Lactating Porcine Mammary Tissue Catabolizes Branched-Chain Amino Acids for Glutamine and Aspartate Synthesis$^{1-3}$

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Abstract

The uptake of branched-chain amino acids (BCAA) from plasma by lactating porcine mammary gland substantially exceeds their output in milk, whereas glutamine output is 125% greater than its uptake from plasma. In this study, we tested the hypothesis that BCAA are catabolized for glutamine synthesis in mammary tissue. Mammary tissue slices from sows on d 28 of lactation were incubated at 37°C for 1 h in Krebs buffer containing 0.5 or 2 mmol/L L-[1-14C]- or L-[U-14C]-labeled leucine, isoleucine, or valine. Rates of BCAA transport and degradation in mammary tissue were high, with ~60% of transaminated BCAA undergoing oxidative decarboxylation and the remainder being released as branched-chain α-ketoisocaproate (BCKA). Most (~70%) of the decarboxylated BCAA were oxidized to CO$_2$. Rates of net BCAA transamination were similar to rates of glutamate, glutamine, aspartate, asparagine, and alanine synthesis. Consistent with the metabolic data, mammary tissue expressed BCAA aminotransferase (BCAT), BCKA dehydrogenase, glutamine synthetase (GS), glutamate-oxaloacetate aminotransferase, glutamate-pyruvate aminotransferase, and asparagine synthetase, but no phosphate-activated glutaminase, activity. Western blot analysis indicated relatively high levels of mitochondrial and cytosolic isoforms of BCAT, as well as BCKA dehydrogenase and GS proteins in mammary tissue. Our results demonstrate that glutamine and aspartate (abundant amino acids in milk protein) were the major nitrogenous products of BCAA catabolism in lactating porcine mammary tissue and provide a biochemical basis to explain an enrichment of glutamine and aspartate in sow milk. J. Nutr. 139: 1502–1509, 2009.

Introduction

Lactating sows have high requirements for branched-chain amino acids (BCAA),$^8$ leucine, isoleucine, and valine to support milk production (1). Interestingly, their uptake by porcine mammary glands (76 g/d on d 13–20 of lactation) is much greater than their secretion in milk protein (46 g/d) (2). Thus, the lactating porcine mammary gland may catabolize 30 g BCAA/d (40% of the BCAA taken up from arterial plasma). The possibility that BCAA are degraded by the lactating tissue is supported by the report that BCAA aminotransferase (BCAT) and branched-chain α-ketoisocaproate (BCKA) dehydrogenase (BCKAD) are present in rat mammary tissue, with BCAT being induced ~10-fold during lactation (3). However, little is known about the metabolic fate of BCAA or their nutritional significance in the lactating mammary gland of any species.

Sow milk contains high concentrations of free and peptide-bound glutamine plus glutamate (4,5), which are crucial for the growth, development, and function of the neonatal small intestine (6–8). The available evidence shows that on d 10 of lactation, the lactating porcine mammary gland takes up 16 g/d glutamine from the arterial circulation (2) but produces 36 g/d glutamine in milk (9). Therefore, extraction of glutamine by the lactating mammary gland is much less than its output in milk and a large amount of glutamine (20 g/d) may be synthesized by mammary tissue to support the production of milk proteins.

Because of the absence of pyrroline-5-carboxylate dehydrogenase and proline oxidase (10), lactating porcine mammary tissue cannot convert arginine, ornithine, or proline into glutamine. Alternatively, BCAA nitrogen might be utilized for glutamine synthesis in lactating mammary gland through the sequential reactions of BCAT and glutamine synthetase (GS), as...
previously reported for skeletal muscle (11,12) and placenta (13). We tested this hypothesis in the present study using both radiochemical and chromatographic techniques. To compare capacity for BCAA catabolism between mammary and other tissues, we also determined BCAT and BCKAD proteins in skeletal muscle, small intestine, and liver of lactating sows.

