Branched-Chain Amino Acid Needs in Children with Mild-to-Moderate Chronic Cholestatic Liver Disease1,2

Diana R. Mager,*† Linda J. Wykes,†† Eve A. Roberts,††*** Ronald O. Ball,*‡‡ and Paul B. Pencharz,*†††‡‡‡‡‡‡

*Department of Nutritional Sciences, †Department of Paediatrics, and **Department of Pharmacology, University of Toronto, Toronto, ON, Canada; †The Research Institute, The Hospital for Sick Children, Toronto, ON, Canada; ††School of Dietetics & Human Nutrition, McGill University, Ste-Anne-de-Bellevue, QC, Canada; and ‡‡Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada

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 $\text{L-[1-}^{13}\text{C]}\text{Phe}$ to $^{13}\text{CO}_2$ ($^{13}\text{CO}_2$ in $\mu\text{mol/(kg h)}$), 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, making it important to study the effect of chronic liver disease in children (1–5). Limited information is available in children regarding the effect of chronic liver disease on branched-chain amino acid (BCAA) 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 range of chronic liver diseases. The characteristic amino acid patterns often result in decreased AAA oxidation rates, which may be compensated by increased BCAA oxidation rates. The current study was designed to determine the total BCAA requirement in children with chronic liver disease supplemental 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).

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4 To whom correspondence should be addressed. E-mail: paul.pencharz@sickkids.ca.

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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-y-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, L-[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 estimated 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 kg</th>
<th>LBM kg</th>
<th>FFM kg</th>
<th>RMR kJ/d</th>
<th>Energy Intake kJ/d</th>
<th>RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<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>
<td>3922</td>
<td>6667</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>4.1</td>
<td>13.9</td>
<td>99.8</td>
<td>89.1</td>
<td>12.3</td>
<td>11.1</td>
<td>8.1</td>
<td>3797</td>
<td>6492</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>11.9</td>
<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>
<td>0.78</td>
</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>3314</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
chronic MCC (Table 1). Activity patterns were reviewed by the investigator on each study day to ensure that activity patterns were maintained over the course of the 1- to 2-mo study period.

The study diet consisted of a flavored protein-free liquid formula (Protein-Free Powder; product 80056; Mead Johnson; Tang and Kool-Aid, Kraft Foods), a crystalline L-amino acid mixture study mixture, and protein-free cookies (15,16). The experimental diet provided ~14% of total energy from protein, 50% of total energy from carbohydrate, and 36% of total energy from fat. All diets were prepared and weighed (scale model PE2000, Mettler) in the research kitchen at the HSC. Protein was provided as an L-amino acid mixture based on the amino acid composition of egg protein. BCAA were provided in the same proportion as in egg protein (38.5% leucine, 29% isoleucine, and 32.5% valine) to minimize potential interactive effects of the BCAA on determining the requirement (33-35). The experimental diet included 25 mg/(kg-d) Phe to ensure adequacy of dietary intake. This was provided in the presence of excess tyrosine [40 mg/(kg-d)]. This level of tyrosine in the diet was shown to minimize the delay of Phe in the liver because both are metabolized in the liver. We chose to use [1-13C]-labeled Phe for 3 main reasons: 1) little is known about the effect of liver disease on lysine metabolism and its potential to affect study results, 2) [1-13C]-labeled lysine is expensive, and 3) the effect of liver disease on Phe oxidation is well documented. Although reductions in Phe oxidation in chronic liver disease range between 30 and 60%, the effect in mild liver disease tends to be much smaller (36).

