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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.

Keywords

branched chain amino acids; catabolism; diabetes; cancer; heart disease

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.

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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 (α KG), 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- μ M 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 α_2/β_2 heterotetramer (21), encoded by the *BCKDHA* and *BCKDHB* genes, respectively; a lipoyl-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 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 μ M). 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 CO₂ or enter the tricarboxylic acid (TCA) cycle. Specifically, valine (5C) loses 2 carbons to CO₂ and contributes 3 carbons to the TCA as succinyl-CoA; leucine (6C) loses 1 carbon to CO₂ and contributes 5 to the TCA in acetyl-CoAs; and isoleucine (6C) loses 1 carbon to CO₂ 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- μ M 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 μ M (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, keto-genesis 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 2a). 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 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 α KG (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 α KG 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). α KIC is also a strong insulin secretagogue, in part via its transamination, which yields both leucine to activate GDH and α KG to enter the TCA cycle (63). In addition, α KIC 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 2c). 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- μ M 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 PPAR α . 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 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 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 BCAAs 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-1 α (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 α GDH. Plasma BCAAs, α -ketoacids, and hydroxy-BCAAs are high, and elevations in lalloisoleucine 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 neuro-transmitter, 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 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 ec-topic 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 α KG, leading to inactivation of α KG-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-1 α 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 α KG 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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LITERATURE CITED

1. Davis TA, Fiorotto ML, Reeds PJ. 1993 Amino acid compositions of body and milk protein change during the suckling period in rats. *J. Nutr* 123(5):947–56 [PubMed: 8487106]
2. Harper A, Block K, Cree T. 1983 Branched-chain amino acids: nutritional and metabolic interrelationships In Proceedings of the Fourth Symposium on Protein Metabolism and Nutrition, ed. Pion R, Arnal M, Bonin D, pp. 159–81. Paris: INRA
3. Moura A, Savageau MA, Alves R. 2013 Relative amino acid composition signatures of organisms and environments. *PLOS ONE* 8(10):e77319 [PubMed: 24204807]
4. Chou PY, Fasman GD. 1973 Structural and functional role of leucine residues in proteins. *J. Mol. Biol* 74(3):263–81 [PubMed: 4692853]
5. Dill KA. 1990 Dominant forces in protein folding. *Biochemistry* 29(31):7133–55 [PubMed: 2207096]
6. Schweigert BS, Bennett BA, Guthneck BT. 1954 Amino acid composition of organ meats. *J. Food Sci* 19(1–6):219–23

7. Brosnan JT, Brosnan ME. 2006 Branched-chain amino acids: enzyme and substrate regulation. *J. Nutr* 136(1):207S–11S [PubMed: 16365084]
8. Shimomura Y, Obayashi M, Murakami T, Harris RA. 2001 Regulation of branched-chain amino acid catabolism: nutritional and hormonal regulation of activity and expression of the branched-chain α -keto acid dehydrogenase kinase. *Curr. Opin. Clin. Nutr. Metab. Care* 4(5):419–23 [PubMed: 11568504]
9. McCourt JA, Duggleby RG. 2006 Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. *Amino Acids* 31(2):173–210 [PubMed: 16699828]
10. Amorim Franco TM, Blanchard JS. 2017 Bacterial branched-chain amino acid biosynthesis: structures, mechanisms, and drugability. *Biochemistry* 56(44):5849–65 [PubMed: 28977745]
11. Ichihara A, Koyama E. 1966 Transaminase of branched chain amino acids. *J. Biochem* 59(2):160–69 [PubMed: 5943594]
12. Ichihara A, Yamasaki Y, Masuji H, Sato J. 1975 Isozyme patterns of branched chain amino acid transaminase during cellular differentiation and carcinogenesis In *Isozymes*, Vol. 3, ed. Markert CL, pp. 875–89. Amsterdam: Elsevier
13. Kadowaki H, Knox WE. 1982 Cytosolic and mitochondrial isoenzymes of branched-chain amino acid aminotransferase during development of the rat. *Biochem. J* 202:777–83 [PubMed: 7092844]
14. Krebs HA, Lund P. 1977 Aspects of the regulation of the metabolism of branched-chain amino acids. *Adv. Enzyme Regul* 15:375–94
15. Goto M, Shinno H, Ichihara A. 1977 Isozyme patterns of branched-chain amino acid transaminase in human tissues and tumors. *GANN Jpn. J. Cancer Res* 68(5):663–67
16. Patel MS, Nemeria NS, Furey W, Jordan F. 2014 The pyruvate dehydrogenase complexes: structure-based function and regulation. *J. Biol. Chem* 289(24):16615–23 [PubMed: 24798336]
17. Wieland OH. 1983 The mammalian pyruvate dehydrogenase complex: structure and regulation In *Reviews of Physiology, Biochemistry and Pharmacology*, Vol. 96, ed. Adrian RH, zur Hausen H, Helmreich E, Holzer H, Jung R, et al., pp. 123–70. Berlin: Springer
18. Johnson WA, Connelly JL, Glynn MT. 1972 Cellular localization and characterization of bovine liver branched-chain α -keto acid dehydrogenases. *Biochemistry* 11(10):1967–73 [PubMed: 4337197]
19. Paxton R, Harris RA. 1982 Isolation of rabbit liver branched chain α -ketoacid dehydrogenase and regulation by phosphorylation. *J. Biol. Chem* 257(3):14433–39 [PubMed: 7142221]
20. Pettit FH, Yeaman SJ, Reed LJ. 1978 Purification and characterization of branched chain α -keto acid dehydrogenase complex of bovine kidney. *PNAS* 75(10):4881–85 [PubMed: 283398]
21. Danner DJ, Lemmon SK, Elsas LJ II. 1978 Substrate specificity and stabilization by thiamine pyrophosphate of rat liver branched chain α -ketoacid dehydrogenase. *Biochem. Med* 19(1):27–38 [PubMed: 203270]
22. Babady NE, Pang Y-P, Elpeleg O, Isaya G. 2007 Cryptic proteolytic activity of dihydrolipoamide dehydrogenase. *PNAS* 104(15):6158–63 [PubMed: 17404228]
23. Harris RA, Popov KM, Shimomura Y, Zhao Y, Jaskiewicz J, et al. 1992 Purification, characterization, regulation and molecular cloning of mitochondrial protein kinases. *Adv. Enzyme Regul* 32:267–84 [PubMed: 1496922]
24. Popov KM, Shimomura Y, Harris RA. 1991 Purification and comparative study of the kinases specific for branched chain α -ketoacid dehydrogenase and pyruvate dehydrogenase. *Protein Expr. Purif* 2(4):278–86 [PubMed: 1821799]
25. Popov KM, Zhao Y, Shimomura Y, Kuntz MJ, Harris RA. 1992 Branched-chain α -ketoacid dehydrogenase kinase. Molecular cloning, expression, and sequence similarity with histidine protein kinases. *J. Biol. Chem* 267(19):13127–30 [PubMed: 1377677]
26. Shimomura Y, Nanaumi N, Suzuki M, Popov KM, Harris RA. 1990 Purification and partial characterization of branched-chain α -ketoacid dehydrogenase kinase from rat liver and rat heart. *Arch. Biochem. Biophys* 283(2):293–99 [PubMed: 2177326]
27. Tuor U, Simone C, Bascaramurty S. 1992 Local blood-brain barrier in the newborn rabbit: postnatal changes in α -aminoisobutyric acid transfer within medulla, cortex, and selected brain areas. *J. Neurochem* 59(3):999–1007 [PubMed: 1494922]

