Branched-Chain Amino Acid Metabolism: Implications for Establishing Safe Intakes¹,²

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ABSTRACT There are several features of the metabolism of the indispensable BCAAs that set them apart from other indispensable amino acids. BCAA catabolism involves 2 initial enzymatic steps that are common to all 3 BCAAs; therefore, the dietary intake of an individual BCAA impacts on the catabolism of all 3. The first step is reversible transamination followed by irreversible oxidative decarboxylation of the branched-chain α-keto acid transamination products, the branched chain α-keto acids (BCKAs). The BCAA catabolic enzymes are distributed widely in body tissues and, with the exception of the nervous system, all reactions occur in the mitochondria of the cell. Transamination provides a mechanism for dispersing BCAA nitrogen according to the tissue’s requirements for glutamate and other dispensable amino acids. The intracellular compartmentalization of the branched-chain aminotransferase isozymes (mitochondrial branched-chain aminotransferase, cytosolic branched-chain amino-transferase) impacts on intra- and interorgan exchange of BCAA metabolites, nitrogen cycling, and net nitrogen transfer. BCAAs play an important role in brain neurotransmitter synthesis. Moreover, a dysregulation of the BCAA catabolic pathways that leads to excess BCAAs and their derivatives (e.g., BCKAs) results in neural dysfunction. The relatively low activity of catabolic enzymes in primates relative to the rat may make the human more susceptible to excess BCAA intake. It is hypothesized that the symptoms of excess intake would mimic the neurological symptoms of hereditary diseases of BCAA metabolism. J. Nutr. 135: 1557S–1564S, 2005.

KEY WORDS: • branched-chain aminotransferases • nitrogen cycles • glutamate • alanine • mitochondria

Amino acids are the building blocks of protein. Twenty amino acids are required for protein synthesis. In healthy adults, nine of these amino acids, including the branched-chain amino acids (BCAAs) (leucine, isoleucine, and valine) cannot be synthesized endogenously or in sufficient amounts. Therefore, they must be acquired in the diet and are classified as nutritionally essential or indispensable. The need of the organism for each essential amino acid for protein synthesis and for nonprotein functions defines the individual daily requirement for an essential amino acid. Clearly, a major part of the daily requirement is to meet the body’s needs for protein synthesis. Adequate intake of dietary BCAAs is required for normal growth and development, and intake of BCAAs below the requirements will limit growth. In the adult, BCAAs are required to maintain body protein at steady state, BCAAs taken in excess of their requirement are degraded. Inborn errors of BCAA metabolism have shown that dysregulation of the BCAA catabolic pathways that results in excess BCAAs or their derivatives is toxic to the central nervous system. Therefore, regulation of key BCAA catabolic enzymes serves to limit irreversible BCAA oxidation when dietary intake of these amino acids is inadequate and serves to clear BCAAs efficiently when dietary intake is in excess of the body’s needs.

There are several hallmarks of BCAA metabolism and their nonprotein functions that set them apart from other indispensable amino acids. a) BCAA catabolism involves 2 initial common enzymatic steps, transamination and oxidative decarboxylation of the branched-chain α-keto acid (BCKA)⁴ products of transamination (1). As a result, the amount of individual dietary BCAAs impacts on the catabolism of all 3

