



Branched Chain Amino Acids in Metabolic Disease

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Abstract

Purpose of Review Elevations in circulating branched chain amino acids (BCAAs) have gained attention as potential contributors to the development of insulin resistance and diabetes.

Recent Findings Epidemiological evidence strongly supports this conclusion. Suppression of BCAA catabolism in adipose and hepatic tissues appears to be the primary drivers of plasma BCAA elevations. BCAA catabolism may be shunted to skeletal muscle, where it indirectly leads to FA accumulation and insulin resistance, via a number of proposed mechanisms.

Summary BCAAs have an important role in the development of IR, but our understanding of how plasma BCAA elevations occur, and how these elevations lead to insulin resistance, is still limited.

Keywords Branched chain amino acids · Catabolism · Diabetes · Insulin resistance

Introduction

Branched chain amino acids (BCAAs) are fundamental building blocks of mammalian life, comprising ~20% of the amino acids incorporated into protein [1]. All three BCAAs, leucine, isoleucine, and valine, are essential, and thus must be ingested. Plants, fungi, and bacteria synthesize BCAAs. BCAAs are thus generally abundant, and BCAA deficiency is rare. Mammals contain large amounts of BCAAs, mostly in muscle protein. Excess protein intake does not, however, lead to equivalent increase in muscle protein; excess protein intake thus obligatorily leads to its catabolism, in contrast to carbohydrates and fats that can be stored as glycogen and adipose tissue, respectively. A tightly regulated system has thus evolved to control BCAA catabolism.

The first two steps of BCAA catabolism are shared by all three BCAAs (Fig. 1). The initial transamination to branched chain keto acids (BCKAs) by BCAA transaminase (BCAT) is followed by decarboxylation and dehydrogenation by the BCAA dehydrogenase complex (BCKDH) [2, 3]. The BCKDH complex is rate-limiting for BCAA catabolism, and is tightly regulated by inhibitory phosphorylation by BCKDK and reciprocal dephosphorylation (activating) by PPM1K [4–6]. Subsequent steps of BCAA catabolism are unique to each BCAA. Valine ultimately yields propionyl-CoA that enters the TCA, and is thus potentially gluconeogenic, while leucine yields acetyl-CoA and is thus ketogenic, and isoleucine yields both propionyl-CoA and acetyl-CoA. The BCKDH complex resides on the inner side of the inner membrane of mitochondria, and all BCAA catabolism thus occurs inside the mitochondrial matrix.

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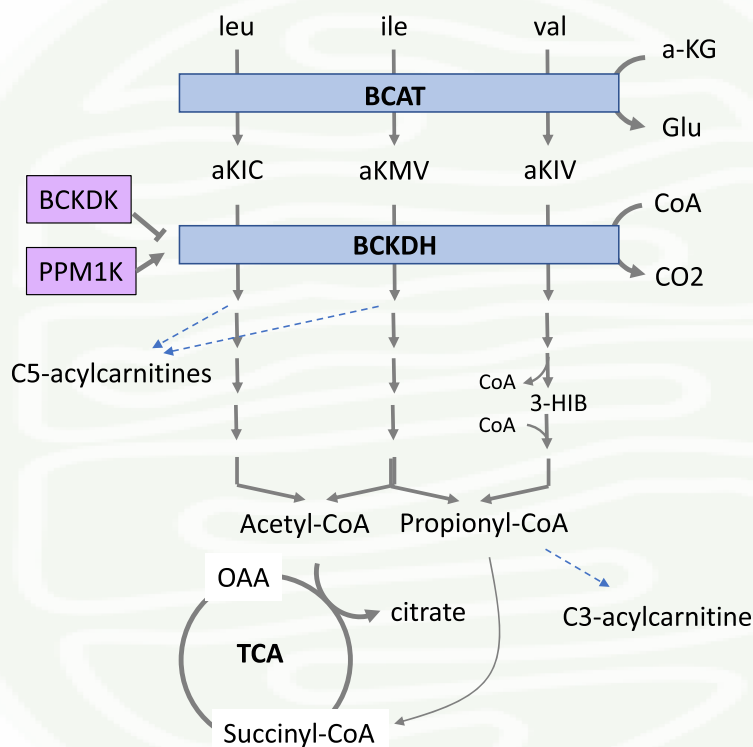
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Do Elevations in BCAAs Cause Insulin Resistance?