28. Damuni Z, Reed LJ. 1987 Purification and properties of the catalytic subunit of the branched-chain α -keto acid dehydrogenase phosphatase from bovine kidney mitochondria. *J. Biol. Chem* 262(11): 5129–32 [PubMed: 3031042]
29. Damuni Z, Merryfield ML, Humphreys JS, Reed LJ. 1984 Purification and properties of branched-chain α -keto acid dehydrogenase phosphatase from bovine kidney. *PNAS* 81(14):4335–38 [PubMed: 6589597]
30. Crowell PL, Block KP, Repa JJ, Torres N, Nawabi MD, et al. 1990 High branched-chain α -keto acid intake, branched chain α -keto acid dehydrogenase activity, and plasma and brain amino acid and plasma keto acid concentrations in rats. *Am. J. Clin. Nutr* 52(2):313–19 [PubMed: 2375298]
31. Frick G, Tai L, Blinder L, Goodman H. 1981 l-leucine activates branched chain α -keto acid dehydrogenase in rat adipose tissue. *J. Biol. Chem* 256(6):2618–20 [PubMed: 7009595]
32. Lau KS, Fatania HR, Randle PJ. 1982 Regulation of the branched chain 2-oxoacid dehydrogenase kinase reaction. *FEBS Lett* 144(1):57–62 [PubMed: 6980796]
33. Waymack P, DeBuysere M, Olson M. 1980 Studies on the activation and inactivation of the branched chain α -keto acid dehydrogenase in the perfused rat heart. *J. Biol. Chem* 255(20):9773–81 [PubMed: 7430101]
34. Islam MM, Nautiyal M, Wynn RM, Mobley JA, Chuang DT, Hutson SM. 2010 Branched-chain amino acid metabolon, interaction of glutamate dehydrogenase with the mitochondrial branched-chain amino-transferase (BCATm). *J. Biol. Chem* 285(1):265–76 [PubMed: 19858196]
35. Green CR, Wallace M, Divakaruni AS, Phillips SA, Murphy AN, et al. 2016 Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat. Chem. Biol* 12(1):15–21 [PubMed: 26571352]
36. Liebich HM, Först C. 1984 Hydroxycarboxylic and oxocarboxylic acids in urine: products from branched-chain amino acid degradation and from ketogenesis. *J. Chromatogr. B Biomed. Sci. Appl* 309:225–42
37. Anderson KA, Huynh FK, Fisher-Wellman K, Stuart JD, Peterson BS, et al. 2017 SIRT4 is a lysine deacylase that controls leucine metabolism and insulin secretion. *Cell Metab* 25(4):838–55 [PubMed: 28380376]
38. Jones JM, Morrell JC, Gould SJ. 2000 Identification and characterization of HAOX1, HAOX2, and HAOX3, three human peroxisomal 2-hydroxy acid oxidases. *J. Biol. Chem* 275(17):12590–97 [PubMed: 10777549]
39. Van Koevering M, Nissen S. 1992 Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate in vivo. *Am. J. Physiol. Endocrinol. Metab* 262(1):E27–31
40. Long JZ, Svensson KJ, Bateman LA, Lin H, Kamenecka T, et al. 2016 The secreted enzyme PM20D1 regulates lipidated amino acid uncouplers of mitochondria. *Cell* 166(2):424–35 [PubMed: 27374330]
41. Crown SB, Marze N, Antoniewicz MR. 2015 Catabolism of branched chain amino acids contributes significantly to synthesis of odd-chain and even-chain fatty acids in 3T3-L1 adipocytes. *PLOS ONE* 10(12):e0145850 [PubMed: 26710334]
42. Wallace M, Green CR, Roberts LS, Lee YM, McCarville JL, et al. 2018 Enzyme promiscuity drives branched-chain fatty acid synthesis in adipose tissues. *Nat. Chem. Biol* 14:1021–31 [PubMed: 30327559]
43. Mika A, Stepnowski P, Kaska L, Proczko M, Wisniewski P, et al. 2016 A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight: odd- and branched-chain fatty acids in obesity. *Obesity* 24(8):1669–76 [PubMed: 27355152]
44. Ran-Ressler RR, Devapatla S, Lawrence P, Brenna JT. 2008 Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. *Pediatr. Res* 64(6):605–9 [PubMed: 18614964]
45. Buse MG, Reid SS. 1975 Leucine. A possible regulator of protein turnover in muscle. *J. Clin. Investig* 56(5):1250–61 [PubMed: 1237498]
46. Chang Hong S-O, Layman DK. 1984 Effects of leucine on in vitro protein synthesis and degradation in rat skeletal muscles. *J. Nutr* 114(7):1204–12 [PubMed: 6737085]
47. Li JB, Jefferson LS. 1978 Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochim. Biophys. Acta* 544(2):351–59 [PubMed: 719005]

48. Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, et al. 2015 Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science* 351:43–48 [PubMed: 26449471]
49. Saxton RA, Knockenhauer KE, Wolfson RL, Chantranupong L, Pacold ME, et al. 2016 Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. *Science* 351(6268):53–58 [PubMed: 26586190]
50. Bonfils G, Jaquenoud M, Bontron S, Ostrowicz C, Ungermann C, De Virgilio C. 2012 Leucyl-tRNA synthetase controls TORC1 via the EGO complex. *Mol. Cell* 46(1):105–110 [PubMed: 22424774]
51. Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, et al. 2012 Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* 149(2):410–24 [PubMed: 22424946]
52. Yoon M-S, Son K, Arauz E, Han JM, Kim S, Chen J. 2016 Leucyl-tRNA synthetase activates Vps34 in amino acid-sensing mTORC1 signaling. *Cell Rep* 16(6):1510–17 [PubMed: 27477288]
53. Efeyan A, Zoncu R, Sabatini DM. 2012 Amino acids and mTORC1: from lysosomes to disease. *Trends Mol. Med* 18(9):524–33 [PubMed: 22749019]
54. Jewell JL, Russell RC, Guan K-L. 2013 Amino acid signalling upstream of mTOR. *Nat. Rev. Mol. Cell Biol* 14(3):133–39 [PubMed: 23361334]
55. Laplante M, Sabatini DM. 2012 mTOR signaling in growth control and disease. *Cell* 149(2):274–93 [PubMed: 22500797]
56. Wolfson RL, Sabatini DM. 2017 The dawn of the age of amino acid sensors for the mTORC1 pathway. *Cell Metab* 26(2):301–9 [PubMed: 28768171]
57. Christensen HN, Hellman B, Sehlin J, Tager HS, Täljedal I-B. 1971 In vitro stimulation of insulin release by non-metabolizable, transport-specific amino acids. *Biochim. Biophys. Acta Biomembr* 241(2):341–48
58. Fajans SS, Quibrera R, Pek S, Floyd J, Christensen HN, et al. 1971 Stimulation of insulin release in the dog by a nonmetabolizable amino acid. Comparison with leucine and arginine. *J. Clin. Endocrinol. Metab* 33(1):35–41 [PubMed: 4934141]
59. Sener A, Malaisse WJ. 1980 l-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* 288(5787):187–89 [PubMed: 7001252]
60. Gao ZY, Li G, Najafi H, Wolf BA, Matschinsky FM. 1999 Glucose regulation of glutaminolysis and its role in insulin secretion. *Diabetes* 48(8):1535–42 [PubMed: 10426370]
61. Wilson DF, Cember ATJ, Matschinsky FM. 2018 Glutamate dehydrogenase: role in regulating metabolism and insulin release in pancreatic β -cells. *J. Appl. Physiol* 125(2):419–28 [PubMed: 29648519]
62. Stanley CA, Lieu YK, Hsu BYL, Burlina AB, Greenberg CR, et al. 1998 Hyperinsulinism and hyper-ammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N. Engl. J. Med* 338(19):1352–57 [PubMed: 9571255]
63. Gao Z, Young RA, Li G, Najafi H, Buettger C, et al. 2003 Distinguishing features of leucine and α -ketoisocaproate sensing in pancreatic β -cells. *Endocrinology* 144(5):1949–57 [PubMed: 12697702]
64. Bränström R, Efendic S, Berggren P-O, Larsson O. 1998 Direct inhibition of the pancreatic β -cell ATP-regulated potassium channel by α -ketoisocaproate. *J. Biol. Chem* 273(23):14113–18 [PubMed: 9603910]
65. Jang C, Oh SF, Wada S, Rowe GC, Liu L, et al. 2016 A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat. Med* 22(4):421–26 [PubMed: 26950361]
66. Roberts LD, Boström P, O’Sullivan JF, Schinzel RT, Lewis GD, et al. 2014 β -Aminoisobutyric acid induces browning of white fat and hepatic β -oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab* 19(1):96–108 [PubMed: 24411942]
67. Kitase Y, Vallejo JA, Gutheil W, Vemula H, Jahn K, et al. 2018 β -Aminoisobutyric acid, l-BAIBA, is a muscle-derived osteocyte survival factor. *Cell Rep* 22(6):1531–44 [PubMed: 29425508]
68. Shi C-X, Zhao M-X, Shu X-D, Xiong X-Q, Wang J-J, et al. 2016 β -Aminoisobutyric acid attenuates hepatic endoplasmic reticulum stress and glucose/lipid metabolic disturbance in mice with type 2 diabetes. *Sci. Rep* 6:21924 [PubMed: 26907958]

