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Whole-body metabolic fate of branched-chain amino acids

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Abstract

Oxidation of branched-chain amino acids (BCAAs) is tightly regulated in mammals. We review here the distribution and regulation of whole-body BCAA oxidation. Phosphorylation and dephosphorylation of the rate-limiting enzyme, branched-chain α -ketoacid dehydrogenase complex directly regulates BCAA oxidation, and various other indirect mechanisms of regulation also exist. Most tissues throughout the body are capable of BCAA oxidation, and the flux of oxidative BCAA disposal in each tissue is influenced by three key factors: 1. tissue-specific preference for BCAA oxidation relative to other fuels, 2. the overall oxidative activity of mitochondria within a tissue, and 3. total tissue mass. Perturbations in BCAA oxidation have been implicated in many disease contexts, underscoring the importance of BCAA homeostasis in overall health.

Basic biochemistry of branched-chain amino acids

Intake and loss

The three branched-chain amino acids (BCAAs: leucine, valine, and isoleucine) are essential for animals, meaning they cannot be synthesized by the organism and must be obtained from dietary protein. BCAAs are synthesized by plants, fungi, and bacteria. Despite being essential, BCAAs are surprisingly abundant and make up ~35% of essential amino acids and 18% of all amino acids in animal protein [1-3]. Because BCAAs are essential, animals must balance their intake and loss. All intake comes from diet, and loss occurs through oxidation. The relative abundance of BCAAs in protein is almost always 2.2 : 1.6 : 1.0 leucine : valine : isoleucine, illustrating that the synthesis and oxidation of each individual BCAA are linked to one another [4]. Circulating plasma levels of BCAAs in mammals remain consistent at ~200 μ M valine, 100 μ M leucine, and 60 μ M isoleucine in the fasted state; after feeding, these levels rise briefly but fall back to baseline after a few hours. Together this indicates homeostatic regulation of BCAAs [5].

In the fasted state, protein breakdown releases BCAAs from tissues into circulation [6-8]. The average rate of BCAA appearance in a fasted human is ~0.75 g/kg/day, reflecting a

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

rate of protein breakdown of ~250 g/day for a 70 kg man. The main source of this protein breakdown is not known, and >75% of it is likely recycled into protein synthesis, while the remainder is oxidized [9-11]. This rate of oxidation, ~150 mg/kg/day, is on par with the recommended minimum daily intake of BCAAs [12]. In the fed state, BCAAs are taken up by tissues and both protein synthesis and oxidation are increased [4].

Both rates of intake and loss can be altered under physiological or pathological conditions. For example, BCAAs have become a popular exercise supplement [4] because protein intake coupled with exercise promotes muscle protein synthesis, a process likely largely driven by leucine. Numerous studies, typically using infusions of amino acids, BCAAs, or leucine during rest versus acute resistance exercise, have shown that the ensuing hyperaminoacidemia or hyperleucinemia boosts amino acid transport into skeletal muscle and muscle protein synthesis, and that repeated exercise training coupled with protein ingestion leads to skeletal muscle hypertrophy [13]. It is important to note, however, that the magnitude of protein/amino acid/leucine's effects on stimulating muscle protein synthesis is much smaller than the effect of acute exercise itself. Conversely, limiting BCAA intake has received increasing attention as a potential mediator of the widely recorded benefits of calorie restriction on lifespan and healthspan. Recent work has strengthened the notion that BCAAs are a key component of these benefits [14]. Finally, rates of BCAA loss can also change significantly and are typically tied to rates of overall protein catabolism, as can occur in the often dramatic increases in protein catabolism that occur in prolonged fasting, or cachexia associated with for example AIDS, sepsis, heart failure, or cancer.

Oxidation overview

The BCAA oxidative pathway is conserved in animals, plants, fungi, and bacteria. BCAAs are initially transaminated by branched-chain amino transferases (BCATs 1 and 2) to form branched-chain α -ketoacids (BCKAs: α -KIC, α -KMV, and α -KIV), and this reaction is reversible. *BCAT1* encodes a protein that localizes to the cytoplasm and is expressed primarily in the brain, while *BCAT2* encodes a protein that localizes to mitochondria and is expressed in most tissues. BCKAs are then decarboxylated and dehydrogenated by the branched-chain α -ketoacid dehydrogenase (BCKDH) complex [15-17] (Figure 1). BCKDH is the rate-limiting enzyme in this pathway, and it lies on the inner mitochondrial membrane. Its oxidative decarboxylation of BCKAs releases carbon dioxide, while covalently linking a coenzyme A (CoA) group to the oxidized product (Figure 1). The bulky hydrophilic CoA group traps oxidized BCKAs and all downstream intermediates inside the mitochondria [18-20]. Only at two steps is the CoA moiety transiently removed, enabling exit from the mitochondria: 3-hydroxyisobutyrate (3-HIB) [21], a product of valine, and acetoacetate [4], a product of leucine (Figure 1). 3-HIB can be significantly secreted from cells and tissues [21], while, except in the liver, acetoacetate is likely not [4]. After decarboxylation and dehydrogenation by BCKDH, intermediate products from the three BCAAs each follow different catabolic pathways, with each terminating at the tricarboxylic acid (TCA) cycle either via acetyl-CoA (leucine and isoleucine) or succinyl-CoA (isoleucine and valine) (Figure 1) [4,22]. Rarely, BCAA oxidation does not terminate in the TCA cycle, generating instead monomethyl branched-chain fatty acids (mmBCFAs) from the