Materials and Methods

Materials. HPLC-grade water and methanol were procured from Fisher Scientific. L-[U-14C]-Leucine, L-[1-14C]-Isoleucine, L-[1-14C]-Valine, L-[U-14C]-Ileucine, L-[U-14C]-Isoleucine, L-[U-14C]-Valine, and 1-[$^{14}$C]-glutamate were purchased from American Radiochemicals. L-[U-14C]-Beta-ketoisocaproate (KIC) was obtained from Amersham. Immediately before use, $^{14}$C-labeled BCAA were purified using AG 1-X8 (acetate form, 200–400 mesh) as resin bed (0.6 × 6 cm) and deionized water (2 mL) as eluting solvent (14). 1-[$^{14}$C]-KIC was purified by incubation at 25°C with 100 mL of 1.5 mol/L HClO$_4$ for 30 min, followed by neutralization with 50 mL of 2 mol/L K$_2$CO$_3$ (15). Soluene-350, a strong organic base formulated for compatibility with liquid scintillation cocktails and wet tissue solubilization, was obtained from Perkin Elmer. AG 1-X8, Triton X-100, Tween-20, and nitrocellulose membranes were obtained from Bio-Rad. MOPS, SDS running buffer (20×) and NuPage 10% Bis-Tris gel (15-lane) were purchased from Invitrogen. The BCA Protein assay kit and SuperSignal West Dura Extended Duration Substrate were purchased from Pierce. Rabbit anti-mitochondrial and cytosolic BCAT were prepared as described by Hutson et al. (16). Rat anti-BCKAD E1α antibody and rat anti-phosphorylated form of BCKAD E1α antibody were prepared as described by Lynch et al. (17). Mouse anti-GS was obtained from BD Biosciences, Parmingen. All other chemicals were purchased from Sigma Chemicals.

Lactating sows and dissection of tissues. This study was approved by the Texas A&M University Animal Care and Use Committee. Seven multiparous sows obtained from the Texas A&M University Swine Center and were offspring of Yorkshire × Landrace sows and Duroc × Hampshire boars. During gestation, sows had free access to water and a grain sorghum-soybean meal–based diet (5) providing 13.24 MJ metabolizable energy/kg, 14.1% crude protein, 0.6% lysine, 0.8% calcium, and 0.8% phosphorus. During gestation, sows had free access to water and a grain sorghum-soybean meal–based diet (5) providing 13.24 MJ metabolizable energy/kg, 14.1% crude protein, 0.6% lysine, 0.8% calcium, and 0.8% phosphorus. Immediately after collection of mammary tissue, samples of skeletal muscle, liver, small intestine, kidney, and adipose tissue were rinsed thoroughly with fresh KHB buffer and then solubilized in 0.5 mL Soluene-350. The solution was measured for 14C radioactivities using a dual-channel counting program (13). The specific activity of each 1-[$^{14}$C]-labeled BCAA in the medium was used to calculate BCAA uptake by mammary tissue. Results from preliminary experiments established that BCAA uptake was linear over a 5-min period.

Analysis of amino acids in mammary and other tissues. Amino acids were determined using HPLC methods involving precolumn derivatization with ophthaldialdehyde (18). Briefly, 100 mg of mammary gland, skeletal muscle, liver, small intestine, kidney, and adipose tissue was homogenized in 2 mL of 1.5 mol/L HClO$_4$ and then neutralized with 1 mL of 2 mol/L K$_2$CO$_3$. The extract was diluted 10 times with HPLC-grade water and an aliquot (50 μL) was directly used for analysis. Amino acids in the samples were quantified on the basis of authentic standards using the Millennium-32 workstation (Waters).