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⁴ Abbreviations used: α-KG, α-ketoglutarate; BCAT, branched chain aminotransferase; BCATc, cytosolic branched chain aminotransferase; BCATm, mitochondrial branched chain aminotransferase; BCKA, branched chain α-keto acid; BCKD, branched chain α-keto acid dehydrogenase; E1, branched chain α-keto acid decarboxylase; GABA, γ-amino butyric acid; GDH, glutamate dehydrogenase; KIC, α-ketoisocaproatate; KIV, α-ketoisovalerate; KMW, α-keto-β-methylvalerate; MSUD, maple syrup urine disease; PLP, pyridoxal phosphate; PMP, pyridoxamine phosphate; TCA, tricarboxylic acid.
BCAAs. b) Instead of being restricted to the liver, the BCAA catabolic enzymes are distributed widely in body tissues (2,3). With one exception, which occurs in the central nervous system, all reactions occur in the mitochondria of the cell (1,4). c) The widespread distribution of BCAA catabolic enzymes and differences in expression within a tissue result in significant inter- and intraorgan exchange of BCAA metabolites (2,5). d) The BCAA leucine is a precursor of cholesterol and one of 2 solely ketogenic amino acids (leucine and lysine). e) In the brain and in peripheral tissues, BCAAs are nitrogen donors (2,6–9). BCAAs probably participate as nitrogen donors for moving nitrogen in the form of alanine and glutamine from muscle amino acid oxidation to the liver for urea synthesis. They are also important nitrogen donors for synthesis of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter γ-amino butyric acid (GABA) in brain. f) The BCAA leucine acts as an anabolic nutrient signal influencing both insulin secretion by the β cells of the pancreas (10–12) and protein synthesis in skeletal muscle and selected other tissues (13–16).

**BCAA catabolism**

The first step in BCAA catabolism is transamination catalyzed by the branched-chain aminotransferase (BCAT) isozymes (Fig. 1). There are 2 mammalian BCATs—a mitochondrial (BCATm) and a cytosolic (BCATc) isozyme (1,17,18). Aminotransferases, which are vitamin B-6, pyridoxal phosphate (PLP), dependent enzymes, exhibit ping pong kinetic mechanisms (Fig. 2). In the first half-reaction, the PLP form of the enzyme reacts with the α-amino group of a BCAA. Then the reaction proceeds to the pyridoxamine [pyridoxine monophosphate (PMP)] form of the enzyme, releasing the respective BCKA, α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KVM), or α-ketoisovalerate (KIV). The PMP-enzyme then aminates a second glutamate, with substrate preferences for isoleucine (Ile), one of 2 solely ketogenic amino acids (leucine and lysine). The BCAA leucine acts as an anabolic nutrient signal influencing both insulin secretion by the β cells of the pancreas (10–12) and protein synthesis in skeletal muscle and selected other tissues (13–16).

![FIGURE 1](https://example.com/figure1.png)  
**FIGURE 1** Scheme of the BCAA catabolic pathways. The first step is transamination of the BCAAs [leucine (Leu), isoleucine (Ile), valine (Val)], and is catalyzed by the BCAT isozymes (BCATm and BCATc), producing the BCKA, KIC, KVM, and KIV. α-KG is the α-keto acid acceptor of the BCAA nitrogen group and glutamate (Glu) is the product. The second step is oxidative decarboxylation, catalyzed by the BCKD enzyme complex. Other abbreviation, Gln, glutamine.

> valine ≫ glutamate (17–19). Published kinetic data for both human BCATm and BCATc show that α-KG and all BCKAs are substrates for the second half-reaction (17–19). Although K<sub>m</sub> values indicate KIC is preferred over the other 2 BCKAs and over α-KG, the specificity constants for human BCATm (<i>k</i><sub>d</sub>/<i>K</i><sub>m</sub>) show that α-KG is actually the preferred substrate for the second half-reaction (M. Islam and S. M. Hutm, Wake Forest University School of Medicine, 2004, unpublished results). Thus, the kinetic data suggest that BCKAs and glutamate are the favored products of the BCAT reaction. A unique feature of the mammalian BCATs is the presence of a redox-sensitive CXXC [(C) cysteine (X,X) any amino acid (C) cysteine] center that plays a structural role in catalysis (20–22). Oxidation and reduction of this center permit reversible modulation of BCAT activity (22).