CoA-bound intermediates of mitochondrial BCAA oxidation and exporting them to the cytoplasm. This process occurs mostly in white and brown adipocytes [23,24].

Structure and regulation of BCKDH

BCKDH is a member of the mitochondrial α -ketoacid dehydrogenase complex family, along with PDH and OGDH, which together share primary and tertiary structure, as well as likely evolutionary origin. BCKDH is a multienzyme complex made up of three components, E1, E2, and E3 (Figure 1) [19,20]. E1 is a heterotetramer composed of two α subunits and two β subunits, encoded by *BCKDHA* and *BCKDHB*, respectively. E1 functions as a thiamin-dependent decarboxylase. E2 forms BCKDH's structural core, made up of 24 identical subunits, encoded by *DBT*. E2 functions as a dihydrolipoyl transacylase. E3 is a homodimer, encoded by *DLD* and shared with the other α -ketoacid dehydrogenase complexes, that functions as a dihydrolipoamide dehydrogenase. E1 decarboxylates BCKAs and transfers an acyl group to the lipoamide cofactor on E2. E2 transfers the acyl group from lipoamide to CoA, which reduces lipoamide and forms an acyl-CoA. Finally, E3 re-oxidizes lipoamide, using FAD as a cofactor and NAD^+ as an electron acceptor to form NADH [4,19,20]. Crystal structures of the BCKDH complex have recently been described [19,20].

BCKDH activity is regulated by phosphorylation and dephosphorylation. Phosphorylation of E1 by the BCKDH kinase (BCKDK) inhibits BCKDH activity [25,26]. Human BCKDH has two phosphorylation sites targeted by BCKDK, Ser292- α and Ser302- α , that both reside in the E1 catalytic site. Phosphorylation of Ser292- α alone inactivates BCKDH, while phosphorylation of Ser302- α appears to have no impact [27]. Dephosphorylation of E1 is carried out by the protein phosphatase $\text{Mg}^{2+}/\text{Mn}^{2+}$ dependent 1K (PP2Cm, encoded by *PPMIK*), thereby activating BCKDH [28,29] (Figure 1). Numerous additional mechanisms of regulation of BCKDH exist. Product inhibition of BCKDH by NADH and acyl-CoA can occur, as well as allosteric inhibition of BCKDK by high levels of BCKAs, thereby promoting their oxidation [30-33]. Interestingly, BCAT2 and BCKDH can associate to form a metabolon, a supramolecular complex that allows substrates to channel from enzyme to enzyme [34]. BCAT2 binding to BCKDH also increases BCKDH activity, while phosphorylation of BCKDH destabilizes BCAT2's interaction with BCKDH [34].

Whole-body oxidation of branched-chain amino acids

Foundational infusions of stable isotopes of BCAAs quantified systemic flux of transamination and oxidative decarboxylation in humans and rodents (Figure 2A,B). Transamination is faster than oxidation and rapidly reversible [35]. Oxidation accounts for 10–15% of BCAA disposal in the fasted state (the remainder is deposited into protein) [36]. Upon feeding, oxidation as much as triples and accounts for a greater fraction of BCAA disposal [37,38].

Although most tissues are capable of BCAA oxidation, BCAAs only account for roughly 1% of total calories consumed. Tissues have a low preference for BCAA oxidation relative to other fuels like glucose, glutamine, lactate, and fatty acids [39]. An interesting exception is the pancreas, which, at least in mice, fills nearly a third of its TCA cycle with carbons derived from BCAAs [39,40]; the reason for this predilection is not known. In mice housed

at normal room temperature, the greatest fraction of total BCAA oxidation occurs in skeletal muscle, liver, and brown fat [40] (Figure 2B). Skeletal muscle and liver are well-known sinks for BCAAs [41], but brown fat is a novel contributor to BCAA disposal. Genetic deletion in mice of *BCKDHA* or of a BCAA transporter specifically in brown fat is sufficient to raise plasma BCAAs [42]. Using similar genetic tools in mice, ongoing work will test the contribution of BCAA oxidation to systemic BCAA disposal in more tissues.