Determination of enzyme activities. The activities of BCAT, BCKAD, glutamate-pyruvate aminotransferase (GPT), glutamate-oxaloacetate aminotransferase (GOT), asparagine synthetase (AS), GS, and glutamine in mammary gland, muscle, liver, and small intestine were determined as we previously described (13,21). For assays of BCAT, BCKAD, GOT, and GPT, samples (~200 mg) were homogenized in 1 mL of freshly prepared buffer consisting of 50 mMol/L HEPES (pH 7.5), 3 mMol/L EDTA, 5 mMol/L dithiothreitol, 2% (v/v) Triton X-100, and 0.1% (wt/v) protease inhibitor (aprotinin, chymostatin, pepstatin A, and phenylmethylsulfonyl fluoride). For BCKAD assay, the homogenization buffer also contained 1 mMol/L potassium fluoride. Homogenates were
centrifuged at 600 × g; 10 min and the supernatant fluid subjected to 3 cycles of freezing in liquid nitrogen and thawing in a 30°C water bath. The BCKAD assay mixture consisted of 0.5 mL of 50 mmol/L Tris/HCl buffer (pH 8.6), 0.1 mL of 1.6 mmol/L pyridoxal phosphate, 0.2 mL of 50 mmol/L α-ketoglutarate, 1 mL of 10 mmol/L leucine plus 0.1 μCi purified I-1-[14C]leucine, and 0.2 mL of tissue extract. The BCKAD assay mixture consisted of 0.1 mL of 20 mmol/L MgCl2, 0.1 mL of 10 mmol/L dihydroheptitol, 0.1 mL of 4 mmol/L thiamine pyrophosphate plus 4 mmol/L coenzyme A plus 10 mmol/L NAD+, 0.4 mL of 50 mmol/L potassium phosphate buffer (pH 7.5), 0.1 mL of tissue extract, and 0.1 mL of 1 mmol/L potassium fluoride. Potassium fluoride (an inhibitor of phosphoprotein phosphatase) was used to assess BCKAD activity in its active state. All samples were preincubated for 10 min in a 30°C water bath, after which 0.1 mL of 10 mmol/L KCl plus 0.1 μCi [1-14C]KIC was added into all tubes. Exogenous dihydrolipoamide dehydrogenase was not supplemented to the BCKAD assay mixture.

The assay mixture (3 mL) for GOT consisted of 50 mmol/L alanine, 7 mmol/L α-ketoglutarate, 0.16 mmol/L NADH, 25 μg/l-lactate dehydrogenase, cell extracts (0.2–0.5 mg protein), and 80 mmol/L sodium phosphate buffer (pH 7.6). The assay mixture (3 mL) for GPT consisted of 33 mmol/L aspartate, 7 mmol/L α-ketoglutarate, 0.16 mmol/L NADH, 50 μg t-malate dehydrogenase, cell extracts (0.2–0.5 mg protein), and 80 mmol/L sodium phosphate buffer (pH 7.6). The assay mixture for AS consisted of 20 mmol/L glutamine, 10 mmol/L aspartate, 10 mmol/L MgCl2, 10 mmol/L ATP, cell extracts (0.5–1 mg protein) and 100 mmol/L Tris/HCl buffer (pH 7.5).

For GS and glutaminase assays, ~200 mg of samples of various tissues were homogenized in 1 mL buffer consisting of 50 mmol/L Tris (pH 7.9), 2 mmol/L EDTA, and 2 mmol/L dithiothreitol. Homogenates were centrifuged at 10,000 × g; 10 min at 4°C. Then 100 μL of supernatant was mixed with 100 μL of 100 mmol/L MgCl2, plus 75 mmol/L ATP plus 50 mmol/L phosphocreatine, 100 μL of 100 mmol/L Na3HCl, 100 μL of 100 mmol/L NH4Cl, 100 μL of 100 mmol/L creatine kinase (100 U/mL), and 100 μL of 100 mmol/L l-glutamate plus 0.05 μCi I-1-[14C]l-glutamate. The glutaminase assay mixture (0.2 mL) consisted of 20 mmol/L glutamine, 150 mmol/L potassium phosphate buffer (pH 8.2), and cell extracts (0.5–1 mg protein). Protein concentrations in tissue homogenates were measured using the BCA Protein Assay kit (Pierce). Enzyme activities are expressed on the basis of protein content.

**Western blot analysis of BCAT, BCKAD, and GS.** Mammary gland, skeletal muscle, liver, and small intestine were pulverized in liquid nitrogen and homogenized lysis buffer containing 20 mmol/L Tris-HCl (pH 7.4), 50 mmol/L NaCl, 50 mmol/L NaF, 50 mmol/L of EDTA, 1% Triton X-100, 1× protease inhibitor cocktail, and 1× phosphatase inhibitor cocktail (Calbiochem). Proteins in homogenates were analyzed using the BCA Protein Assay kit (Pierce). Enzyme activities are expressed on the basis of protein content.

