 4 metabolites were elevated in the urine of the children with CRI, with Cys concentrations significantly (P < 0.05) higher at all test intakes of Met.

Discussion
To our knowledge, this study is the first to report TSAA and minimum MET requirements in children with CRI with the use of the indicator amino acid oxidation method. The major finding was that the mean TSAA requirement (in the absence of dietary Cys) and population-safe (95% CI) intake of children with CRI were found to be 12.6 and 15.9 mg · kg⁻¹ · d⁻¹, respectively. These values are similar to the mean TSAA requirement and population-safe (95% CI) intake of healthy children (12.9 and 17.2 mg · kg⁻¹ · d⁻¹, respectively) as determined previously with the use of a similar study protocol (10). The second major finding was that the mean minimum Met requirement (with a Cys intake of 21 mg · kg⁻¹ · d⁻¹) and population-safe (95% CI) intake of children with CRI were found to be 7.3 and 10.9 mg · kg⁻¹ · d⁻¹, respectively and are higher than our previous study of healthy children (11). The mean Met requirement of 7.3 mg · kg⁻¹ · d⁻¹ found in this study represents the minimum obligatory Met requirement at a Cys intake of 21 mg · kg⁻¹ · d⁻¹. The minimum obligatory Met requirement is defined as the intake of Met that cannot be replaced by Cys (30).

The rate of label baseline oxidation (F¹³CO₂) in this study was found to be 0.50 μmol · kg⁻¹ · h⁻¹ at the mean TSAA requirement concentration of 12.6 mg · kg⁻¹ · d⁻¹ when the diet was devoid of Cys. This F¹³CO₂ value was similar to that found in the group of healthy children when we determined their mean TSAA requirement as being 12.9 mg · kg⁻¹ · d⁻¹ (10). The same level of baseline label oxidation between the 2 groups of children suggests that the level of protein synthesis was similar between healthy children and those with CRI. Thus, if children with CRI ingest adequate protein and energy, the TSAA requirements would not be altered, and they could maintain a similar growth pattern and nutritional status as seen in healthy children.

In this study, the minimum mean obligatory Met requirement was found to be 7.3 mg · kg⁻¹ · d⁻¹ with the intake of 21 mg · kg⁻¹ · d⁻¹ Cys. These reductions in Met requirements caused by the intake of excess Cys represent the Cys-sparing effect. Cys sparing is defined as the proportion of dietary requirement for sulfur amino acids (above the minimum obligatory Met requirement) that can be fulfilled by dietary Cys (30). In CRI, the Cys-sparing effect of 31–43% on Met requirements is significantly lower than the 55% determined previously in healthy children (11). The lower Cys-sparing effect in children with CRI is not clear. Previous stable isotope–based work in adults with ESRD showed that remethylation rates were lower in patients than in healthy controls (31, 32), suggesting a greater need for dietary Met. It also might be that 21 mg · kg⁻¹ · d⁻¹ Cys may not be sufficient to meet Cys requirements compared with healthy children. Children with CRI may have increased requirements for antioxidant glutathione synthesis (33, 34), for which Cys is a precursor. Because Cys is also a substrate for protein synthesis, a lower level of protein synthesis for the intake of 21 mg · kg⁻¹ · d⁻¹ Cys suggests that glutathione synthesis may be preferable to protein synthesis. Further studies may shed light on whether providing >21 mg · kg⁻¹ · d⁻¹ Cys results in the same

FIGURE 3 Urinary excretion of sulfur metabolites in children with CRI and healthy children in response to increasing intakes of Met in the absence of Cys and in the presence of 21 mg · kg⁻¹ · d⁻¹ Cys. Values are means ± SDs (n = 5 [4 boys, 1 girl] or n = 4 [3 boys, 1 girl] in children with CRI; n = 6 [6 boys, 1 girl]) in healthy children. *Different from healthy children at that dose, P < 0.05. Urine samples were collected from healthy children as part of previous studies to determine TSAAs (10) and minimum Met requirements (11). (A and E) Urinary Met; (B and F) urinary HCY; (C and G) urinary Cys; and (D and H) urinary sulfate. CRI, chronic renal insufficiency; HCY, homocysteine; TSAA, total sulfur amino acid.
level of protein synthesis and the Cys-sparing effect as seen in healthy children.