Tissue concentrations of BCAT substrates and products suggest that the BCAT enzymes operate near equilibrium in most cells (2,23). Because under most physiological conditions tissue substrate and product concentrations are at or below their K<sub>m</sub> values, the BCAT would be expected to respond rapidly to changes in substrate and product concentrations, i.e., mass action control. The reversibility of the transamination reaction (Fig. 2) and the substrate specificity of BCAT isozymes (4 amino acid and 4 α-keto acid substrates) also lead to the prediction that exchange of nitrogen between BCAAs and glutamate is likely unless the α-keto acid product of the first half-reaction is removed efficiently (one way transfer). Indeed when <sup>15</sup>N-labeled leucine is used in vivo to measure leucine kinetics and protein metabolism, most of the <sup>15</sup>N-label is found in glutamate, but label is also found in other BCAAs and amino acids that are formed by transamination with glutamate (aspartate, alanine, etc.) (24–28). The size of the tissue glutamate pool relative to the BCAA pool will limit the amount of reincorporation of <sup>15</sup>N-label from glutamate into leucine during short-term [<sup>15</sup>N]leucine infusions. For example, in the brain the glutamate pool is large and the ratio of glutamate mass to total BCAA mass is ~30:1 (27,29). Kanamori et al. (27) calculated that during 4.6 h of [<sup>15</sup>N]leucine infusion, reamination of brain BCKAs by [<sup>15</sup>N]glutamate was negligible. The degree to which <sup>15</sup>N-label from leucine is incorporated into tissue amino acids other than glutamate also provides information on the pattern of aminotransferase expression within a tissue. Thus, transamination provides a
mechanism for dispersing BCAA amino acid nitrogen according to the tissue's need for glutamate and other dispensable amino acids.

Although transamination and reamination of BCAAs will label the dispensable amino acid pool and will permit cycling of BCAA nitrogen, removal of the BCKAs by release from the cell or oxidation of the carbon skeleton within the cell is required for net transfer to occur from BCAAs to glutamate and other dispensable amino acids. Release of the BCKAs from one tissue and reamination in a different tissue does provide a mechanism for transfer of BCAA nitrogen between organs, tissues, and even different cell types within a tissue (BCAA nitrogen cycling). However, only oxidation and removal of the carbon skeleton will actually result in a net transfer of BCAA nitrogen to body dispensable amino acids. Thus, widespread expression of BCAA catabolic enzymes allows nitrogen cycling, transfer, and net transfer to occur in different tissues and cells.

It is the activity of the second enzyme in BCAA catabolism, the mitochondrial branched-chain α-keto acid dehydrogenase (BCKD) (Fig. 1), that determines the rate of oxidation of the BCAA carbon skeletons (irreversible loss), which is equivalent to the rate of net nitrogen transfer from BCAAs to glutamate. The mammalian BCKD enzyme complex contains multiple copies of 3 enzymes (30): a branched-chain α-keto acid decarboxylase (E1), a dihydrolipoyl transacylase, and a dihydrolipoyl dehydrogenase. The overall structure of the BCKD complex resembles the structures of the mammalian pyruvate dehydrogenase and α-KG dehydrogenase enzyme complexes. In the BCKD reaction, BCKA substrate is oxidatively decarboxylated, forming the respective branched-chain acyl-CoA derivative and NADH. BCKD is highly regulated, both by short-term mechanisms and by long-term mechanisms (31,32). Short-term regulation is mediated by the highly specific 44 kDa BCKD kinase that can associate and dissociate from the complex (33,34). The kinase phosphorylates and inactivates the E1 enzyme. Short-term regulation of the kinase is achieved by changes in levels of KIC (leucine α-keto acid), which inhibits the kinase (33). In the presence of excess KIC, the kinase is inactivated, facilitating oxidation of the BCKAs and net nitrogen transfer. A specific BCKD phosphatase has also been reported, but the significance of this phosphatase and its inhibitor protein is not known (35). Mutations in one or more proteins in the BCKD complex result in the inborn error of metabolism, maple syrup urine disease (MSUD), which is characterized by elevated plasma BCAAs and BCKAs (36).

The remaining enzymes of the BCAA catabolic pathways are also mitochondrial. The next step in the oxidation of BCAAs is oxidation of the branched-chain acyl-CoA BCKD reaction products, catalyzed by 2 different dehydrogenases (36). After this step, the individual BCAA catabolic pathways diverge. Leucine is unique in that its catabolic pathway contains an ATP- and biotin-dependent carboxylation step (β-methyl crotonyl-CoA carboxylase) and an intermediate that is a precursor of cholesterol. Leucine is ketogenic, forming acetyl-CoA and acetoacetate, whereas valine is glucogenic, entering the tricarboxylic acid (TCA) cycle as succinyl-CoA. Both isoleucine and valine are metabolized to succinate via methylmalonyl-CoA. Free 3-hydroxyisobutyrate is formed during valine metabolism, and this BCAA metabolite can be released from tissues. The other product of isoleucine metabolism is acetoacetate. Mutations in these pathway enzymes usually result in neurological consequences (36).