69. Jung TW, Park HS, Choi GH, Kim D, Lee T. 2018 β -Aminoisobutyric acid attenuates LPS-induced inflammation and insulin resistance in adipocytes through AMPK-mediated pathway. *J. Biomed. Sci* 25(1):27 [PubMed: 29592806]
70. Everman S, Mandarin LJ, Carroll CC, Katsanos CS. 2015 Effects of acute exposure to increased plasma branched-chain amino acid concentrations on insulin-mediated plasma glucose turnover in healthy young subjects. *PLOS ONE* 10(3):e0120049 [PubMed: 25781654]
71. Louard RJ, Barrett EJ, Gelfand RA. 1990 Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. *Clin. Sci* 79(5):457–66 [PubMed: 2174312]
72. Wahren J, Felig P, Hagenfeldt L. 1976 Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. *J. Clin. Investig* 57(4):987–99 [PubMed: 947963]
73. Grimble G 2000 Mechanisms of peptide and amino acid transport and their regulation In *Proteins, Peptides and Amino Acids in Enteral Nutrition*, ed. Fürst P, Young V, pp. 63–88. Basel: Karger
74. Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois J-L, Beaufrère B. 1997 Slow and fast dietary proteins differently modulate postprandial protein accretion. *PNAS* 94(26):14930–35 [PubMed: 9405716]
75. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, et al. 2001 The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am. J. Physiol. Endocrinol. Metab* 280(2):E340–48 [PubMed: 11158939]
76. Wolfe RR, Cifelli AM, Kostas G, Kim I-Y. 2017 Optimizing protein intake in adults: interpretation and application of the recommended dietary allowance compared with the acceptable macronutrient distribution range. *Adv. Nutr. Int. Rev. J* 8(2):266–75
77. Fulgoni VL. 2008 Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am. J. Clin. Nutr* 87(5):1554S–57S [PubMed: 18469286]
78. Bier DM. 1989 Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. *Diabetes/Metab. Rev* 5(2):111–32
79. Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, et al. 2017 Glucose feeds the TCA cycle via circulating lactate. *Nature* 551:115–18 [PubMed: 29045397]
80. Matthews DE, Motil KJ, Rohrbach DK, Burke JF, Young VR, Bier DM. 1980 Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-¹³C] leucine. *Am. J. Physiol. Endocrinol. Metab* 238(5):E473–79
81. Wolfe RR, Goodenough RD, Wolfe MH, Royle GT, Nadel ER. 1982 Isotopic analysis of leucine and urea metabolism in exercising humans. *J. Appl. Physiol* 52(2):458–66 [PubMed: 7061300]
82. Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS. 2004 Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am. J. Physiol. Endocrinol. Metab* 286(1):E92–101 [PubMed: 14506079]
83. Waterlow JC. 2006 *Protein Turnover* Wallingford, UK: CABI
84. May ME, Buse MG. 1989 Effects of branched-chain amino acids on protein turnover. *Diabetes/Metab. Rev* 5(3):227–45
85. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. 2000 Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J. Nutr* 130(10):2413–19 [PubMed: 11015466]
86. Anthony JC, Lang CH, Crozier SJ, Anthony TG, MacLean DA, et al. 2002 Contribution of insulin to the translational control of protein synthesis in skeletal muscle by leucine. *Am. J. Physiol. Endocrinol. Metab* 282(5):E1092–101 [PubMed: 11934675]
87. Wang X, Proud CG. 2006 The mTOR pathway in the control of protein synthesis. *Physiology* 21(5):362–69 [PubMed: 16990457]
88. Nygren J, Nair KS. 2003 Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. *Diabetes* 52(6):1377–85 [PubMed: 12765947]
89. James HA, O'Neill BT, Nair KS. 2017 Insulin regulation of proteostasis and clinical implications. *Cell Metab* 26(2):310–23 [PubMed: 28712655]
90. Rooyackers OE, Nair KS. 1997 Hormonal regulation of human muscle protein metabolism. *Annu. Rev. Nutr* 17(1):457–85 [PubMed: 9240936]

91. Biolo G, Tipton KD, Klein S, Wolfe RR. 1997 An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol. Endocrinol. Metab* 273(1):E122–29
92. Shad BJ, Thompson JL, Breen L. 2016 Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am. J. Physiol. Endocrinol. Metab* 311(5):E803–17 [PubMed: 27555299]
93. Stokes T, Hector A, Morton R, McGlory C, Phillips S. 2018 Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. *Nutrients* 10(2):180
94. Fryburg DA, Barrett EJ, Louard RJ, Gelfand RA. 1990 Effect of starvation on human muscle protein metabolism and its response to insulin. *Am. J. Physiol. Endocrinol. Metab* 259(4):E477–82
95. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA. 1987 Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. Role of substrate availability on estimates of whole body protein synthesis. *J. Clin. Investig* 80(6):1784–93 [PubMed: 3316280]
96. Hole ek M, Šprongl L, Skopec F, Andryš C, Pecka M. 1997 Leucine metabolism in TNF- α and endotoxin-treated rats: contribution of hepatic tissue. *Am. J. Physiol. Endocrinol. Metab* 273(6):E1052–58
97. Riis ALD, Jørgensen JOL, Gjedde S, Nørrelund H, Jurik AG, et al. 2005 Whole body and forearm substrate metabolism in hyperthyroidism: evidence of increased basal muscle protein breakdown. *Am. J. Physiol. Endocrinol. Metab* 288(6):E1067–73 [PubMed: 15657093]
98. Riis ALD, Jørgensen JOL, Ivarsen P, Frystyk J, Weeke J, Møller N. 2008 Increased protein turnover and proteolysis is an early and primary feature of short-term experimental hyperthyroidism in healthy women. *J. Clin. Endocrinol. Metab* 93(10):3999–4005 [PubMed: 18628521]
99. Kobayashi R, Shimomura Y, Otsuka M, Popov KM, Harris RA. 2000 Experimental hyperthyroidism causes inactivation of the branched-chain α -ketoacid dehydrogenase complex in rat liver. *Arch. Biochem. Biophys* 375(1):55–61 [PubMed: 10683248]
100. el-Khoury AE, Forslund A, Olsson R, Branch S, Sjodin A, et al. 1997 Moderate exercise at energy balance does not affect 24-h leucine oxidation or nitrogen retention in healthy men. *Am. J. Physiol. Endocrinol. Metab* 273(2):E394–407
101. Phillips SM, Atkinson SA, Tarnopolsky MA, MacDougall JD. 1993 Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J. Appl. Physiol* 75(5):2134–41 [PubMed: 8307870]
102. Wagenmakers AJM, Brookes JH, Coakley JH, Reilly T, Edwards RHT. 1989 Exercise-induced activation of the branched-chain 2-oxo acid dehydrogenase in human muscle. *Eur. J. Appl. Physiol. Occupat. Physiol* 59(3):159–67
103. White TP, Brooks GA. 1981 [U- 14 C]-glucose, -alanine, and -leucine oxidation in rats at rest and two intensities of running. *Am. J. Physiol. Endocrinol. Metab* 240(2):E155–65
104. McKenzie S, Phillips SM, Carter SL, Lowther S, Gibala MJ, Tarnopolsky MA. 2000 Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. *Am. J. Physiol. Endocrinol. Metab* 278(4):E580–87 [PubMed: 10751189]
105. Hamadeh MJ, Devries MC, Tarnopolsky MA. 2005 Estrogen supplementation reduces whole body leucine and carbohydrate oxidation and increases lipid oxidation in men during endurance exercise. *J. Clin. Endocrinol. Metab* 90(6):3592–99 [PubMed: 15755861]
106. Overmyer K, Evans C, Qi N, Minogue C, Carson J, et al. 2015 Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation. *Cell Metab* 21(3):468–78 [PubMed: 25738461]
107. Dohm GL, Hecker AL, Brown WE, Klain GJ, Puente FR, et al. 1977 Adaptation of protein metabolism to endurance training. *Biochem. J* 164:705–8 [PubMed: 883961]
108. Hatazawa Y, Tadaishi M, Nagaike Y, Morita A, Ogawa Y, et al. 2014 PGC-1 α -mediated branched-chain amino acid metabolism in the skeletal muscle. *PLOS ONE* 9(3):e91006 [PubMed: 24638054]
109. Hatazawa Y, Senoo N, Tadaishi M, Ogawa Y, Ezaki O, et al. 2015 Metabolomic analysis of the skeletal muscle of mice overexpressing PGC-1 α . *PLOS ONE* 10(6):e0129084 [PubMed: 26114427]

