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Branched Chain Amino Acids

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Abstract

Branched chain amino acids (BCAAs) are building blocks for all life-forms. We review here the fundamentals of BCAA metabolism in mammalian physiology. Decades of studies have elicited a deep understanding of biochemical reactions involved in BCAA catabolism. In addition, BCAAs and various catabolic products act as signaling molecules, activating programs ranging from protein synthesis to insulin secretion. How these processes are integrated at an organismal level is less clear. Inborn errors of metabolism highlight the importance of organismal regulation of BCAA physiology. More recently, subtle alterations of BCAA metabolism have been suggested to contribute to numerous prevalent diseases, including diabetes, cancer, and heart failure. Understanding the mechanisms underlying altered BCAA metabolism and how they contribute to disease pathophysiology will keep researchers busy for the foreseeable future.

THE BIOCHEMISTRY OF BRANCHED CHAIN AMINO ACIDS

Branched chain amino acids (BCAAs) cannot be synthesized by metazoans. Despite this, they are abundant components of animals, constituting approximately 35% of essential amino acids in most mammals (1–3). The functional R groups of all three BCAAs are branched (hence their name), small, and hydrophobic, rendering them critical components of most protein (4, 5). Together, BCAAs account for about 18% of amino acids and 63% of hydrophobic amino acids in protein across many life-forms (1–3, 6). The molar relative abundance of BCAAs to each other is nearly always approximately 1.6:2.2:1.0 Val:Leu:Ile, reflecting the linked nature of their synthesis and oxidation (see below). The three BCAAs are thus almost always eaten and combusted together (7, 8), and as such are also almost always studied as one entity, despite significant differences in their biological effects as outlined below. This tendency has often led to erroneous assumptions.

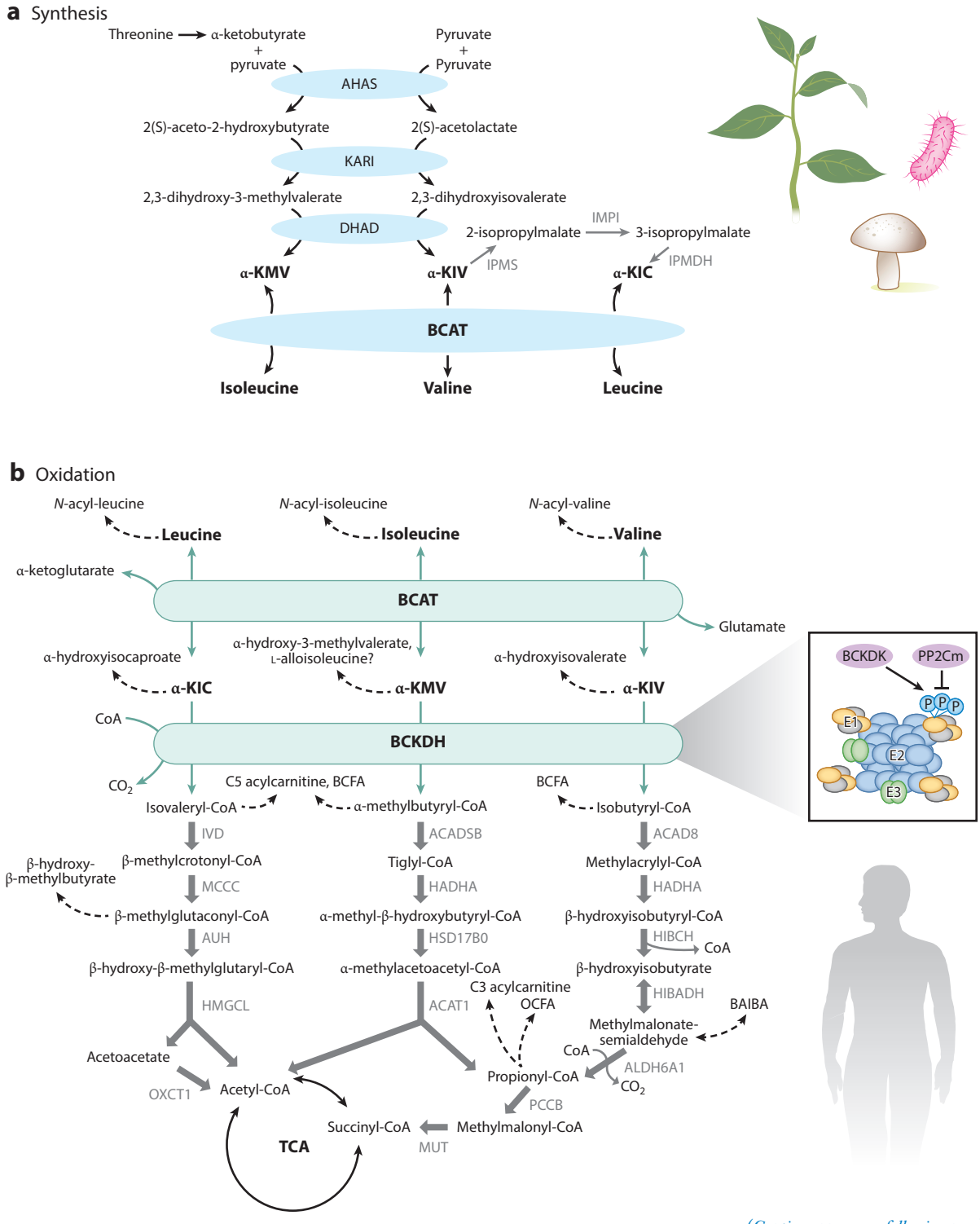
Synthesis

BCAAs are synthesized in bacteria, plants, and fungi, but not in animals. The synthesis of valine and isoleucine is carried out by the same enzymes, and leucine is created from α-ketoisovalerate, a transamination precursor of valine (**Figure 1**) (9). The carbons in valine (and leucine) are derived from the readily available and abundant pyruvate, but isoleucine carbons are derived from the relatively rare threonine, again reflecting the conserved ratio of abundance in protein. Because it does not occur in animals, BCAA synthesis has been successfully targeted for antimicrobials, herbicides, and antifungal agents (10).

Catabolism

All life-forms catabolize BCAAs in a similarly linked process (**Figure 1**). In mammals, all three BCAAs are initially transaminated by branched chain amino transferases (BCATs) to form branched chain α-ketoacids (BCKAs) (11). The most common nitrogen acceptor is α-ketoglutarate (αKG), yielding glutamate (12). This reaction is rapid with a low free energy change and is thus likely in equilibrium in most cases. There is, however, a preference for the reverse reaction because (*a*) the *K*m for BCKAs is in the 100-μM range, while that for BCAAs is in the 1-mM range (somewhat higher for valine) (12, 13); and (*b*) the concentration of intracellular glutamate is typically relatively high (14). Two genes encode BCATs: *BCAT1* (or *cBCAT*) encodes a cytoplasmic protein and is primarily expressed in the brain, whereas *BCAT2* (or *mBCAT*) encodes a mitochondrial protein and is ubiquitously expressed (12, 15).

