Effects of Branched Chain Amino Acid-enriched Total Parenteral Nutrition on Amino Acid Utilization in Rats Bearing Yoshida Sarcoma

Lisa E. Crosby, Bruce R. Bistrian, Pei-ru Ling, Nawfal W. Istfan, George L. Blackburn, and Stacy B. Hoffman

ABSTRACT

We have studied the ability of branched chain amino acid-enriched total parenteral nutrition solutions to improve nutritional status without stimulating tumor growth. Protein kinetics, nitrogen balance, tumor kinetics, fractional synthetic rates of individual tissues, and albumin synthesis were compared in male Sprague-Dawley rats (125-145 g) that had either s.c. Yoshida sarcoma \( (n = 15) \) or sham implantations \( (n = 18) \). Ten days postinjection, rats were randomly assigned to 2 diet groups and given parenteral infusions of 4 days at 170 kcal/kg-body wt.-day as dextrose and 2 g N/kg-body wt.-day as either 19 or 50% branched chain amino acid-enriched diet. During the last 4 h of feeding, protein kinetic values were studied using a constant infusion of \(^{14} \)C]tyrosine. Plasma tyrosine appearance, synthesis, and breakdown were unchanged by branched chain amino acid infusion. Percentage of tyrosine flux oxidized and tyrosine oxidation decreased \( (P < 0.05) \) and net tyrosine balance improved \( (P < 0.05) \) in rats receiving the branched chain amino acid-enriched diet. Greater nitrogen balance and lower tumor growth rates were also found in branched chain amino acid-infused rats although not statistically significant. Tumor intracellular specific activity was significantly higher in tumor animals receiving crystalline infusions, suggesting greater tumor protein breakdown with branched chain amino acid-enriched infusion. Fractional synthetic rates of liver, muscle, and tumor were unchanged. Hence, branched chain amino acid-enriched total parenteral nutrition increases amino acid utilization for net protein synthesis principally by reducing oxidation without stimulating tumor growth.

INTRODUCTION

Cancer cachexia is recognized as a primary cause of death in many types of cancer (1). In fact, protein malnutrition is the single most common secondary diagnosis in cancer patients (2). Failure to prevent protein malnutrition increases the risk of developing complications, aggravates antineoplastic therapy, reduces the quality of life, and increases morbidity and mortality (2-5).

Although malnutrition may result from interference with gastrointestinal function, the usual cachexic syndrome developing in the course of malignant disease often cannot be explained by depression of food intake due to local factors alone and behaves as if it were the result of a systemic alteration including anorexia induced by the tumor (6). Furthermore, the weight loss accompanying cancer in humans bears no consistent relationship with the type, duration, site, size, or number of metastases (7).

Recent evidence indicates a similarity between the metabolic response to injury and active tumor growth (8). This similarity raises interest in a possible role for BCAA in modifying the response to injury and active tumor growth (8). This similarity raises interest in a possible role for BCAA in modifying the regulation of tumor growth. Protein synthesis, fractional synthetic rates of individual tissues, and albumin synthesis were compared in male Sprague-Dawley rats (125-145 g) that had either s.c. Yoshida sarcoma \( (n = 15) \) or sham implantations \( (n = 18) \). Ten days postinjection, rats were randomly assigned to 2 diet groups and given parenteral infusions of 4 days at 170 kcal/kg-body wt.-day as dextrose and 2 g N/kg-body wt.-day as either 19 or 50% branched chain amino acid-enriched diet. During the last 4 h of feeding, protein kinetic values were studied using a constant infusion of \(^{14} \)C]tyrosine. Plasma tyrosine appearance, synthesis, and breakdown were unchanged by branched chain amino acid infusion. Percentage of tyrosine flux oxidized and tyrosine oxidation decreased \( (P < 0.05) \) and net tyrosine balance improved \( (P < 0.05) \) in rats receiving the branched chain amino acid-enriched diet. Greater nitrogen balance and lower tumor growth rates were also found in branched chain amino acid-infused rats although not statistically significant. Tumor intracellular specific activity was significantly higher in tumor animals receiving crystalline infusions, suggesting greater tumor protein breakdown with branched chain amino acid-enriched infusion. Fractional synthetic rates of liver, muscle, and tumor were unchanged. Hence, branched chain amino acid-enriched total parenteral nutrition increases amino acid utilization for net protein synthesis principally by reducing oxidation without stimulating tumor growth.