We have proposed a simple model of whole-body BCAA oxidation, highlighting three key factors influencing the flux of oxidative BCAA disposal in each tissue (Figure 2A):

1. First, the tissue-specific preference for BCAA oxidation relative to other fuels (Figure 2A). This factor is impacted by numerous tissue-specific variables, including transcriptional and post-translational mechanisms. Transcriptional regulation of the enzymatic machinery for BCAA oxidation can drive adaptive responses, as exemplified by PGC-1 α or KLF15 driving increased BCAA oxidation [43-45]. Other transcription factors may regulate specific portions of the pathway, e.g. ChREBP regulating transcription of *BCKDK* and *PPMIK* [46], or KLF15 increasing transcription of *BCAT2* in some contexts [44]. Acute and chronic regulation of BCAA oxidation is also mediated by phosphorylation of BCKDHA, as demonstrated by the rapid (within minutes) activation of BCAA oxidation by pharmacologic inhibition of BCKDK and the sustained changes upon whole-body or liver-specific knockouts of BCKDK in mice [40,46,47]. Phosphorylation status, abundance and activity of BCKDH measured by *ex vivo* enzyme preparations was the basis of previous estimates for tissue-specific BCAA oxidation [48]. Regulation beyond the BCKDH complex is almost certainly also at play, including cell- and organelle-specific BCAA transporters, transaminases, and catabolic enzymes downstream of BCKDH, although these aspects of regulation remain understudied.
2. Second, the overall oxidative activity of mitochondria within a tissue. To a first approximation, the mitochondrial oxidative activity can be represented by TCA flux and is proportional to oxygen consumption [40,49,50] (Figure 2A). The variable mitochondrial oxidative activity can have enormous impact on BCAA disposal. For example, the heart has the lowest preference for BCAA oxidation relative to other fuels, but its high mitochondrial activity makes the heart the highest oxidizer of BCAA per weight of tissue [40]. Tissue-specific modulation of mitochondrial oxidative activity will thus similarly impact BCAA oxidation, often accompanied by changes in specific regulation of BCKDH. For example, acute exercise, which can increase oxygen consumption of muscle by an order of magnitude, can also increase BCAA oxidation, and *ex vivo* assays for BCKDH activity show corresponding increases in activity [51]. Similarly, activation of thermogenesis in brown adipose tissue increases BCAA disposal in this tissue [42]. However, it remains to be tested if there is a specific increase in BCAA oxidation relative to other fuels during acute exercise or thermogenesis.
3. And third, total tissue mass. Simply put, the more of a given tissue, the more whole-body BCAA oxidation will be apportioned to that tissue (in the absence

of changes in the two factors above) (Figure 2A). Thus whole-body BCAA oxidation can change when organs increase or decrease in mass. For example, any exercise that builds muscle mass (e.g. anabolic weight lifting) will increase the relative importance of skeletal muscle in systemic BCAA oxidation. In contrast, interestingly, dramatic expansion of white adipose tissue may not significantly impact total body BCAA disposal, in part because the metabolic rate in this tissue is low but also because the overall expression of BCAA catabolism enzymes is decreased in obesity [52-54]. This example underscores that all three components of this simple model of BCAA oxidation must be considered when making predictions about how physiological or pathological events will impact systemic BCAA disposal.

These factors should be considered when investigating mechanisms behind observed changes in BCAA oxidation flux. As mentioned earlier, acute exercise and thermogenesis in brown fat increase BCAA oxidation. Nutritional supply of protein and other macronutrients has complex effects on systemic BCAA oxidation. Compared with a normal overnight fasted state, starvation transiently increases BCAA oxidation, presumably to support gluconeogenesis until the body fully transitions to reliance on ketones [55]. BCAA oxidation is reduced after chronic dietary protein restriction [56], presumably to conserve BCAAs for protein synthesis. In normal conditions, BCAA oxidation increases after a meal [37]. All of this regulation of BCAA oxidation could be mediated or influenced by various hormones: insulin may contribute to increased oxidation during feeding by increasing the preference for BCAA oxidation in striated muscle [40], and other hormones like glucocorticoids and thyroid hormones are known to increase systemic BCAA oxidation [57,58]. In most of these cases, however, the molecular mechanisms behind these changes remain poorly understood, and studies have to date been limited to *ex vivo* measurements of BCKDH phosphorylation status and activity [59].

