Basal Amino Acid Concentrations and the Response to Incremental Glucose Infusion in Tumor Bearing Rats

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ABSTRACT

Plasma amino acid concentrations were measured in fasting nontumor bearing (NTB) and tumor bearing (TB; methylcholanthrene induced sarcoma) male Fischer F344 rats during infusion of 0.9% NaCl solution or glucose at 3.72 or 13.05 µmol/100 g total body weight (TBW)/min. The animals were studied when the tumor comprised only 8% of the TBW at a time when decreased food intake and weight loss were not manifest.

During 0.9% NaCl infusion there were no significant differences between NTB or TB animals in the concentration of alanine (NTB: 152.6 ± 20.1; TB: 150.3 ± 19.0 µM; mean ± SD), branched chain amino acids (BCAA) (NTB: 343.3 ± 48.7; TB: 344.2 ± 20.5 µM), essential amino acids, aromatic amino acids, or total amino acids. During infusion of glucose at 3.72 µmol/100 g TBW/min the alanine levels rose (NTB: 283.6 ± 33.4; TB: 286.7 ± 43.3 µM), and the BCAA levels fell (NTB: 215.9 ± 19.4; TB: 228.7 ± 43.4 µM) to similar concentrations in both NTB and TB animals. Glucose infusion at 13.05 µmol/100 g TBW/min resulted in an additional increase in the alanine concentration (NTB: 344.5 ± 28.7; TB: 382.8 ± 116.6 µM), and a further decrease in the BCAA concentration (NTB: 166.4 ± 30.8; TB: 160.7 ± 30.5 µM) without significant differences between NTB and TB animals. Paired analysis for each animal prior to and during glucose infusion demonstrated a similar absolute micromolar change in alanine and BCAA concentrations during both glucose infusion rates in both NTB and TB animals. The levels of aromatic amino acids and total amino acids were unchanged and the essential amino acid concentrations were decreased only at the higher glucose infusion rate in both NTB and TB groups.

Basal amino acid metabolism appears similar in the NTB and TB animals, prior to the onset of anorexia and weight loss. During exogenous glucose infusion the reciprocal changes in the plasma alanine and BCAA concentrations support the concept of a glucose-alanine-BCAA cycle at the whole body level that appears to respond to a similar extent in NTB and TB animals.

INTRODUCTION

Certain patterns of plasma amino acid concentrations have been demonstrated to exist in various metabolic states; postprandial (1), prolonged fasting (2), kwashiorkor (3), marasmus (4), diabetes (5), hepatic encephalopathy (6), after elective operation (7, 8), fever (9), and sepsis (10). To date there has been little work on the impact of tumor bearing on plasma amino acid concentrations of either 0.9% NaCl solution or glucose at 3.72 or 13.05 µmol/100 g total body weight (TBW)/min. The animals were studied when the tumor comprised only 8% of the TBW at a time when decreased food intake and weight loss were not manifest.

During 0.9% NaCl infusion there were no significant differences between NTB or TB animals in the concentration of alanine (NTB: 152.6 ± 20.1; TB: 150.3 ± 19.0 µM; mean ± SD), branched chain amino acids (BCAA) (NTB: 343.3 ± 48.7; TB: 344.2 ± 20.5 µM), essential amino acids, aromatic amino acids, or total amino acids. During infusion of glucose at 3.72 µmol/100 g TBW/min the alanine levels rose (NTB: 283.6 ± 33.4; TB: 286.7 ± 43.3 µM), and the BCAA levels fell (NTB: 215.9 ± 19.4; TB: 228.7 ± 43.4 µM) to similar concentrations in both NTB and TB animals. Glucose infusion at 13.05 µmol/100 g TBW/min resulted in an additional increase in the alanine concentration (NTB: 344.5 ± 28.7; TB: 382.8 ± 116.6 µM), and a further decrease in the BCAA concentration (NTB: 166.4 ± 30.8; TB: 160.7 ± 30.5 µM) without significant differences between NTB and TB animals. Paired analysis for each animal prior to and during glucose infusion demonstrated a similar absolute micromolar change in alanine and BCAA concentrations during both glucose infusion rates in both NTB and TB animals. The levels of aromatic amino acids and total amino acids were unchanged and the essential amino acid concentrations were decreased only at the higher glucose infusion rate in both NTB and TB groups.