MATERIALS AND METHODS

Animal Preparation and Nutrient Infusion. Thirty-three specific pathogen-free male Sprague-Dawley rats (35-40 g) were obtained from Taconic Farms (Germantown, NY). The study was conducted under the approval of the Animal Care Committee at the New England Deaconess Hospital and in compliance with their established rules and guidelines. Prior to the experiment, rats were housed, for a minimum of 5 days, 2 to a cage and maintained on a 12/12 h light/dark photoperiod at an ambient temperature of \( 27 \pm 1\) °C. Tap water and rodent chow (Charles River D-3000; Agway Agricultural Products, Minneapolis, MN) were provided ad libitum.

The Yoshida sarcoma tumor cell line was used to induce cancer cachexia. The study was conducted from day 10 to 14 which is on the linear portion of the tumor growth curve and was determined to be a cachectic period (Fig. 1). On day 0 of the study, 15 animals were inoculated with \( 10^7 \) cells of viable Yoshida sarcoma into the s.c. area of the right flank. The non-tumor-bearing rats were given an identical sham injection with sterile saline. The animals were weighed, returned to their cages, and allowed to consume standard laboratory chow and tap water ad libitum. The animals were not pair fed and the rats bearing rapidly growing tumors had diminished food intake, reflecting the presence of tumor. On day 10, the tumor-bearing animals weighed less reduced the loss of body protein by 15-80% in rats (9-12) and man (13, 14). In vitro, BCAA inhibit protein degradation and promote protein synthesis (15) and have specific regulatory functions in the turnover of skeletal muscle protein (16). Hence, the regulatory effect of BCAA on muscle protein turnover needs to be further characterized in relationship to host tissues as well as the tumor itself.

Studies by Schaur and his associates demonstrated that continuous p.o. administration of BCAA to rats bearing Yoshida sarcoma significantly increased survival time by 32% and reduced tumor size by 33% after 3 wk of growth (17). Their work also demonstrated that a shift in nitrogen balances was delayed, but not prevented, by BCAA administration (17). In our laboratory, Kawamura et al. found that BCAA-enriched solutions infused into animals bearing RNC-fibrosarcoma limited the reduction in skeletal protein synthesis seen in tumor-bearing animals receiving standard amino acid formulas (18). Also, Tayek and his coworkers gave parenteral infusions containing 50% BCAA-enriched solutions to malnourished patients with intraabdominal metastatic adenocarcinoma and improved whole body leucine flux, oxidation, leucine balance, and fractional synthetic rates of albumin (19). However, there was concern in the latter study that the choice of leucine as the tracer when leucine-enriched solutions are studied might lead to underestimates of leucine oxidation and therefore overestimates of leucine balance. Thus, we sought to confirm the benefits of parenterally administered BCAA for kinetic estimates of protein metabolism using tyrosine as the amino acid tracer. Since the tyrosine content of both formulas is very low and lower in the BCAA-enriched solution, a finding of improvement in parameters of protein metabolism with the BCAA-enriched solution using tyrosine as the tracer amino acid in a second animal tumor model would lend further support to their potential value for nutritional repletion in cancer cachexia.
than the non-tumor-bearing animals, indicating their cachectic state. The rats were anesthetized with ether and a Silastic catheter (0.025 inside diameter x 0.047 in outside diameter; Dow-Corning Laboratories, Corning, NY) was inserted through the internal jugular vein as previously described (20). The animals were placed in metabolic units and i.v. infused with saline (approximately 20 ml of sodium chloride (154 mmol) overnight from a Holler pump (Ritterikon Inc., Tampa, FL)). On day 11, the animals were randomly assigned to 2 groups and i.v. administered diets containing amino acids and one half the dextrose calories (Table 1) to allow adaptation to the glucose. There was no significant difference in the size of the tumors, as indicated by measurements of tumor volume, between the two diet groups. The animals were given the full calorie diet on day 12 and feeding continued for an additional 2 days. At the full calorie level both diets contained 220 calories/kg • body wt-day, of which 75% was hydrous dextrose and 25% was amino acids (2 g amino N/kg • body wt-day as either 19% BCAA or 50% BCAA (Table 1). The diets were isonitrogenous, isovoleric, and isocaloric and were formulated in the hospital pharmacy under aseptic conditions. An infusion of 40 ml per day was sufficient to deliver the total amount of calories. The animals were infused for 3.5 days which has been demonstrated to be a sufficient time to reveal differences if they indeed exist. Hyperalimentation in a small tumor-bearing rat for a longer period of time (i.e., 10–14 days) is difficult to accomplish due to technical failures and an increase in mortality. Twenty four-h urine was collected for nitrogen balance determination.