An important assumption of this model, which is based on tracing labeled BCAAs into the tissue TCA cycle, is that intermediate products of BCAA catabolism do not exchange between tissues, often referred to as secondary labeling [60]. This could be considered at three levels: secondary labeling from BCKAs, secondary labeling from metabolites produced after the BCKDH step but before the TCA cycle, and finally secondary labeling from metabolites derived from the TCA cycle. The first of these, i.e. interchange of BCKAs between tissues, undoubtedly occurs. For example, arterio-venous measurements of consumption and release across skeletal muscle and hepatic beds indicate that the liver does not transaminate BCAAs, instead, it only oxidizes BCKAs released by other tissues (mostly skeletal muscle) [22,61,62]. Isotopic tracing comparing the tissue-specific oxidation of valine vs α -KIV was consistent with this conclusion [40]. It is important to note, however, that the BCAA/BCKA transamination step does not involve oxidation. Therefore, the likely significant (and difficult to quantify) exchange of BCKAs between tissues does not impact conclusions in the above model as to where BCAA *oxidation* occurs.

Under physiological conditions, secondary labeling from products produced after the BCKDH step but before the TCA cycle is minimal, because nearly all intermediates are trapped in the mitochondria by covalent bonding to CoA. The exceptions are 3-HIB [21]

(from valine) and acetoacetate [4] (from leucine), and possibly the mmBCFAs [23,24]. These mmBCFAs synthesized in adipose tissue are likely mixed with a very large pool of lipids within the adipose tissue, and therefore the labeled carbons from BCAAs may effectively ‘disappear’ during the course of a typical infusion experiment. Secondary labeling from mmBCFAs is thus not likely. Acetoacetate is avidly consumed by most tissues, and no ^{13}C -labeled ketones are detectable in plasma of ^{13}C -BCAA-infused animals, strongly arguing against the escape of acetoacetate from tissues. In contrast, 3-HIB likely does contribute to some tissue-exchange and secondary labeling, and 3-HIB secretion may acutely correlate with BCAA oxidation, but precise quantification of this exchange will require studies with infused labeled 3-HIB [63,64]. Under rare pathological conditions such as inborn errors of metabolism affecting enzymes in the BCAA catabolic pathway, massive accumulation of upstream intermediates can also lead to their CoA-free escape from mitochondria, possibly leading to some secondary labeling in other tissues, although these intermediates are likely mostly secreted in the urine. Finally, the impact of secondary labeling from metabolites derived from intermediates of the TCA cycle is also likely minimal, because escape of TCA intermediates into the circulation is low, and little of it is derived from BCAAs [39,40].

System perturbations in disease

Understanding how BCAA metabolism in one tissue impacts systemic BCAA levels is important for studying why BCAAs become elevated in specific diseases.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is caused by autosomal recessive mutations in either the E1 (BCKDHA/BCKDHB) or E2 (DBT) subunits of BCKDH. Because the E3 (DLD) subunit of BCKDH is shared with PDH and OGDH, mutations in DLD cause more complex disease, and though sometimes labeled MSUD, is more appropriately labeled dihydrolipoamide dehydrogenase deficiency. Mutations in PPM1K can cause a mild variant of MSUD. Overall, disease severity is usually inversely related to residual enzyme activity. These mutations lead to elevated plasma BCAA and BCKA levels, as well as elevated urine levels of sotolone, an otherwise rare byproduct of excess leucine and isoleucine that gives urine a maple syrup-like odor [65-67]. If left untreated, MSUD can cause cerebral edema, encephalopathy, and ultimately death, underscoring the importance of tight homeostatic regulation of BCAAs [68,69]. Initial treatment requires patients to eat a protein-restricted diet to maintain BCAAs at a more physiologic level [70]. MSUD can be cured by liver transplantation, which restores BCAA homeostasis, presumably by reintroducing sufficient whole-body BCAA oxidation capacity [71].

Cancer

Alterations in plasma BCAA levels and BCAA metabolism have been noted in a variety of cancers. Plasma BCAAs are increased in mice and humans with pancreatic cancer [72] or hepatocellular carcinoma (HCC) [73]. The metabolism of the tumor itself is unlikely to account for systemic changes in plasma BCAA levels [74], suggesting that cancer can drive a change in whole-body BCAA metabolism. However, few studies have focused on the