Irreversible initiation of BCKA oxidation occurs in the branched chain amino acid dehydrogenase (BCKDH) complex. The BCKDH complex is found on the inner surface of the inner membrane of mitochondria and shares many attributes with the pyruvate dehydrogenase (PDH) complex (16, 17). Like the PDH complex, BCKDH catalyzes an oxidative decarboxylation, releasing CO_2 and covalently adding a coenzyme A (CoA) group to the oxidized BCKA product (18). CoA, a bulky and hydrophilic prosthetic moiety, traps all subsequent intermediates inside the mitochondria with one exemption: 3-hydroxyisobutyrate (3-HIB) in the valine catabolic pathway. The BCKDH complex has three components (19, 20): a thiamin-dependent decarboxylase, existing as an alpha2/beta2 heterotetramer (21), encoded by the *BCKDHA* and *BCKDHB* genes, respectively; a lipoate-dependent dihydrolipoyl transacylase that transfers the acyl groups to CoA, encoded by the *DBT* gene; and a FAD-dependent dihydrolipoyl dehydrogenase that transfers the released electrons to NAD^+ and is encoded by the *DLD* gene (18). DLD also participates in other



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

BCAA synthesis and catabolism. Synthesis (*a*) occurs in plants, bacteria, and fungi. Oxidation (*b*) occurs in plants, bacteria, fungi, and animals. All three BCAAs share the BCAT and BCKDH steps, after which catabolism of each BCAA is unique. The BCKDH complex is composed of a core of 24 E2 subunits, which are docked by E1 heterotetamers and E3 dimers. BCKDK inhibits E1 via phosphorylation, which is reversed by PP2Cm. Abbreviations: ACAD8, acyl-CoA dehydrogenase family member 8; ACADSB, short/branched chain acyl-CoA dehydrogenase; ACAT1, acetyl-CoA acetyltransferase 1; AHAS, acetohydroxyacid synthase; α-KIC, α-ketoisocaproic acid; α-KIV, α-ketoisovaleric acid; α-KMV, α-ketomethylvaleric acid; ALDH6A1, aldehyde dehydrogenase 6 family member A1; AUH, AU RNA-binding protein/enoyl-coenzyme A hydratase; BAIBA, beta-amino-isobutyric acid; BCAA, branched chain amino acid; BCAT, branched chain amino transferase; BCFA, branched chain fatty acid; BCKDH, branched chain amino acid dehydrogenase; BCKDK, BCKDH kinase; CoA, coenzyme A; DHAD, dihydroxyacid dehydratase; HADHA, hydroxyacyl-CoA dehydrogenase subunit alpha; HIBADH, 3-hydroxyisobutyrate dehydrogenase; HIBCH, 3-hydroxyisobutyryl-CoA hydrolase; HMGCL, 3-hydroxymethyl-3-methylglutaryl-CoA lyase; HSD17B0, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase; IPMDH, isopropylmalate dehydrogenase; IPMI, isopropylmalate isomerase; IPMS, isopropylmalate synthase; IVD, isovaleryl-CoA dehydrogenase; MCCC, methylcrotonoyl-CoA carboxylase; MUT, methylmalonyl-CoA mutase; OCFA, odd-chain fatty acid; OXCT1, 3-oxoacid CoA transferase; P, phosphorylation; PCCB, propionyl-CoA carboxylase subunit beta.

complexes, including the PDH complex, and may have moonlighting proteolytic functions (22). The rate of oxidation by the BCKDH complex is thought to be largely proportional to intracellular concentrations of BCKAs, as these are typically well below their *K*m for the BCKDH complex (~15–30 μM). Also similar to PDH, the BCKDH complex is tightly regulated by phosphorylation/dephosphorylation (16, 19). BCKDH kinase (BCKDK) adds phosphate on three residues of BCKDHA, thereby suppressing BCKDH activity (23–27). The complementary activating dephosphorylation is carried out by the recently identified phosphatase, PP2Cm (28, 29). BCKDK is allosterically suppressed by BCKAs [its greatest affinity is for α-ketoisocaproic acid (α-KIC)], thus allowing elevations in BCKAs to promote their own oxidation (30–33). Efficient product inhibition of BCKDH also occurs by nicotinamide adenine dinucleotide hydrate (NADH) and acyl-CoAs.

Like many sequential metabolic enzymes, *BCAT2* and the BCKDH complex can be found physically associated in organized supramolecular complexes, often termed metabolons, allowing for substrate channeling from one enzyme to another. The association of BCKDHA with *BCAT2* and the phosphorylation by BCKDK compete for the same loop on BCKDHA, such that *BCAT2* binding increases BCKDH activity, while conversely, phosphorylation of BCKDHA destabilizes the interaction with *BCAT2* (34).

After BCKDH decarboxylation, subsequent catabolism of BCAAs resembles fatty acid oxidation, and indeed, these two processes share a number of enzymatic subunits. Each set of reactions is mostly unique to each BCAA, and all occur inside the mitochondrial matrix. Ultimately, BCAA carbons are either lost as CO_2 or enter the tricarboxylic acid (TCA) cycle. Specifically, valine (5C) loses 2 carbons to CO_2 and contributes 3 carbons to the TCA as succinyl-CoA; leucine (6C) loses 1 carbon to CO_2 and contributes 5 to the TCA in acetyl-CoAs; and isoleucine (6C) loses 1 carbon to CO_2 and contributes 5 to the TCA as acetyl-CoA and succinyl-CoA (see 35 for a carbon tracing diagram). As a consequence, valine is considered glucogenic (i.e., succinyl-CoA is anaplerotic), whereas leucine is considered ketogenic, and isoleucine is mixed.

Catabolic Intermediates and By-Products

As noted above, all of these reactions occur in the mitochondrial matrix, and essentially all intermediate metabolites are trapped within the matrix by the CoA adduct. The main exception is 3-HIB, an intermediate in the valine catabolic pathway. The CoA adduct is removed in the

preceding reaction and re-added after the generation of methylmalonic semialdehyde. As a result, 3-HIB can be secreted and is detected in plasma at 10–40-μM concentrations. Another possible exception is the last step of leucine oxidation, which yields acetyl-CoA plus acetoacetate, the latter of which could escape the matrix prior to ketone oxidation. In addition, some alternative metabolites can emanate from BCAA catabolism, although they are poorly studied and likely represent a small fraction of overall BCAA catabolic flux. Prior to oxidation by BCKDH, α-ketoacids can be reduced at the α-carbon to form branched chain α-hydroxy ketoacids (36, 37). These are present in healthy adult urine at very low levels (often below detection) and are directly degraded by α-hydroxyacid oxidases, probably in the liver (38). Additionally, a cytosolic dioxygenase converts a small percentage of α-KIC to beta-hydroxy-beta-methylbutyrate (HMB), which is present in human serum at approximately 2 μM (39). After oxidation by BCKDH, and prior to entry into propionyl-CoA or HMG-CoA, species that are normally bound to CoA can be released as 3-hydroxy acids (3-hydroxy-2-methylbutyric acid, 3-hydroxy-2-ethylpropionic acid, and 3-hydroxyisovaleric acid) (36). In healthy adults, ketogenesis is associated with urinary excretion of these 3-hydroxy acids, although in small amounts. Several intermediates of BCAA oxidation may also contribute to acylation of mitochondrial enzymes, but the biological significance of these side products of BCAA catabolism remains unclear (37).