110. Neinast MD, Jang C, Hui S, Murashige DS, Chu Q, et al. 2018 Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. *Cell Metab* In press
111. Hutson S, Wallin R, Hall T. 1992 Identification of mitochondrial branched chain aminotransferase and its isoforms in rat tissues. *J. Biol. Chem* 267(22):15681–86 [PubMed: 1639805]
112. Ding C, Li Y, Guo F, Jiang Y, Ying W, et al. 2016 A cell-type-resolved liver proteome. *Mol. Cell. Proteom* 15(10):3190–202
113. Palacín M, Estévez R, Bertran J, Zorzano A. 1998 Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev* 78(4):969–1054 [PubMed: 9790568]
114. Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H. 1998 Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem* 273(37):23629–32 [PubMed: 9726963]
115. Mastroberardino L, Spindler B, Pfeiffer R, Skelly PJ, Loffing J, et al. 1998 Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* 395(6699):288–91 [PubMed: 9751058]
116. Oxender DL, Christensen HN. 1963 Distinct mediating systems for the transport of neutral amino acids by the Ehrlich cell. *J. Biol. Chem* 238(11):3686–99 [PubMed: 14109206]
117. Verrey F 2003 System L: heteromeric exchangers of large, neutral amino acids involved in directional transport. *Pflügers Arch* 445(5):529–33 [PubMed: 12634921]
118. Newgard CB. 2012 Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 15(5):606–14 [PubMed: 22560213]
119. Smith QR, Momma S, Aoyagi M, Rapoport SI. 1987 Kinetics of neutral amino acid transport across the blood-brain barrier. *J. Neurochem* 49(5):1651–58 [PubMed: 3668544]
120. Gluud LL, Dam G, Les I, Marchesini G, Borre M, et al. 2017 Branched-chain amino acids for people with hepatic encephalopathy. *Cochrane Database Syst. Rev* 9:1–83
121. Silva LS, Poschet G, Nonnenmacher Y, Becker HM, Sapcariu S, et al. 2017 Branched-chain ketoacids secreted by glioblastoma cells via MCT1 modulate macrophage phenotype. *EMBO Rep* 2017:e201744154
122. Harper Alfred E 1989 Chairman's remarks: thoughts on the role of branched-chain α -keto acid dehydrogenase complex in nitrogen metabolism. *Ann. N.Y. Acad. Sci* 573(1):267–73 [PubMed: 2699400]
123. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, et al. 1995 Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J. Clin. Investig* 95(1):272–77 [PubMed: 7814625]
124. Haymond MW, Miles JM. 1982 Branched chain amino acids as a major source of alanine nitrogen in man. *Diabetes* 31(1):86–89 [PubMed: 7152124]
125. Odessey R, Khairallah EA, Goldberg AL. 1974 Origin and possible significance of alanine production by skeletal muscle. *J. Biol. Chem* 249(23):7623–29 [PubMed: 4436328]
126. Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J. 1998 Human kidney and liver gluconeogenesis: evidence for organ substrate selectivity. *Am. J. Physiol. Endocrinol. Metab* 274(5):E817–26
127. Sperringer JE, Addington A, Hutson SM. 2017 Branched-chain amino acids and brain metabolism. *Neurochem. Res* 42(6):1697–709 [PubMed: 28417264]
128. Dancis J, Levitz M, Miller S, Westall RG. 1959 Maple syrup urine disease. *Br. Med. J* 1(5114):90–91 [PubMed: 13608087]
129. Menkes JH. 1959 Maple syrup disease: isolation and identification of organic acids in the urine. *Pediatrics* 23(2):348–53 [PubMed: 13633350]
130. Menkes JH, Hurst PL, Craig JM. 1954 A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. *Pediatrics* 14(5):462–67 [PubMed: 13214961]
131. Schadewaldt P, Bodner-Leidecker A, Hammen H-W, Wendel U. 1999 Significance of l-alloisoleucine in plasma for diagnosis of maple syrup urine disease. *Clin. Chem* 45(10):1734–40 [PubMed: 10508118]

132. Podebrad F, Heil M, Reichert S, Mosandl A, Sewell A, Böhles H. 1999 4,5-Dimethyl-3-hydroxy-2[5H]-furanone (sotolone)—the odour of maple syrup urine disease. *J. Inher. Metab. Dis* 22(2):107–14 [PubMed: 10234605]
133. Sewell AC, Mosandl A, Böhles H. 1999 False diagnosis of maple syrup urine disease owing to ingestion of herbal tea. *N. Engl. J. Med* 341(10):769 [PubMed: 10475807]
134. Adeva-Andany MM, López-Maside L, Donapetry-García C, Fernández-Fernández C, Sixto-Leal C. 2017 Enzymes involved in branched-chain amino acid metabolism in humans. *Amino Acids* 49(6):1005–28 [PubMed: 28324172]
135. Yudkoff M. 1997 Brain metabolism of branched-chain amino acids. *Glia* 21(1):92–98 [PubMed: 9298851]
136. Yudkoff M, Nissim I, Kim S, Pleasure D, Hummeler K, Segal S. 1983 [¹⁵N] leucine as a source of [¹⁵N] glutamate in organotypic cerebellar explants. *Biochem. Biophys. Res. Commun* 115(1):174–79 [PubMed: 6137216]
137. Burrage LC, Nagamani SCS, Campeau PM, Lee BH. 2014 Branched-chain amino acid metabolism: from rare Mendelian diseases to more common disorders. *Hum. Mol. Genet* 23(R1):R1–8 [PubMed: 24651065]
138. Gambello MJ, Li H. 2018 Current strategies for the treatment of inborn errors of metabolism. *J. Genet. Genom* 45(2):61–70
139. Díaz VM, Camarena C, de la Vega Á, Martínez-Pardo M, Díaz C, et al. 2014 Liver transplantation for classical maple syrup urine disease: long-term follow-up. *J. Pediatr. Gastroenterol. Nutr* 59(5):636–39 [PubMed: 24979318]
140. Barshop BA, Khanna A. 2005 Domino hepatic transplantation in maple syrup urine disease. *N. Engl. J. Med* 353(22):2410–11 [PubMed: 16319396]
141. García-Cazorla A, Oyarzabal A, Fort J, Robles C, Castejón E, et al. 2014 Two novel mutations in the BCKDK (branched-chain keto-acid dehydrogenase kinase) gene are responsible for a neurobehavioral deficit in two pediatric unrelated patients. *Hum. Mutat* 35(4):470–77 [PubMed: 24449431]
142. Novarino G, El-Fishawy P, Kayserili H, Meguid NA, Scott EM, et al. 2012 Mutations in *BCKDK* lead to a potentially treatable form of autism with epilepsy. *Science* 338(6105):394–97 [PubMed: 22956686]
143. Trlungeanu DC, Deliu E, Dotter CP, Kara M, Janiesch PC, et al. 2016 Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder. *Cell* 167(6):1481–94.e18 [PubMed: 27912058]
144. Adibi SA. 1968 Influence of dietary deprivations on plasma concentration of free amino acids of man. *J. Appl. Physiol* 25(1):52–57 [PubMed: 5661154]
145. Felig P, Marliss E, Cahill GF. 1969 Plasma amino acid levels and insulin secretion in obesity. *N. Engl. J. Med* 281(15):811–16 [PubMed: 5809519]
146. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, et al. 2011 Metabolite profiles and the risk of developing diabetes. *Nat. Med* 17(4):448–53 [PubMed: 21423183]
147. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. 2009 A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 9(4):311–26 [PubMed: 19356713]
148. Liu J, Semiz S, van der Lee SJ, van der Spek A, Verhoeven A, et al. 2017 Metabolomics based markers predict type 2 diabetes in a 14-year follow-up study. *Metabolomics* 13(9):104 [PubMed: 28804275]
149. Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, et al. 2016 Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. *Diabetes Care* 39(5):833–46 [PubMed: 27208380]
150. Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, et al. 2016 Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of type 2 diabetes: a Mendelian randomisation analysis. *PLOS Med* 13(11):e1002179 [PubMed: 27898682]
151. Harris L-ALS, Smith GI, Patterson BW, Ramaswamy RS, Okunade AL, et al. 2017 Alterations in 3-hydroxyisobutyrate and FGF21 metabolism are associated with protein ingestion-induced insulin resistance. *Diabetes* 66(7):1871–78 [PubMed: 28473464]

