

## Chapter 2

# Metabolism of BCAAs

Jeffrey T. Cole

### Key Points

- Branched chain amino acids are essential amino acids that cannot be synthesized in the body.
- Leucine, isoleucine and valine share enzymes for catabolizing the first two steps in their metabolism.
- The three branched chain amino acids are unique among amino acids in that their first catabolic step cannot occur in the liver.
- Disturbances in branched chain amino acids metabolism are primarily genetic in origin.
- Traumatic brain injury appears to uniquely influence the concentration of branched chain amino acids in the brain.

**Keywords** Traumatic brain injury • BCATm • BCKD • Astroglial-neuronal nitrogen cycle • Transamination • Michaelis constant • Nitrogen

### Introduction

Leucine, isoleucine and valine are collectively known as the branched chain amino acids (BCAAs), a descriptive term derived from the structure of their side chains.

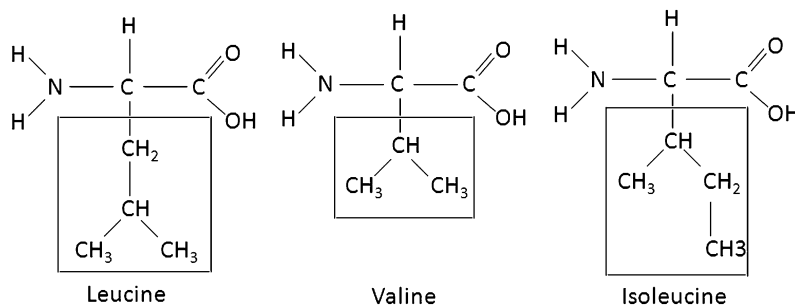
In addition to the structural similarity, and the metabolic synchronization discussed below, the plasma levels of the three BCAAs are remarkably consistent with one another in a variety of physiological conditions, including both Type 1 and Type 2 diabetes [1]. However, despite these remarkable similarities, there are subtle but important differences in their metabolism and function. Despite their similar structure, the side chains differ in the structural conformation and hydrophobicity. These differences account for the variability of roles the three BCAAs play in structural elements. For example, leucine is more common in  $\alpha$ -helices, while valine and isoleucine tend to be found primarily in  $\beta$ -sheets. These slight differences in structure also suggest an underlying reason for why leucine is so often the BCAA involved in helical zippers. Additionally, these differences emphasize why the

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J.T. Cole, Ph.D., M.S. (✉)

Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

e-mail: [Jeffrey.cole@usuhs.edu](mailto:Jeffrey.cole@usuhs.edu)



**Fig. 2.1** The chemical structure of the three branched chain amino acids—leucine, valine and isoleucine. For each amino acid, the square outlines the R-group which gives all amino acids, not just BCAAs, their structural uniqueness and specificity. The slightly different structure of the BCAAs also accounts for their different formula weights (131 for leucine and isoleucine, 117 for valine). Unpublished figure from Cole

BCAAs should be considered, and researched, as separate entities. Often leucine, isoleucine and valine are treated as interchangeable amino acids and frequently, results gained from research into one of the three amino acids is thought to apply automatically to the other two BCAAs.

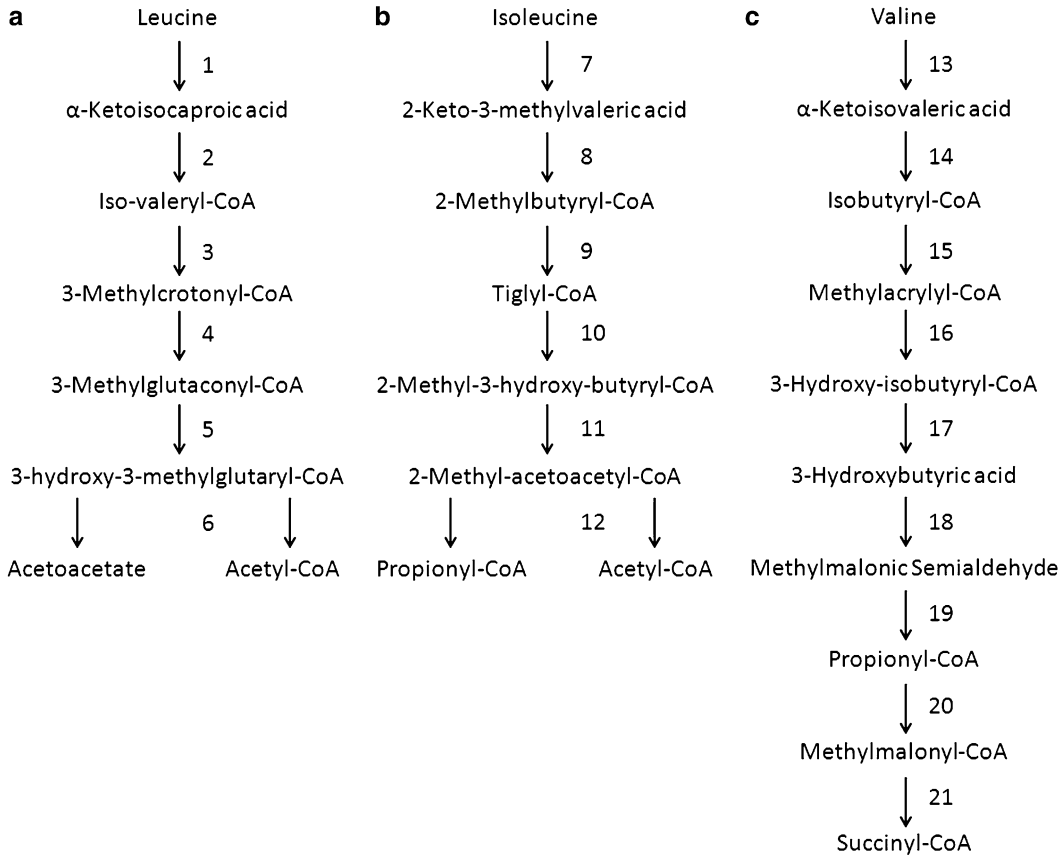
## Structure and Physical Characteristics of BCAAs

