ABSTRACT  Protein-energy malnutrition is prevalent in children with chronic cholestatic liver disease. Supplementation of branched-chain amino acids (BCAA) in infants and children with chronic liver disease has been associated with significant improvement in growth and nitrogen balance, suggesting that BCAA requirements are increased in chronic liver disease. The goal of the present study was to determine the total BCAA requirement in children with mild-to-moderate chronic cholestatic (MCC) liver disease using indicator amino acid oxidation (IAAO). Total BCAA requirements were determined in 6 children (6.3 ± 3.7 y, mean ± SD) with MCC liver disease. Children were randomly assigned to receive 7 graded intakes of total BCAA. Individual BCAA in the test diet were provided in the same proportions as those present in egg protein to minimize the potential interactive effects of individual BCAA on assessment of requirement. The total BCAA requirement was determined by measuring the oxidation of L-[1-13C] Phe to 13CO2 [F13CO2 in μmol/(kg·h)], after a primed, continuous oral administration of the tracer and using a 2-phase linear regression crossover regression analysis. The estimated mean requirement and the upper limit of the 95% CI for total BCAA establishing using the IAAO in children with MCC liver disease were 209 and 272 mg/(kg·d), respectively. Total BCAA estimated average requirements using the IAAO were significantly higher than mean requirements established previously for healthy children (P < 0.05). J. Nutr. 136: 133–139, 2006.

KEY WORDS: • amino acid • children • liver disease

Malnutrition occurs in ~70% of children with chronic liver disease (1). Inadequate dietary intake, increased resting energy expenditure, and malabsorption appear to be the major factors contributing to the development of malnutrition in these children (2). Whole-body protein metabolism and energy expenditure may be different in children with cholestatic liver diseases compared with adults with compensated liver cirrhosis (1,2). Specifically, a higher proportion of children with cholestatic liver diseases have increased energy expenditure, depleted lean body mass, and increased lipid utilization in the postabsorptive state (2–5). These changes in metabolic environment may not occur in adults with compensated cirrhosis (1–5). Limited information is available in children regarding the effect of chronic liver disease on branched-chain amino acid (BCAA)4 metabolism (6,7). Chin et al. (1) demonstrated significant increases in growth and nitrogen balance in children with cholestatic liver disease supplemented with BCAA, suggesting that liver disease results in increased BCAA requirements. In adults, BCAA supplementation during liver cirrhosis has also been associated with improvements in liver function, and nitrogen balance (8,9).

The characteristic amino acid patterns observed in chronic liver disease are lower concentrations of plasma BCAA (leucine, valine, and isoleucine) and elevated concentrations of aromatic amino acids (AAA; phenylalanine and tyrosine) (7). The etiology of these alterations in plasma amino acid patterns remains unclear. However, these changes in plasma concentrations of BCAA and AAA are observed in a wide

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4 Abbreviations used: AAA, aromatic amino acid; APE, atom % excess; BCAA, branched-chain amino acid; Brate, Phe released from proteolysis; EAR, estimated average requirement; F13CO2, rate of release of 13CO2 from 13C-phenylalanine oxidation; FFM, fat-free mass; γ-GT, γ-glutamyl transpeptidase; HSC, Hospital for Sick Children; IAAO, indicator amino acid oxidation; %IBW, percentage of ideal body weight; IGF-1, insulin growth factor-1; IGFBP, IGF binding protein; HOMA, homeostasis model assessment; LBM, lean body mass; MCC, mild-to-moderate chronic cholestatic; MPE, mol % excess; NOPD, nonoxidative phenylalanine disposal; RDA, recommended daily allowance; RMR, resting metabolic rate; RQ, respiratory quotient.
variety of liver diseases (6). Studies of BCAA kinetics and protein turnover in adults with compensated liver cirrhosis have given conflicting results regarding potential causes for these changes. Increased oxidation and decreased leucine released from endogenous breakdown of protein in skeletal muscle are thought to be major contributors to the lower plasma levels of BCAA observed in liver disease and may be indicative of an increased need for the BCAA in chronic liver disease (10–14).