**Statistical analysis.** Values expressed as mean ± SEM, n = 7. Within each concentration of BCAA, among 3 BCAA groups or among 4 amino acid products, data were analyzed by 1-way ANOVA. For data with unequal variance, as assessed using the Levene’s test, log transformation of data was performed before ANOVA. Differences among means were determined by the Tukey’s multiple comparison test. Between 0.5 and 2 mmol/L BCAA or between 0 (control) and 1.5 mmol/L l-cycloserine, paired t test was employed, because tissue slices used for incubation were obtained from the same sow. All statistical analyses were performed using SAS (SAS Institute). P-values ≤ 0.05 were considered significant.

**Results**

**BCAA transport and catabolism in mammary tissue.** The porcine mammary tissue had a high capacity to take up BCAA from the incubation medium and rates of leucine transport were higher (P < 0.05) than those for isoleucine and valine (Fig. 1). Increasing extracellular concentrations of BCAA from 0.5 to 2 mmol/L dose dependently increased (P < 0.05) their uptake by mammary tissue (Fig. 1).

Using leucine as a prototype BCAA, we found that its transamination, oxidative decarboxylation, and KIC release were linear (P < 0.01) during a 60-min period of incubation in the presence of 5 mmol/L L-glucose (Supplemental Fig. 1). In the absence of glucose from the medium, net leucine transamination was reduced (P < 0.01) by 72% (data not shown).

All BCAA were actively transaminated in this tissue, resulting in the production of BCKA (Table 1). Approximately 60% of transaminated BCAA underwent oxidative decarboxylation, with the remainder (~40%) being released as BCKA into the incubation medium. Most (~70%) of the decarboxylated BCAA were oxidized to CO2. Rates of BCAA catabolism increased (P < 0.05) with their increasing extracellular concentrations from 0.5 to 2 mmol/L. Nitrogenous products of BCAA transamination included alanine, aspartate, asparagine, glutamate, and glutamine, with glutamine and aspartate being the predominant ones (Table 2). Inhibition of BCAA transamination by L-cycloserine reduced (P < 0.05) the production of glutamate, glutamine, alanine, and aspartate in mammary tissue (Fig. 2). Likewise, the absence of glucose from incubation medium reduced (P < 0.01) the synthesis of glutamate, glutamine, alanine, and aspartate by 65–70% (data not shown).
Activities of BCAA-metabolic enzymes in mammary and other tissues. All the tissues, including mammary tissue, skeletal muscle, liver, and small intestine, expressed BCAT, BCKAD, GS, GOP, GPT, and AS (Table 3). The BCAT activity in mammary tissue was in the range of 258, 538, and 219% higher \((P < 0.01)\) than that in skeletal muscle, liver, and small intestine, respectively. In contrast, the activities of BCKAD and GS were higher \((P < 0.01)\) in the liver than in other tissues. Notably, GS activity in mammary tissue was 2.3 times \((P < 0.01)\) that in skeletal muscle and small intestine. The activities of GOT and GPT were high, but those of AS were low in mammary gland and other tissues. Glutaminase activity was not detectable in mammary tissue \(< 0.1 \text{ nmol/(mg tissue} \cdot 15 \text{ min})\).

Isoforms of BCAT in mammary and other tissues. The mitochondrial and cytosolic isoforms of BCAT were detected in the mammary tissue, skeletal muscle, liver, and small intestine of lactating sows (Fig. 3). The protein level of total BCKAD in mammary gland was higher \((P < 0.05)\) than that in skeletal muscle and liver, but was lower \((P < 0.05)\) than that in the small intestine (Table 4). Phosphorylated BCKAD E1a was found in mammary gland and small intestine but was not detectable in skeletal muscle and liver. The level of phosphorylated BCKAD was relatively high in the small intestine but nondetectable in skeletal muscle and liver (Table 4). The ratio of phosphorylated BCKAD:total BCKAD in mammary gland \((0.205 \pm 0.02)\) differed from that in small intestine \((0.408 \pm 0.03)\) \((P < 0.01)\).

GS protein levels in mammary and other tissues. GS protein was detected in the mammary tissue, skeletal muscle, liver, and small intestine of lactating sows (Fig. 3). The protein level of GS in mammary tissue was higher \((P < 0.05)\) than that in skeletal muscle and the small intestine but was lower \((P < 0.05)\) than that in liver. Based on quantification of western blots, GS protein in liver was 11.5, 183-, and 135-fold higher than that in mammary tissue, skeletal muscle, and small intestine, respectively (Table 4).