The side chains of amino acids are what give each of the more than 20 amino acids their unique characteristics. In the case of leucine, isoleucine and valine, these side chains referred to as aliphatic, a general term which designates organic compounds composed of hydrogen and carbon atoms, that do not contain a benzene-like ring. The 3 BCAAs (Fig. 2.1) contain a 3 or 4 carbon side chain and are of virtually the same molecular weight (leucine 131.17 g/mol; isoleucine 131.17 g/mol; valine 117.15 g/mol). Collectively, the three branched chain amino acids make up approximately 33 % of all the amino acids in the body. A great proportion of these three amino acids are found in skeletal muscle, where they act as both a structural element and as a store for systemic nitrogen.

These amino acids are “essential” amino acids, meaning that mammals cannot de novo synthesize them, necessitating their intake from external sources. As essential amino acids, the human body must consume approximately 40, 20 and 19 mg/kg/day of leucine, valine and isoleucine, respectively. Collectively, this amounts in total to approximately 5.5 g/day for a 70 kg adult. The best sources of these amino acids are red meat or dairy products. However, it is possible for vegans to consume sufficient amounts to meet their needs by judicious consumption of soy protein and other vegetarian sources. In the typical Western diet, approximately 20 % of all dietary protein consists of the three BCAAs which makes deficiency an exceptionally rare occurrence. In fact, metabolic and physiological disorders associated with BCAAs typically involve the genetic disruptions in their metabolism or as secondary to other primary health problems and not due to deficiency (see Chap. 12 on MSUD and below for further discussion)[2–5].

## Catabolism of BCAAs

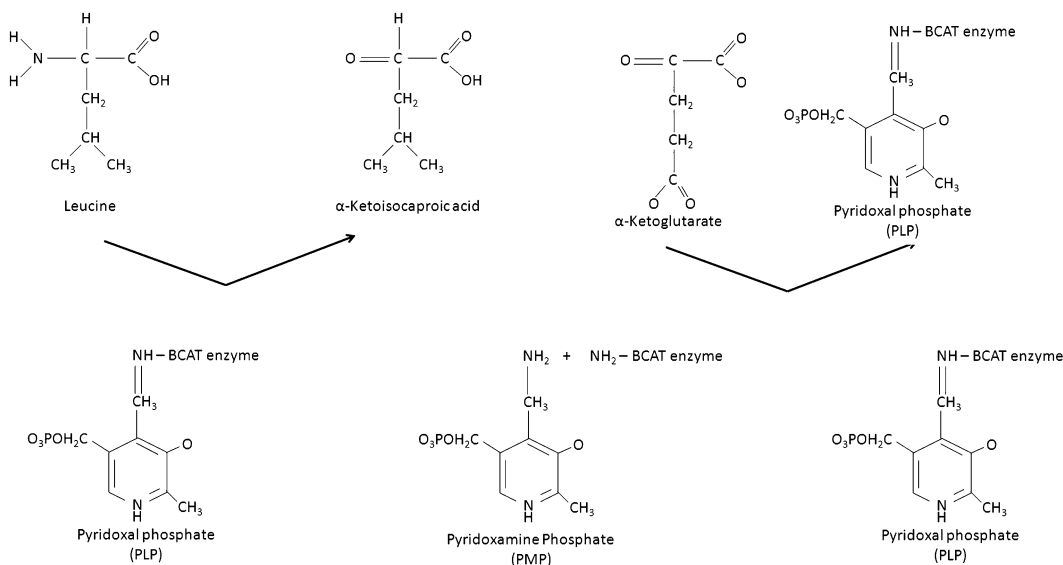
To completely catabolize BCAAs it requires a number of enzymatic steps (Fig. 2.2), most of which occur in the mitochondria [6–8]. As with most amino acids, the final catabolic step results in the production of acetyl-CoA, propionyl-CoA and succinyl-CoA. However, it should be remembered that at