BCAAs also contribute to the synthesis of several unique lipid species, broadly categorized as *N*-acyl amino acids, branched chain fatty acids, and odd-chain fatty acids. Mammals appear to be capable of synthesizing all three types. In mammals, at least some *N*-acyl amino acids are synthesized by the secreted enzyme PM20D1, which covalently couples fatty acids to amino acids by an amide bond (40). Adipocytes and perhaps other cell types can synthesize odd-chain fatty acids by combining propionyl-CoA (with carbons derived from valine or isoleucine) and malonyl-CoA, followed by fatty acid chain extension via fatty acid synthase (35, 41). Fatty acid synthase can also elongate isobutyryl-CoA, isovaleryl-CoA, or 2-methylbutyryl-CoA to form branched chain fatty acids (42). These unique fatty acids are mostly synthesized in brown adipocytes, but their role remains unclear. All of these particular lipid species are present in normal serum but at low concentrations (43). Strikingly, branched chain fatty acids are found at very high levels in vernix caseosa, the white waxy substance found on newborn human skin, constituting 30% of fatty acid content (44).

SIGNALING BY BRANCHED CHAIN AMINO ACIDS AND THEIR CATABOLITES

mTOR

In addition to their structural and metabolic roles, BCAAs and many of their metabolites also have important allosteric regulatory and signaling effects. The best studied of these is the regulation of the mechanistic target of rapamycin (mTOR) pathway by leucine. Numerous cellular processes, including most notably protein synthesis and cellular growth, are controlled by the ubiquitous multiunit mTORC1 complex, and leucine is a potent activator of mTORC1 activity. The role of leucine as a growth-regulatory signal was first established in early experiments demonstrating that leucine stimulates muscle protein synthesis in vitro (45–47) and in perfused skeletal muscle preparations (47). Leucine (but not valine, isoleucine, or the BCKAs) promotes mTORC1 activation by directly binding Sestrin2 a negative regulator of mTORC1 activity (48) (**Figure 2*a***). In the absence of leucine, Sestrin2 binds and inhibits GATOR2, a positive regulator of mTORC1 activity. When leucine is available at physiologically relevant concentrations, Sestrin2 releases

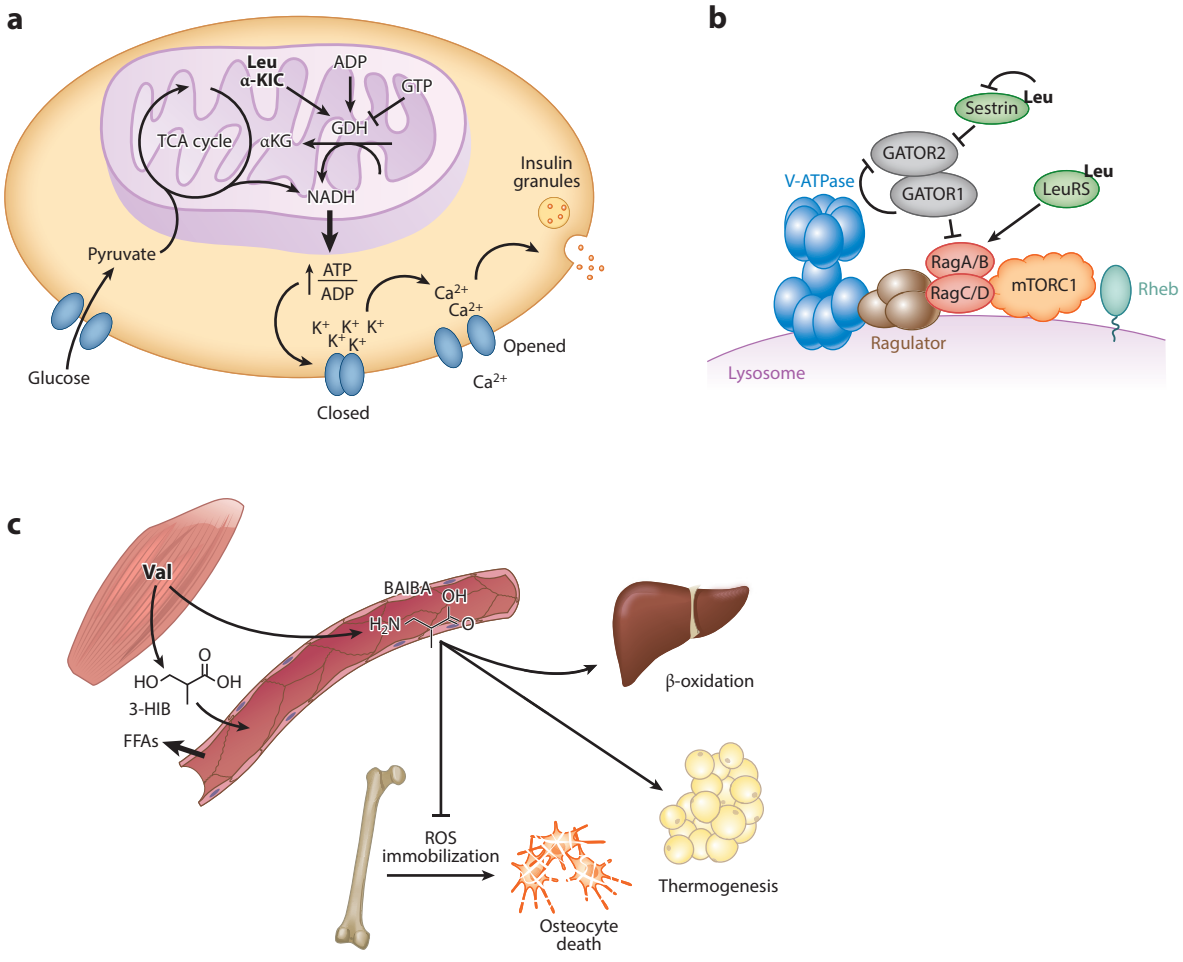


Figure 2

(*a*) Leucine and α-KIC promote insulin release from pancreatic B cells via activation of glutamate dehydrogenase. (*b*) Leucine promotes mTORC1 activity by relieving Sestrin2-mediated inhibition and promoting LeuRS-mediated pathway activation. (*c*) Skeletal muscle secretes valine catabolites BAIBA and 3-HIB. BAIBA promotes hepatic B oxidation, adipocyte thermogenesis, and osteocyte survival; 3-HIB induces fatty acid transport across the endothelium and into skeletal muscle. Abbreviations: 3-HIB, 3-hydroxyisobutyrate; ADP, adenosine 5′-diphosphate; αKG, α-ketoglutarate; ATP, adenosine 5′-triphosphate; BAIBA, beta-amino-isobutyric acid; FFA, free fatty acid; GATOR1, GAP activity toward the Rag GTPases 1; GATOR2, GAP activity toward the Rag GTPases 2; GDH, glutamate dehydrogenase; GTP, guanosine triphosphate; Leu, leucine; LeuRS, leucyl tRNA synthetase; mTORC1, mechanistic target of rapamycin complex 1; NADH, nicotinamide adenine dinucleotide; TCA, tricarboxylic acid; ROS, reactive oxygen species; Val, valine; v-ATPase, vacuolar H+-adenosine triphosphatase ATPase.

GATOR2, promoting full mTORC1 activation (49). Upon activation, mTORC1 promotes protein synthesis and inhibits autophagy by phosphorylating several targets, including S6K, 4E-BP1, Ulk1, and TFEB/3. Leucine likely activates mTORC1 via other mechanisms as well, including via loaded leucyl tRNA synthetase (50–52). For a full discussion of mTOR signaling, we refer the reader to a number of excellent reviews (53–56).