Concentrations of amino acids in mammary and other tissues. In mammary tissue, concentrations of BCAA and all other essential amino acids, as well as serine, cysteine, tyrosine, \(\beta\)-alanine, asparagine, ornithine, and citrulline, were relatively low \(< 0.25 \text{ nmol/mg tissue}\), but concentrations of taurine, glutamate, aspartate, glycine, and alanine were particularly high \((2.2–4.9 \text{ nmol/mg tissue})\), with arginine, glutamine, and proline being in between \([0.6–0.86 \text{ nmol/mg tissue}]\) (Supplemental Table 1). For comparison, concentrations of glutamine, glutamate, aspartate, alanine, and taurine in semitendinosus muscle of nonlactating sows was 3.87 ± 0.35, 2.94 ± 0.31, 0.96 ± 0.15, 4.52 ± 0.43, and 4.05 ± 0.37 nmol/mg tissue, respectively. Consistent with a low activity of hepatic BCAT and a high activity of hepatic GS, concentrations of BCAA and glutamine were higher \((P < 0.05)\) in the liver than in mammary tissue, skeletal muscle, and small intestine. Interestingly, concentrations of aspartate were much lower \((P < 0.05)\) in skeletal muscle compared with the other tissues (Supplemental Table 1).

Discussion
The mammary gland of lactating sows produces 125% more glutamine in milk than its uptake from arterial plasma (2,9),

### Table 1: Concentration-dependent increase in BCAA catabolism in lactating porcine mammary tissue

<table>
<thead>
<tr>
<th>BCAA</th>
<th>(\text{CO}_2) from all carbons (A)</th>
<th>(\text{CO}_2) from carbon-1 (B)</th>
<th>Net release of BCKA</th>
<th>Net transamination (D = B + C)</th>
<th>Transaminated BCAA released as BCKA</th>
<th>Decarboxylated BCAA oxidized as BCKA</th>
<th>(\text{CO}_2) from (A/D) × 100</th>
<th>(\text{CO}_2) from (A/B) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (\text{mmol/L BCAA})</td>
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<tr>
<td>Isoleucine</td>
<td>1.67 ± 0.11(^a)</td>
<td>0.38 ± 0.02(^b)</td>
<td>0.26 ± 0.02(^b)</td>
<td>0.64 ± 0.04(^b)</td>
<td>40.3 ± 0.62</td>
<td>67.6 ± 2.7</td>
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<tr>
<td>Leucine</td>
<td>2.26 ± 0.14(^a)</td>
<td>0.51 ± 0.03(^b)</td>
<td>0.36 ± 0.02(^b)</td>
<td>0.87 ± 0.05(^a)</td>
<td>40.8 ± 0.57</td>
<td>68.2 ± 3.0</td>
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<tr>
<td>Valine</td>
<td>1.31 ± 0.08(^c)</td>
<td>0.35 ± 0.02(^b)</td>
<td>0.24 ± 0.02(^b)</td>
<td>0.59 ± 0.04(^b)</td>
<td>40.5 ± 0.49</td>
<td>68.8 ± 3.4</td>
<td></td>
<td></td>
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<tr>
<td>2 (\text{mmol/L BCAA})</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.02 ± 0.24(^a)</td>
<td>0.89 ± 0.05(^b)</td>
<td>0.61 ± 0.04(^b)</td>
<td>1.50 ± 0.07(^b)</td>
<td>40.7 ± 0.52</td>
<td>69.7 ± 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>5.14 ± 0.32(^a)</td>
<td>1.16 ± 0.08(^b)</td>
<td>0.75 ± 0.06(^b)</td>
<td>1.91 ± 0.13(^a)</td>
<td>39.5 ± 0.69</td>
<td>68.5 ± 2.5</td>
<td></td>
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</tr>
<tr>
<td>Valine</td>
<td>3.19 ± 0.21(^c)</td>
<td>0.85 ± 0.05(^b)</td>
<td>0.58 ± 0.04(^b)</td>
<td>1.44 ± 0.08(^b)</td>
<td>40.1 ± 0.66</td>
<td>69.2 ± 3.1</td>
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</tbody>
</table>

1 Values are means ± SEM, \(n = 7\). Within each concentration of BCAA, means in a row without a common letter differ, \(P < 0.05\).