Elevations in plasma levels of BCAAs have been noted to associate with obesity and diabetes since the 1960s, and have been repeatedly reported since then [7–10]. More recently, unbiased metabolomics profiling of obese insulin resistant versus lean insulin sensitive subjects revealed that elevations in BCAAs were in fact the metabolic signature most strongly associated with insulin resistance [11–17].

Fig. 1 Biochemical pathways of BCAA catabolism. See text for details. Leu leucine, Ile isoleucine, Val valine, A-KG alpha-ketoglutarate, Glu glutamate, aKIC alpha keto isocaproate, aKMV alpha keto methyl valerate, aKIV alpha keto isovalerate, BCAT branched chain amino acid aminotransferase, BCKDK branched chain alpha keto acid dehydrogenase kinase, BCKDH branched chain alpha keto acid dehydrogenase, PPM1K protein phosphatase, Mg²⁺/Mn²⁺-dependent 1 K, CoA coenzyme A, CO₂ carbon dioxide. 3-HIB 3-hydroxyl isobutyrate, OAA oxaloacetate, TCA tricarboxylic acid cycle



The association has often assumed to be consequence rather than cause of insulin resistance, reflecting inability to properly suppress protein breakdown in the context of insulin resistance. However, several recent lines of evidence have strongly suggested that elevations in BCAAs contribute to insulin resistance:

1. Infusions of BCAAs in humans acutely worsen insulin sensitivity, as typically measured by hyperinsulinemic euglycemic clamps [18–21]. Similarly, co-administration of BCAAs with high-fat diet (HFD) to rodents generally worsens the ensuing insulin resistance [11]. Conversely, low BCAA diets generally improve insulin resistance [22•, 23, 24]. Importantly, in all these rodent models, the presence of lipids or HFD was critical, and BCAAs alone had little or no effect, strongly suggesting that mechanistically, BCAAs interact with lipids in some way to promote insulin resistance. In general, accumulation of incompletely esterified or oxidized lipid species can be demonstrated in rodent models of insulin resistance or diabetes, usually in skeletal muscle [11, 22•, 25•]. These species include acyl-carnitines, acyl-CoAs, and diacylglycerols (DAG), although which species primarily drives insulin resistance remains actively debated.
2. Elevations in plasma BCAA levels can be detected in people more than 10 years before developing diabetes. This was first demonstrated in an unbiased metabolomics profiling of banked blood samples from 189 subjects in the Framingham Heart Study who subsequently developed diabetes, compared to 189 controls who did not develop diabetes [26]. In this study, BCAAs were the signature most strongly associated with later development of diabetes. At the time of the initial blood sampling, none of the subjects had diabetes or detectable insulin resistance. Similar findings have been reported in an independent study [27], although not in a third study [28].
3. A genetic Mendelian randomization study identified a locus near PPM1K that was associated with BCAA levels with genome-wide significance, and that in turn also correlated with incidence of diabetes, strongly suggesting that the former leads to the latter [29]. However, other genetic studies have identified similar close relationships, but suggested opposite direction of causality [30, 31]. Importantly, genetic studies may be undermined by the fact that genetic variants affect all tissues, while BCAA oxidation may have opposing effects in different tissues (see below).

4. Pharmaceutical activation of BCKDH, leading to oxidative disposal of BCAA and reversal of BCAA elevations in the Zucker rat model of obesity and diabetes, leads to marked improvement in insulin resistance [32••]. However, this study also identified an alternate explanation for improvement in insulin resistance (see below).

Although none of these studies individually provides undeniable proof of causality, in aggregate, the studies provide robust support for the hypothesis that chronic elevations in BCAAs in humans and in rodent models contribute to the development of insulin resistance and diabetes. As noted, insulin resistance conversely likely predisposes to BCAA elevations by failing to fully suppress protein breakdown, thus potentially activating a vicious cycle.