The work of Chin et al. (1) provides clear evidence that BCAA requirements may be increased in infants and children with mild-to-moderate chronic cholestatic (MCC) liver disease. However there are no data on the optimal amount of BCAA needed. Recently we modified the indicator amino acid oxidation (IAAO) method to make it minimally invasive so that it could be used in children (15,16); we applied it to determine the total BCAA requirements in healthy 6- to 10-year-old children and in children with MCC liver disease (17). In addition, because the body composition of patients with chronic liver disease was comparable to that of healthy children whose BCAA requirements we reported earlier, and because the same methods were used to determine BCAA requirements, we compared our BCAA requirement results from children with liver disease to the values obtained in healthy children. This is the first study to directly determine dietary essential amino acid needs in children with chronic liver disease. Because of concerns regarding the potential interactive effects of individual BCAA, the model used in this study included feeding dietary BCAA in the same proportions as those present in egg protein (17–20). We hypothesized that total BCAA needs in children with MCC liver disease would be increased compared with needs in healthy children with normal liver function.

SUBJECTS AND METHODS

Subjects. Children with MCC liver disease (n = 6) between the ages of 3 and 11 y (6.3 ± 3.7 y) were recruited for this study (Table 1). All subjects were born with chronic liver disease. Hence, the mean duration of having the disease was 6.3 ± 3.7 y. One subject dropped out of the study due to travel and compliance restraints after completing the baseline study day and 1 level of total BCAA intake (100 mg/(kg·d)). The data from this subject were excluded. Subjects were studied on an outpatient basis in the Clinical Investigation Unit at The Hospital for Sick Children (HSC), Toronto, Canada. Inclusion criteria were as follows: 1) known diagnosis of chronic cholestatic liver, 2) mild-to-moderate liver disease, 3) clinical presentation of jaundice and/or laboratory evidence of cholestasis [total serum bile salts > 8.2 µmol/L, and/or conjugated bilirubin > 20 µmol/L, and/or elevations in γ-glutamyl transpeptidase (γ-GT)] (Table 2). The severity of liver disease in this study was assessed clinically using the modified Child-Turcotte classification (Table 2) (21). Tanner staging assessment was done by one of the investigators at the time of subject recruitment (22).

None of the children participating in the study had a recent history of variceal hemorrhage or ascites; all were ambulatory and appeared clinically stable. Subjects were excluded if they were taking medications that alter energy or protein metabolism (e.g., corticosteroid therapy), were clinically unstable, or were known to have any other primary diagnosis such as endocrine and/or metabolic disorders that cause liver disease (e.g., hereditary tyrosinemia type I).

Written consent and/or assent was obtained from study participants and their responsible caregivers. The purpose of these studies and potential risks were explained before obtaining written consent/assent. All study procedures were approved by the Research Ethics Board at HSC. Study participants and their responsible caregivers were provided with financial compensation for costs incurred in participating in these studies.

Experimental design. The minimally invasive IAAO model was used (15,16). In this study, t-1-[13C]Phe was used as the indicator and a mixture of BCAA (based on the profile of egg protein) was used as the test amino acid. Each subject randomly received 7 dietary intakes of the total BCAA [75, 100, 150, 200, 225, 250, and 300 mg/(kg·d)] on 7 different test days. Dietary levels of the total BCAA were selected to match intake levels used in healthy children to ensure that appropriate comparisons of total BCAA requirements could be made between healthy children and children with chronic MCC liver disease. We also added 2 additional dietary levels of the total BCAA [250 and 300 mg/(kg·d)] because we hypothesized that the breakpoint [representative of the estimated average requirement (EAR)] in children with liver disease would be between 180 and 200 mg/(kg·d) (17).