question of how cancer affects whole-body BCAA metabolism. Instead, most studies have focused on BCAA metabolism in the tumor itself, whether in tumor cells or in surrounding stroma. The majority of these studies focused specifically on BCAT1, which is found to be increased in breast cancer [75], both acute and chronic myeloid leukemia (AML and CML [76]), glioblastoma [77], ovarian cancer [78], and hepatocellular cancer [73]. Pro-tumorigenic mechanisms likely differ in different contexts. In AML, for example, by virtue of transferring nitrogen from BCAAs to α -ketoglutarate (α -KG), BCAT1 decreases the availability of α -KG needed as a cofactor for cellular diogenases, including histone and DNA demethylases [76,79]; BCAT1 overexpression thus mimics mutations in IDH1, which also inhibit demethylases via the production of the potent α -KG-like inhibitor (R)-2-hydroxy-glutarate (2HG), as well as mutations in TET2, a key target demethylase. Similar mechanisms are likely at play in glioblastoma, which bear similar genetic profiles [77]. In contrast, in pancreatic cancer, BCAT1 has recently been suggested to be required in stromal cancer-associated fibroblasts, serving to provide BCKAs to the tumor cells [80]. Other members of BCAA catabolism have received less attention. HCC is associated with low BCAA catabolic capacity, and pharmaceutical or nutritional reduction in plasma BCAAs is protective in a mouse model of HCC; in this setting, reduced BCAA catabolism in the tumor is hypothesized to increase leucine, in turn, activating mTOR [73]. And three recent reports have implicated BCAT2 in pancreatic cancer progression, although the mechanism of action is not clear [81-83]. In sum, BCAA metabolism has profound effects on multiple cancers and cell types, in ways that are only beginning to be understood. For further details, we refer the reader to Sivanand and Vander Heiden [74].

BCAAs and insulin resistance

Elevated plasma BCAAs have been observed in obese, insulin-resistance patients compared with age- and sex-matched controls since the 1960s [84,85]. More recently, metabolite profiles taken from the blood of over 2000 individuals with normal glucose homeostasis who were followed for 12 years revealed that elevated BCAAs correlated with future type 2 diabetes development [86]. A similar study evaluated metabolite profiles and insulin resistance at baseline and after 6 years, revealing a strong association between BCAAs and the development of insulin resistance, especially in men [87]. A systematic review of multiple metabolomics studies including a total of 8000 people revealed that the risk of type 2 diabetes development increased by ~35% per standard deviation increase in circulating BCAA [88]. Another study demonstrated through metabolomic profiling that a BCAA-related metabolite signature was suggestive of increased BCAA catabolism in obese, insulin-resistant people compared with lean controls [89]. Together, these studies leave little doubt that elevated BCAAs and/or increased BCAA catabolism correlate with future insulin resistance and type 2 diabetes, strongly suggesting a causal role of BCAAs in the development of insulin resistance.

A genetic Mendelian randomization study further supported the conclusion that BCAAs cause type 2 diabetes [90]. This type of study proposes that if a biomarker, e.g. elevated BCAAs, is causal in the development of a disease, e.g. type 2 diabetes, then genetic variants associated with the biomarker should also associate with the disease. Metabolomic measurements were coupled with genome-wide association studies (GWASs) to obtain

genetic signatures that associate with elevated plasma BCAAs, and these signatures were then shown also to associate with type 2 diabetes. The SNP with the strongest association with type 2 diabetes lay near, and alters the expression of, *PPM1K*, the gene that encodes the BCKDH phosphatase, adding mechanistic plausibility to the observation [90].

Finally, studies involving either infusion [91-93] or ingestion [64] of BCAAs followed by assessment of insulin resistance using hyperinsulinemic-euglycemic clamps also support a causal role for BCAAs in the development of insulin resistance. In these studies, healthy patients given BCAAs became acutely insulin resistant in less than 6 h [64,91-93]. Similarly, supplementing a high-fat diet with BCAAs worsens insulin resistance in rats [94], while restricting BCAAs improves insulin resistance in mice and rats [95,96]. Together, these numerous epidemiological studies, Mendelian randomization studies, and intervention studies, strongly support a causal role for BCAAs in the development of insulin resistance and type 2 diabetes. It is important to note that insulin resistance could also, conversely, drive elevations in BCAA levels. A different Mendelian randomization study than the one discussed above suggested the converse conclusion, i.e. that insulin resistance may drive elevated BCAA levels [97]. Similar conclusions were made in a rodent study of gastric bypass in which surgery improved glucose handling independent of various BCAA challenges [98]. Importantly, these relationships between BCAAs and insulin sensitivity are not mutually exclusive, and may in fact create a disease-worsening positive feedback loop.

The mechanism by which BCAAs drive insulin resistance remains unclear and the subject of active investigation. In 2016, White et al. demonstrated in Zucker-fatty rats (ZFRs) that BCKDH activity was higher in skeletal muscle and lower in liver from obese rats compared with lean controls [96]. Others have also noted elevated phosphorylation, and lower activity, of liver BCKDH in various models of diabetes [99,100]. The same group later found that inhibition of BCKDK, either systemically with the small-molecule BCKDK inhibitor 3,6-dichlorobrenzo(b)thiophene-2-carboxylic acid (BT2) or primarily in the liver with adenovirus-mediated delivery of PPM1K, increased BCKDH activity in the livers of ZFRs, lowered hepatic triacylglyceride levels, and improved insulin resistance [46]. These data further support the causality of aberrant BCAA metabolism in insulin resistance, and strongly implicated suppressed liver BCAA oxidation in this process.