Glutamate Dehydrogenase

Leucine also directly regulates protein-mediated insulin secretion in pancreatic islet beta cells. Leucine and α-KIC are strong insulin secretagogues in low-glucose states. The secretogenic activity is direct and not dependent on leucine oxidation because nonhydrolyzable analogs of leucine are equally secretogenic (57, 58). Instead, leucine promotes insulin release via activation of glutamate dehydrogenase (GDH), which catalyzes the oxidative deamination of glutamate to αKG (59). Under high-glucose states, GDH is inactive and suppressed by high GTP levels. When glucose drops below 5 mM, adenosine 5′-diphosphate (ADP) levels rise and can activate GDH, thereby providing reducing equivalents and promoting αKG entry into the TCA cycle. Both of these promote adenosine 5′-triphosphate (ATP) production, subsequent inhibition of KATP channels, depolarization of plasma membrane, and vesicular release of insulin (59–61). Leucine allosterically activates GDH under these conditions by increasing its affinity for ADP, thus further increasing the ATP energy charge, and consequently, insulin secretion. How leucine allosterically activates GDH is not known. Leucine can be a low-affinity substrate for GDH, so the catalytic may also be a site of allosteric activation (GDH functions as a homohexamer). Patients with mutations in GDH that cause hyperactivation in response to leucine (via loss of inhibition by GTP) lead to protein meal–induced hypoglycemia and hyperinsulinemia-hyperammonia syndrome (62). αKIC is also a strong insulin secretagogue, in part via its transamination, which yields both leucine to activate GDH and αKG to enter the TCA cycle (63). In addition, αKIC may directly inhibit KATP channels (64).

Valine Catabolites

Metazoans likely evolved to use leucine as a sensor for activation of mTOR signaling and insulin secretion because leucine is the most abundant essential amino acid; it thus serves well as an indicator of access to protein-derived amino acids. Nevertheless, other BCAA metabolites can also serve as signaling molecules. Two salient examples are 3-HIB and beta-amino-isobutyric acid (BAIBA), both related to valine catabolism (**Figure 2*c***). As noted above, 3-HIB is the only intermediate metabolite of BCAAs that is separated from its covalent attachment to CoA; consequently, it is the only such metabolite that can easily leave the mitochondrial matrix. 3-HIB is thus in a position to report on rates of mitochondrial BCAA catabolic flux. 3-HIB is secreted by muscle and likely other tissues and is present in plasma in 30–50-μM concentrations (65). In muscle, secreted 3-HIB acts, in a paracrine fashion, on surrounding microvascular endothelial cells, where it promotes the transport of fatty acids out of the circulation, across the endothelial capillary wall, and to the myofibers. The pathway thus provides an important cross-regulatory link between BCAA and fatty acid consumptions, which represent two dominant fuel sources. Much remains to be learned about this pathway, including the receptor (if any) for 3-HIB and the mechanisms of transendothelial fatty acid transport.

BAIBA (technically also a BCAA) is not a direct intermediate of valine catabolism, but rather a potential side product, derived from methylmalonic semialdehyde, itself in rapid equilibrium with 3-HIB. Importantly, BAIBA can also be derived from thymine breakdown, and it is not always clear which source of BAIBA, thymine, or valine predominates under various studied conditions. Secretion of BAIBA, probably from muscle, has both paracrine and endocrine effects on muscle adipocytes and distal fat tissues, respectively (66). In these cells, BAIBA induces expression of the uncoupling protein UCP1, likely in part via activation of the nuclear receptor PPARα. BAIBA has also been reported to promote osteocyte survival, prevent bone loss (67), suppress renal fibroblast proliferation, prevent endoplasmic reticulum stress in hepatocytes (68), and suppress

inflammation in adipocytes (69). Similar to 3-HIB, how BAIBA signals to its target cells remains ill defined, although in the case of osteocytes, the Mas-related G protein–coupled receptor type D appears to be directly targeted by BAIBA, preventing apoptosis in osteocytes (67). Both the 3-HIB and BAIBA pathways thus uncover novel extracellular metabolites with paracrine or endocrine signaling functions. They also underscore the important concept that the three BCAAs should not always be freely interchanged, conceptually or experimentally. Only valine oxidation can yield 3-HIB and BAIBA, whereas only leucine powerfully activates mTOR and GDH, for example.

ORGANISMAL PHYSIOLOGY

As noted, BCAAs are essential amino acids and cannot be synthesized by animals. Therefore, under homeostatic conditions, animals must maintain a precise balance between intake and loss of BCAAs. The diet is likely the only significant source of BCAAs; synthesis of BCAAs by gut microbiota has also been proposed, but it likely contributes a minor component. In terms of losses, oxidative catabolism of BCAAs dominates, as no appreciable amounts of BCAAs are lost in the urine. Circulating levels of BCAAs (approximately 200 μM of valine, 100 μM of leucine, and 60 μM of isoleucine) are maintained in the fasted state and return to these levels within hours after feeding; thus, the balance of BCAA intake/loss is under homeostatic control (70–72). Broadly, whole-animal BCAA physiology can be divided into a circulating pool and a tissue pool (**Figure 3**). BCAAs derived from the diet or released from protein breakdown appear in circulation. BCAAs are then disposed from circulation into tissues where they can be oxidized or incorporated into newly synthesized protein.

Dietary Intake

Dietary BCAA uptake is generally very efficient. Ingested BCAAs are usually derived from protein and are absorbed in the gut predominantly by short peptide carriers rather than by single amino acid carriers (73). After a protein-rich meal, circulating BCAA levels rise about 2–3 fold and decline back to baseline within 3 h, and the uptake kinetics differ depending on the protein source (74, 75). The classic recommended protein intake to maintain minimal muscle mass is 0.8 g/kg/day, but modern recommendations for a healthy diet are higher (76). The range of protein intake in the United States varies widely from 0.9 g/kg/day in the fifth percentile to 2.2 g/kg/day in the ninety-fifth percentile for young adult males (77), perhaps reflecting the variety of popular diets. The average protein intake in males of 1.7 g/kg/day translates to approximately 88, 145, and 66 mg/kg/day of valine, leucine, and isoleucine. Protein intake varies by age and sex: It is on average higher in males than females and declines with age but comprises close to 15% of calories in all groups (77). Notably, typical laboratory rodent diets used for research contain 30% protein by calories, although the typical Western diet contains about 20%. BCAAs account for only 2–5% of dietary energy sources.

Protein Breakdown

Isotope tracing studies in the fasted state have consistently demonstrated that BCAAs and other essential amino acids appear in circulation at rates proportional to their concentration in protein (78, 79), consistent with protein breakdown being the primary source of BCAA in the circulation. Typically, the combined rate of appearance of BCAAs from normal protein breakdown is approximately 0.76 g/kg/day in overnight fasted adults, which is more than double the average intake of 0.35 g/kg/day, reflecting significant cycling in and out of the protein pool. Most of the BCAAs