**Compartmentalization of catabolic enzymes in peripheral tissues**

Research on the metabolism of BCAAs in body organs and tissues (2,3,37) led to the hypothesis that BCAAs play an important role in body nitrogen metabolism, particularly as nitrogen donors for the major nitrogen carriers alanine and glutamine (6,38). As shown in Figure 3, the enzymes for degradation of the BCAAs are expressed in tissues throughout the body (1,3,4,39). BCATm is either not expressed or is expressed only at low levels in the liver but at higher levels in other organs, leaving the initial step in BCAA degradation to tissues other than liver (7,37,40). BCAAs represent about 50% of skeletal-muscle amino acid uptake, and most of the other plasma amino acids do not undergo catabolism in muscle. The BCAAs can be used for maintenance of the nitrogen in muscle pools of glutamate, alanine, and glutamine, and to provide nitrogen for alanine and glutamine release from muscle, with subsequent uptake by the liver, the kidneys, and the intestines. In rat and human skeletal muscle, BCAT activity is high compared with BCKD activity (ratio of measured BCAT...
to actual BCKD activities ≥90 and ratio of BCAT to total BCKD activities ≥25) (41). Actual and total activities refer to BCKD activity measured in tissue extracts before (actual) and after dephosphorylation (total). Total activity reflects tissue oxidative capacity, which is often higher than actual activity. The high ratios of skeletal muscle BCAT:BCKD activities favor a) release of BCKAs instead of their oxidation (2,3,5) and b) efficient transfer of BCAA nitrogen. In hepatocytes, the absence of the BCATm isoform and high activity of BCKD favors liver oxidation of circulating BCKAs. This would account for the low concentrations of BCKAs found in liver (42). This complex metabolic scheme also permits leucine to act as an effective nutrient signal both in liver and peripheral tissues. Leucine can stimulate protein synthesis in skeletal muscle, liver, and adipose tissues (13) via an as yet incompletely characterized signaling cascade(s).

Another unique feature of BCAA metabolism is the compartmentalization of BCAT isozymes and BCKD within organs and tissues. The BCATm is expressed ubiquitously, whereas the cytosolic isozyme BCATc is found almost exclusively in nervous tissue. Recently, Sweat et al. (43,44) have shown that in the rat, BCATc is expressed in neurons of the central nervous system and in peripheral nerves. Neither BCATm nor BCKD were found in peripheral nerves. In most peripheral tissues BCATm is by far the predominant isoform expressed. Its expression, however, is cell specific. It is often localized in secretory epithelial cells (43). Surprisingly, the enzyme is highly expressed in the acinar cells of the exocrine pancreas and in parietal and chief cells of the stomach; however, the enzyme was not found in the absorptive epithelial cells (enterocytes) of the intestine. The physiological significance of BCAT metabolism in the digestive system is not known, but there is the potential for BCAA metabolism or leucine signaling.

The consequences of intracellular compartmentalization in peripheral tissue

BCATm is the BCAT isoform expressed in most body tissues, so transamination of BCAAs occurs in mitochondria, where the TCA cycle intermediate, α-KG, is the α-keto acid substrate for BCAT-catalyzed glutamate synthesis (1) (see Fig. 4). The BCAA nitrogen can be transferred from glutamate to alanine, or glutamate can be converted to glutamine, and these amino acids are then released. Thus, the fate of the BCAA nitrogen in a particular tissue depends on the pattern of expression of the mitochondrial transporters of glutamate, on other transaminases that use glutamate as a substrate, and on glutamine synthetase (see Fig. 4). Furthermore, if product glutamate exits the mitochondria, there is a net loss of a TCA cycle intermediate (α-KG) that must be replenished.