Dietary protein and energy intakes. Before each study day, participants were acclimated to a dietary protein intake of 2.0 g protein/(kg·d) for 2 d. The level of protein intake was chosen because it exceeded the recommended protein intake in both healthy children and those with chronic liver disease (23,24). In addition, the level of protein and energy provided in this diet approximated the subjects’ habitual protein [≈2.05 g/(kg·d)] and energy intakes [resting metabolic rate (RMR) × 1.71]. Menu plans provided by the investigator consisted of typical foods consumed by the child, and food records were collected to ensure consistency of dietary intake before each study day. The dietary study periods were separated by ≥1 wk; all subjects completed all study days within 2 mo.

Energy needs were determined by measuring RMR after a 12-h overnight fast, using open-circuit indirect calorimetry (2900 Computerized Energy Measurement System; Sensormedics). The RMR was multiplied by an activity factor of 1.7 to reflect energy needs related to growth and the metabolic stress associated with children having

### TABLE 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age y</th>
<th>Weight kg</th>
<th>Height cm</th>
<th>% IBW</th>
<th>FFM 1,4 kg</th>
<th>LBM 1,4 kg</th>
<th>FFM 3,4 kg</th>
<th>RMR kJ/d</th>
<th>Intake 5 kJ/d</th>
<th>RQ</th>
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<td>3.7</td>
<td>16.6</td>
<td>100.4</td>
<td>100.5</td>
<td>12.8</td>
<td>14.1</td>
<td>9.8</td>
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<td>6667</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
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<td>13.9</td>
<td>99.8</td>
<td>89.1</td>
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<td>8.1</td>
<td>3797</td>
<td>6492</td>
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<tr>
<td>3</td>
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<td>54.1</td>
<td>155.1</td>
<td>112.7</td>
<td>42.2</td>
<td>39.9</td>
<td>36.5</td>
<td>7510</td>
<td>12842</td>
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</tr>
<tr>
<td>4</td>
<td>3.4</td>
<td>13.4</td>
<td>89.7</td>
<td>111.7</td>
<td>11.7</td>
<td>9.7</td>
<td>8.5</td>
<td>8314</td>
<td>5667</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>8.5</td>
<td>22.6</td>
<td>123.1</td>
<td>98.3</td>
<td>15.9</td>
<td>19.7</td>
<td>12.5</td>
<td>4855</td>
<td>8350</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Mean ± SD: 6.3 ± 3.7, 24.1 ± 17.2, 113.6 ± 26.2, 102.5 ± 9.8, 18.9 ± 13.1, 18.9 ± 12.4, 15.1 ± 12.1, 4680 ± 1678, 8004 ± 2875, 0.78 ± 0.02

1 FFM was determined from the sum of skin fold thickness.
2 LBM was determined from bioelectrical impedance analysis.
3 FFM was determined by isotope dilution methodology from total body water data.
4 The methodologies for determination of FFM by Bland-Altman (55) did not differ.
5 Total dietary intake, is RMR × 1.71
Liver biochemistry and disease severity in study subjects

<table>
<thead>
<tr>
<th>Sub</th>
<th>Bile acids¹</th>
<th>ALT²</th>
<th>γ-GT³</th>
<th>Albumin⁴</th>
<th>Leucine⁵</th>
<th>Isoleucine⁵</th>
<th>Valine⁵</th>
<th>Phe⁶</th>
<th>Tyrosine⁶</th>
<th>DX⁶</th>
<th>Child-Turcotte Classification⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>67</td>
<td>214</td>
<td>40</td>
<td>18.1</td>
<td>18.1</td>
<td>104.1</td>
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<td>A</td>
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<td>130</td>
<td>63</td>
<td>36</td>
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<tr>
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<td>210</td>
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<td>40</td>
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<td>44.8</td>
<td>25.7</td>
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<tr>
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<td>69.4</td>
<td>5</td>
<td>33</td>
<td>39</td>
<td>23.7</td>
<td>23.7</td>
<td>111.4</td>
<td>40.9</td>
<td>78.5</td>
<td>CHF</td>
<td>A</td>
</tr>
</tbody>
</table>

Mean ± SD 92.1 ± 62.6 86.6 ± 87.8 181 ± 227 39.6 ± 2.5 31.7 ± 16.9 31.7 ± 16.9 130.1 ± 68.2 35.7 ± 6.7 51.3 ± 24.5