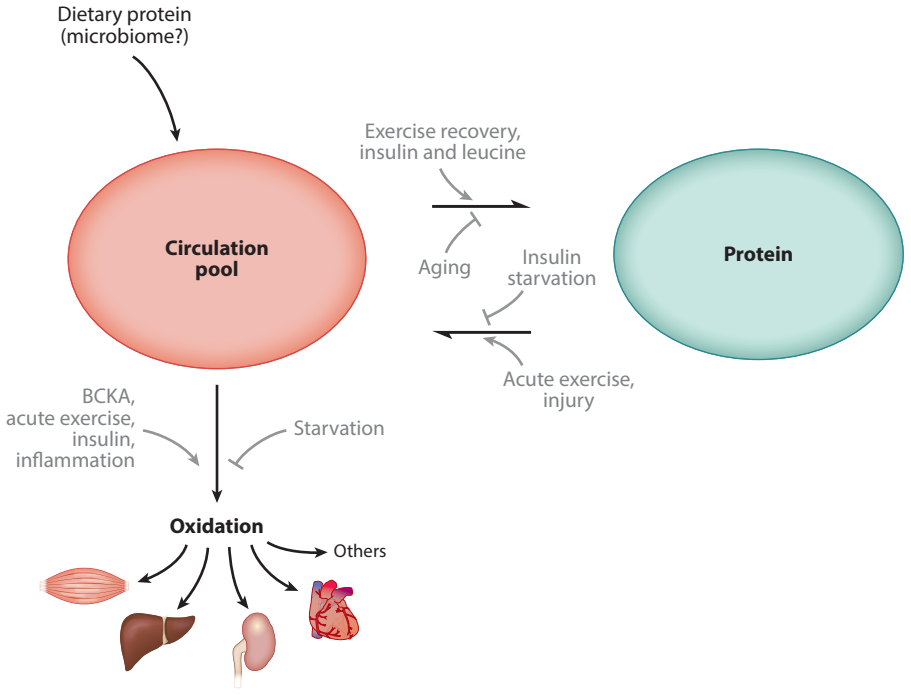


Figure 3

Two-compartment model of whole-organism branched chain amino acid (BCAA) physiology. BCAAs appear in circulation when released from protein, from either the diet or tissues. BCAAs can leave the circulation to be deposited into new protein. All BCAAs are ultimately cleared from the system when oxidized in tissues. Many factors promote or inhibit each of these processes (*grey arrows*). The tissue-specific regulation of protein turnover and oxidation is poorly understood.

that appear in circulation are reincorporated into newly synthesized protein, typically accounting for 70–90% of disposal in the fasted state (80–82). Which tissues serve as the source of BCAAs is difficult to measure directly, and it likely differs under different conditions. However, current estimates suggest that skeletal muscle, the liver, and the gut account for most protein breakdown, reflecting both the large mass of skeletal muscle protein (about 38% of whole-body protein) and the fast turnover in the liver and gut (83).

Protein Synthesis

In the absence of significant secretion or absorption of protein, rates of protein synthesis and breakdown in each tissue must be equal under homeostatic conditions. Most studies aimed at measuring protein synthesis in vivo have focused on skeletal muscle. A summary of these collective findings establishes that protein synthesis requires both an anabolic signal and the amino acid building blocks to make new protein. Importantly, BCAAs, and specifically leucine, contribute to the anabolic signal. Perfusion of isolated skeletal muscle with BCAAs stimulates protein synthesis as efficiently as a complete amino acid mixture, and conversely, perfusion of muscle with an amino acid mixture lacking BCAAs fails to promote synthesis (84). Leucine exerts the most potent

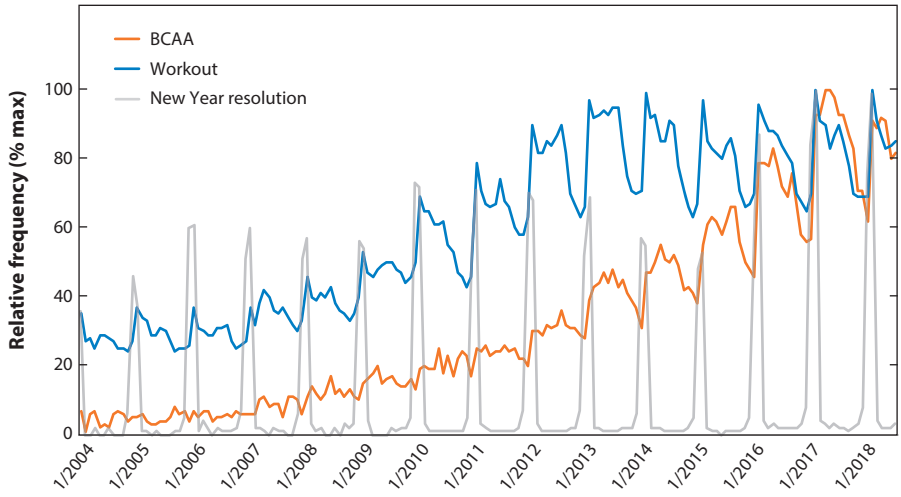


Figure 4

The graph illustrates increasing public interest in branched chain amino acids (BCAAs) since 2004. The relative frequency of Google searches for the indicated keyword is shown, revealing the rising cyclical interest in "BCAA" in close correlation with the term "workout" and rising each year in January, coincident with the term "New Year resolution."

growth-promoting effect as evidenced by the fact that oral gavage with leucine, but not isoleucine or valine, stimulates protein synthesis in skeletal muscle (85). Incubation of skeletal muscle with leucine, but not isoleucine or valine, stimulates protein synthesis nearly as well as a complete BCAA mixture (85). Importantly, in vivo, other anabolic signals such as insulin must accompany leucine in order to promote protein synthesis. For example, oral administration of leucine leads to increased plasma insulin and stimulates protein synthesis, but when plasma insulin levels are maintained at fasting levels by somatostatin infusion, protein synthesis is inhibited (86). Many of these effects are likely mediated by mTOR, whose maximal activation requires both hormonal signals (e.g., insulin, or insulin-like growth factor) and amino acid signals (e.g., leucine via Sestrin2) (55, 56, 87). Interestingly, the relative importance of insulin versus amino acid is likely different in the splanchnic bed or viscera, where insulin has little effect while amino acids powerfully inhibit breakdown and activate synthesis (88). More detail can be found in the extensive literature on protein synthesis (83, 89, 90). The potent anabolic effects of BCAAs have led to growing interest in their use as a supplement to exercise (**Figure 4**), typically in the form of a whey protein shake consumed immediately after exercise. Resistance exercise is a powerful anabolic signal, which synergizes with protein or BCAA intake; combining exercise and protein intake thus leads to maximum protein synthesis (91). The literature on this topic is vast, and we refer the reader to detailed discussions (92, 93).

BCAA Oxidation

BCAAs that are not reincorporated in the protein pool are instead oxidized to maintain homeostasis. Oxidation must occur at appreciable amounts, because at a steady state of protein maintenance where there is no net gain or loss of BCAAs, disposal must match intake.

Because BCKAs can activate their own oxidation, oxidation increases after feeding (74, 75). Conversely, briefly restricting food reduces BCAA oxidation causing plasma BCAA levels to rise

(interestingly, more so than other amino acids). If fasting continues into starvation, however, BCAA oxidation once again increases, likely in large part to provide gluconeogenic precursors to the liver (94). In severe starvation, BCAA oxidation rates fall again, presumably to conserve essential amino acids. Various factors in addition to BCAA availability modulate BCAA oxidation rates. For example, insulin increases whole-body BCAA oxidation when amino acid concentration is maintained (95). Inflammatory cytokines can double whole-body BCAA oxidation in rats (96). Moreover, thyroid hormone increases BCAA oxidation before it changes energy expenditure, glucose metabolism, or fat metabolism (97, 98), although interestingly, it appears to inhibit oxidation in the liver (99).