152. Cummings NE, Williams EM, Kasza I, Konon EN, Schaid MD, et al. 2017 Restoration of metabolic health by decreased consumption of branched-chain amino acids: a low BCAA diet restores metabolic health. *J. Physiol* 596(4):623–45 [PubMed: 29266268]
153. Mahendran Y, Jonsson A, Have CT, Allin KH, Witte DR, et al. 2017 Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. *Diabetologia* 60(5):873–78 [PubMed: 28184960]
154. Wang Q, Holmes MV, Davey Smith G, Ala-Korpela M. 2017 Genetic support for a causal role of insulin resistance on circulating branched-chain amino acids and inflammation. *Diabetes Care* 40(12):1779–86 [PubMed: 29046328]
155. Herman MA, She P, Peroni OD, Lynch CJ, Kahn BB. 2010 Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. *J. Biol. Chem* 285(15):11348–56 [PubMed: 20093359]
156. Lips MA, Van Klinken JB, van Harmelen V, Dharuri HK, 't Hoen PAC, et al. 2014 Roux-en-Y gastric bypass surgery, but not calorie restriction, reduces plasma branched-chain amino acids in obese women independent of weight loss or the presence of type 2 diabetes. *Diabetes Care* 37(12):3150–56 [PubMed: 25315204]
157. Pietiläinen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, et al. 2008 Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. *PLOS Med* 5(3):e51 [PubMed: 18336063]
158. Sears DD, Hsiao G, Hsiao A, Yu JG, Courtney CH, et al. 2009 Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. *PNAS* 106(44):18745–50 [PubMed: 19841271]
159. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. 2007 Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. *AJP Endocrinol. Metab* 293(6):E1552–63
160. Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, et al. 2016 Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Sci. Rep* 6:24540 [PubMed: 27080554]
161. Burrill JS, Long EK, Reilly B, Deng Y, Armitage IM, et al. 2015 Inflammation and ER stress regulate branched-chain amino acid uptake and metabolism in adipocytes. *Mol. Endocrinol* 29(3):411–20 [PubMed: 25635940]
162. Lo KA, Labadorf A, Kennedy NJ, Han MS, Yap YS, et al. 2013 Analysis of in vitro insulin-resistance models and their physiological relevance to in vivo diet-induced adipose insulin resistance. *Cell Rep* 5(1):259–70 [PubMed: 24095730]
163. Hsiao G, Chapman J, Ofrecio JM, Wilkes J, Resnik JL, et al. 2011 Multi-tissue, selective PPAR γ modulation of insulin sensitivity and metabolic pathways in obese rats. *Am. J. Physiol. Endocrinol. Metab* 300(1):E164–74 [PubMed: 20959535]
164. Lian K, Du C, Liu Y, Zhu D, Yan W, et al. 2015 Impaired adiponectin signaling contributes to disturbed catabolism of branched-chain amino acids in diabetic mice. *Diabetes* 64(1):49–59 [PubMed: 25071024]
165. White PJ, Lapworth AL, An J, Wang L, McGarrah RW, et al. 2016 Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. *Mol. Metab* 5(7):538–51 [PubMed: 27408778]
166. White PJ, McGarrah RW, Grimsrud PA, Tso S-C, Yang W-H, et al. 2018 The BCKDH kinase and phosphatase integrate BCAA and lipid metabolism via regulation of ATP-citrate lyase. *Cell Metab* 27(6):1281–93.e7 [PubMed: 29779826]
167. DeFronzo RA, Tripathy D. 2009 Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32(Suppl. 2):S157–63 [PubMed: 19875544]
168. Pedrosa J, Zampieri T, Donato J. 2015 Reviewing the effects of l-leucine supplementation in the regulation of food intake, energy balance, and glucose homeostasis. *Nutrients* 7(5):3914–37 [PubMed: 26007339]
169. Mardinoglu A, Gogg S, Lotta LA, Stancakova A, Nerstedt A, et al. 2018 Elevated plasma levels of 3-hydroxyisobutyric acid are associated with incident type 2 diabetes. *EBioMedicine* 27:151–55 [PubMed: 29246479]

170. Budhathoki S, Iwasaki M, Yamaji T, Yamamoto H, Kato Y, Tsugane S. 2016 Association of plasma concentrations of branched-chain amino acids with risk of colorectal adenoma in a large Japanese population. *Ann. Oncol* 28(4):818–23
171. Mayers JR, Wu C, Clish CB, Kraft P, Torrence ME, et al. 2014 Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat. Med* 20(10):1193–98 [PubMed: 25261994]
172. Nezami Ranjbar MR, Luo Y, Di Poto C, Varghese RS, Ferrarini A, et al. 2015 GC-MS based plasma metabolomics for identification of candidate biomarkers for hepatocellular carcinoma in Egyptian cohort. *PLOS ONE* 10(6):e0127299 [PubMed: 26030804]
173. Watanabe A, Higashi T, Sakata T, Nagashima H. 1984 Serum amino acid levels in patients with hepatocellular carcinoma. *Cancer* 54(9):1875–82 [PubMed: 6090002]
174. Mayers JR, Torrence ME, Danai LV, Papagiannakopoulos T, Davidson SM, et al. 2016 Tissue of origin dictates branched-chain amino acid metabolism in mutant *Kras*-driven cancers. *Science* 353(6304):1161–65 [PubMed: 27609895]
175. Bi X, Henry CJ. 2017 Plasma-free amino acid profiles are predictors of cancer and diabetes development. *Nutr. Diabetes* 7(3):e249 [PubMed: 28287627]
176. Wei J, Xie G, Zhou Z, Shi P, Qiu Y, et al. 2011 Salivary metabolite signatures of oral cancer and leukoplakia. *Int. J. Cancer* 129(9):2207–17 [PubMed: 21190195]
177. Chang IW, Wu WJ, Wang YH, Wu TF, Liang PI, et al. 2015 BCAT1 overexpression is an indicator of poor prognosis in patients with urothelial carcinomas of the upper urinary tract and urinary bladder. *Histopathology* 68(4):520–32 [PubMed: 26173071]
178. Xu Y, Yu W, Yang T, Zhang M, Liang C, et al. 2018 Overexpression of BCAT1 is a prognostic marker in gastric cancer. *Hum. Pathol* 75:41–46 [PubMed: 29447920]
179. Zheng YH, Hu WJ, Chen BC, Grahn THM, Zhao YR, et al. 2016 BCAT1, a key prognostic predictor of hepatocellular carcinoma, promotes cell proliferation and induces chemoresistance to cisplatin. *Liver Int* 36(12):1836–47 [PubMed: 27246112]
180. Tönjes M, Barbus S, Park YJ, Wang W, Schlotter M, et al. 2013 BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. *Nat. Med* 19(7):901–8 [PubMed: 23793099]
181. Xu W, Yang H, Liu Y, Yang Y, Wang P, et al. 2011 Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases. *Cancer Cell* 19(1):17–30 [PubMed: 21251613]
182. Hattori A, Tsunoda M, Konuma T, Kobayashi M, Nagy T, et al. 2017 Cancer progression by reprogrammed BCAA metabolism in myeloid leukaemia. *Nature* 545(7655):500–4 [PubMed: 28514443]
183. Oktyabri D, Ishimura A, Tange S, Terashima M, Suzuki T. 2016 DOT1L histone methyltransferase regulates the expression of BCAT1 and is involved in sphere formation and cell migration of breast cancer cell lines. *Biochimie* 123:20–31 [PubMed: 26783998]
184. Eden A, Simchen G, Benvenisty N. 1996 Two yeast homologs of *ECA39*, a target for c-Myc regulation, code for cytosolic and mitochondrial branched-chain amino acid aminotransferases. *J. Biol. Chem* 271(34):20242–45 [PubMed: 8702755]
185. Zhou W, Feng X, Ren C, Jiang X, Liu W, et al. 2013 Over-expression of BCAT1, a c-Myc target gene, induces cell proliferation, migration and invasion in nasopharyngeal carcinoma. *Mol. Cancer* 12(1):53 [PubMed: 23758864]
186. Raffel S, Falcone M, Kneisel N, Hansson J, Wang W, et al. 2017 BCAT1 restricts α KG levels in AML stem cells leading to IDH^{mut}-like DNA hypermethylation. *Nature* 551:384–88 [PubMed: 29144447]
187. McBrayer SK, Mayers JR, DiNatale GJ, Shi DD, Khanal J, et al. 2018 Transaminase inhibition by 2-hydroxyglutarate impairs glutamate biosynthesis and redox homeostasis in glioma. *Cell* 175(1):101–16 [PubMed: 30220459]
188. Zhang L, Han J. 2017 Branched-chain amino acid transaminase 1 (BCAT1) promotes the growth of breast cancer cells through improving mTOR-mediated mitochondrial biogenesis and function. *Biochem. Biophys. Res. Commun* 486(2):224–31 [PubMed: 28235484]