How might the suppression of liver BCAA catabolism promote whole-body insulin resistance? Work by our laboratory has implicated whole-body redistribution of BCAA oxidation towards skeletal muscle as a driver of insulin resistance (Figure 3). Steady-state *in vivo* heavy isotopic tracing has shown that the majority of BCAA oxidation occurs in muscle, and that, in *db/db* mice, a model of severe insulin resistance, BCAA oxidation is significantly further increased in skeletal muscle and decreased in the liver and adipose tissue [40]. The decreased adipose BCAA oxidation in models of insulin resistance appears to be mediated by suppressed transcriptional expression of BCAA catabolic genes [101], perhaps driven by hypoxic activation of HIF-1 α [23], while the decreased liver BCAA oxidation is likely mediated by post-translational inhibition of BCKDH via phosphorylation [25-27]. Thus, in states of insulin resistance, BCAAs appear to be shunted away from the liver and adipose tissue and towards skeletal muscle [4]. We and others have suggested that alleviating this shunt via activation of BCKDH with BT2 improves insulin sensitivity

[40,46,101]. Decreased BCAA metabolism in adipose tissue or the liver may also relate to reduced insulin sensitivity in other more indirect ways as well. For example, BCKDK and PPM1K in the liver have been suggested to target metabolic enzymes outside of BCAA metabolism [46]. And it is possible that the near complete loss of mmBCFA synthesis in adipose tissue may somehow promote insulin resistance.

Several theories have been proposed to explain how excess BCAA oxidation in skeletal muscle may link to insulin resistance. Insulin resistant patients and type 2 diabetics often have increased free fatty acid levels, as well as higher triglyceride content within their skeletal muscle, suggesting defects in lipid metabolism. An accumulation of incompletely oxidized lipid species, known as lipotoxicity, has been implicated in the development of insulin resistance, but how lipotoxicity occurs is incompletely understood [102,103]. One recently proposed mechanism directly implicates BCKA as inhibitors of insulin signaling [104]. Another hypothesis, supported by the observation of increased circulating levels of downstream catabolites of BCAA oxidation, such C3 and C5 acylcar-nitines, in patients with insulin resistance [89], is that increased BCAA oxidation may out-compete fatty acid oxidation, causing the accumulation of lipotoxic, incompletely oxidized lipid species [99,105]. Support for this notion includes studies in ZFRs that demonstrate that when fed a BCAA-restricted diet, fatty acyl-CoA content in the skeletal muscle of the rats decreased and insulin resistance was improved [96]. The same group also proposed the intriguing additional hypothesis that BCAA overload in muscle also presents a nitrogen burden, which is relieved by pyruvate conversion to alanine, at the expense of glycine conversion to pyruvate [106]. The consequent decrease in glycine may prevent the buffering of the excess of acyl (especially acetyl) CoAs as acyl-glycines to be secreted in urine, thus contributing to a bottleneck in lipid metabolism [96]. This hypothesis is consistent with the established observation that levels of glycine are inversely correlated with high circulating BCAA levels in cohorts with insulin resistance [107,108]. Glycine supplementation did not, however, improve glucose tolerance in HF-fed ZFRs, nor decrease muscle long-chain acyl CoAs [106]. The interesting connection between BCAAs, glycine, and lipid metabolism thus needs further investigation.

Work from our laboratory also supports the model that excess BCAA oxidation alters lipid metabolism and drives insulin resistance. We found that BCAA catabolism in skeletal muscle causes the secretion of 3-HIB, a catabolic intermediate of valine, which in turn promotes fatty acid uptake and transport to the muscle by the endothelium. Mice treated with 3-HIB were more insulin resistance than vehicle-treated controls [21]. Increased BCAA oxidation can thus promote lipid accumulation in skeletal muscle via paracrine effects on the vasculature. 3-HIB levels are elevated in *db/db* mice, as well as human subjects with type 2 diabetes compared with healthy controls [21]. How FA transport by the endothelium is mediated and regulated by 3-HIB remains unclear, but appears to involve a small family of ATP-dependent acyl-CoA synthetases and the production of ATP specifically from mitochondria [109].