**Fig. 2.2** Schematic depicting the complete metabolism of leucine, isoleucine and valine. The numbers correspond to the appropriate enzymes for each reaction. 1. Branched Chain AminoTransferase; 2. Branched Chain Keto-Acid Dehydrogenase; 3. Isovaleryl-CoA Dehydrogenase; 4. 3-Methylcrotonyl-CoA Carboxylase; 5. 3-Methylglutaconyl-CoA Hydratase; 6. 3-Hydroxy-3-Methylglutaryl-CoA lyase; 7. Branched Chain AminoTransferase; 8. Branched Chain Keto-Acid Dehydrogenase; 9. 2-Methylbutyryl-CoA Dehydrogenase; 10. Enol-CoA Dehydrogenase; 11. 2-Methylhydroxybutyryl CoA Thiolase; 12. 3-Methylacetoacetyl-CoA Thiolase; 13. Branched Chain AminoTransferase; 14. Branched Chain Keto-Acid Dehydrogenase; 15. 2-Methylbutyryl-CoA Dehydrogenase; 16. Enol-CoA Dehydrogenase; 17. 3-Hydroxyisobutyryl-CoA Deacylase; 18. 3-Hydroxyisobutyryl-CoA Dehydrogenase; 19. Methylmalonic semialdehyde Dehydrogenase; 20. Propionyl-CoA Carboxylase; 21. Methylmalonyl-CoA Mutase. Unpublished figure from Cole

virtually every catabolic step, the metabolite can be diverted for other uses, such as fatty acid or cholesterol synthesis. Additionally, while some reactions are reversible, leucine, isoleucine and valine cannot be synthesized *de novo* by the body.

*Two shared enzymes catabolize the first step:* BCAA catabolism begins with a transamination reaction catalyzed by the branched chain aminotransferase (BCATs). The distribution of these enzymes will be discussed in greater depth in Chap. 3; briefly however, there are two isoforms—mitochondrial BCATm and cytosolic BCATc. In fact, the cytosolic isoform catalyzes the only catabolic step in BCAA metabolism that occurs outside of the mitochondria [9–12]. These isozymes transfer the  $\alpha$ -amino group from leucine, isoleucine or valine to  $\alpha$ -ketoglutarate [13, 14]. This metabolic step is unique to BCAA metabolism as the BCAT isozymes (and the second step, catabolized by BCKD) are common to three different amino acids, while no other amino acids share a primary first step enzyme. The products resulting from this reaction are glutamate, which now contains a nitrogen-containing group provided



**Fig. 2.3** An example of the first step of BCAA catabolism, using leucine as an example. In the first half of this “ping-pong” reaction, leucine is deaminated, forming  $\alpha$ -ketoisocaproic acid, with the amino group being temporarily transferred to the BCAT isozyme. Both the mitochondrial and cytosolic isozymes function identically in this reaction. Accepting the amino group converts BCAT-PLP to BCAT-PMP. In the second half of the reaction, BCAT-PMP donates the amino group to an acceptor molecule, typically  $\alpha$ -ketoglutarate, to form the amino acid glutamate. The BCAT-PMP is thus returned to its initial BCAT-PLP state. PLP—pyridoxal 5' phosphate. PMP—pyridoxamine 5' phosphate. Unpublished figure from Cole

by the BCAAs and a branched chain keto-acid (BCKA) [15–17]. Once deaminated, leucine, isoleucine and valine form  $\alpha$ -ketoisocaproic acid,  $\alpha$ -keto- $\beta$ -methylvaleric acid and  $\alpha$ -ketoisovaleric acid, respectively.

Both BCATm and BCATc use Vitamin B-6 cofactors to catalyze the reaction. During the first half of reaction, pyridoxal-5-phosphate (PLP) acts as a temporary acceptor of the  $\alpha$  amino group being donated from the BCAA and becomes pyridoxamine-5-phosphate, which converts BCAT-PLP to BCAT-PMP. In the second half of the reaction, BCAT-PMP donates the amino group to  $\alpha$ -ketoglutarate. This converts the BCAT-PMP back to BCAT-PLP, while simultaneously transaminating  $\alpha$ -ketoglutarate to glutamate (Fig. 2.3). This is often referred to as “ping-pong metabolism”, as the enzyme and cofactor revert back to their initial state following release of the temporarily attached amino group.