Isotopic Turnover Design. On day 14, 14C]tyrosine (350 mCi/mmol; ICN, Irvine, CA) was added to the diets and a 4-h constant infusion was conducted to investigate protein kinetics. Each animal received 1.7 µCi/h of [14C]tyrosine at a volume rate of 1.67 ml/h through a syringe pump (±1%; Harvard Apparatus Co., Natick, MA). Total carbon dioxide production, O2 consumption, respiratory quotient, and resting energy expenditure were determined during the infusion as previously described (20). VO2 and VCO2 were measured by methods previously published by our laboratory (20).

At the end of the infusion, the animals were sacrificed by decapitation and the blood was collected in heparinized tubes. Plasma was separated by centrifugation and stored at —25°C. Immediately following decapitation, the body was quickly dissected, and the liver, tumor, and portions of the abdominal rectus muscle were removed. The liver was weighed and 1-g pieces were placed in 5 ml of 10% sulfosalicylic acid and 5 ml of saline and then completely frozen in liquid nitrogen (~180°C) to halt all metabolic processes. Two 1-g pieces of muscle were similarly frozen. The tumor was weighed and also frozen intact in liquid nitrogen. All samples were stored at —25°C until analysis. The total time between decapitation and sample freezing in liquid nitrogen was 2–3 min.

Analytical Methods. VO2, VCO2, respiratory quotient, and resting energy expenditure were derived from the equations of Weir (21). Total urinary nitrogen was determined following a micro-Kjeldahl digestion.

In preparation for the tyrosine and albumin specific activity assays, the plasma was thawed and deproteinized with 10% trichloroacetic acid (2:1 v/v; plasma:trichloroacetic acid). Following centrifugation, the supernatant was analyzed for free tyrosine specific activity as previously described (22). Tyramine concentration was determined by the method of Waalkes and Underfriend (23).

The protein pellet was analyzed for albumin specific activity by means of an ethanol-water extraction. One ml of the extracted supernatant was added to 10 ml of commercial scintillant (Monофluoroc; National Diagnostics, Manville, NJ) with 100 µl of glacial acetic acid to suppress chemiluminescence and analyzed, in duplicate, for 14C radioactivity (LS-8000 spectrometer; Beckman, Fullerton, CA). To determine ammin concentration, 25 µl of the ethanol supernatant were transferred, in duplicate, into test tubes containing 6 ml of a bromocresol green solution (Folch substrate; General Diagnostics, Morris Plains, NJ) and distilled water (1:5 v/v solution) and vortexed for 10 sec. The samples were analyzed spectrophotometrically at a wavelength of 630 nm. Albumin concentrations were determined by linear regression of known standards, and unknowns were determined from the regression line.

The tissue samples, liver, abdominal rectus muscle, and tumor were thawed and homogenized (Polytron; Brinkmann Instruments, Westbury, NY). Nitrogen content of tissue samples stored in saline was determined by micro-Kjeldahl digestion. Homogenized tissue samples stored in sulfosalicylic acid were centrifuged at 4500 rpm to separate the acid-soluble amino acids (Si) from protein-bound precipitate (Sb). The acid-soluble amino acid supernatant for each sample was analyzed spectrophotometrically at a wavelength of 630 nm. Albumin concentrations were determined by linear regression of known standards, and unknowns were determined from the regression line.

The tissue samples, liver, abdominal rectus muscle, and tumor were thawed and homogenized (Polytron; Brinkmann Instruments, Westbury, NY). Nitrogen content of tissue samples stored in saline was determined by micro-Kjeldahl digestion. Homogenized tissue samples stored in sulfosalicylic acid were centrifuged at 4500 rpm to separate the acid-soluble amino acids (Si) from protein-bound precipitate (Sb). The acid-soluble amino acid supernatant for each sample was analyzed spectrophotometrically at a wavelength of 630 nm. Albumin concentrations were determined by linear regression of known standards, and unknowns were determined from the regression line.

Table 1 Dietary intake

<table>
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<tr>
<td>Control</td>
<td>45.6</td>
<td>174.4</td>
<td>220</td>
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<tr>
<td>Test</td>
<td>51.6</td>
<td>168.4</td>
<td>220</td>
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<tr>
<td>Diets with one half dextrose calories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular diet</td>
<td>Amino acid = 3.7%</td>
<td>Dextrose = 10.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branched chain enriched diet</td>
<td>Amino acid = 4.2%</td>
<td>Dextrose = 10.4%</td>
<td></td>
<td></td>
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<tr>
<td>Diets with full dextrose calories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular diet</td>
<td>Amino acid = 3.7%</td>
<td>Dextrose = 21.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branched chain enriched diet</td>
<td>Amino acid = 4.2%</td>
<td>Dextrose = 20.9%</td>
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Additive per 1500 ml