It is well known that patients with CRI and ESRD exhibit higher plasma homocysteine concentrations. However, the causes of high homocysteine concentrations are not clear. Hyperhomocysteinemia in patients with chronic renal failure may be related to either disturbed metabolism or the retention of TSAA, or both. Because homocysteine is a key intermediate in the biosynthesis of Cys (via the transsulfuration of homocysteine to Cys), the finding of a similar TSAA requirement in healthy children and those with CRI suggests that the transsulfuration pathway is sufficient in children with CRI and that the cause of hyperhomocysteinemia in CRI and ESRD may be something other than the blockage of the transsulfuration pathway. Indeed, with the use of a stable isotope tracer of Met (\(^{2}H_{3}\)-methyl-\(^{13}C\)-Met), van Guldener et al. (31) and Stam et al. (32) previously showed that transsulfuration was not affected as a result of renal disease in adult patients with ESRD.

Studies have also shown that plasma Cys concentrations were either similar (35) or significantly higher in hemodialysis patients (36) compared with controls, suggesting that one of the reasons for the increase in homocysteine concentrations in CRI and ESRD might be the decreased urinary clearance of the end products of sulfur metabolism caused by a decreased renal flow and GFR, with the net effect of increased concentrations of sulfate, homocysteine, and TSAA. This is supported by several studies (35, 37–39) that have shown that plasma homocysteine, total Cys, and sulfate concentrations were significantly higher in patients with chronic renal failure than in healthy controls and patients with no renal failure. Univariate correlations have suggested that plasma homocysteine concentrations are significantly and positively correlated with plasma sulfate and negatively correlated with the urinary excretion of sulfate (37). Similarly, a multiple regression analysis has shown that plasma homocysteine concentrations in patients with renal disease was significantly predicted by plasma and urinary sulfate (37). Dialysis (35) or frequent nocturnal hemodialysis treatments (3 sessions/wk) (40, 41). These studies seem to indicate that the transsulfuration pathway is intact in patients with ESRD and that higher homocysteine concentrations may be related to the decreased excretion of sulfate in the urine, thus resulting in homocysteine and TSAA buildup. In our study, we did not observe a decreased urinary clearance of sulfur metabolites. In fact, in children with CRI with an excess load of dietary Cys, we saw an increased excretion of sulfur metabolites, including sulfate. This could be because of the fact that children in this study had a moderate estimated GFR of \(\sim 28\) mL \(\cdot\) min\(^{-1}\) \(\cdot\) 1.73 m\(^{-2}\) and did not require dialysis, in contrast to the previously discussed studies in which the patients had ESRD. Evidence suggests that high urinary concentrations of sulfate in aging patients with nephropathy because of type 2 diabetes have a decreased risk of renal disease progression (42). In renal transplant recipients, urinary sulfate concentrations were associated with an improved cardiovascular profile and survival benefit (43), presumably via \(H_{2}S\) production, which is also an end product of Cys metabolism (43). Whether a similar beneficial role for Cys exists in pediatric patients with CRI or ESRD is unknown and needs to be explored in the future.

In conclusion, the mean TSAA (in the absence of dietary Cys) and minimum Met (with a Cys intake of 21 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\)) requirements in this study were found to be 12.6 and 7.3 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\), respectively, for children with CRI. These values are similar to the mean TSAA requirements of 12.9 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) in healthy children (10) but higher than the minimum Met requirements of 5.8 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) (11) for healthy children. That the TSAA requirements were similar to healthy children suggests that Cys synthesis via transsulfuration is sufficient, and if adequate protein and amino acids are ingested by children with CRI then they could maintain a similar growth pattern and nutritional status as seen in healthy children. However, care should be taken to ensure Met intakes are sufficient in children with CRI.

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