As mentioned, studies of the BCAAs frequently “lump” the three amino acids together and use one BCAA, usually leucine, as a representative for the other two BCAAs. However, there is a marked substrate preference for the BCAT enzymes for isoleucine, followed by leucine and then valine. Typically,  $\alpha$ -ketoglutarate is presumed to be the default amino group acceptor although other ketoacids can serve this function. Indeed, kinetic data indicates that BCKAs themselves can act as substrates for the second half of the reaction (i.e. the donation of amino group from BCAT-PMP), with  $K_m$  values indicating that KIC is preferred to KIV and KMV, and even  $\alpha$ -ketoglutarate. The Michaelis constant ( $K_m$ ) characterizes the affinity of an enzyme for a substrate. In an enzymatic reaction, the  $K_m$  value is the substrate concentration at which the reaction proceeds at half its maximum speed. A low  $K_m$  value means that the enzyme reaction has a high affinity for its substrates, with just a small amount of substrate needed to make the reaction proceed at its maximum rate. However, despite this marked preference, Islam and Hutson clearly demonstrate that specificity constant ( $k_{cat}/K_m$ ) for BCATm determine that  $\alpha$ -ketoglutarate is the major substrate in vivo for the second half reaction. A specificity constant

is the efficiency of the enzyme reaction and is determined by the  $K_{\text{cat}}$  (maximum rate at which an enzyme can function) divided by the  $K_m$  (the rate of substrate-enzyme interaction) [18].

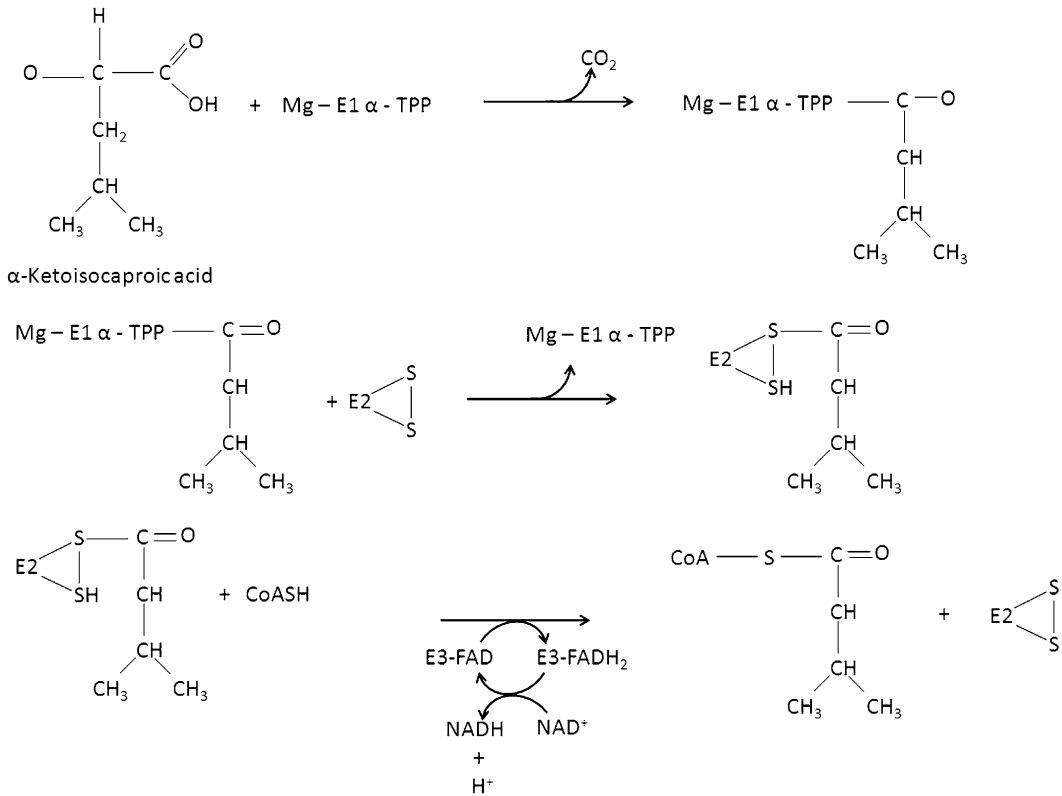
*Enzymatic characteristics of BCATc and BCATm:* There are many unique features of BCAT enzymes, one of which is a redox-sensitive CXXC center that plays a major role in catalytic reactions. In this case, the C's represent the amino acid cysteine, while the X's can be any amino acid. Both isozymes of BCAT are reversible, and it is this redox center that permits this reversibility. In most cells, BCAT enzymes operate near equilibrium, with both substrate and product concentrations being at or below their  $K_m$  values. This allows BCAT isozymes to quickly respond to changes in tissue concentrations of substrate or product, which allows BCAAs to be an ideal reserve for both carbon skeletons and nitrogen for glutamate synthesis.

However, this near equilibrium status also means that for the reaction to proceed, rather than cycle between BCAAs and BCKAs, BCKAs must be eliminated. This can occur via simple removal from the cell which is not typical as demonstrated by the exceptionally low circulating levels of BCKAs in the serum [19]. However, the movement of BCKAs between tissues (or cell types, as seen in the brain, discussed above) would provide a mechanism for the transfer of BCAA-derived nitrogen between organs and tissues. The other, more prevalent, mechanism by which BCKAs can be eliminated is through simple oxidation within the cells. Removal of the BCKA carbon skeleton following transamination results in the net transfer of the amino group from BCAAs to glutamate.