189. Bhattacharya S, Granger CB, Craig D, Haynes C, Bain J, et al. 2014 Validation of the association between a branched chain amino acid metabolite profile and extremes of coronary artery disease in patients referred for cardiac catheterization. *Atherosclerosis* 232(1):191–96 [PubMed: 24401236]
190. Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, et al. 2010 Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circ. Cardiovasc. Genet* 3(2):207–14 [PubMed: 20173117]
191. Shah SH, Sun J-L, Stevens RD, Bain JR, Muehlbauer MJ, et al. 2012 Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease. *Am. Heart J* 163(5):844–50 [PubMed: 22607863]
192. McGarrah RW, Crown SB, Zhang G-F, Shah SH, Newgard CB. 2018 Cardiovascular metabolomics. *Circ. Res* 122(9):1238–58 [PubMed: 29700070]
193. Li T, Zhang Z, Kolwicz SC, Abell L, Roe ND, et al. 2017 Defective branched-chain amino acid catabolism disrupts glucose metabolism and sensitizes the heart to ischemia-reperfusion injury. *Cell Metab* 25(2):374–85 [PubMed: 28178567]
194. Sun H, Olson KC, Gao C, Prosdocimo DA, Zhou M, et al. 2016 Catabolic defect of branched-chain amino acids promotes heart failure. *Circulation* 133(21):2038–49 [PubMed: 27059949]
195. Wang W, Zhang F, Xia Y, Zhao S, Yan W, et al. 2016 Defective branched chain amino acid catabolism contributes to cardiac dysfunction and remodeling following myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol* 311(5):H1160–69 [PubMed: 27542406]
196. McNulty PH, Jacob R, Deckelbaum LI, Young LH. 2000 Effect of hyperinsulinemia on myocardial amino acid uptake in patients with coronary artery disease. *Metab. Clin. Exp* 49(10):1365–69 [PubMed: 11079831]
197. Schwartz RG, Barrett EJ, Francis CK, Jacob R, Zaret BL. 1985 Regulation of myocardial amino acid balance in the conscious dog. *J. Clin. Investig* 75(4):1204–11 [PubMed: 2859300]
198. Turer AT, Stevens RD, Bain JR, Muehlbauer MJ, van der Westhuizen J, et al. 2009 Metabolomic profiling reveals distinct patterns of myocardial substrate use in humans with coronary artery disease or left ventricular dysfunction during surgical ischemia/reperfusion. *Circulation* 119(13):1736–46 [PubMed: 19307475]
199. Buse MG, Biggers JF, Friderici KH, Buse JF. 1972 Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat: the effect of fatty acids, glucose, and pyruvate respiration. *J. Biol. Chem* 247(24):8085–96 [PubMed: 4640937]
200. De Jong KA, Lopaschuk GD. 2017 Complex energy metabolic changes in heart failure with preserved ejection fraction and heart failure with reduced ejection fraction. *Can. J. Cardiol* 33(7):860–71 [PubMed: 28579160]
201. Lopaschuk GD. 2017 Metabolic modulators in heart disease: past, present, and future. *Can. J. Cardiol* 33(7):838–49 [PubMed: 28279520]
202. McMullen JR. 2004 Inhibition of mTOR signaling with rapamycin regresses established cardiac hyper-trophy induced by pressure overload. *Circulation* 109(24):3050–55 [PubMed: 15184287]
203. Völkers M, Toko H, Doroudgar S, Din S, Quijada P, et al. 2013 Pathological hypertrophy amelioration by PRAS40-mediated inhibition of mTORC1. *PNAS* 110(31):12661–66 [PubMed: 23842089]
204. Sciarretta S, Forte M, Frati G, Sadoshima J. 2018 New insights into the role of mTOR signaling in the cardiovascular system. *Circ. Res* 122(3):489–505 [PubMed: 29420210]
205. Jackson RH, Singer TP. 1983 Inactivation of the 2-ketoglutarate and pyruvate dehydrogenase complexes of beef heart by branched chain keto acids. *J. Biol. Chem* 258(3):1857–65 [PubMed: 6822537]