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2 The abbreviations used in this paper are: TB, tumor bearing; NTB, nontumor bearing; TBC, total body weight; BCAA, branched chain amino acids; AAA, aromatic amino acids; EAA, essential amino acids; TAA, total amino acids; ANOVA, analysis of variance.
TBW/min. These glucose infusion rates were the glucose turnover rate and 3.2 times the glucose turnover rate of a NTB rat as determined by d-[3-3H]glucose (18). Six h later, four arterial blood samples were obtained at 5, 20, 45, and 90 min posttracer injection (18). A pretreatment sample of arterial blood was obtained for insulin levels, the animals were killed by air embolization, and the TBWs were determined.

Preparation and Analysis of Blood Samples. The samples (220 µl) were placed in chilled tubes containing 22 µl of sodium heparin (1000 units/ml; Upjohn, Kalamazoo, MI), centrifuged, and the plasma was deproteinized by membrane centrifugation (Centriflo Cones Type CF50A; Amicon, Lexington, MA). A 35-µl aliquot of filtrate was diluted with 60 µl of 0.15% lithium citrate buffer, pH 2.20 (34) (Beckman Instruments, Palo Alto, CA), and 10 µl of norleucine (Sigma Chemical Co., St. Louis, MO) were added as an internal standard and placed on an amino acid analyzer (Beckman Instruments Model 121 MB). Variability in the sample aspiration was corrected by multiplying each amino acid concentration by a normalization factor obtained from the known quantity of added internal standard.

The serum insulin concentration was determined by a radioimmunoassay technique (35). Data Analysis. The individual amino acids were organized into groups; TAA, BCAA (leucine, isoleucine, and valine), AAA (phenylalanine, tyrosine, and tryptophan), and EAA (BCAA, lysine, arginine, histidine, phenylalanine, tryptophan, methionine, and threonine) to facilitate recognition of patterns of response (28).

The concentration of each amino acid or group of amino acids is the mean ± SD of each of the sample time points of each animal during infusion; after preliminary analysis of variance (36) showed no statistical differences between time points. Statistical significance between groups of animals was determined by the two-tailed Student’s t test for paired and unpaired data and two-way ANOVA where appropriate (36).

RESULTS

The characteristics of the animal model are displayed in Table 1. The TB animals were used in an early stage of tumor growth when the tumor burden was only about 8% of the total body weight (Table 1). Food intake measured during the 48-h period prior to the 24-h fast was similar, but the carcass weight was still significantly decreased in the TB compared to the NTB animals (Table 1). The significantly decreased urinary nitrogen excretion exhibited by the TB animals suggested that the decrement in carcass weight was not due to accelerated nitrogen loss in this group (Table 1).

During 0.9% NaCl infusion there were small but statistically significant lower levels of aspartate, citrulline, and serine in the TB animals (Table 2). There were again no significant differences in the concentration of glutamine was 35% lower in both NTB and TB animals compared to values during 0.9% NaCl infusion (Table 2). The alanine level was 86 and 90% higher, and the concentration of leucine, isoleucine, and valine each were significantly decreased to a similar degree in NTB and TB animals (Table 2), yielding BCAA levels that were 46 and 34% lower in the NTB and TB groups, respectively, compared to values during 0.9% NaCl infusion (Table 2). Paired analysis for each animal of the difference in alanine and BCAA concentrations at base line and during glucose infusion at 3.72 µmol/100 g TBW/min showed that the alanine concentration rose 85.5 ± 35.7 and 130.5 ± 45.3 µM, while the BCAA fell 186.8 ± 52.10 and 118.2 ± 112.2 µM in the NTB and TB animals, respectively (Chart 1).