As shown by analysis of the tissue and organ distribution of mRNAs of the mitochondrial and cytosolic isozymes of alanine aminotransferase, isoform expression is organ specific (45). This suggests that alanine synthesis from glutamate occurs in mitochondria in skeletal muscle and brain, but it occurs in the cytosol in intestine, colon, and brown adipose tissue. Both compartments can generate alanine in liver, white adipose tissue, and kidney (45). High levels of expression of mitochondrial alanine aminotransferase and BCATm in skeletal muscle suggest that efficient transfer of BCAA nitrogen to alanine occurs in the mitochondria. When alanine and BCKA are released from mitochondria, it occurs without depletion of a TCA cycle intermediate (Fig. 4A).

To form glutamine, glutamate must be generated in the cytosol, which is the site of expression of glutamine synthetase.

It is not clear how this can be accomplished without active synthesis of extra citric acid cycle intermediates (anaplerosis). Loss of either glutamate or α-KG from the mitochondria and then from the cell causes a net loss of TCA cycle intermediates (Fig. 4B), which must be replaced by an anaplerotic reaction. Thus, if the tissue expresses the Glu/OH carrier, extra mitochondrial aminotransferases can transfer the nitrogen from glutamate to form other amino acids such as alanine, aspartate, γ-aminobutyric acid, etc., in the cytosolic compartment. The example shown in Panel B is for a tissue expressing cytosolic alanine aminotransferase. Alternatively, the α-KG can be regenerated by the action of GDH.

![FIGURE 4 Metabolic consequences of intracellular compartmentalization of BCAA metabolism in mitochondria. Panel A. In tissues (such as muscle) that express the mitochondrial form of alanine aminotransferase (AlaATm), glutamate (Glu) produced by BCATm is recycled back to α-KG through transamination with pyruvate, and alanine (Ala) is released from the mitochondria. Panel B. In tissues that do not contain a second mitochondrial aminotransferase, exit of BCAA-derived Glu on the Glu-hydroxyl carrier (Glu/OH) results in loss of a TCA cycle intermediate that must be replaced by an anaplerotic reaction. Thus, if the tissue expresses the Glu/OH carrier, extramitochondrial aminotransferases can transfer the nitrogen from glutamate to form other amino acids such as alanine, aspartate, γ-aminobutyric acid, etc., in the cytosolic compartment. The example shown in Panel B is for a tissue expressing cytosolic alanine aminotransferase. Alternatively, the α-KG can be regenerated by the action of GDH.](image-url)
portant role in glutamine synthesis (Fig. 4B). GDH in humans is thought to be present in all tissues but levels vary (48–50). The glutamate formed by BCATm-catalyzed transamination is oxidized by GDH to form α-KG and ammonia. The ammonia, after diffusing out of the mitochondria, is used to aminate cytosolic glutamate via glutamine synthetase. This BCATm–GDH couple would account for observations (27,28) that [15N]leucine can label both nitrogen atoms of glutamine formed in vivo, in brain and in skeletal muscle.

BCAA requirements and toxicity

BCAA imbalance in growing animals on low-protein diets has been documented but only a high ratio of leucine to valine + isoleucine affects growth (51). In rats fed diets high in leucine, Dixon and Harper (52) found increases in the BCKD activity state and increased oxidation of valine and isoleucine, presumably limiting their availability for protein synthesis. These special conditions are unlikely to occur in children and in adults in the general population. The widespread expression of the BCAA catabolic enzymes would facilitate redistribution of the imbalance across tissues, and there is little evidence in adult rats or humans that BCAAs are toxic except at very high doses. In young rats fed a standard nonpurified commercial diet and supplied with leucine in the drinking water, leucine intake doubled and plasma leucine concentrations were twice those of control rats (53). Growth over a 10-d period was not compromised, and the rats showed no signs of toxicity. Postprandial protein synthesis was increased over controls in the supplemented rats, and the results suggested that protein turnover may have been increased (53), raising the possibility of an increased energy cost because of excessive leucine intake without evidence of toxicity.