Exercise also strongly affects BCAA oxidation. The flux of BCAA oxidation increases during a bout of acute endurance exercise in proportion to (submaximal) intensity (81, 100–103). It is unclear if the relative preference for BCAA oxidation versus other substrates is also increased. This increase in BCAA oxidation is not accompanied by increases in oxidation of other essential amino acids. Females oxidize less leucine than males during exercise (101, 104), and this is at least in part mediated by estrogen (105). In animals, endurance training clearly drives an adaptation for increased BCAA oxidation in skeletal muscle (106), probably through induction of the transcriptional activator PGC-1α (106–110). However, results from human studies testing the hypothesis that endurance training actually increases BCAA oxidation during exercise are controversial (104).

In all of these cases, the molecular mechanisms driving changes in BCAA oxidation—and in which tissues oxidation is being modulated—are unclear. In fact, there is no good consensus on the relative distribution of BCAA oxidation between different tissues. The oxidation of BCAAs occurs after transamination to BCKAs, and these two processes can occur in different tissues. In fact, some have argued that liver lacks BCAT activity and that BCAAs are largely transaminated in the muscle and then shuttled to the liver for oxidation (111), although it should be noted that nonhepatocyte cells in the liver do express BCAT enzymes (112). Regardless, the relative distribution of BCKA oxidation between different tissues has been challenging to address. Enzymes of BCAA catabolism are expressed throughout the body, in contrast to those of all other essential amino acids, which are largely confined to the liver (111). To estimate BCAA oxidation flux in various tissues, many groups have used ex vivo assays of BCAT and BCKDH activities in extracts or slices from different tissues (7). In general, such studies indicate that BCAT enzyme activity is highest in heart, kidney, stomach, and pancreas, and is lowest in liver; BCKDH enzyme activity is highest in liver, less in heart and kidney, and lowest in muscle, adipose tissue, and brain. For BCKDH, these measured activities typically reflect the phosphorylation status of BCKDH and, interestingly, correlate poorly with mRNA or protein expression of BCKDH enzymes.

However, these studies with cell extracts or purified enzymes fail to account for the numerous in vivo regulatory factors (e.g., availability of BCAAs, product inhibition, redox state, subcellular compartmentalization). Direct measurements of BCAA oxidation can be made in vivo using isotopic tracer contributions to each tissue, but such studies are not practical in humans. In mouse studies, such steady-state heavy isotope infusion studies in vivo have recently demonstrated that specific rates of BCAA oxidation in fact actively occur in all tissues examined (110). Interestingly, in the pancreas, BCAAs appear to be a dominant source of oxidative fuel, accounting for >20% of carbons incorporated into the TCA cycle. Overall, skeletal muscle oxidizes more BCAAs than any other tissue. Strikingly, oxidative flux correlates poorly with extent of phosphorylation of BCKDH, again reflecting the likely numerous other factors that dictate BCAA catabolic flux in vivo.

Finally, it should be noted that an implicit assumption in most whole-body studies to date has been that BCAAs and BCKAs are transported easily and quickly in and out of cells. This area of BCAA physiology, however, remains poorly understood. There are many amino acid transporters that are often capable of transporting a suite of amino acids, with significant redundancy

(reviewed in 113). The most prominent transporter of BCAAs into cells is the large neutral amino acid (LNAA) transporter, a heterodimer composed of LAT1 and its molecular chaperone CD98 (SLC7A5 and SLC3A2, respectively) (114–116). Genetic and pharmacologic inhibition studies indicate that, at least for some cell types in cell culture, LNAA mediates most BCAA uptake (117). To what extent these transporters are rate limiting under physiological conditions is not clear. The LNAA also transports aromatic amino acids (AAAs), and the frequent correlation between plasma levels of BCAAs and AAAs (e.g., in prediabetes) has been ascribed to competition for these transporters (118). The transporter is highly expressed in the blood-brain barrier, where it has been estimated to be 96% saturated with LNAAs, mostly leucine and phenylalanine (119). Based on this observation, treatment with BCAAs has been proposed to competitively prevent uptake of AAAs into the brain in, for example, hepatic encephalopathy, with variable results (120). If so, then LNAA transport does likely contribute a rate-limiting step in systemic BCAA homeostasis, although the LNAA transporter may have lower affinity for BCAAs in tissues other than the brain. BCKAs may be transported by nonspecific monocarboxylate transporters (121).

BCAAs are also important for interorgan nitrogen exchange, most often studied between muscle and liver (122). BCAT enzymes operate near equilibrium in most tissues, and rates of transamination generally far outstrip rates of BCKA oxidation, thus allowing BCAA amino groups to contribute significantly to the transamination pool. Alanine is a major circulating metabolite (with higher turnover than glutamine, glycerol, or pyruvate) (79), which is in large part secreted by skeletal muscle; it is also an important source for gluconeogenesis in the liver (123). Muscle secretion of alanine requires amination of pyruvate, and strikingly, leucine alone accounts for 20% of this nitrogen (124). Valine and isoleucine likely contribute proportionally. BCAAs thus participate as nitrogen donors both to move nitrogen to the liver for urea synthesis and to facilitate moving carbons to the liver for gluconeogenesis. Of note, net transfer of nitrogen from BCAAs requires the concomitant removal of BCKAs to prevent the reverse reaction, which is achieved via either BCKA secretion or oxidation (125). BCAA nitrogen can also be transferred to glutamine, a substrate for gluconeogenesis in the kidney (126) and in the brain; to the synthesis of both excitatory glutamate and inhibitory GABA neurotransmitters; and to the neuroprotective astrocyte/neuron glutamine/glutamate shuttle (127).

In summary, whole-body BCAA metabolism reflects a balance between protein ingestion, cycling of protein synthesis and breakdown, and BCAA oxidation. Large gaps still exist in our understanding of how these processes are regulated and how they differ between tissues.

BRANCHED CHAIN AMINO ACIDS IN DISEASE

Inborn Errors of BCAA Metabolism

Inborn errors of BCAA metabolism have demonstrated the importance of evolutionarily honed BCAA homeostatic mechanisms to prevent excess of BCAAs or their derivatives. Maple syrup urine disease (MSUD), first described in the 1950s (128–130), is an autosomal recessive disease caused by mutations in the first two subunits of BCKDH (the BCKDHA/BCKDHB heterotetramer or DBT) and occurs in approximately 1:200,000 births. Mutations in the third subunit of BCKDH, DLT, lead to more severe and distinct disease because DLT is shared with PDH and αGDH. Plasma BCAAs, α-ketoacids, and hydroxy-BCAAs are high, and elevations in L-alloisoleucine are pathognomonic (131). Accumulation of a rare catabolic product, sotolone, gives the urine its characteristic odor (132, 133). The disease presentations are variable, in part depending on which subunit of BCKDH is affected (134). Untreated, MSUD leads to encephalopathy, cerebral edema, and death. The mechanism of encephalopathy remains unclear, but it likely

involves disturbed neurotransmission. BCAAs, especially leucine, donate via BCAT transamination one-third or more of the amino groups in brain glutamate, the major excitatory neurotransmitter, and are critical to maintain nitrogen homeostasis in the astrocyte/neuron glutamate/glutamine cycle (127, 135, 136); elevations in α-KIC may thus contribute to glutamate depletion. In addition, as noted above, BCAAs (especially leucine) compete with AAAs for transport across the blood-brain barrier, thus potentially limiting important neurotransmitter precursors. Numerous other mechanisms have been proposed (137). Upon diagnosis, patients are treated by aggressive protein withdrawal, and then amino acid–defined diets are slowly reintroduced to maintain BCAA levels as close to normal as possible (138). Liver transplantation is curative, which demonstrates that providing ~10% of total body BCKDH activity is sufficient to restore BCAA homeostasis (139). Conversely, MSUD patients can serve as liver donors (140), indicating that BCKDH activity outside the liver is also sufficient to maintain BCAA homeostasis. Gene therapy to deliver functional BCKDH or edit the endogenous mutations may provide viable alternatives to liver transplant.