2 In the equation, \(n\) represents the number of noncarboxyl carbons \((n = 5\) for leucine and isoleucine; \(n = 4\) for valine). *Different from corresponding 0.5 \(\text{mmol/L BCAA}, \ P < 0.01\).

### Table 2: Concentration-dependent increase in synthesis of amino acids from BCAA in lactating porcine mammary tissue

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentrations of BCAA in incubation medium</th>
<th>(\text{nmol/mg tissue} \cdot h)</th>
<th>(0)</th>
<th>(0.5)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.21 ± 0.02(^c)</td>
<td>0.39 ± 0.03(^b)</td>
<td>0.64 ± 0.05(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.06 ± 0.01(^c)</td>
<td>0.08 ± 0.01(^b)</td>
<td>0.15 ± 0.02(^a)</td>
<td></td>
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</tr>
<tr>
<td>Aspartate</td>
<td>0.22 ± 0.02(^c)</td>
<td>0.58 ± 0.04(^b)</td>
<td>1.26 ± 0.07(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.35 ± 0.02(^c)</td>
<td>0.79 ± 0.08(^b)</td>
<td>1.82 ± 0.12(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.84 ± 0.07(^c)</td>
<td>1.48 ± 0.10(^b)</td>
<td>2.56 ± 0.24(^a)</td>
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</table>

1 Values are means ± SEM, \(n = 7\). Means in a row without a common letter differ, \(P < 0.05\).

2 Incubation medium contained 0, 0.5, or 2 \(\text{mmol/L}\) each of BCAA.
whereas the uptake of arterial glutamate by the mammary gland is fairly matched by the amount of glutamate in milk (23). It is unlikely that the extra glutamine is derived from proline, because porcine mammary tissue lacks the proline oxidase enzyme that would be needed for this activity (10). There was increased synthesis of glutamine when BCAA were provided to porcine mammary tissue, suggesting a de novo synthesis from α-ketoglutarate and BCAA nitrogen. Rates of transamination of BCAA with α-ketoglutarate [primarily from glucose via pyruvate carboxylase and possibly other anaplerotic reactions (24,25)] in porcine mammary tissue (Table 1) were high relative to perfused skeletal muscle of rats (26). This reaction generated glutamate, which was subsequently amidated to form glutamine (Fig. 4). The gland appeared to contain sufficient BCAT activity to support glutamine synthesis when ammonia was provided in the medium (Table 1). Interestingly, phosphate-activated glutaminase, the major enzyme initiating glutamine catabolism in the mitochondria, was absent from the mammary tissue of lactating sows (Table 3), as previously reported for the porcine placenta (13). Of particular note, the generation of products of GAT appears to predominate in lactating mammary tissue (Table 3). In contrast to the skeletal muscle of nonlactating mammals (11) and chickens (12), GAT activity exceeds GAT activity and alanine is a major product of skeletal muscle metabolism.

The synthesis of aspartate and asparagine is also of nutritional importance, because the uptake of these 2 amino acids by the mammary gland of lactating sows accounts for only 50% of their output in milk (2). Aspartate plus asparagine are the 3rd most abundant nonessential amino acids in porcine milk protein (4,15). Thus, by stimulating their synthesis, BCAA play a role in supporting protein synthesis in lactating mammary tissue. Modulation of BCAA catabolism and, therefore, glutamine and aspartate synthesis may play a hitherto unrecognized important role in regulating milk synthesis.

There is a distinct pattern of distribution of BCAT activity in the mammary gland, skeletal muscle, liver, and small intestine of lactating sows (Table 3) compared with that in nonlactating rats, humans, and monkeys (3,29,30). The lactating porcine mammary gland had a particularly high BCAT activity (Table 3), which explains our findings that BCAA were transaminated extensively in this tissue and promoted glutamine synthesis (Table 3). Consistent with this notion, concentrations of free BCAA were low, but concentrations of free glutamate and glutamine were high in mammary tissue (Supplemental Table 1). The mammary tissue also had a relatively high activity of BCAT that oxidatively decarboxylated BCKA to yield acetyl-CoA (Table 3), which was further oxidized via the Krebs cycle (31). Consistent with a high BCAT activity, 60% of transaminated BCAA were decarboxylated in lactating porcine mammary tissue (Table 1). Furthermore, the use of [1-14C]- and [U-14C]-labeled BCAA reveals that most (70%) of the decarboxylated BCKA were oxidized to CO$_2$ via the Krebs cycle in the lactating porcine mammary tissue. Thus, during lactation, the mammary gland is a physiologically significant site of BCAA catabolism. Acetyl-CoA derived from leucine and isoleucine may be utilized for the synthesis of fatty acids in mammary tissue, whereas succinyl-CoA produced from isoleucine and valine catabolism may provide carbon skeletons for the formation of nonessential amino acids such as glutamine (32).