⁴ Bile acids: Normal reference range: 0–8.2 μmol/L (fasting).
⁵ ALT, alanine aminotransferase; Normal reference range: 0–45 U/L.
⁶ γ-GT: Normal reference range: 0–45 U/L.
⁷ Albumin: Normal reference range: 33–58 g/L.
⁸ Reference Healthy Ranges: leucine 115 ± 29; isoleucine 66 ± 18; valine 218 ± 45; phenylalanine 60 ± 14; tyrosine 71 ± 21.
⁹ DX, disease diagnosis; BA, extrahepatic biliary atresia; AS, Alagille Syndrome; CHF, congenital hepatic fibrosis/Caroly Syndrome.
¹⁰ Child-Turcotte Classification: A, mild disease; B, moderate disease; C, severe disease.
A stochastic model was used to calculate the EAR for total BCAA in healthy school-aged children studied previously (17). Data were compared with healthy children by repeated-measures ANOVA.

### Phenylalanine kinetics

A stochastic model was used to calculate Phe kinetics with a constant oral administration of isotope to study amino acid oxidation (48). Flux [μmol/(kg·h)] was calculated from isotope dilution of the administered tracer in the metabolic pool at steady state (urinary enrichment) using standard equations (49,50). \( F^{13}CO_2 \) was calculated and the rate of tracer oxidation [μmol/(kg·h)] determined according to the model of Mathews et al. (51). The rate of \( L\text{-}[1^{13}C]\text{Phe} \) oxidation [μmol/(kg·h)] was calculated from urinary Phe enrichment and from \( F^{13}CO_2 \) (26,49).

### Statistical analysis

A mixed model (proc mixed; ANOVA) was performed to assess the relation of \( F^{13}CO_2 \), Phe flux, oxidation, nonoxidative Phe disposal (NOPD), and Phe released from endogenous proteolysis (B\(_{\text{Phe}}\)) to the following variables: total BCAA intake, order of intake, subject, and potential interactions (52). Potential interactions included the subject and diet interactions, diet, and order of consumption effects. Changes in body weight and body composition during the study period were compared by repeated-measures ANOVA. A comparison of FFM between the study subjects and healthy subjects was done using repeated-measures ANOVA. The breakpoint in phenylalanine enrichment (B\(_{\text{Phe}}\)) was calculated from \( F^{13}CO_2 \) (representing the EAR) was determined using Fieller’s theorem (53). A post hoc Student-Newman-Keuls test was done to determine any significant effect of dietary BCAA intake on Phe kinetic parameters (NOPD, flux, B\(_{\text{Phe}}\), and oxidation). The statistical difference between the EAR for total BCAA in healthy school-aged children studied previously and in children with MCC liver disease was assessed using the unpaired 2-sample \( t \) procedure (17,54). These analyses were conducted using SAS statistical software (SAS, Version 9.1; SAS Institute). Differences were considered significant at \( P < 0.05 \). Values are expressed as means ± SD.

### RESULTS

All subjects received 7 randomly allocated graded intakes of total BCAA on different test days. Body composition measures [weight, height, percentage of ideal body weight (%IBW), FFM, and LBM] were all within the normal ranges for age and did not change over the study period (Table 1) (28,30). Measured FFM did not differ among the 3 methods used or between the children in this study and the healthy children studied previously (\( P > 0.05 \)) (17,55). The Tanner stage of all study participants ranged between 1 and 2 (1.2 ± 0.4). RMR and the fasting respiratory quotient (RQ) were 4480 ± 2159 (kJ/d) and 0.78 ± 0.02, respectively. All children had MCC liver disease as indicated by elevations in bile acids, conjugated bilirubin, and/or \( \gamma \)-GT (Table 2). All children had normal synthetic liver function (as assessed by serum levels of albumin and international normalized ratio) and were clinically free of visible jaundice, with the exception of one child with Alagille Syndrome (47).