More recent work shows that mutations in *BCKDK*, leading to excess, rather than restricted BCAA oxidation may lead to autism spectrum disorder with epilepsy (141, 142). In addition, homozygous mutations in *SLC7A5*, a component of the LNAA transporter (see above), have been found in patients with autistic traits. Deletion of *Slc7A5* in endothelial cells of mice leads to low brain BCAAs and severe neurological symptoms, and intracerebroventricular administration of BCAAs partially reverses these abnormalities (143). Thus, excess or insufficient BCAAs/BCKAs in the brain contributes to neurologic diseases, underscoring the importance of BCAA homeostasis for normal brain function, although in both cases the mechanisms remain unclear.

Diabetes

Unlike the clear neurotoxic effects of large excesses in BCAAs that are seen in MSUD patients, the possible pathogenic consequences of milder elevations in BCAAs are only slowly coming to light. Elevations of BCAA levels in blood of patients with obesity and insulin resistance were first noted in the 1960s (144, 145). Recent work has revitalized these observations and supports the notion that elevations in BCAAs in fact contribute causally to insulin resistance, as supported by the following observations: (*a*) Unbiased metabolomic studies with plasma from normal subjects with normal insulin sensitivity identified elevations in plasma BCAAs as the strongest predictor for developing diabetes in the subsequent decade or more, indicating that changes in BCAA metabolism precede detectable insulin resistance (146–149); (*b*) Mendelian genetics studies revealed that polymorphisms near the *PPM2* gene (encoding for the BCKDH phosphatase) that affect BCAA levels also increase the risk of insulin resistance (150); and (*c*) BCAAs infused into the circulation of healthy adults are sufficient to impair glucose disposal (151). Moreover, adding BCAAs to a high-fat diet worsens the development of glucose intolerance in rodents (147), whereas limiting BCAAs improves glucose tolerance and insulin sensitivity (152). Together, these data support the notion that BCAAs contribute to insulin resistance, likely via a mechanism dependent on excess lipid availability. Conversely, insulin resistance itself likely can cause elevations in BCAAs, establishing a feed-forward loop (153, 154).

Identifying the mechanisms that lead to BCAA elevations may present novel targets for interventions early in the progression to insulin resistance. Multiple mechanisms are likely at play, involving multiple organs (**Figure 5**). To date, nearly all mechanistic studies are largely based on rodent studies. In adipose tissue of insulin resistant people and animals, gene expression of nearly every enzyme required for BCAA oxidation is suppressed (155–160). Cell culture studies

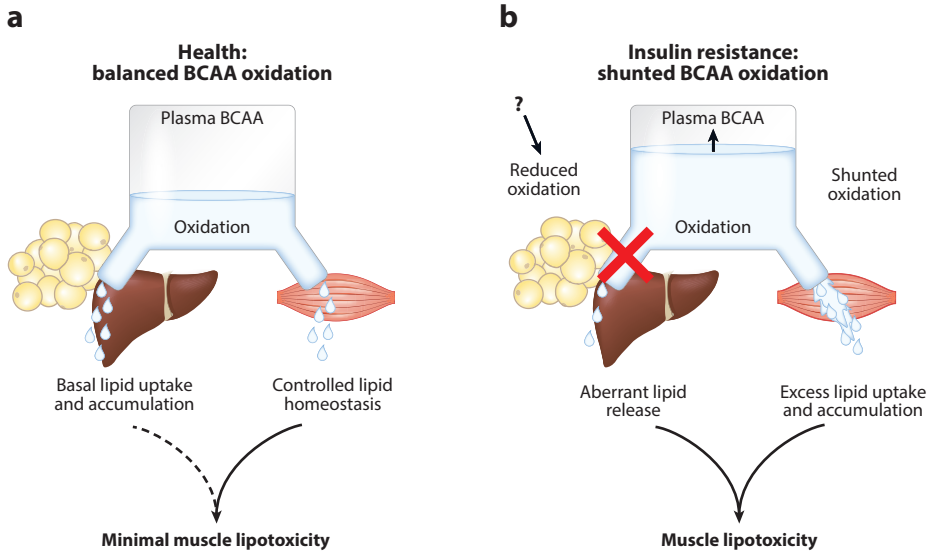


Figure 5

Proposed model of casual relationship between altered tissue branched chain amino acid (BCAA) oxidation and elevated circulating BCAA levels in insulin resistance. In healthy conditions (*a*), BCAA oxidation is balanced among different organs. Genetic and environmental factors suppress BCAA oxidation in the liver and adipose tissues (*b*), causing increased circulating BCAA levels and overflow of BCAAs into skeletal muscle, which results in lipotoxicity and insulin resistance. Adapted with permission from Reference 110.

suggest that hypoxia, endoplasmic reticulum stress, and inflammation contribute to this suppression (161, 162), and thiazolidinediones rescue expression in vivo (158, 163). In the liver, BCKDH phosphorylation is increased, which is likely driven by high BCKDK expression (159, 164–166). Suppression of BCAA oxidation in liver and adipose tissue promotes elevations in plasma BCAAs, likely shunting BCAA oxidation to permissive organs (118). Consistent with these observations, recent studies in whole animals with steady-state heavy isotope infusions revealed in *db/db* mice blunted BCAA oxidation in fat and liver, with consequent significant shunting of oxidation to skeletal muscle (110). Recent work also showed that fructose ingestion induces the hepatic transcription factor ChREBP-β, which in turn activates BCKDK transcription, thus linking fructose and BCAA metabolism. This suggests a mechanism by which the modern dietary choices of high fructose and protein consumption synergistically conspire to elevate plasma BCAAs (166). Reversing the effects of BCKDK by liver-targeted overexpression of the PPCM phosphatase PPM1K, or by systemic pharmacological inhibition of BCKDK, improved glucose tolerance in Zucker fatty rats (166), strongly supporting the causal role of reduced liver BCAA oxidation in the development of insulin resistance.

How elevations in BCAAs cause insulin resistance remains unclear. In fact, it remains uncertain if elevations in BCAAs per se promote insulin resistance; alternative explanations include the consequences of decreased oxidation in some tissues (e.g., adipose) or shunted increased oxidation in others (e.g., muscle). Few mechanisms have been proposed to connect impaired BCAA oxidation in the liver with direct effects within the liver. Interestingly, BCKDK in the liver appears to also phosphorylate and inhibit ATP citrate lyase, a rate-limiting step for de novo lipogenesis, possibly providing an alternative explanation for insulin resistance in the face of elevated BCKDK (166). The near complete loss of BCAA oxidation in adipose tissue may also have important

cell-autonomous consequences. In cultured adipocytes, leucine and isoleucine contribute 30% of lipogenic acetyl-CoA, and BCAA oxidation is required for differentiation (35). These observations predict that loss of BCAA oxidation could impair lipid storage in adipocytes, thus contributing to ectopic lipid deposition and insulin resistance. Adipocytes also use BCAAs to synthesize odd-chain fatty acids (35, 41), but the role for these unique lipids is unknown.