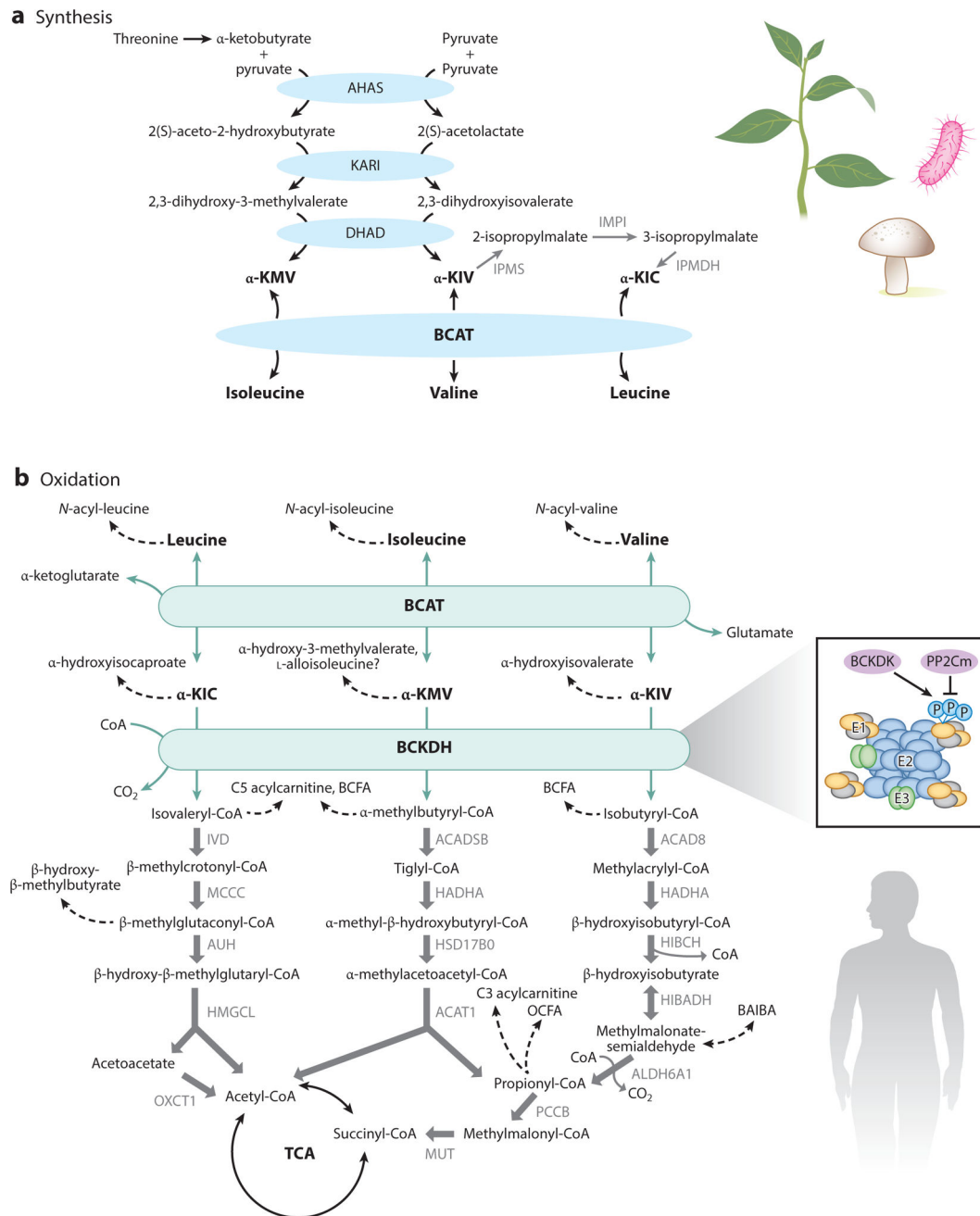
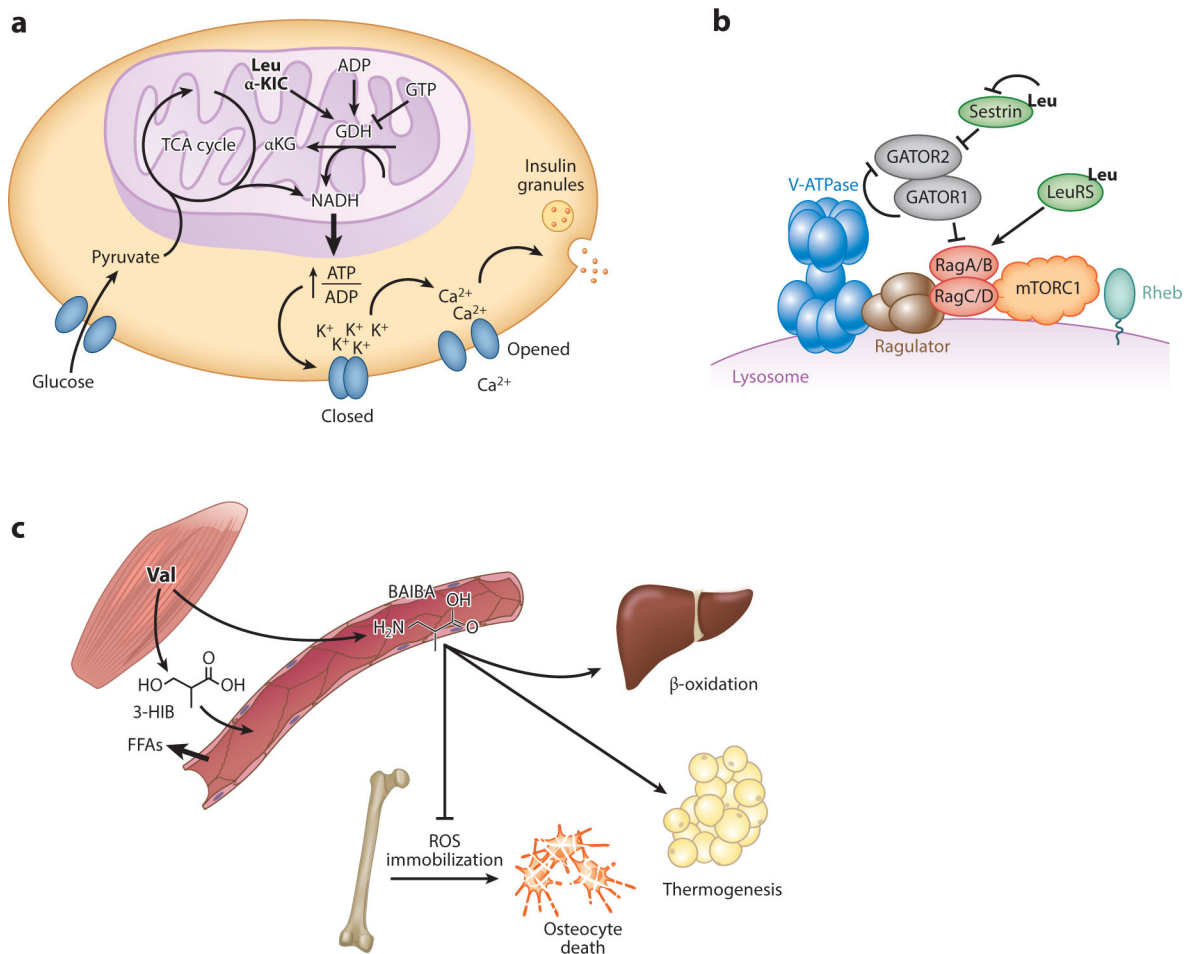


Figure 1.

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.

**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; α -KG, 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.

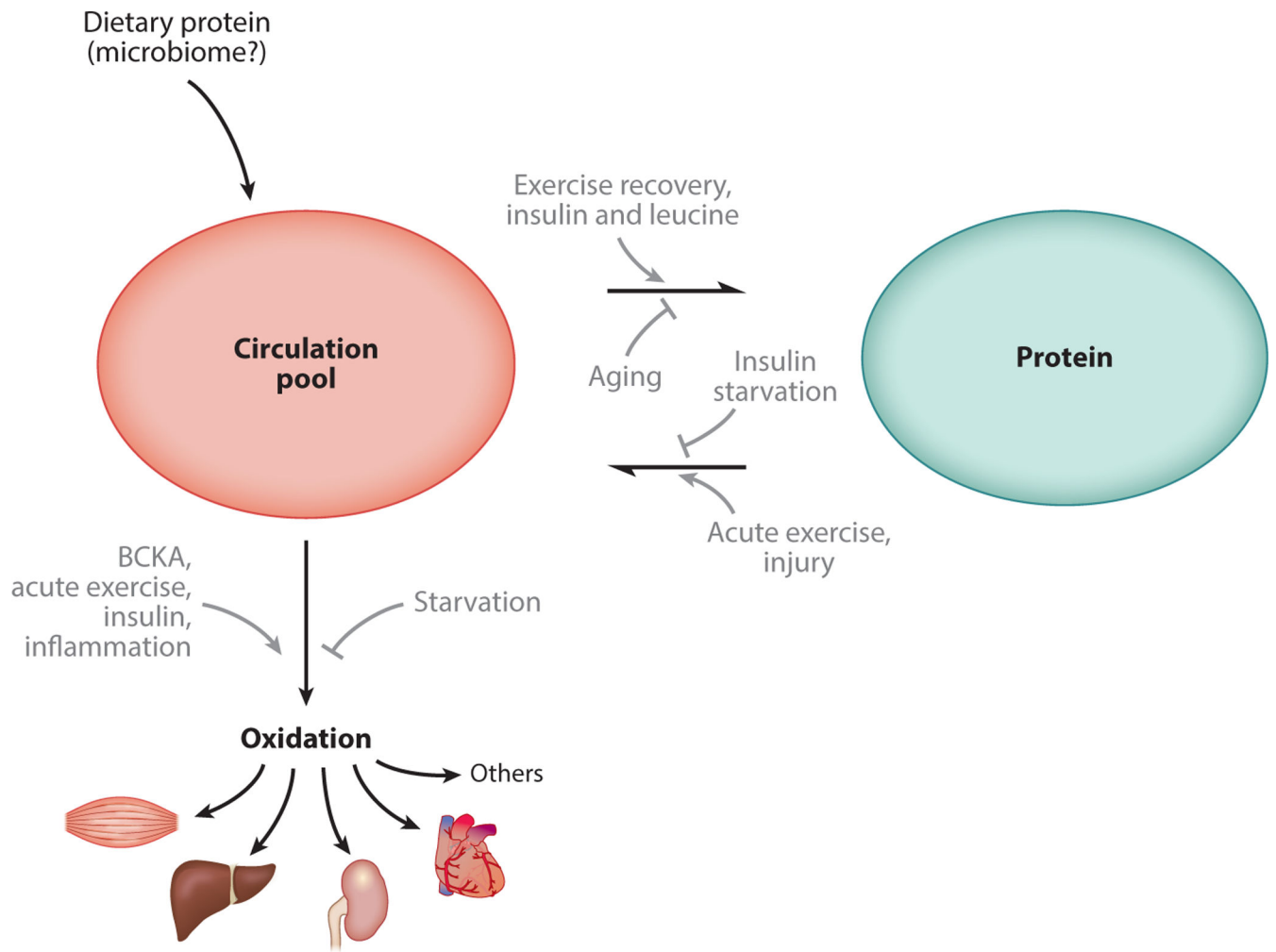


Figure 3.