The increasing evidence that BCAA elevations contribute to insulin resistance has led to concerted ongoing investigations by numerous labs into how this process occurs mechanistically. Two overarching questions arise: how do plasma BCAA elevations occur? And how do elevated BCAA levels lead to insulin resistance?

How Do Elevations in BCAAs Arise?

The best evidence to date argues for the inhibition of BCAA catabolism in specific tissues. Which tissues primarily contribute to BCAA catabolism under normal conditions is, surprisingly, controversial. Studies to date have largely relied on ex vivo measurements of BCAA catabolism, using tissue sections or extracts [1, 33]. These approaches, however, do not incorporate the numerous regulatory factors that exist in vivo, including physiological availability of substrates, presence of product inhibitors like NADH and acetyl-CoA, and subcellular localization and compartmentalization of key enzymes. More definitive studies using heavy isotope steady-state infusion studies in awake mice have recently demonstrated that in fact most tissues actively oxidize BCAAs, with the largest contribution likely from skeletal muscle and liver [34••]. Similar studies in humans have not been performed. In this context, adipose tissue and liver have been proposed to be the primary contributors to BCAA elevations (Fig. 2).

1. Adipose tissue. Adipose-specific expression of Glut4 in mice led to systemic insulin resistance, decreased mRNA expression of BCAA catabolic genes, and a strong increase in circulating BCAA levels [35]. BCAA catabolic genes are also suppressed in adipose tissue in other models of obesity and insulin resistance [36], and in humans with diabetes and insulin resistance [37, 38]. Conversely, in humans, bariatric surgery-induced weight loss is associated with both reduced plasma BCAA and rises in adipose BCAT and BCKDH expression [39, 40].

Thus, a concerted suppression of BCAA catabolism in adipose tissue has been proposed as a cause of systemic elevations of BCAAs. ER stress, inflammation, and tissue hypoxia may all contribute to this suppression [41, 42], and TZDs, well-established insulin sensitizers, reverse this suppression in rats and humans [36, 43]. However, an important limitation of these models is that adipose tissue oxidizes only minimal quantities of BCAAs, likely less than 5% of whole-body oxidation even in obese models [34], suggesting that the increase in circulating BCAA levels must have additional contributors.

2. Hepatic tissue. BCKDH activity is also decreased in the liver of obese and diabetic mice, although the mechanism differs from that in adipose tissue: BCKDK expression is increased, leading to increased inhibitory phosphorylation of BCKDH [22•, 32••, 39, 44]. Expression of BCKDK appears to be in part under regulation of ChREBP-beta, a carbohydrate-responsive transcription factor sensitive in particular to ingestion of dietary carbohydrates (including fructose), thus potentially linking modern high-fructose diets to elevations in plasma BCAAs [32••]. Adiponectin and other hormones may also modulate liver BCKDH activity [44], as well as neuronal circuits in response to brain insulin [45].
3. Skeletal muscle. Interestingly, none of the studies above have detected changes in mRNA or protein expression, or of phosphorylation, of BCAT or BCKDH in skeletal muscle, despite the fact that muscle is the primary sink for glucose after a glucose load, and a predominant site of insulin resistance. To the contrary, elevations of C3 and C5 carnitines have been noted in skeletal muscle of diabetic mice, suggesting that skeletal muscle may in fact be a site of increased BCAA catabolism in states of insulin resistance [46]. This inference was recently strongly supported by the use of heavy isotope steady-state infusion studies in *db/db* mice, demonstrating a shift in tissue-specific BCAA catabolism away from adipose and hepatic tissues, and towards skeletal muscle [34••]. One recent study did, however, observe suppression of some genes of BCAA catabolism in skeletal muscle of patients with insulin resistance [47].