Pilot studies to assess the effect of MCC liver disease in children on Phe metabolism were done before the initiation of the indicator studies. These studies showed that Phe oxidation was reduced by <15% in children with MCC liver disease (4.39 ± 2.94 compared with 5.03 ± 1.24 μmol/L in healthy children). In addition, we were able to demonstrate in the current study that the presence of MCC liver disease in children did not result in any significant differences in whole-body estimates of Phe flux (P = 0.98), Phe oxidation (P = 0.54), nonoxidative Phe disposal (P = 0.98), and Phe released from endogenous protolysis (P = 0.70) compared with estimates in healthy school-aged children reported previously by our group (Table 3) (17). The oxidation also followed the same response to increases in total BCAA intake, with similar levels of intersubject variability at each level of dietary intake as those reported previously in healthy children and adults, suggesting that the effect of liver disease on isotope kinetics did not affect study conclusions.

**TABLE 2**

<table>
<thead>
<tr>
<th>Sub</th>
<th>Bile acids1 μmol/L</th>
<th>ALT2 U/L</th>
<th>γ-GT3 μmol/L</th>
<th>Albumin4 g/L</th>
<th>Leucine5 μmol/L</th>
<th>Isoleucine5 μmol/L</th>
<th>Valine5 μmol/L</th>
<th>Phe5 μmol/L</th>
<th>Tyrosine6 μmol/L</th>
<th>DX6</th>
<th>Child-Turcotte Classification7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.8</td>
<td>67</td>
<td>214</td>
<td>40</td>
<td>18.1</td>
<td>18.1</td>
<td>104.1</td>
<td>36.1</td>
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<td>2</td>
<td>79.6</td>
<td>130</td>
<td>63</td>
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<td>36.2</td>
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<td>151.7</td>
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<td>565</td>
<td>40</td>
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<td>B</td>
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<tr>
<td>5</td>
<td>69.4</td>
<td>11</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>

1 Bile acids: Normal reference range: 0–8.2 μmol/L (fasting).
2 ALT, alanine aminotransferase; Normal reference range: 0–45 U/L.
3 γ-GT, normal reference range: 0–45 U/L.
4 Albumin: Normal reference range: 33–58 g/L.
5 Reference Healthy Ranges: leucine 115 ± 29; isoleucine 66 ± 18; valine 218 ± 45; phenylalanine 60 ± 14; tyrosine 71 ± 21.
6 DX, disease diagnosis; BA, extrahepatic biliary atresia; AS, Alagille Syndrome; CHF, congenital hepatic fibrosis/Carolyi Syndrome.
7 Child-Turcotte Classification: A, mild disease; B, moderate disease; C, severe disease.
Effect of total BCAA intake on Phe kinetics in children with chronic liver disease

<table>
<thead>
<tr>
<th>Total BCAA intake (mg/(kg d))</th>
<th>13CO2 Phe oxidation</th>
<th>NOPD</th>
<th>Flux</th>
<th>Bphe</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>1.094 ± 0.581</td>
<td>7.61 ± 1.95</td>
<td>62.3 ± 23.7</td>
<td>69.8 ± 27.7</td>
</tr>
<tr>
<td>100</td>
<td>0.828 ± 0.076</td>
<td>4.84 ± 1.92</td>
<td>65.3 ± 27.4</td>
<td>70.2 ± 29.3</td>
</tr>
<tr>
<td>150</td>
<td>0.741 ± 0.121</td>
<td>4.07 ± 2.61</td>
<td>60.2 ± 28.7</td>
<td>64.3 ± 31.2</td>
</tr>
<tr>
<td>200</td>
<td>0.669 ± 0.141</td>
<td>4.21 ± 2.11</td>
<td>68.2 ± 29.7</td>
<td>72.4 ± 31.6</td>
</tr>
<tr>
<td>250</td>
<td>0.663 ± 0.299</td>
<td>3.06 ± 2.17</td>
<td>47.4 ± 28.9</td>
<td>50.4 ± 31.2</td>
</tr>
<tr>
<td>300</td>
<td>0.588 ± 0.220</td>
<td>3.70 ± 1.99</td>
<td>62.2 ± 16.3</td>
<td>61.3 ± 12.1</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 5. Data were analyzed by repeated-measures ANOVA, followed by Student-Newman-Keuls post hoc test.
2 Values are not different ($P > 0.05$) from those of healthy 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 ($\mu$mol/(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). $F^{13}$CO2 was calculated and the rate of tracer oxidation ($\mu$mol/(kg·h)) determined according to the model of Mathews et al. 1980 (51). The rate of L-[1-13C]Phe oxidation ($\mu$mol/(kg·h)) was calculated from urinary Phe enrichment and from $F^{13}$CO2 (26,49).