BCAA concentrations in plasma were lower than normal reference ranges, whereas AAA concentrations were within normal range (Table 2). These results represent the characteristic amino acid patterns observed in MCC liver disease in children (7). The HOMA score for evaluating insulin resistance [3.47 ± 5.16 (mmol/L)] was elevated, suggesting that children in this study were mildly insulin resistant. Fasting levels of insulin (80 ± 97.4 pmol/L) and C-peptide (180 ± 12 pmol/L) were within normal ranges (38,56).

Results of the \( L\text{-}[1^{13}C]\text{Phe} \) \( F^{13}CO_2 \) for the individual subjects participating in this study are shown in Table 4. \( F^{13}CO_2 \) production was related to total BCAA intake (\( P = 0.0063 \)) and was affected by the interaction of individual study subjects (\( P = 0.018 \)). The rate of the release of the \( ^{13}CO_2 \) varied among the subjects, although the pattern of response to varying intakes of the total BCAA was consistent. Phe flux (\( P = 0.8932 \)) and Phe oxidation (\( P = 0.2396 \)), NOPD (\( P = 0.9088 \)), and B\(_{\text{Phe}}\) (\( P = 0.8272 \)) were not affected by total BCAA intake or order of test but differed among study subjects (\( P < 0.0001 \)). There was no significant effect of order of total BCAA intake on any of the outcomes measured.

Breakpoint analysis of \( F^{13}CO_2 \) production using a mixed-model ANOVA indicated an EAR for total BCAA of 209 mg/
DISCUSSION

This is the first study done to determine directly the total BCAA needs in children with chronic liver disease. We determined previously that the mean requirement of total BCAA using the IAAO in healthy school-aged children was 147 mg/(kg·d) (17), which is significantly lower than the mean requirement determined in this study. We studied healthy school-aged children previously in part to enable comparison of total BCAA requirements in health and disease in school-aged children. Although the children in this study were not matched precisely with the school-aged children studied previously, they were similar in age and did not differ in body composition and resting energy expenditure (19). Thus, the healthy school-aged children studied previously are an appropriate comparison group with which to assess the effect of chronic liver disease on total BCAA requirements in children. The current study demonstrates that the mean requirement of total BCAA, as determined by IAAO, in children with mild-to-moderate liver disease is 207 mg/(kg·d), which is greater than the mean requirements for the total BCAA established in the healthy children studied previously (17).

The amount of dietary protein provided in both studies was in excess of estimated requirements to ensure that all subjects were in positive nitrogen balance (2,17,20). The model used in this study provided BCAA in the same proportions as those present in egg protein to minimize the potential confounding effects of interactions of the individual BCAA on assessment of requirement (18,19,57). We used this model previously in our laboratory for determining the total BCAA requirements of healthy children and adults (17,20). A limitation of this model is that it assumes that the BCAA composition of egg protein represents the "ideal" pattern of dietary BCAA in children (17,58). Because recent studies of Riazi et al. (58) in adults suggested that valine is limiting in egg protein, overestimation of isoleucine and leucine requirements (by ~10%) may have occurred in the present study.

Alterations in leucine metabolism were attributed in part to the differences in body composition that were observed in cirrhotic patients (10–12,14,59). Because potential differences in body composition may affect the estimate of total BCAA requirements in this population, we used a variety of methods to assess body composition to minimize the potential for this to occur in our study. MCC liver disease may significantly alter body composition, even when weight and height are within normal ranges (6). Isotope dilution was shown to detect changes in extracellular fluid in adults with mild liver disease and permits more accurate measurement of FFM in this population (59). Total BCAA requirements in the children with MCC liver disease were increased to the same extent compared with requirements in healthy children whether the requirement was expressed on a per unit FFM or per kilogram total mass basis, indicating that increases in total BCAA requirements in MCC liver disease were not due to differences in body composition induced by liver disease.