Infusion of glucose at 13.05 µmol/100 g TBW/min resulted in concentrations of arginine, asparagine, proline, glycine, lysine, tyrosine, serine, and taurine that were at a level closer to that seen during infusion of 0.9% NaCl in both NTB and TB animals, with serum still significantly lower in the TB group (Table 2). The alanine concentration was an additional 40% and 65% higher in the NTB and TB groups, respectively, compared to the groups infused with glucose at the lower rate, without a significant difference between NTB and TB animals (Table 2). The individual BCAA each fell significantly to a similar degree (Table 2), and there was an additional 15 and 20% decrease in the total BCAA levels in the NTB and TB animals compared to levels in these groups at the lower glucose infusion rate (Table 2). Paired analysis for each animal of the alanine concentration rose 162.2 ± 56.6 and 221.8 ± 93.8 µM, and that the BCAA fell 251.5 ± 34.4 and 198.5 ± 48.3 µM in the NTB and TB animals (Chart 1). There were again no significant differences in either the absolute micromolar alanine concentration rise compared to the micromolar fall in the BCAA concentration, or between NTB and TB animals (two-way ANOVA, P > 0.05). The concentration of glutamine was 35% lower in both NTB and TB animals compared to levels seen in the groups during 0.9% NaCl infusion (Table 2). The AAA and TAA concentrations were again similar to the values during the 0.9% NaCl and lower glucose infusion rate (Table 2).

Serum insulin was barely detectable in both NTB and TB groups during 0.9% NaCl infusion (Chart 2). Glucose infusion at 3.72 µmol/100 g TBW/min resulted in a small elevation, while...
infusion at 13.05 μmol/100 g TBW/min produced a marked and significant response (Chart 2). There were no significant differences in insulin levels between the NTB and TB animals during any of the infusions.

**DISCUSSION**

At an early stage of tumor development, after a 24-h fast, there are minor differences in basal plasma amino acid levels between NTB and TB animals. At two different rates of exogenous glucose infusion, the pattern of response of individual and groups of amino acids are similar in NTB and TB animals.

The arterial-venous cannulated animal model (18) utilized in this study has been shown to be in good health with return to preoperative weight and resumption of normal food intake postcannulation (Table 1). The TB animals were chosen for use at a point of tumor growth prior to the onset of marked cachexia (31), when the measured food intake was similar to, and the nitrogen retention more avid, compared to NTB animals.

The amino acid levels in NTB and TB animals during 0.9% NaCl infusion were lower than those found previously for fed rats (37), but similar to those reported for rats fasted for 24 h (13, 38), emphasizing the importance of antecedent food intake on plasma amino acid metabolism (13).

The concentrations of the aromatic amino acids in both the NTB and TB groups were similar to values found for fasted rats without arterial-venous cannulas (39). Since phenylalanine and the phenylalanine-tyrosine ratio have been reported to be elevated in shock (40), sepsis (9, 10), and trauma (8), the low values in this study are further support for the physiologically basal state of the animal model in general, and that in particular the TB animals lack this element of a stress pattern in their plasma amino acid profiles.

Previous studies in our laboratory and others have shown decreased plasma alanine and glutamine levels (11, 12) and elevated rates of gluconeogenesis from alanine (16, 17, 29, 30) in TB compared to NTB animals and humans. Prior work in this animal model has shown that the percentage of glucose derived from alanine is not increased in TB compared to NTB animals (18). These results, together with the similar levels of basal plasma alanine and glutamine in the NTB and TB groups in this study, further suggest that gluconeogenesis from amino acid precursors is not increased in TB animals without anorexia, since these amino acids account for the majority of skeletal muscle release (41, 42), hepatic uptake and glucose production (43).

The biphasic concentration response of many of the nonessential amino acids to the incremental glucose infusion displayed by NTB and TB animals implies that amino acid-synthesis (44). The similar response to exogenous glucose infusion could be due to a combination of hormone and substrate availability effects. Previous work has shown that mild increases in serum insulin levels would inhibit amino acid release substantially (41). Thus at the low infusion rate exogenous glucose would provide additional substrate for nonessential amino acid formation via stimulation of transamination reactions (44). The similar response to exogenous glucose would provide additional substrate for nonessential amino acid formation via stimulation of transamination reactions (44), while the low insulin levels seen during this infusion rate would be inadequate to prevent release of those de novo and essential amino acids to the incremental glucose infusion displayed by NTB and TB animals.