There are other possible causes for increased BCAA levels. As noted above, mammals cannot synthesize BCAA. The diet may thus contribute, as the overall per capita daily consumption of calories has increased by ~20% over the last 50 years. This increase, however, is largely due to increased carbohydrates, rather than protein. Moreover, epidemiological studies are varied, with some studies indicating that a diet high in BCAAs can be associated with circulating BCAAs, while others not [14, 26, 46]. Recent work has also suggested a role for BCAA arising from synthesis by the intestinal

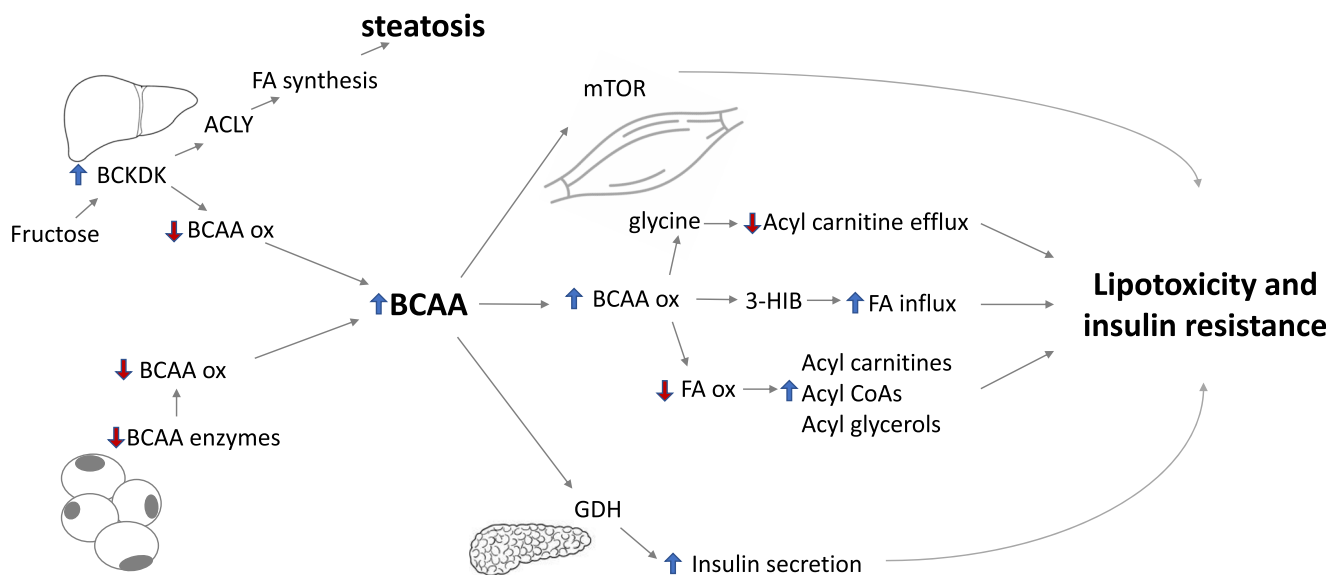


Fig. 2 Integration of proposed mechanistic models for BCAA-induced insulin resistance. See text for details. BCKDK branched chain alpha keto acid dehydrogenase kinase, FA fatty acid, ACLY ATP citrate lyase,

BCAA branched chain amino acid, Ox oxidation, mTOR mechanistic target of rapamycin, 3-HIB 3-hydroxyl isobutyrate, GDH glutamate dehydrogenase

microbiome. A small study in four monozygotic twin pairs discordant for obesity showed that obesity-associated microbiota had higher synthesis and lower catabolism of BCAAs [48]. Moreover, transfer of fecal matter from obese twins into germ-free mice led to increased body weight and plasma BCAAs, compared to fecal matter from lean siblings. A more recent study in 277 subjects supported these findings: elevated BCAAs in insulin resistant subjects correlated with a gut microbiome with enriched BCAA biosynthetic capacity, and inoculation of mice with one of these species induced insulin resistance and increased circulating BCAA levels [49]. Thus, microbiota appear able to modulate host circulating BCAA levels. However, none of these studies have demonstrated that plasma BCAA detected in the host were synthesized by the bacteria, leaving the equally likely possibility of indirect effects by the microbiome.

In summary, though diet and microbiome may contribute to elevations in BCAAs, the current data best support the model that suppression of BCAA catabolism in adipose and liver tissues, via still poorly defined mechanisms, leads to increased plasma BCAA levels, in turn shunting BCAA catabolism to skeletal muscle.