Animal models of BCAA metabolism

Because BCAA metabolism is less active in primates than in rats, primates may be a better animal model than rats for testing susceptibility of humans to overdoses of BCAA. As shown in Tables 1 and 2, the tissue distribution of BCAA

### TABLE 1

Distribution of BCAA transamination capacity in rat, human, and monkey tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat</th>
<th>Human</th>
<th>Monkey</th>
<th>Tissue</th>
<th>Rat</th>
<th>Human</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/100 g body weight</td>
<td>% of total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>63.9</td>
<td>5.1</td>
<td>10.17</td>
<td>81.6</td>
<td>65.4</td>
<td>79.8</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3.8</td>
<td>0.3</td>
<td>0.26</td>
<td>4.8</td>
<td>3.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.4</td>
<td>0.6</td>
<td>0.73</td>
<td>0.5</td>
<td>7.7</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>3.3</td>
<td>1.2</td>
<td>0.73</td>
<td>4.2</td>
<td>15.4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Stomach + intestine</td>
<td>7.0</td>
<td>0.8</td>
<td>0.85</td>
<td>8.9</td>
<td>7.7</td>
<td>6.7</td>
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</tr>
<tr>
<td>Total</td>
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<td>7.8</td>
<td>12.74</td>
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</tr>
</tbody>
</table>

1 Data are from Suryawan et al. (41).

### TABLE 2

Distribution of BCKD (oxidative) capacity in rat, human, and monkey tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat</th>
<th>Human</th>
<th>Monkey</th>
<th>Tissue</th>
<th>Rat</th>
<th>Human</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/100 g body weight</td>
<td>% of total</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Actual activity2</td>
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<td></td>
<td></td>
<td>Total activity2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.15</td>
<td>0.04</td>
<td>0.24</td>
<td>2.03</td>
<td>0.21</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Liver</td>
<td>3.76</td>
<td>0.01</td>
<td>0.17</td>
<td>4.28</td>
<td>0.03</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.47</td>
<td>0.006</td>
<td>0.02</td>
<td>0.62</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Brain</td>
<td>0.05</td>
<td>0.015</td>
<td>0.01</td>
<td>0.07</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Stomach + intestine</td>
<td>0.07</td>
<td>0.003</td>
<td>0.004</td>
<td>1.11</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>4.50</td>
<td>0.074</td>
<td>0.444</td>
<td>7.11</td>
<td>0.32</td>
<td>0.70</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1 Data are from Suryawan et al. (41)

2 Actual activity reflects the activity state of BCKD in the tissue, whereas total BCKD complex activity is an estimate of enzyme amount measured after activation (dephosphorylation) of the complex. The activity state is the ratio of actual activity before activation to total activity obtained after activation by phosphatase treatment.
enzymes in nonhuman primates (monkeys) is similar to that in humans (41) and is dissimilar to that observed in rats. Although the ratios of BCAA:BCKD enzyme activities are similar in the 3 different species, total activities of both branched-chain catabolic enzymes are much higher in rats than in primates. Therefore, the capacity for disposal is higher in rats than in primates. Another striking difference between the patterns of enzyme expression in rats compared with primates is the stringent organ specificity in rats. In rats, 82% of the total transaminase activity is expressed in skeletal muscle but only 0.5% in liver and 4.2% in brain, whereas 83% of the BCKD of rats is expressed, not in muscle, but in liver. Only 3% appears in skeletal muscle and 1% in brain. The enzyme expression pattern is less organ specific in humans. In the case of the transaminase, 65% is in human muscle, 7.7% in liver, and 15% in brain. In the case of the BCKD, only 13% is expressed in human liver but 54% in skeletal muscle and 20% in brain. It is physiologically significant that in rats most of the transaminase is in the muscle; however, actual BCKD activity is quite low in rat skeletal muscle, whereas in humans both activities are present in significant amounts. Thus, in rats the branched-chain carbon skeleton produced mainly in muscle must pass through the vascular space before it can be oxidized in the liver. This makes it available to all organs before irreversible oxidation in the liver.