*BCKD catabolizes the second step:* The oxidation of BCKAs following BCAA deamination is regulated by the second enzyme in BCAA catabolism. As with the BCAT isozymes, this enzyme is also shared among the three BCAAs. The mitochondrial branched chain  $\alpha$ -keto acid dehydrogenase (BCKD) irreversibly catabolizes BCKAs and regulates the rate of BCAA metabolism. Interestingly, the rate at which BCAA carbon skeletons are oxidized mirrors the rate of net transfer of nitrogen to glutamate from BCAAs. BCKD, also sometimes referred to as BCKDC (the "C" designating "complex"), is one of a family of macromolecular proteins which include pyruvate dehydrogenase (PDH) and  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ -KDH).

*Structural arrangement of BCKD:* BCKD contains within its complex 3 enzymes, each of which also has multiple copies. The three subunits have been identified as branched chain  $\alpha$ -keto acid decarboxylase (E1), a dihydrolipoyl transacylase (E2) and a dihydrolipoyl dehydrogenase (E3). Ultimately, BCKD oxidatively decarboxylates any of the three BCKAs, forming isovaleryl-CoA from KIC, isobutyryl-CoA from KIV, and  $\alpha$ -methylbutyryl-CoA from KLV, with NADH being also produced. This reaction is both rate limiting and highly regulated, with BCKD kinase (BCKD-K) mediating the reaction by associating and dissociating from the complex. BCKD-K association with BCKD results in phosphorylation and inactivation of E1 enzyme. The regulation of BCKD-K is in turn regulated by changes in the local concentrations of KIC. Elevated levels of KIC inactivate BCKD-K, which facilitates oxidation of all three BCKAs. When levels of KIC drop, the BCKD-K is reactivated, signaling it to inhibit BCKD activity. Some research groups have speculated about the existence of a BCKD phosphatase. However, it has yet to be identified and the exact function and role of this enzyme has not been determined, nor have the possible protein regulators of this phosphatase been identified [20–22].

All three enzymes catalyze multi-step reactions and irreversibly oxidatively decarboxylate  $\alpha$ -keto acids. Due to its complexity, the structure and function of BCKD enzyme has only been partially elucidated [18]. However, the enzyme complex is assembled around a scaffolding consisting of a dihydrolipoamide transacylase (E2 subunit). This 24-meric core is the noncovalent attachment site for multiple copies each of the BCKA dehydrogenase (E1 subunit) and the dihydrolipoamide dehydrogenase (E3 subunit). It is the E1 subunit that catalyzes the decarboxylation of BCKAs, using as a cofactor thiamine diphosphate (ThDP). The reaction produces carbanion ThDP, which is then reduced by E1, transferring the carbanion to the lipoyl moiety bound to the E2 subunit. The acyl group is transferred to the active site of E2 by the sacyldihydrolipoamide domain. The FAD moiety of E2 re-oxidizes the dihydrolipoyl residue, while NAD<sup>+</sup> serves as the electron acceptor. In turn, the mitochondria will oxidize the NADH to provide energy (Fig. 2.4).



**Fig. 2.4** Branched chain ketoacid dehydrogenase catabolizes the second, irreversible, step in BCAA catabolism. While any of the three ketoacids generated during BCAA catabolism can act as a substrate, this figure uses  $\alpha$ -ketoisocaproic acid as an example. In step A, the E1 subunit of BCKD, in a thiamine pyrophosphate-mediated reaction temporarily binds to the BCKA, releasing a carbon dioxide ( $\text{CO}_2$ ). This complex then interacts with the E2 subunit (step B), transferring the decarboxylated BCKA and releasing the E1 subunit. This E2 complex then transfers the BCKA derivative to CoASH, forming the third product in BCAA metabolism (after the BCAA and BCKA), which are isovaleryl-CoA, 2-methylbutyryl-CoA and isobutyryl-CoA. During this release, the FAD-linked E3 subunit acts as a temporary proton acceptor, mediating the conversion of  $\text{NAD}^+$  to NADH. FAD—flavin adenine dinucleotide. NAD—nicotinamide adenine dinucleotide. Unpublished figure from Cole

*BCATm and BCKD— a metabolon?*: The efficiency of BCATm and BCKD is enhanced not just by the close physical proximity of the enzymes, but through an actual structural relationship referred to as a metabolon. Islam and Hutson demonstrated channeling of the BCKAs formed via BCATm activity directly to the E1 component of the BCKD complex [18, 23]. This direct channeling enhanced the capacity of BCKD to decarboxylate BCKA and the overall complex activity. Using isothermal calorimetry, Islam et al. determined that BCATm and the E1 subunit of BCKD directly, structurally, interact. However, this association, with a binding constant of 6 mM, is relatively weak. Despite this weak association, when a BCAA donates its amino group to BCATm-PLP, the resultant BCKA is channeled directly to the active site of E1, while the BCATm-PMP, having transaminated  $\alpha$ -ketoglutarate, is released. These results suggest that not only is the activity of BCATm and BCKD dependent on substrate availability, but also on the ratio of BCATm to BCKD E1. This close relationship drives transamination and glutamate synthesis while providing additional carbon skeletons for energy generation.