How Do Elevated BCAA Levels Lead to Insulin Resistance?

Numerous fundamental questions remain unanswered on this issue, including the following: Do elevations in BCAAs per se promote insulin resistance, or is the increased BCAA catabolism that results from elevated BCAAs to blame? What tissue bed is primarily responsible for promotion of

insulin resistance in the face of elevated BCAAs? Which BCAA, if only one, is primarily responsible? We discuss below a number of proposed mechanisms and their implications for these questions (Fig. 2).

1. mTOR. The lysosome-associated mTORC1 complex promotes cellular growth pathways in most cells, and is exquisitely sensitive to metabolic cues, including amino acids [50]. Of these, leucine most potently activates mTORC1, via direct binding to Sestrin2, itself a modulator of the mTORC1-associated GATOR complex [51, 52]. Elevated BCAAs can thus be expected to promote mTORC1 association, and indeed BCAA supplementation to rodent diet leads to activation of mTOR and downstream targets [11], and infusions of AAs in humans decreases insulin sensitivity in both muscle and liver, in parallel with activation of mTOR [18–21]. Moreover, treatment with rapamycin, a specific inhibitor of mTOR, partially reversed the insulin resistance caused by addition of BCAAs to HFD in rodents [11]. However, a primary role for leucine-activated mTOR in driving insulin resistance is not consistent with a number of other observations. For example, while diets supplemented with BCAAs generally worsen insulin resistance [11], diets supplemented with only leucine generally do not, and in fact often promote insulin sensitivity [21, 53–56]. The latter could be explained by the fact that leucine supplementation activates BCKDH, leading to lower levels of the other two BCAAs. If so, then the observations suggest that isoleucine or valine is primarily responsible for driving insulin resistance, rather than leucine, and thus not mTOR activation (which is primarily mediated by leucine).