Although we did not determine urine nitrogen excretion and hence cannot calculate the nonprotein respiratory quotient, the reduced respiratory quotient (0.78) measured in the children with MCC liver disease compared with that of healthy children studied previously (0.87), suggests that the children with MCC liver disease utilize fat more than glucose in the fasted state (4,17). Increased protein and lipid utilization along with hypermetabolism was shown to occur in children with more advanced liver disease (4). This is in contrast to healthy children who rely more heavily on glucose and protein oxidation (RQ = 0.87) to meet energy needs in the postabsorptive state (19).
Assessing insulin resistance using HOMA, the children participating in this study were found to be mildly insulin resistant (56). Fasting plasma levels of glucose, C-peptide, and insulin were normal. Although we did not measure plasma levels of insulin growth factor-1 (IGF-1) and the related IGF binding proteins (IGFBP), there is evidence that changes in IGF-1 and IGFBP levels that reflect growth hormone resistance occur in children with biliary atresia and Alagille Syndrome (4,60,61). Growth hormone resistance and insulin resistance in MCC liver disease likely contribute to the shift toward lipid and protein oxidation in the postabsorptive state. The extent to which this contributes to an increase in total BCAA requirements in children with MCC liver disease remains unclear. There is evidence in animal models of liver disease for increased activity of the branched chain keto-acid dehydrogenase complex in both liver and skeletal muscle, suggesting that increased oxidation of the BCAA may occur (62). Leucine, as a ketogenic amino acid, may be preferentially used as an energy substrate, and the carbon skeletons of valine and isoleucine diverted toward gluconeogenesis. This is currently under investigation by our group (63).

Changes in BCAA metabolism typically observed include depressed plasma levels of BCAA in the presence of normal levels of the AAA, although increasing levels of the AAA may be observed with disease progression (6,7). The children in the current study had depressed plasma levels of the BCAA with normal levels of the AAA in the presence of comparatively mild cholestasis and normal liver synthetic function. These alterations in BCAA metabolism may occur in children with even mild liver dysfunction. Portal hypertension, which was present in at least 3 of the 5 study participants, may contribute to alterations in metabolism of the BCAA by the shunting of insulin and other trophic factors from the liver to the periphery (61). Studies demonstrating increased whole-body leucine oxidation in the postabsorptive state in adults with compensated liver cirrhosis support the premise that leucine is diverted for oxidative utilization in liver disease (4,60,61). These studies are in contrast to other work demonstrating decreased or no changes in leucine oxidation in patients with chronic liver disease in both the fed and fasted states (1,5,8,64,65). Differing techniques for assessment of body composition and precursor choice for leucine kinetic studies, along with varying severity of liver disease in study participants, are likely contributors to the heterogeneity in study conclusions (10–12,14,59).

Children with chronic liver disease often experience lower intakes of macronutrients because of anorexia, particularly with disease progression (2). This places them at high risk for becoming deficient in the BCAA and suggests that routine dietary supplementation of BCAA may be warranted in this population. Significant improvements in weight gain, liver function, and quality of life were observed in adults and children with end-stage liver disease who received dietary BCAA supplements (1,8). Supplementation of the BCAA in adult patients with liver cirrhosis provided at 206 mg/(kg * C1)* over 1 y resulted in significant improvements in liver function and quality of life markers (8). Because this level of BCAA supplementation did not differ from the measured EAR of total BCAA in the present study, it is likely that BCAA supplementation at the level of 272 mg/(kg * C1)* (upper 95% CI) in children with MCC liver disease would result in similar improvements in growth, liver function, and quality of life.

In summary, this study demonstrates that total BCAA needs in children with MCC liver disease, as determined by the IAAO, are significantly higher than the requirements of healthy school-aged children previously studied by our group (17). This was likely due to the fact that the children ingested sufficient dietary BCAA, protein, and energy in their daily diets. The current study provides evidence that children with relatively mild liver disease have increased dietary needs for the BCAA, and is consistent with the work of Chin et al. (1).

Further studies examining the effect of dietary BCAA supplementation (at the level of the determined RDA) on growth in children with more advanced liver disease are warranted because malnutrition and growth failure are prevalent in this population (2,66).

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LITERATURE CITED