Statistical analysis. A mixed model (proc mixed: ANOVA) was performed to assess the relation of $F^{13}$CO2, Phe flux, oxidation, nonoxidative Phe disposal (NOPD), and Phe released from endogenous protein ($B_{phe}$) to the following variables: total BCAA intake, breakpoint in healthy subjects was done using repeated-measures ANOVA. The ANOVA. A comparison of FFM between the study subjects and during the study period were compared by repeated-measures ANOVA. FFM steady state (urinary enrichment) using standard equations (49,50).

FIGURE 1 Effect of total BCAA intake on breath $^{13}$CO2 enrichment and urinary L-[1-13C]Phe enrichment in children with MCC liver disease. Values are means ± SD at one representative level of BCAA intake [100 mg/(kg·d)], n = 5.

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 ± 1859 (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 γ-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-[1-13C]Phe $F^{13}$CO2 for the individual subjects participating in this study are shown in Table 4. $F^{13}$CO2 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 $^{13}$CO2 varied among the subjects, although the pattern of response to varying intakes of the total BCAA was consistent. Phe flux ($P = 0.8932$), Phe oxidation ($P = 0.2396$), NOPD ($P = 0.9088$), and $B_{phe}$ ($P = 0.8272$) 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 $F^{13}$CO2 production using a mixed-model ANOVA indicated an EAR for total BCAA of 209 mg/
TABLE 4

Individual $^{13}$CO$_2$ data at all total BCAA intake levels for children with chronic liver disease

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total BCAA intake, mg/(kg·d)</th>
<th>$^{13}$CO$_2$ (μmol/(kg·h))</th>
<th>$^{13}$CO$_2$ (μmol/(kg·h))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>1</td>
<td>1.726</td>
<td>0.928</td>
<td>0.689</td>
</tr>
<tr>
<td>2</td>
<td>1.429</td>
<td>0.765</td>
<td>0.867</td>
</tr>
<tr>
<td>3</td>
<td>0.724</td>
<td>0.875</td>
<td>0.706</td>
</tr>
<tr>
<td>4</td>
<td>0.799</td>
<td>0.830</td>
<td>0.868</td>
</tr>
<tr>
<td>5</td>
<td>0.803</td>
<td>0.712</td>
<td>0.866</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.094 ± 0.581</td>
<td>0.828 ± 0.078</td>
<td>0.741 ± 0.121</td>
</tr>
</tbody>
</table>

1 $^{13}$CO$_2$ is the rate of release of $^{13}$CO$_2$ from $^{13}$C-Phe oxidation.

kg and an RDA (upper 95% confidence limit) of 272 mg/(kg·d) in children with liver disease ($P < 0.05$) (Fig 2). The regression lines represent the partitioning of the data that provided the best fit of the data ($r^2 = 0.36$, SE of the breakpoint = 36.2). These results demonstrate that the EAR for total BCAA using the IAAO was significantly higher in children with MCC liver disease than in healthy children studied previously ($P < 0.05$) (17).

**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 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 urinary 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).

FIGURE 2 Effect of total BCAA intake on oxidation of L-[1-$^{13}$CO$_2$]Phe determined from the rate of release of $^{13}$CO$_2$ ($F^{13}$CO$_2$). Values are means ± SE for oxidation at each of the 7 levels of total BCAA for 5 children with MCC liver disease ($n = 35$ observations); $r^2 = 0.36$, $P < 0.01$. 

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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-d) over 1 yr 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-d) (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