If rats are used as the experimental animal to test toxicity, then transgenic models with a block of BCAA catabolism (e.g., BCATm knockout) might allow for shorter-term feeding studies and determination of toxic intermediates. As outlined above, rats have a very high capacity to metabolize BCAAs compared with humans (41), so previous studies using rats may have underestimated the toxic potential of high BCAA intake by humans. Indeed the pathological symptoms observed in humans with genetic diseases that impair BCKA oxidation (MSUD) suggest that there is a potential for toxicity when intake exceeds an as yet undefined limit. The pathological symptoms of MSUD are neurological. Although recent investigations have provided some insight into possible mechanisms of toxicity (54,55), it is still not clear why excesses of BCAAs or BCKAs cause the specific neurological symptoms. The essential role of BCAAs in the brain is being actively investigated and that role has become clearer in recent years (56,57), though numerous questions remain.

The role of BCAAs in the central nervous system

Recent studies of BCAA and glutamate metabolism suggest that the role of BCAAs involves maintenance of neurotransmitter glutamate at a relatively constant level. There are parallels between the proposed function of BCAAs in skeletal muscle and their role in the central nervous system. However, in the central nervous system, the glutamate that is formed is an excitatory neurotransmitter, as well as a precursor of glutamine (glutamate–glutamine cycle) and a precursor of the inhibitory neurotransmitter GABA. As in skeletal muscle, there is substantial uptake of BCAAs into the brain in rats (58), and, in humans, the cerebral arteriovenous difference for leucine exceeds that of other amino acids (59). Reported Michaelis constants for uptake of BCKA into rat brain (60) would be consistent with limited transport of BCKA across the blood–brain barrier at physiological concentrations of these α-keto acids (42). Nevertheless, release of BCKAs from the brain has been reported by Matsuo et al. (61). The low concentrations of BCKAs observed in the brain (61) and liver (42) are consistent with efficient metabolism or release of BCKAs. Whether or not BCKAs are oxidized in situ or are released to other tissues, net transfer of BCAA nitrogen can occur in the brain. In the brain, as in skeletal muscle, maximal BCAT activity exceeds total BCKD activity (BCAT/total BCKD activity ≈ 50) (41,56). This suggests that the BCAAs are not consumed for some important role but instead can act in a cyclic way to maintain glutamate levels. Isotope studies have shown that there is efficient transfer of nitrogen from BCAA to glutamate (glutamine) in subcellular brain fractions and primary cell cultures in vitro and in animal models in vivo (27,28,62–65). Serum glutamate cannot be the major source of neurotransmitter glutamate, because interstitial glutamate is highly neurotoxic in the brain. In vivo incorporation of 13N from leucine into glutamate, glutamine, and GABA has been reported in mice (62), and Kanamori et al. (27) estimated that leucine provides ≈25% of glutamate nitrogen in the rat brain. During 9 h of continuous intragastric feeding in rats, Sakai et al. (28) estimated that at least 50% of brain glutamate α-amino nitrogen was derived from leucine. Not surprisingly, there was significant reamination of the leucine α-keto acid, KIC (28). Although the relative rates of brain KIC reamination (nitrogen cycling) vs. oxidation (net nitrogen transfer) remain to be determined, human brain BCAT and actual BCKD capacities are second only to skeletal muscle (41). Thus, the brain may be a quantitatively important site of BCAA metabolism in humans, especially because the brain represents a higher percentage of total body weight than in rats and nonhuman primates.

In the brain, the mitochondrial BCATm is expressed in astrocytes, whereas the brain specific isozyme BCATc is found in neurons. BCATc is the predominant isozyme in the brain, accounting for 60–70% of total brain BCAT activity (4,18,39,66). In the rat nervous system, BCATc is expressed in selected populations of glutamatergic and GABAergic neurons (44,67). Yudkoff (57) has suggested that because the BCAAs can provide nitrogen for the synthesis of glutamate and because transamination of glutamate with BCKAs can lower glutamate levels, the BCAT isozymes act as a buffering system for glutamate. Consequently, disruption of the buffer may interfere with normal neurotransmission.