**AMINO ACID METABOLISM IN TB RATS**

### Table 2

<table>
<thead>
<tr>
<th>Amino acid (μmol)</th>
<th>NTB (n = 5)</th>
<th>TB (n = 5)</th>
<th>NTB (n = 5)</th>
<th>TB (n = 4)</th>
<th>NTB (n = 4)</th>
<th>TB (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>152.6 ± 20.4</td>
<td>150.3 ± 19.0</td>
<td>283.6 ± 33.2</td>
<td>286.7 ± 42.3</td>
<td>344.5 ± 28.1</td>
<td>382.8 ± 116.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>68.6 ± 12.1</td>
<td>65.7 ± 5.3</td>
<td>97.1 ± 4.0</td>
<td>97.7 ± 16.2</td>
<td>79.9 ± 3.9</td>
<td>74.2 ± 8.8</td>
</tr>
<tr>
<td>Aspartate</td>
<td>30.0 ± 7.1</td>
<td>21.1 ± 1.0</td>
<td>32.0 ± 6.9</td>
<td>27.2 ± 7.2</td>
<td>34.2 ± 4.9</td>
<td>20.7 ± 8.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>4.5 ± 0.4</td>
<td>4.3 ± 0.6</td>
<td>6.0 ± 0.9</td>
<td>5.7 ± 1.2</td>
<td>5.3 ± 0.4</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>Citrulline</td>
<td>26.1 ± 3.1</td>
<td>21.5 ± 2.6</td>
<td>33.0 ± 1.9</td>
<td>27.5 ± 4.8</td>
<td>36.4 ± 7.4</td>
<td>29.1 ± 13.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>61.2 ± 8.8</td>
<td>56.3 ± 4.9</td>
<td>69.0 ± 6.8</td>
<td>75.8 ± 3.8</td>
<td>54.9 ± 4.8</td>
<td>64.4 ± 11.4</td>
</tr>
<tr>
<td>Glutamine</td>
<td>392.8 ± 54.5</td>
<td>394.9 ± 46.7</td>
<td>389.3 ± 64.4</td>
<td>412.3 ± 53.7</td>
<td>255.4 ± 20.1</td>
<td>281.3 ± 23.5</td>
</tr>
<tr>
<td>Glutamate</td>
<td>100.4 ± 14.9</td>
<td>124.5 ± 22.7</td>
<td>136.2 ± 32.0</td>
<td>153.8 ± 35.1</td>
<td>127.2 ± 24.4</td>
<td>149.2 ± 44.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>120.8 ± 31.6</td>
<td>110.3 ± 11.0</td>
<td>147.2 ± 7.6</td>
<td>145.2 ± 20.2</td>
<td>140.4 ± 12.7</td>
<td>131.0 ± 17.9</td>
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<tr>
<td>Histidine</td>
<td>46.2 ± 6.9</td>
<td>44.9 ± 2.1</td>
<td>46.6 ± 5.3</td>
<td>48.1 ± 4.7</td>
<td>49.2 ± 3.2</td>
<td>45.2 ± 6.5</td>
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<tr>
<td>Isoleucine</td>
<td>65.0 ± 6.8</td>
<td>67.0 ± 2.7</td>
<td>32.6 ± 4.3</td>
<td>38.2 ± 6.2</td>
<td>24.5 ± 2.6</td>
<td>22.4 ± 2.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>104.0 ± 13.1</td>
<td>104.0 ± 4.0</td>
<td>58.1 ± 7.1</td>
<td>61.9 ± 9.9</td>
<td>44.2 ± 5.9</td>
<td>38.5 ± 5.1</td>
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<td>Lysine</td>
<td>260.0 ± 45.2</td>
<td>275.8 ± 31.0</td>
<td>353.3 ± 50.0</td>
<td>369.6 ± 50.0</td>
<td>304.7 ± 30.0</td>
<td>310.5 ± 61.6</td>
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<tr>
<td>Methionine</td>
<td>35.3 ± 4.9</td>
<td>31.3 ± 2.3</td>
<td>38.7 ± 3.5</td>
<td>40.9 ± 4.7</td>
<td>37.6 ± 3.4</td>
<td>38.8 ± 3.4</td>
</tr>
<tr>
<td>Ornithine</td>
<td>24.9 ± 5.8</td>
<td>27.1 ± 3.5</td>
<td>25.2 ± 1.9</td>
<td>23.4 ± 5.4</td>
<td>18.6 ± 0.8</td>
<td>16.8 ± 2.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>47.8 ± 6.2</td>
<td>47.9 ± 4.3</td>
<td>51.5 ± 5.4</td>
<td>58.7 ± 5.0</td>
<td>50.8 ± 5.9</td>
<td>53.7 ± 6.5</td>
</tr>
<tr>
<td>Proline</td>
<td>64.6 ± 7.0</td>
<td>67.4 ± 6.7</td>
<td>88.6 ± 6.5</td>
<td>92.6 ± 3.9</td>
<td>81.3 ± 3.2</td>
<td>86.6 ± 20.2</td>
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<tr>
<td>Serine</td>
<td>111.8 ± 15.3</td>
<td>89.1 ± 12.4</td>
<td>132.4 ± 9.1</td>
<td>100.1 ± 19.2</td>
<td>111.7 ± 2.6</td>
<td>78.8 ± 10.5</td>
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<tr>
<td>Taurine</td>
<td>95.1 ± 15.8</td>
<td>93.3 ± 13.4</td>
<td>109.9 ± 67.9</td>
<td>113.4 ± 10.5</td>
<td>82.8 ± 12.4</td>
<td>74.7 ± 11.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>113.6 ± 27.4</td>
<td>111.9 ± 12.1</td>
<td>127.1 ± 20.0</td>
<td>137.1 ± 20.7</td>
<td>113.1 ± 1.9</td>
<td>114.8 ± 17.0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>22.9 ± 14.5</td>
<td>20.5 ± 18.4</td>
<td>11.0 ± 3.1</td>
<td>17.7 ± 4.5</td>
<td>11.6 ± 1.3</td>
<td>12.5 ± 14.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>57.8 ± 6.6</td>
<td>55.8 ± 5.8</td>
<td>73.6 ± 6.4</td>
<td>69.6 ± 3.9</td>
<td>70.9 ± 11.8</td>
<td>73.6 ± 10.8</td>
</tr>
<tr>
<td>Valine</td>
<td>174.1 ± 30.3</td>
<td>173.0 ± 15.0</td>
<td>125.1 ± 11.5</td>
<td>128.4 ± 27.5</td>
<td>97.6 ± 24.5</td>
<td>99.7 ± 22.9</td>
</tr>
</tbody>
</table>