GLUTAMINE DEGRADATION VIA THE GLUTAMINASE PATHWAY

This result was not due to an artifact, because we readily detected glutaminase activity in other tissues (Table 3). The lack of glutamine degradation via the glutaminase pathway maximizes the availability of newly synthesized glutamine for the production of milk proteins and indicates that ammonia needed for the amidation of glutamate to glutamine is likely to be obtained from the circulation or nonglutaminase pathways. Glutamine made in the alveolar cells is secreted into the mammary alveolar lumen and duct system and then released to piglets after an oxytocin surge (7). The presence of the glutamine-synthetic pathway in the absence of glutamine degradation is consistent with the high abundance of glutamine in the milk of mammals, including pigs (e.g. 2–4 mmol/L free glutamine vs. 0.4–1 mmol/L free glutamate) (5,9) and humans (e.g. 1.4 mmol/L free glutamine vs. 0.3 mmol/L free glutamate) (28), despite insufficient uptake of glutamine from the arterial circulation.

Glutamate transaminates with pyruvate and oxaloacetate to form alanine and aspartate, respectively, in mammary gland (Table 2). Aspartate is then converted into asparagine (Fig. 4), as reported for porcine enterocytes (9). The synthesis of these amino acids is consistent with the presence of GPT, GOT, and AS in the mammary gland of lactating sows (Table 3). Interestingly, although the activities of GPT and GOT were greater than those of GS, glutamate and glutamine synthases were higher than formation of alanine or aspartate in mammary tissue (Table 2), as previously reported for the porcine placenta (13). Of particular note, the generation of products of GAT appears to predominate in lactating mammary tissue (Table 3). In contrast to the skeletal muscle of nonlactating mammals (11) and chickens (12), GAT activity exceeds GAT activity and alanine is a major product of skeletal muscle metabolism.

The synthesis of aspartate and asparagine is also of nutritional importance, because the uptake of these 2 amino acids by the mammary gland of lactating sows accounts for only 50% of their output in milk (2). Aspartate plus asparagine are the 3rd most abundant nonessential amino acids in porcine milk protein (4,15). Thus, by stimulating their synthesis, BCAA play a role in supporting protein synthesis in lactating mammary tissue. Modulation of BCAA catabolism and, therefore, glutamine and aspartate synthesis may play a hitherto unrecognized important role in regulating milk synthesis.