Hutson and co-workers have taken this concept a step further, emphasizing the role of BCAAs in de novo synthesis of glutamate as opposed to a purely buffering role (29,56,68,69). Because aminotransferases catalyze reversible reactions and operate under near equilibrium conditions in cells, buffering could be provided by a single isozyme in a single compartment. But these enzymes are not in a single compartment. Instead BCATm is not usually found in neurons where glutamate is stored but in the mitochondria of brain astrocytes (29,56,70,71), where pyruvate carboxylase is expressed exclusively (46,47). Pyruvate carboxylase is an anaplerotic enzyme that carboxylates pyruvate to form an excess of TCA cycle intermediates, including α-KG. This will increase mitochondrial α-KG levels in the same cellular compartment as BCATm, thereby driving glutamate synthesis through transamination with BCAAs. Glutamate can enter the astrocyte cytosol, be aminated by glutamine synthetase to glutamine, and then enter the well-known glutamate–glutamine cycle that provides substrate for glutamate neurotransmission (72,73). Therefore, Hutson and co-workers have postulated the existence of a regulated glial–neuron shuttle as opposed to a buffering system (29,56,68,69). Driven by pyruvate carboxylase and glutamine synthetase, the net direction of the shuttle would usually promote glutamate synthesis but could reverse direction. The synthesis is needed to balance oxidation of glutamate in the astrocyte TCA cycle after its release from neurons as a neurotransmitter.
Efficient, smooth operation of the shuttle could be altered by an unphysiological change in BCAA or BCKA levels or by a change in the BCAA:BCKA ratio. BCKAs, after synthesis in the astrocytes, must be oxidized or reaminated to BCAAs in neurons, and then returned to the astrocytes. Transamination to produce BCAAs in the neurons would involve production of excess α-KG, and this would have to be reaminated, probably with NH₃ via GDH. Excess substrate in any of these steps might saturate the system, allow a build-up of intermediates, and would perturb the steady-state operation of neurotransmission in discrete brain areas, providing a rationale for determining the regional and the cellular distribution of BCAA catalytic enzymes in the human brain.

Summary and conclusions

It is clear that BCAAs play an essential role in whole-body nitrogen metabolism. First, they provide an important transport system to move nitrogen throughout the body for the synthesis of dispensable amino acids. Second, they provide hormone-like signals to tissues, e.g., skeletal muscle, indicating control systems to move nitrogen throughout the body for the synthesis of dispensable amino acids. Furthermore, they can serve as a neurotransmitter in the central nervous system in a rat brain that has been studied. Therefore, it is clear that BCAAs play an essential role in whole-body nitrogen metabolism.

LITERATURE CITED


The first word in the title of this manuscript was spelled incorrectly in the print version of this article. The corrected title, authors, and affiliations follow.

**Branched-Chain Amino Acid Metabolism: Implications for Establishing Safe Intakes**

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Please note: the online version of this article has been corrected, in departure from print, to include the correct spelling of the word “Branched” in the manuscript title.


The first name of author Bohdan Luhovyy was spelled incorrectly in the print version of this article. The corrected author and affiliation lines follow:

Marc Yudkoff, Yevgeny Daikhin, Ilana Nissim, Oksana Horyn, Bohdan Luhovyy, Adam Lazarow, and Itzhak Nissim

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Please note: the online version of this article has been corrected, in departure from print, to include the correct spelling of the author’s name.

On page 1395, in Table 1, “Nutritional composition of the dairy products used in the study,” the values in the “Enriched dairy product” column for “EPA, g/100 g total fat” and “DHA, g/100 g total fat” were transposed. EPA should be 2.1 and DHA should be 1.4. The entire table is printed below.

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<th>Component</th>
<th>Semiskimmed milk</th>
<th>Enriched dairy product</th>
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<td>Protein, g/L</td>
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<td>MUFAs, g/100 g total fat</td>
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<td>α-Linolenic acid, g/100 g total fat</td>
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<td>Retinyl acetate, μmol/L</td>
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<td>Folic acid, nmol/L</td>
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1 U, undetected.