*Downstream BCAA Catabolism:* The remaining catabolic steps are, like BCKD, mitochondrial in location. Two different dehydrogenases (isovaleryl-CoA dehydrogenase for leucine products and 2-methyl-butryl-CoA dehydrogenase for isoleucine and valine products) oxidize the branched chain acyl-CoA products to form 3-methylcrotonyl CoA, tigloyl-CoA and methylacryl-CoA [24]. Following this step, the catabolic pathways of leucine, isoleucine and valine, which had shared enzymes and pathways, diverge (Fig. 2.2) The ketogenic leucine product Isovaleryl-CoA is ultimately catabolized to form acetyl-CoA and acetoacetate. Its metabolism involves  $\beta$ -methylcrotonyl-CoA carboxylase, which is both ATP and biotin dependent. In contrast, both valine and isoleucine are considered to be glucogenic, with both BCAAs being metabolized to propionyl-CoA (and Acetyl-CoA in the case of isoleucine) then succinate via methylmalonyl-CoA. Additionally, valine catabolism results in the production of 3-hydroxybutyrate (3-HB) which can be released from tissue. As a ketone body, 3-HB can be used as an alternative fuel source when glucose is depleted.

## Distribution of BCAA Metabolism

*Unique characteristics of BCAA metabolism:* The metabolism of BCAAs is relatively well-understood and straightforward. However, it is the cellular and tissue distribution of their primary metabolizing enzymes that gives leucine, isoleucine and valine such an important role in nitrogen metabolism [25]. Significantly, neither isoform of BCAT is expressed in the liver. BCATc is expressed primarily in the brain, testes and ovaries, while BCATm is found throughout the body. The distribution of BCAT and BCKD will be discussed further in Chap. 3. This makes BCAAs somewhat unique in comparison to the other amino acids in that not only is their first catabolic step not occurring in the liver, but that its primary catabolism occurs in the skeletal muscle [26, 27]. Despite the fact that dietary amino acids typically contain about 20 % BCAAs, amino acids in the blood exiting the splanchnic bed typically consist of about 50 % BCAAs. In fact, approximately 50 % of all skeletal muscle amino acid uptake consists of BCAAs, since leucine, isoleucine and valine all avoid “first pass metabolism”. The phrase “first pass metabolism” refers to hepatic metabolism of substrates immediately following their absorption from the intestine [25]. Indeed, following consumption of a protein rich meal (beef) in which BCAAs make up about 20 % of the amino acids, the splanchnic output consisted of about 50 % BCAAs [28–30]. This caused speculation that about half the capacity to catabolize BCAAs is found in the skeletal muscle, with a substantial portion also being seen in adipose tissue. This prompted experiments in which forearm metabolism was monitored after the ingestion of a steak. Following consumption of this high protein meal, BCAA uptake into the muscle was significantly increased. However the release of BCKAs was only minimally affected. This suggests that BCAAs are being completely catabolized in the muscle, or contributing to protein deposition, which may account for the popularity of these amino acids as muscle building supplements.

A key function of the BCAAs in the skeletal muscle is to provide the nitrogen needed to maintain muscle pools of glutamate, alanine and glutamine. In the skeletal muscle, BCAT activity is substantially higher than BCKD activity. The high ratio of BCAT:BCKD activity indicates a tissue capacity for efficiently transferring nitrogen from BCAAs to alpha-ketoglutarate and also possibility of a high release of BCKAs from the tissue instead of their being oxidized in situ. Despite the extensive release of BCKAs from the skeletal muscle, the concentrations of circulating BCKAs are exceptionally low. This is due to the complete absence of BCAT isozymes in the liver, while the hepatocytes demonstrate an extremely high level of BCKD activity. This allows virtually complete oxidation of BCKAs and maintains low levels.

*Interorgan BCAA metabolism:* Since muscle is not a gluconeogenic tissue, valine and isoleucine cannot be completely oxidized in this tissue if they are to be converted to glucose. Studies in cardiac



muscle have demonstrated that KIC is almost completely oxidized, while KIV is not. In fact, in both cardiac and skeletal muscle, the primary end product of valine metabolism is not complete oxidation but instead beta-hydroxyisobutyrate. This metabolite is a key indicator for the fate of valine in the muscle. While in a fed state, plasma beta-hydroxyisobutyrate levels are approximately 20 mM, there is a fivefold increase in fasted humans. This is due to the role of  $\beta$ -hydroxyisobutyrate as a key inter-organ gluconeogenic substrate, and is in fact, an ideal gluconeogenic substrate in hepatocytes and renal cortical tubules.