2. Insulin secretion. Chronic hyperinsulinemia has been suggested to be an early contributor to insulin resistance and diabetes, both by exerting constant pressure on the beta-cell leading to early failure, and by exerting pressure on peripheral insulin signaling leading to compensatory desensitization [57]. Elevations in insulin levels, especially post-prandial, occur early in the development of insulin resistance, before changes in glucose levels [58, 59]. BCAAs, in particular leucine, are potent insulin secretagogues, especially under conditions of normal or low glucose levels [60]. Thus, elevated BCAAs may contribute to chronic hyperinsulinemia, potentially promoting compensatory insulin resistance. As with the mTOR hypothesis, however, the model predicts that leucine would be a more potent driver of insulin resistance than the other two BCAAs, while experimentally the opposite appears to be true.
3. Competition for fatty acid oxidation (FAO). The BCAA signatures observed in numerous epidemiological studies are often accompanied by elevations in C3 and C5 acylcarnitines, which are in equilibrium with incompletely oxidized products of BCAA catabolism, including propionyl CoA. These observations thus suggest that BCAA catabolism in insulin resistance is elevated, or at least improperly balanced with combustion of these carnitines, i.e., with TCA cycling. Excess BCAA catabolism may in turn compete with fatty acid beta-oxidation for entry of carbons into the TCA cycle, leading to accumulation of incompletely oxidized lipid intermediates and mitochondrial stress that promote insulin resistance in muscle and liver [46, 61]. This model, however, is difficult to reconcile with the relatively small contribution that BCAAs make to TCA carbons in muscle and liver even under normal physiological conditions [34••]. The model also predicts that levels of acetyl-CoA should increase, which to date has not been demonstrated in insulin resistant states.
4. Glycine. BCAA restriction efficiently reversed muscle insulin resistance in fatty Zucker rats [22•]. Various metabolomics screens in these experiments yielded two prominent observations: BCAA restriction reversed the increase of muscle levels of even chain-length acyl-CoAs in ZF rats, and BCAA restriction similarly rescued the suppressed levels of glycine in ZF rat muscles. Low plasma levels of glycine have been repeatedly noted in human studies of insulin resistance, concordant with the elevations in BCAAs. Glycine can act as an extracellular carrier of incompletely oxidized fatty acids, in the form of acylglycines, and indeed levels of acylglycines were lower in urine of ZF rats [22•]. These observations led the authors to propose that less glycine in skeletal muscle compounds the accumulation of acyl-CoAs caused by competition between BCAA and fatty acid oxidation, because accumulated acyl-CoAs can less easily escape muscle as acylglycines. As above, the model depends on competition of BCAA oxidation with FAO to be sufficient to cause accumulation of acyl-CoAs.
5. 3-HIB. All BCAA catabolic products subsequent to the BCKDH step are covalently bound to CoA and thus trapped within the mitochondria, with the single exception of 3-HIB (Fig. 2). 3-HIB can thus escape mitochondria, and be secreted from the cell, likely in some proportion to active BCAA catabolic flux. Secreted 3-HIB stimulates capillary endothelial cells to promote trans-endothelial fatty acid transport, thereby increasing fatty acid flux to skeletal muscle [25•]. Thus, one potential model would suggest that elevated plasma BCAAs lead to increased muscle BCAA catabolism, which promotes 3-HIB secretion and trans-endothelial fatty acid transport, leading to accumulation of lipid species in muscle that drives insulin resistance. Administration of 3-HIB to mice promotes muscle accumulation of diacyl glycerols and insulin resistance [25•], and elevations of 3-HIB in plasma and urine correlate with present and future insulin resistance [62, 63]. 3-HIB is derived from valine. This model is thus consistent with the idea that catabolism of BCAAs, rather than levels per se, drives insulin resistance. The model is also consistent with the observations that leucine alone tends not to promote insulin resistance, while all three BCAAs together do. Interestingly, the effects of valine alone have not been reported, either in humans or animal models. On the other hand, this model is not immediately consistent with the observation that pharmacological inhibition of BCKDK improves insulin sensitivity, because the resulting increase in BCAA catabolism in skeletal muscle would be predicted to increase 3-HIB production, rather than decrease it [32••, 34••].
6. Indirect effects. Recent work has suggested that liver BCKDK, in addition to regulating BCKDH, may also directly phosphorylate and activate ATP citrate lyase (ACLY), a rate-limiting enzyme for fatty acid synthesis [32••]. Like BCKDH, phosphorylation of ACLY is reversed by PPM1K. Thus, elevated BCKDK in the liver may promote fatty acid synthesis directly, thereby promoting steatosis and insulin resistance independently of its effects on BCAA catabolism. How these data relate to elevated BCAAs is not clear however. BCKAs, in particular aKIC (from leucine), allosterically suppress BCKDK activity on BCKDH. If elevated BCKAs also suppressed the activity of BCKDK on ACLY, as predicted, then high BCAAs would be predicted to improve insulin resistance and steatosis, rather than worsen it.

In summary, numerous mechanisms have been proposed to explain how elevations in BCAA promote insulin resistance, but none of them is yet proven. It is important to realize that in

all likelihood multiple mechanisms occur simultaneously, perhaps explaining some of the apparent contradictions noted above. Returning to the three questions posed at the beginning of this section: the data thus far best support the notion that increased catabolism, in particular in skeletal muscle, and perhaps not of leucine but rather of isoleucine or valine, is the dominant mechanism contributing to BCAA-related insulin resistance and diabetes risk, but this notion is far from conclusive. Proof will at least require tissue-specific genetic modification of BCAA catabolism in skeletal muscle.

Conclusions

Strong evidence indicates that chronic elevations in BCAAs causally contribute to the development of insulin resistance in humans. How elevations in BCAAs occur remains poorly defined, but current data point to tissue-specific suppression of BCAA catabolism in adipose and hepatic tissues. How elevations in BCAAs cause insulin resistance also remains poorly understood, but current evidence points to excess BCAA catabolism in skeletal muscle, possibly simultaneously suppressing FAO and promoting fatty acid influx, leading to FA accumulation and lipotoxicity. Deeper understanding of either of these processes might identify new targets to get at the heart of insulin resistance.

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Compliance with Ethical Standards

Conflict of Interest Zolt Arany and Michael Neinast declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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