<table>
<thead>
<tr>
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<th>mEq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>45</td>
</tr>
<tr>
<td>NaAc</td>
<td>45</td>
</tr>
<tr>
<td>KCI</td>
<td>45</td>
</tr>
<tr>
<td>KAc</td>
<td>37</td>
</tr>
<tr>
<td>KPhos</td>
<td>23</td>
</tr>
<tr>
<td>Ca Glu</td>
<td>12.5</td>
</tr>
<tr>
<td>MgSO4</td>
<td>12.0</td>
</tr>
<tr>
<td>Trace minerals</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Plus 1.0 ml of MVC 9 + 3 vitamins per 100 ml of hyperalimentation solution.
dried protein-bound precipitate and the samples were incubated overnight at 50°C. Ten ml of commercial scintillant (Beckman NA) were added to the vials with 200 µl of glacial acetic acid to suppress chemiluminescence and analyzed for 14C radioactivity (dpm/mg) (Beckman LS-8000 spectrometer).

Rate of whole-body tyrosine appearance, oxidation, percentage of tyrosine oxidized, synthesis, breakdown, and tyrosine balance were estimated from the equations of Waterlow (24). It was assumed that a plateau labeling (steady state) of the plasma compartment was achieved when the S_app was reached in the expired breath (between 2 to 3 h of continuous [14C]tyrosine infusion) (25). Plasma tyrosine appearance can be attributed to the degradation of body protein, dietary intake, or hydroxylation of phenylalanine (Fig. 1). It was previously shown in our laboratory that approximately 22% of tyrosine flux is derived from phenylalanine (26). Studies done by other investigators (27) have shown similar proportions of phenylalanine-derived plasma tyrosine, suggesting that this fraction is relatively stable in animals with normal liver function. In this experiment, most of the plasma phenylalanine flux is derived from protein breakdown since dietary intake was negligible (Table 1). Under these conditions, it can be shown that a 50% increase in the rate of conversion to tyrosine will result in only a 10% increase in tyrosine appearance in excess of what is derived from breakdown. Therefore, it is appropriate to consider that changes in plasma tyrosine appearance in this study are closely related to changes in whole-body protein breakdown. The protein fractional synthetic rates in the liver, abdomen, rectus muscle, and tumor were derived from the equations of Garlick et al. (28).

Estimates of fractional tumor growth, Kf, were derived from tumor volume measurements on days 10 and 14. Tumor volumes were estimated from measurements of tumor length, width, and depth in millimeters as previously described (29). These measurements bear a close relationship to tumor weight. Tumor protein breakdown rates were estimated as the difference between tumor protein synthesis, measured isotopically, and tumor growth.

Statistical Analysis. Data are presented as mean ± SEM. The data from groups one through four were compared for statistical differences using two-way and one-way analysis of variance using statistical software (BMDP Statistical Software, Los Angeles, CA).

RESULTS

During a constant infusion of L-[U-14C]tyrosine, radioisotope excretion in expired breath generally reached a steady state within 2–4 h. Whole-body tyrosine kinetic values are shown in Table 2. There was no significant difference in mean whole-body protein flux due to BCAA infusion. The use of BCAA-enriched diets significantly reduced whole-body tyrosine oxidation and percentage of flux oxidized compared to animals given a standard formula containing 19% BCAA in both tumor and non-tumor-bearing animals. Although tyrosine incorporation into protein and release by tissue breakdown were not statistically different between the two groups, tyrosine balance was improved (P < 0.05) by BCAA administration in both tumor and non-tumor-bearing animals. The greater sensitivity of tyrosine balance to detect differences over the individual components incorporation and breakdown has been previously noted (30). The effectiveness of BCAA in non-tumor-bearing animals may be attributable to the presence of a stress response related to the catheterization.

Nitrogen analysis revealed significantly higher nitrogen balance in tumor-bearing rats versus the nontumor controls (Fig. 2). The greater nitrogen retention in the tumor-bearing animals may be due to the tumor functioning as a "nitrogen trap" (29) particularly as the animals were studied during a rapid tumor growth phase. Also, the nitrogen balance of both the tumor and non-tumor-bearing animals infused with BCAA-enriched solutions was numerically higher than those infused with crystalline solutions.

All of the animals in the study lost weight, but there were no significant differences in final body weight or weight change. The weight loss may be explained by the small size of the animals, the effects of surgical stress, and the administration of only half of the dextrose calories the first night of feeding to allow for adaptation to parenteral glucose.