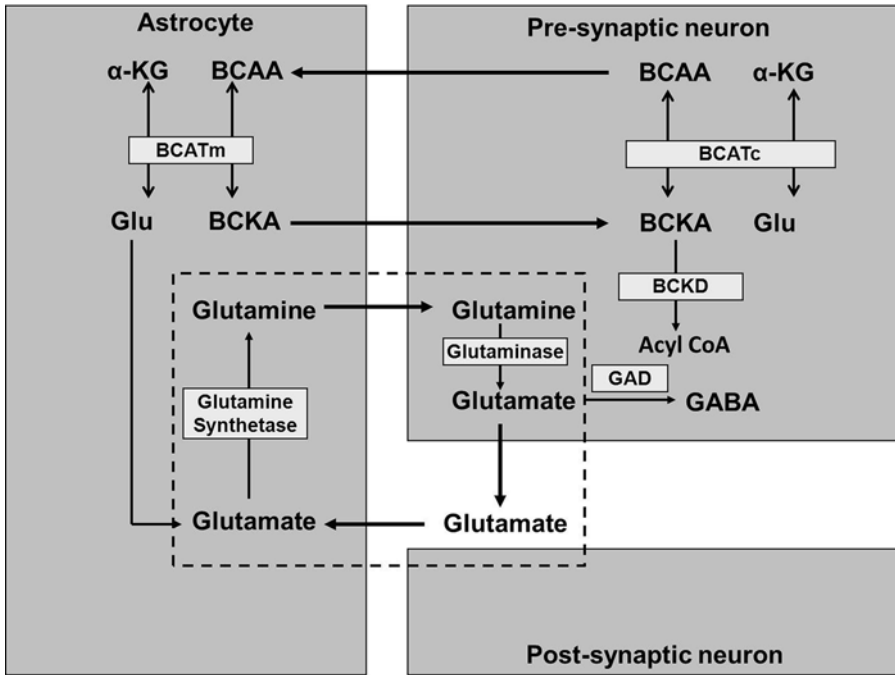
*BCAA metabolism in the brain:* The metabolism of BCAAs in the brain represents a somewhat unique case, in comparison to the rest of the body. This is due to the unique distribution of the BCAA metabolizing enzymes, with the brain being the only tissue (along with the ovaries and testes) that express BCATc, BCATm and BCKD. In the central nervous system (CNS), BCATm and BCKD are expressed in different cell types, despite convincing evidence that elsewhere the two enzymes form a metabolon to facilitate BCAA catabolism. This suggests, perhaps, a slightly different role for BCAAs in the brain than in the rest of the body. In the CNS, BCATc is expressed primarily in neurons, as is BCKD, while in BCATm it appears to be limited to astrocytes [31, 32]. This will be discussed more in depth in Chap. 3.

## Metabolic Disorders

While BCAA deficiency is exceptionally rare, there are several disorders in their metabolism that can cause significant health problems. Perhaps the most well-known is Maple Syrup Urine Disease, which is caused by mutations in the BCKD enzyme complex. This will be discussed completely in Chap. 12. There is another, equally rare, autosomal recessive disease known as Isovaleric Acidemia. This disorder is caused by a mutation in the enzyme isovaleryl-CoA dehydrogenase, which converts isovaleryl CoA to 3-methylcrotonyl-CoA. The accumulation of isovaleryl-CoA results in many of the symptoms of this disorder, which may prove fatal. As with MSUD, this disorder can best be treated by strict regulation of the dietary intake of BCAAs.

*Extra-metabolic functions of BCAAs in the brain:* In the brain, the BCAAs play a key role in maintaining the supply of the neurotransmitter glutamate. To understand this contribution, a brief discussion of the glutamate:glutamine cycle and the astroglial:neuronal nitrogen hypothesis are in order. In the brain, glutamate serves as an excitatory neurotransmitter and is released from the synaptic cleft. After interacting with receptors on the post-synaptic neuron, it is removed from the synapse by astrocytes. Collectively, the astrocyte and pre- and post-synaptic neuron are referred to as a “tripartite synapse”. The glutamate is removed from the synaptic cleft to prevent unrestrained, uncontrolled hyperactivation of the post-synaptic neuron. The astrocyte aminates the glutamate in a glutamine synthetase-mediated reaction to form glutamine. This amino acid is then returned to the pre-synaptic neuron where it can be deaminated by glutaminase to re-enter the pool of neurotransmitter glutamate. This process has been named the “glutamate:glutamine cycle”, and allows for the conservation of excitatory neurotransmitters within the brain. However, as with any physiological processes, the glutamate:glutamine cycle is not 100 % efficient [15–17]. At each step of the process, glutamate can be lost or diverted for other purposes. In the astrocyte, glutamate can be aminated to form glutamine. However, astrocytes also convert a significant fraction of the glutamate to alpha-ketoglutarate via glutamate dehydrogenase, which then enters the TCA cycle and is catabolized eventually to lactate. In fact, the flow of TCA cycle intermediates to glycolytic products means that significant glutamate synthesis must occur to replace the lost glutamate. Additionally, the glutamine produced in the astrocyte can be used both within the astrocyte or neuron for purine and pyrimidine synthesis, or for nitrogen fixation. When glutamine has been returned to the pre-synaptic neuron, the glutamate produced by its deamination by glutaminase can similarly be scavenged for other uses, notably for energy