BCAAs and heart failure

Elevations in plasma BCAAs are also associated with heart failure [45]. The expression of BCAA catabolic genes has been shown to be reduced in tissue from rodent and human failing hearts [110-112], suggesting that a BCAA catabolic defect exists in heart failure, although this has not yet been demonstrated directly. As noted above, the heart accounts for <4% of whole-body BCAA oxidation in wildtype mice and therefore defective BCAA oxidation in the heart is unlikely to explain elevated BCAA levels observed in heart failure, suggesting that this increase in plasma BCAAs may be due to a defect in BCAA metabolism elsewhere — e.g. skeletal muscle or liver. Heart failure is recognized to be, in part, caused by a failure of the heart to produce the energy it needs to pump blood, i.e. that the heart is ‘an engine out of fuel’ [113]. It has thus been suggested that catabolic defect of BCAAs in heart failure may contribute to this fuel insufficiency. However, numerous studies, including our measurements in rodents with isotopic infusions *in vivo*, and our more recent studies in humans, indicate the loss of BCAA oxidation would starve the heart of less than 2% of its energy production [39,114-116]. It may be that, instead, the healthy heart uses BCAA oxidation either to regulate BCAA levels *per se*, or to produce BCAA-derived signaling metabolites. No matter the mechanism, it is striking that BT2 treatment in multiple different mouse models of heart failure markedly improves outcomes [111,112,115], indicating that systemic BCAA metabolism is somehow critical in heart failure. Considering the diverse literature surrounding BCAAs and heart failure, we hypothesize that BT2 might improve outcomes in heart failure either by activating oxidation in another tissue or by impacting signaling from BCAA-derived metabolites within the heart.

Future directions

In conclusion, BCAA oxidation is distributed among most organs in healthy mice, and likely humans. Oxidation is regulated by both transcriptional and post-translational mechanisms (Figure 1). The impact of oxidation in a given tissue on systemic BCAA levels can be modeled by the tissue-specific relative preference for BCAA oxidation over other fuels, the metabolic rate of the tissue, and the total tissue mass in the organism (Figure 2). Alterations in BCAA metabolism have been implicated in a variety of diseases. Overexpression of BCAT1 is associated with a variety of cancers. In models of insulin resistance, BCAA oxidation is altered in adipose tissue, liver, and skeletal muscle. Increased oxidation in skeletal muscle and defective oxidation in the liver and/or adipose tissue likely contribute to insulin resistance (Figure 3). Altered oxidation of BCAAs have also been noted in heart failure, but the implications remain poorly understood. Future work is needed to test the contribution of altered BCAA oxidation in muscle, liver, and adipose tissue to the development of insulin resistance, as well as the role of BCAA oxidation in heart failure.

Abbreviations

3-HIB	3-hydroxyisobutyrate
BCAAs	branched-chain amino acids
BCKDH	branched-chain α -ketoacid dehydrogenase

BCKDK	BCKDH kinase
HCC	hepatocellular carcinoma
MSUD	maple syrup urine disease
TCA	tricarboxylic acid
ZFRs	Zucker-fatty rats
mmBCFAs	monomethyl branched-chain fatty acids
BCKAs	branched-chain α -ketoacids

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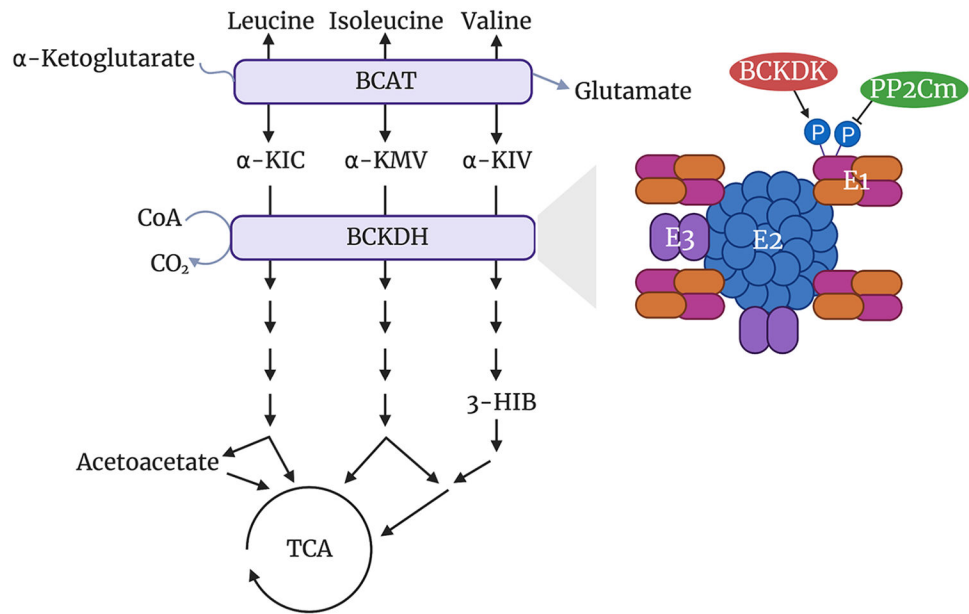


Figure 1. Basic BCAA oxidative pathway, including a simplified structure showing the regulation of the rate-limiting enzyme, BCKDH.