Tumor measurements and kinetics are displayed in Table 3. There were no significant differences in tumor volume, weight, percentage of body weight, or fractional synthetic rates due to diet manipulation. The growth rate of the Yoshida sarcoma, as indicated by measurements of tumor volume, was decreased to an insignificant degree by BCAA infusion. Tumor breakdown rates, determined by the difference between tumor synthesis and tumor growth, were higher but also not significant in the animals receiving BCAA-enriched infusions. Of note, however, tumor intracellular specific activity was significantly higher (P < 0.05) in animals infused with crystalline amino acids which is consistent with greater tumor breakdown with BCAA infusion.

The rates of fractional protein synthesis were calculated using the specific radioactivities of the plasma and the protein bound precipitate. No change in the synthetic rates of liver, rectus

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**Table 2** Whole-body tyrosine kinetics

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<tr>
<th></th>
<th>Tumor</th>
<th>Nontumor</th>
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<tbody>
<tr>
<td></td>
<td>BCAA (7)</td>
<td>Crystalline amino acids (8)</td>
</tr>
<tr>
<td>Flux</td>
<td>42.1 ± 4.9</td>
<td>40.3 ± 5.8</td>
</tr>
<tr>
<td>% of flux</td>
<td>14.4 ± 2.5</td>
<td>16.1 ± 1.3</td>
</tr>
<tr>
<td>Oxidation</td>
<td>5.7 ± 0.8*</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td>Synthesis</td>
<td>36.4 ± 4.8</td>
<td>33.7 ± 4.7</td>
</tr>
<tr>
<td>Breakdown</td>
<td>40.0 ± 4.8</td>
<td>38.1 ± 5.8</td>
</tr>
<tr>
<td>Tyrosine balance</td>
<td>-3.6 ± 0.8*</td>
<td>-4.4 ± 1.2</td>
</tr>
</tbody>
</table>

* Mean ± SEM (µmol/h/g body weight). P < 0.05 two-way analysis of variance (BCAA versus CAA).

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**Fig. 2.** Nitrogen balance. Nitrogen balance was determined to be significantly higher in tumor-bearing animals as compared to non-tumor-bearing animals. The animals infused with BCAA-enriched solutions were in a more positive state than those infused with crystalline solutions, but this did not reach a level of statistical significance. Mean ± SEM, P < 0.05 tumor versus nontumor.
There were no significant differences in tumor volume, weight, percentage as body weight, or fractional synthetic rate due to diet manipulation. Growth rate as indicated by measurements of tumor volume was decreased, but not at a significant level. Tumor protein breakdown rates (fractional synthetic rate - growth rate) increased in rats infused with BCAA-enriched diets, but not significantly. Intracellular specific activity (normalized for total infusate volume) was significantly lower in rats receiving BCAA infusions suggesting greater tumor protein breakdown with BCAA-enriched infusions.

There were no changes in albumin specific activity or plasma albumin levels, suggesting no significant alterations in albumin synthetic rates.

<table>
<thead>
<tr>
<th>Specific activity (dpm/μmol)</th>
<th>Plasma levels (g/100 ml)</th>
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<tbody>
<tr>
<td>Tumor BCAA</td>
<td>19,541 ± 4,497⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Tumor crystalline amino acids</td>
<td>16,244 ± 2,241⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Nontumor BCAA</td>
<td>17,058 ± 818⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Nontumor crystalline amino acids</td>
<td>16,386 ± 721⁺⁺⁺⁺</td>
</tr>
</tbody>
</table>

⁺⁺⁺⁺ Mean ± SEM (g/100 ml).
Thus, the data suggest that administration of TPN solutions enriched with BCAA does not stimulate tumor growth but does support host protein metabolism and may play a role in retarding tumor growth by increasing tumor protein breakdown rates. In conclusion, our results suggest that BCAA-enriched TPN solutions increase amino acid utilization for net protein synthesis by the host principally by reducing oxidation without stimulating tumor growth. The site of the improved protein balance was not specifically identified but presumably was in skeletal muscle due to the large size of this component as well as being the usual major site of net nitrogen balance during feeding. Such a location would also be consistent with the mode of action of BCAA to reduce net skeletal protein catabolism. These observations may be important for the development of optimal nutritional support therapies for cancer patients in the treatment of cancer cachexia. Improved support regimens might afford a heretofore undetected benefit of nutritional support in randomized trials of cancer patients receiving chemotherapy (37) and might also be an effective means to slow the progression of the cachexia syndrome and prolong life where effective chemotherapy does not exist. Finally, the suggestive effect of BCAA-enriched total parenteral nutrition to increase tumor breakdown rates deserves further investigation over a longer study period.

REFERENCES
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