Skeletal muscle is the predominant site of glucose disposal after a carbohydrate load (167), and thus it represents a critical site of insulin resistance. Several mechanisms have been proposed to connect elevated BCAAs or increased BCAA oxidation to insulin resistance in skeletal muscle. mTOR activation by leucine has been investigated, but mTOR does not appear to be responsible for the effects of BCAAs on insulin resistance, because treating rats with rapamycin does not abrogate the effects of BCAAs (147). In fact, in general, diets supplemented with leucine only, rather than all three BCAAs, tend to improve insulin resistance rather than worsen it (168). Competition of elevated BCAA oxidation with oxidation of other substrates, notably glucose, has also been proposed, but this mechanism is unlikely because the relative contribution of BCAAs to total muscle fuel oxidation is small (110). In one proposed mechanism that connects BCAAs and ectopic lipid accumulation, high BCAA oxidation in skeletal muscle depletes the intracellular pool of glycine, thereby impairing lipid export of acyl-glycine adducts, resulting in accumulation of acyl-CoA species (165). Glycine levels frequently correlate inversely with BCAAs (145–147), and a low-BCAA diet raised glycine back to normal levels (165). Finally, elevated oxidation of valine likely increases production of 3-HIB, increasing fatty acid uptake via paracrine promotion of transendothelial fatty acid transport (65). Plasma concentrations of 3-HIB are associated with the future development of diabetes, even after adjusting for body mass index and plasma BCAAs (169). Despite this multitude of potential mechanisms, no studies have definitively demonstrated that any of these changes in BCAA oxidation in specific tissues are sufficient to cause insulin resistance.

Cancer

Because BCAAs are essential amino acids, a growing tumor must obtain them from either the circulation or surrounding tissue. Alterations in circulating BCAA levels in patients diagnosed with cancer have long been noted (170–173). Recent retrospective metabolomic studies demonstrated that elevated plasma BCAA levels are associated with a greater than twofold increased risk in pancreatic cancer and precede clinical presentation by many years. The observation was recapitulated in mice genetically engineered to develop pancreatic ductal adenocarcinoma and is likely caused by subclinical systemic protein breakdown during early tumorigenesis, which is presumed to service the BCAA needs of the growing tumor (171). Interestingly, the same appears not to be true of other tumors, even when driven by the same mutations in *KRAS* and *p53* (174). Whether these alterations in systemic BCAA metabolism contribute to tumor growth or metastasis remains unclear. Regardless, opportunities for biomarker development are an area of intense investigation (175, 176).

A recent surge of studies on tumor BCAA metabolism has focused largely on *BCAT1*, the expression of which is altered in numerous cancers, and in many cases correlates with poor outcome (177–179). Notably, in glioblastomas containing wild-type isocitrate dehydrogenase (IDH), half express high levels of *BCAT1*, whereas IDH^{mut} tumors suppress *BCAT1* expression (180). The latter suppression may be mediated by the oncometabolite 2-hydroxyglutarate (2-HG) that is generated by mutant IDH. 2-HG potently inhibits dioxygenases, including histone demethylases (181), leading to widespread suppressive hypermethylation of promoters, including that of *BCAT1* (180). Conversely, several mechanisms to explain elevated *BCAT1* expression in various

cancers have been proposed, including increased binding of *BCAT1* mRNA transcript to RNA-binding protein MSI2 (182) chromatin hyperacetylation of the *BCAT1* gene by the MLL1 fusion protein (183), and transcriptional activation by the myc oncogene (184, 185). It remains unclear precisely how *BCAT1* expression promotes tumor growth, but it likely differs between tumors. In IDH^{wt} acute myeloid leukemia, *BCAT1* expression correlates with shorter survival and has been proposed to mimic IDH^{mut} acute myeloid leukemia by virtue of depleting αKG, leading to inactivation of αKG-dependent dioxygenases, which is analogous to inhibition of the same dioxygenases by 2-HG in IDH^{mut} cells. Suppression of dioxygenases ultimately promotes growth via HIF-1α stabilization and by altering the epigenomic landscape (186). Conversely, 2-HG produced in IDH^{mut} glioma cells inhibits *BCAT1*, thus limiting the supply of glutamate and opening a vulnerability to treatment with inhibitors of glutaminase, the other main source of glutamate (187). In chronic myeloid leukemia, *BCAT1* is proposed to promote blast crisis by aminating BCKAs to produce BCAAs, leading to progrowth mTOR activation; however, it is unclear how *BCAT1* enzymatic activity should be limited to only one direction (182). *BCAT1* overexpression also promotes mTORC1 activity in breast cancer through unclear mechanisms (188). In summary, data strongly point to an important role for *BCAT1* in multiple cancer types, likely via multiple different mechanisms unique to each cancer.

Heart Failure

BCAA metabolism has also garnered much attention in the context of cardiovascular disease and heart failure. Elevations in plasma BCAAs and their metabolites are independently associated with cardiovascular disease risk (189–191), a topic reviewed elsewhere (192). Additionally, circulating and cardiac BCAAs and BCKAs rise in response to ischemic and hemodynamic murine models of heart failure (193–195). In these studies, the increase in cardiac and plasma BCAAs coincides with diminished expression of multiple components of the BCAA catabolic pathway. However, the heart consumes far fewer BCAAs than other organs (196–198), making it unlikely that diminished cardiac BCAA catabolism alone accounts for increased plasma BCAAs. Suppression of whole-body BCAA catabolism via deletion of PP2Cm elevates circulating and cardiac BCAA levels and worsens cardiac response to aortic constriction and ischemia/reperfusion injury (193, 194). Additionally, dietary BCAA supplementation worsens contractility and increases infarct size following myocardial infarction (195). Conversely, pharmacological promotion of systemic BCAA catabolism lowers circulating and cardiac BCAA levels and improves cardiac function in both hemodynamic and ischemic challenges (193–195). These data strongly support the notion that alterations of BCAA metabolism contribute to heart failure in numerous contexts.

The mechanisms by which BCAAs affect cardiac function, however, remain poorly understood. As in the case of insulin resistance, multiple mechanisms likely contribute. Diminished cardiac BCAA catabolism per se is not likely to compromise ATP production in heart failure because BCAA oxidation contributes to a negligible amount (<5%) of ATP production even in the healthy heart (110, 199; reviewed in 200, 201). It is thus more likely that altered concentrations of BCAAs or BCKAs in the heart affect function. High levels of intracellular cardiac leucine may activate mTOR, thus promoting cardiac insulin resistance and hypertrophy. Indeed, mTOR inhibition mitigates cardiac dysfunction in multiple heart failure models (195, 202, 203; reviewed in 204). Conversely, even transient exposure of isolated rodent hearts to high concentrations of BCAAs impairs contractility. This may occur in part via inhibition of mitochondrial ATP production, as high levels of BCAAs inhibit both pyruvate and αKG dehydrogenases (193, 205). Overall, however, how alterations of systemic BCAA metabolism alter heart failure remains unclear.

CONCLUSION

BCAAs have been the subject of often intense study since their discovery in the mid-nineteenth century. More than 50,000 studies are reported in PubMed. Space constraints invariably precluded us from covering all that is to be said about these fascinating molecules. We have succinctly reviewed here the basic biochemistry and physiology of mammalian BCAA metabolism, much of which was elucidated in the latter part of the twentieth century. This is followed by examples of more recent investigations pointing to a potentially important role for BCAAs in the development of numerous prevalent diseases that increasingly afflict the modern world. Armed with improved understanding of BCAA physiology and pathophysiology, we anticipate that interventions appropriately targeting BCAA metabolism will help improve treatment of these modern ailments.

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