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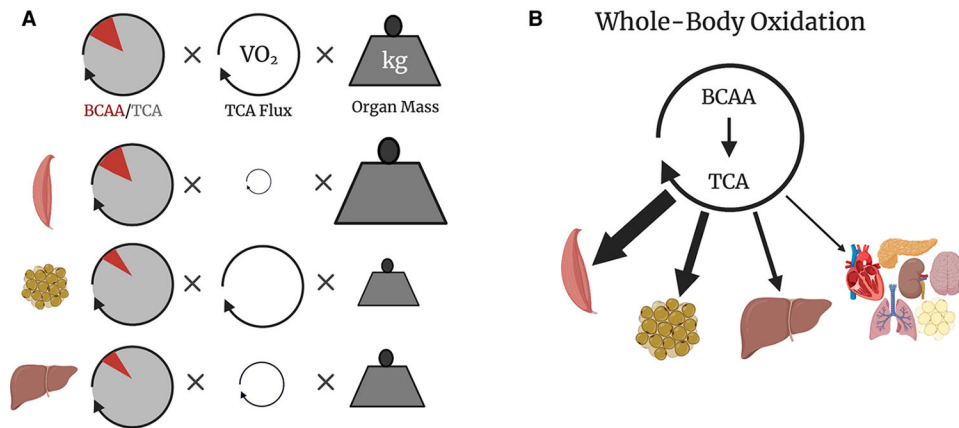


Figure 2. Quantifying Whole-Body BCAA Oxidation.

(A) BCAA oxidation flux into muscle, brown fat, and liver calculated from the fraction of TCA labeling from BCAAs, TCA turnover flux inferred from tissue oxygen consumption rate (VO_2), and normalized to organ mass. (B) Whole-body distribution of measured BCAA oxidation. Figure created with [BioRender.com](https://www.biorender.com).

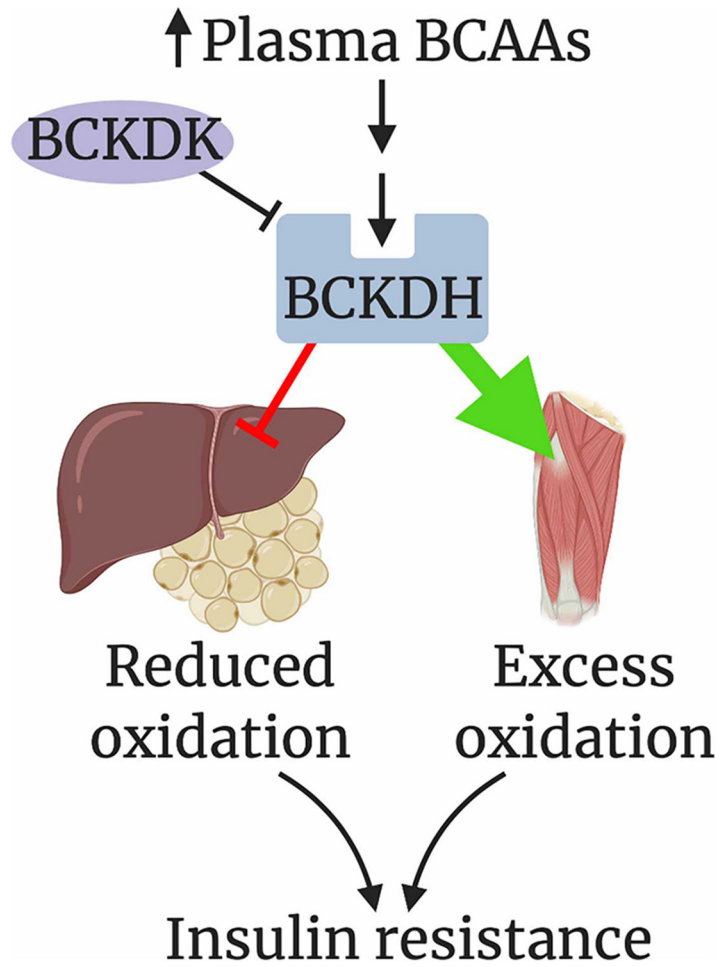


Figure 3. Proposed model of how the redistribution of BCAA oxidation towards skeletal muscle drives insulin resistance. Figure created with [BioRender.com](https://www.biorender.com).