* Mean ± SD.  
  * P < 0.05; comparison of values during each glucose infusion with levels during 0.9% NaCl infusion within each group, Student’s t test.  
  * P < 0.05; comparison of values during glucose infusion at 13.05 μmol/100 g TBW/min and at 3.72 μmol/100 g TBW/min.  
  * P < 0.05; comparison of TB to NTB during each infusion.  

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The most prominent findings in this study were the elevated alanine and decreased BCAA levels present during incremental glucose infusion. These results have been demonstrated in a number of studies at the whole body (24, 27, 28), limb (41, 42, 48), and tissue (25, 26, 49) level. A glucose-alanine-BCAA cycle has been proposed to explain these data, whereby glucose produced in the liver during fasting is converted to pyruvate in skeletal muscle, transaminated with amino nitrogen derived from the BCAA to produce alanine, and then converted back to glucose again in the liver by gluconeogenesis (26). Sixty to 90% of the alanine carbon has been estimated to be derived from glucose (50), with the remainder being supplied from other amino acids via the Krebs cycle (51, 52) or from proteolysis (52). A minimum of 60% of the amino nitrogen has been demonstrated to be derived from the BCAA (53). In this study, the similar micromolar rise in alanine and fall in BCAA plasma concentrations is consistent with the theory of stoichiometric alanine transamination by the BCAA (26). However, the 35% decrease in plasma glutamine concentration at the high glucose infusion rate is suggestive of its additional role as an amino group alanine donor which is postulated to occur both at the tissue (48, 52) and interorgan (54) level. Further, the whole body plasma alanine concentration response to exogenous glucose may be even more complex as illustrated by work demonstrating depressed fractional hepatic uptake of this amino acid after p.o. glucose challenge (55).

Given that the glucose-alanine-BCAA cycle accounts for a major proportion of alanine metabolism, the similar plasma alanine and BCAA response suggests that this cycle is not preferentially increased in TB compared to NTB animals during exogenous glucose infusion. These results are in contrast to previous work in this animal model, which showed significantly elevated lactate levels indicative of increased Cori cycle activity in TB compared to NTB animals during incremental glucose infusion (18).

In conclusion, basal plasma amino acid metabolism appears similar in NTB and TB animals prior to anorexia and marked cachexia. The similar pattern of plasma amino acid concentration response to incremental glucose infusion in NTB and TB animals suggests that whole body amino acid-insulin sensitivity is similar, and that the glucose-alanine-BCAA cycle is not preferentially increased in the TB animals.

REFERENCES

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