There is a distinct pattern of distribution of BCAT activity in the mammary gland, skeletal muscle, liver, and small intestine of lactating sows (Table 3) compared with that in nonlactating rats, humans, and monkeys (3,29,30). The lactating porcine mammary gland had a particularly high BCAT activity (Table 3), which explains our findings that BCAA were transaminated extensively in this tissue and promoted glutamine synthesis (Table 3). Consistent with this notion, concentrations of free BCAA were low, but concentrations of free glutamate and glutamine were high in mammary tissue (Supplemental Table 1). The mammary tissue also had a relatively high activity of BCAT that oxidatively decarboxylated BCKA to yield acetyl-CoA (Table 3), which was further oxidized via the Krebs cycle (31). Consistent with a high BCAT activity, 60% of transaminated BCAA were decarboxylated in lactating porcine mammary tissue (Table 1). Furthermore, the use of [1-14C]- and [U-14C]-labeled BCAA reveals that most (70%) of the decarboxylated BCKA were oxidized to CO$_2$ via the Krebs cycle in the lactating porcine mammary tissue. Thus, during lactation, the mammary gland is a physiologically significant site of BCAA catabolism. Acetyl-CoA derived from leucine and isoleucine may be utilized for the synthesis of fatty acids in mammary tissue, whereas succinyl-CoA produced from isoleucine and valine catabolism may provide carbon skeletons for the formation of nonessential amino acids such as glutamine (32).
The activity of BCAT in the small intestine of lactating sows (Table 3) was lower than that of postweaning pigs (15), enterally-fed infant pigs (33), and adult humans (29). Interestingly, the activity of BCKAD in the small intestine of lactating sows (Table 3) was lower than that of postweaning pigs (15) and adult humans (29) but higher than that of enterally-fed infant pigs (33). A large proportion of BCKAD in the small intestine of lactating sows was in the phosphorylated form (inactive form) (Table 4) as reported for other species (32), which would further limit the irreversible catabolism of transaminated BCAA (34). These results suggest that the entry of dietary BCAA and their carbons into the portal circulation may be higher in lactating sows compared with nonlactating counterparts. Because of a very low activity of hepatic BCAT (Table 3), it is likely that BCAA catabolism is limited or negligible in the liver of lactating sows, as reported for rats (31) and humans (29). This would maximize the escape of BCAA or their carbon skeletons from the liver into the systemic circulation for uptake by extrahepatic tissues. In nonlactating mammals (including growing pigs), skeletal muscle is a major site for initiating BCAA catabolism (15,31). However, under the same assay conditions, BCAT and BCKAD activities in the skeletal muscle of sows during lactation (Table 3) were <15 and 10%, respectively, of those in growing pigs (15). Thus, it can be surmised that in lactating sows, circulating BCAA are preferentially channeled into mammary gland rather than skeletal muscle to support glutamine and aspartate synthesis. This also raises a possibility that glutamine synthesis by the muscle of lactating sows may be reduced. In this regard, it is noteworthy that a recent study reported that concentrations of glutamine in skeletal muscle of lactating sows were <50% of that in nonlactating counterparts (35). Because glutamine has a potential to stimulate net protein synthesis in skeletal muscle in vitro, increasing intramuscular levels of glutamine in vivo may be an effective strategy to improve nitrogen balance in lactating sows and other mammals. Collectively, our findings suggest coordinate tissue-specific metabolism of BCAA in lactating mammals to maximize the provision of BCAA for utilization by their mammary glands at the expense of glutamine synthesis in skeletal muscle.

Our findings have important implications for lactation and neonatal nutrition. Milk-derived glutamine is a major substrate for the intestinal synthesis of citrulline and arginine (37), which is a nutritionally essential amino acid for neonates (38) but is remarkably deficient in the milk of most mammals, including pigs and humans (4,5,39). Enterally administered BCAA may effectively increase their concentrations in plasma of lactating sows. There is evidence that supplementing BCAA to the diet of lactating sows can promote piglet growth performance. For example, increasing the content of i-valine from 0.80 to 1.2% in the diet (containing 1.57% l-leucine and 0.68% l-isoleucine) for lactating sows augmented P < 0.04 litter weaning weight by 5.5% (40). Additionally, increasing the content of total BCAA from 2.92 to 3.62% in the diet of lactating sows enhanced P < 0.02 litter weight gain between farrowing and weaning by 5.7% (41). The underlying mechanisms may include in part enhancements of mammary synthesis of glutamine and aspartate, whose uptake from arterial plasma by the lactating gland meets <50% of their requirements for the production of milk proteins (2,9). It should be borne in mind that effects of dietary supplementation with any amino acid on physiological responses (including production performance of lactating sows) depend on supplemental doses and the contents of other amino acids in the diet (42,43). Future studies involving rat and pig models are warranted to determine the effect of BCAA supplementation on concentrations of protein and glutamine in milk, as well as the volume of milk production. Because impaired lactogenesis occurs in women in response to various stress conditions (44), BCAA may provide a solution to this nutritional problem.

In conclusion, the results of this study indicate that BCAA were catabolized extensively and stimulated the synthesis of glutamine and aspartate in lactating porcine mammary tissue in vitro. There was no detectable glutaminase in mammary tissue, suggesting that the lack of glutamine catabolism in the porcine gland maximizes the availability of newly synthesized glutamine.
for the production of milk proteins and for secretion into the mammary alveolar lumen. Our novel findings provide one biochemical explanation for the high abundance of glutamine in sow milk, as well as valuable insight into the role of BCAA in lactation and neonatal nutrition.

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Literature Cited