**Fig. 2.5** Scheme for the Astroglial-Neuronal Shuttle depicts the current model for BCAA function in the brain. The glutamate:glutamine cycle (within the dotted line box) depicts the uptake of glutamate from the synaptic cleft following release from the pre-synaptic neuron. After re-amination, to form glutamine, the synaptically inert glutamine is returned from the astrocyte to the pre-synaptic neuron. It is deaminated to form the excitatory neurotransmitter glutamate. Since this reaction is not 100 % efficient, it requires a continual input of new glutamate (and nitrogen), which led to the development of the astroglial-neuronal nitrogen shuttle hypothesis. Astrocytic BCATm transaminates BCAAs to form glutamate. The resultant BCKA carbon skeleton is returned to the neuron for re-amination, prior to return to the astrocyte. Alternatively, the BCKAs are disposed of in the neuron by irreversible catabolism by branched chain keto acid dehydrogenase (BCKD). BCAT- branched chain aminotransferase. BCKD- branched chain ketoacid dehydrogenase. GAD- glutamic acid decarboxylase. BCAA, branched chain amino acid. Glu- glutamate.  $\alpha$ -kg—ketoglutarate. Unpublished figure from Cole

production via the TCA cycle, as in the astrocyte. Put simply, there is no guarantee that the glutamate released from the pre-synaptic neuron will be returned to the pre-synaptic neuron as a neurotransmitter without being used for other physiological purposes.

**Astroglial-neuronal nitrogen shuttle:** While the glutamate:glutamine pool is well-documented, less well understood is the means by which the glutamate pool is maintained despite losses for other purposes. This led to the creation of the “astroglial-neuronal nitrogen shuttle hypothesis” based on the contribution of BCAAs to glutamate metabolism in the brain (Fig. 2.5). Approximately 40 % of glutamate in the brain contains an amino group that was derived from a BCAA. This hypothesis was originally developed from results obtained in cultured astrocytes in which leucine oxidation is very slow, while release of  $\alpha$ -ketoisocaproic acid is high, as well as studies using radio-labeled leucine to track the nitrogen label.

In the astroglial-neuronal nitrogen shuttle, BCATm-mediated BCAA transamination occurs in the astrocyte, with  $\alpha$ -ketoglutarate being the amino group receptor resulting in glutamate production. The glutamate can then be further aminated as it enters the glutamate:glutamine cycle. However, the BCKAs are not primarily catabolized in the astrocyte. In fact, the BCKAs themselves are also putatively transferred to the neuron. Once in the neuron, the BCKAs are re-aminated by BCATc.

The re-capitulated BCAAs are then shuttled back to the astrocyte to complete the shuttle process and further produce glutamate. However, while the contribution of BCAAs to glutamate synthesis is indisputable, there are several paradoxes about the astroglial neuronal nitrogen shuttle that require further elaboration. First, in the neuron, BCKAs are re-aminated to BCAAs using a glutamate-derived amino group. Thus, neuronal pools of glutamate are being utilized to synthesis BCAAs for the return trip back to the astrocyte to make additional glutamate. This seems to be something of a futile cycle, although the existence of distinct pools of glutamate render this hypothesis less unlikely than it first appears. A number of researchers speculated on the existence of at least two pools of glutamate in the neuron which have little to no communication. The metabolic pool is many orders of magnitude larger than the synaptic pool and can therefore provide the needed amino group to BCKAs without depleting synaptic glutamate. Why the neuron does not simply transfer glutamate from one pool to the other remains to be answered.

## Traumatic Brain Injury and BCAA Metabolism

BCAAs play a major role in maintaining glutamate stores in the brain. However, disruption of their metabolism or the provision of excess BCAAs is only rarely thought to cause neurological problems. While the classical example is Maple Syrup Urine Disease (discussed Chap. 12), a research effort led by Drs. Cole and Verma determined that following a traumatic brain injury (TBI) [33], in the damaged region of the brain, there is a significant decrease in the concentration of BCAAs. Decreases in BCAAs correlated very strongly to impairments in electrophysiological activity and neurological/cognitive function. Interestingly, application of BCAAs restored brain function in the injured areas and performance on memory tasks used to assess cognitive function was improved. Aquilani et al. (2005, 2008) conducted a clinical trial in which severely brain injured patients are given IV BCAAs. Using a Disability Rating Score, these researchers demonstrated a significant improvement in the neurological health of these individuals. See chapter regarding “The branched chain amino acids in the context of other amino acids in traumatic brain injury” for more details.

## Conclusions

Leucine, isoleucine and valine are key amino acids in a number of systemic functions, most particularly nitrogen homeostasis and neurological function. In comparison to other amino acids, the three BCAAs have a unique distribution of enzymes, and, perhaps more uniquely, the property of sharing key enzymes. Understanding these structural, biochemical, and metabolic properties of BCAAs will shed significant light onto the following chapters.

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Branched Chain Amino Acids in Clinical Nutrition  
Volume 1

Rajendram, R.; Preedy, V.R.; Patel, V.B. (Eds.)

2015, XXVIII, 270 p. 91 illus., 21 illus. in color.,

Hardcover

ISBN: 978-1-4939-1922-2

A product of Humana Press