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.

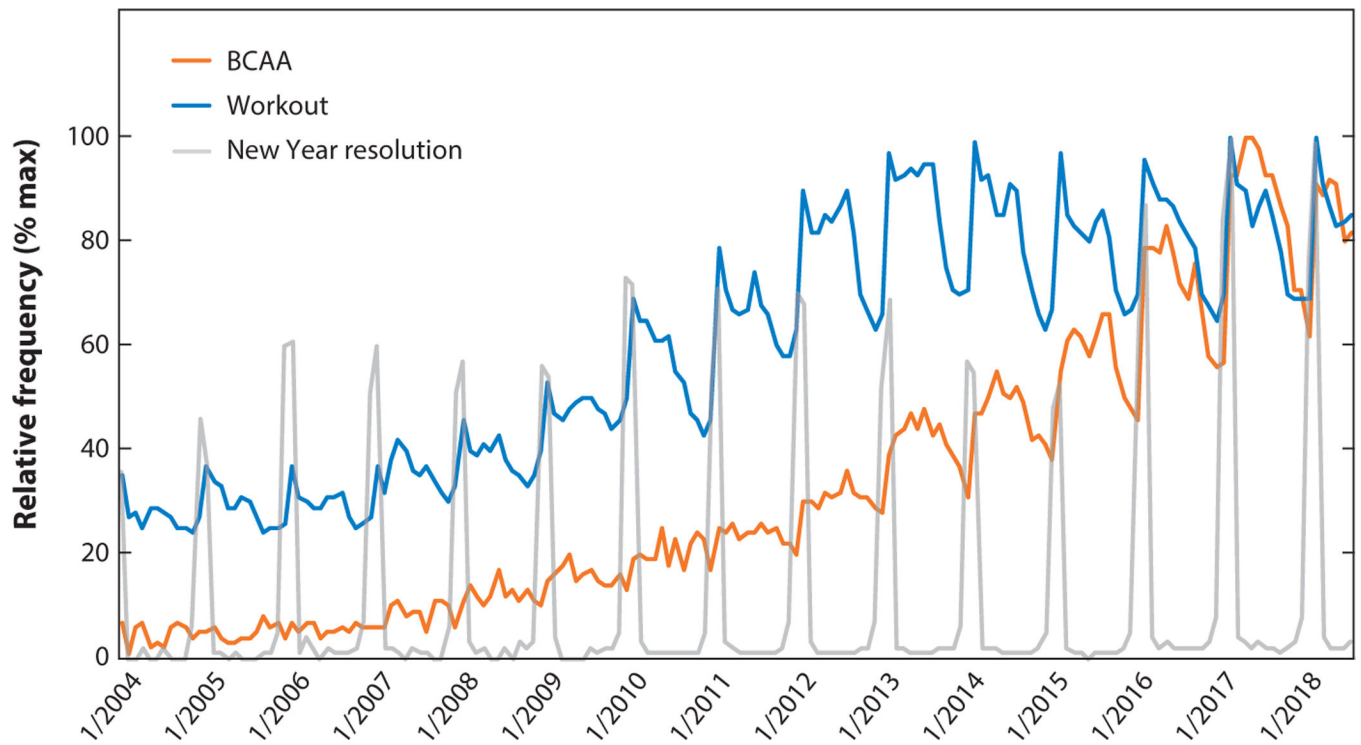


Figure 4.

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.”

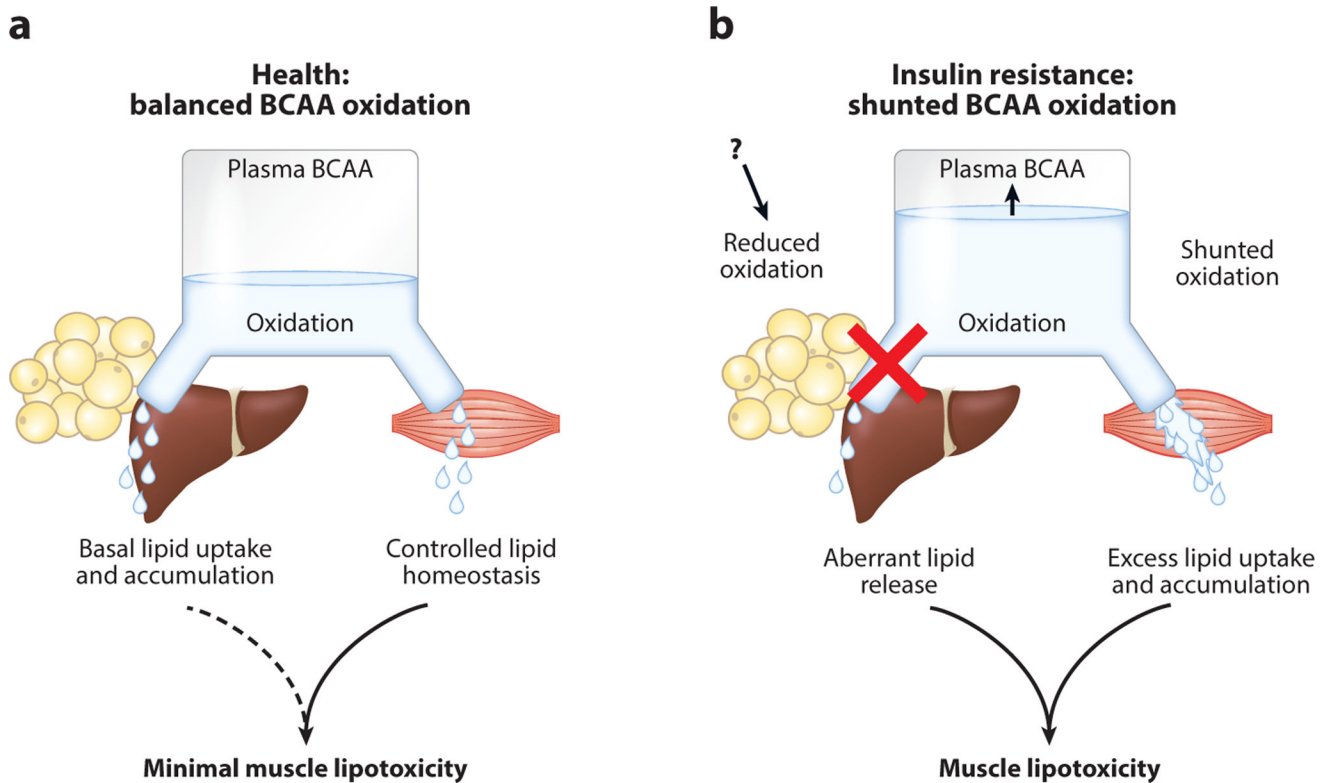


Figure 5. Proposed model of casual relationship between altered tissue branched chain amino acid (BCAA) oxidation and elevated circulating BCAA levels in insulin resistance. In healthy conditions (*a*), BCAA oxidation is balanced among different organs. Genetic and environmental factors suppress BCAA 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.