

Insulin action, type 2 diabetes, and branched-chain amino acids: A two-way street



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ABSTRACT

Background: A strong association of obesity and insulin resistance with increased circulating levels of branched-chain and aromatic amino acids and decreased glycine levels has been recognized in human subjects for decades.

Scope of review: More recently, human metabolomics and genetic studies have confirmed and expanded upon these observations, accompanied by a surge in preclinical studies that have identified mechanisms involved in the perturbation of amino acid homeostasis— how these events are connected to dysregulated glucose and lipid metabolism, and how elevations in branched-chain amino acids (BCAA) may participate in the development of insulin resistance, type 2 diabetes (T2D), and other cardiometabolic diseases and conditions.

Major conclusions: In human cohorts, BCAA and related metabolites are now well established as among the strongest biomarkers of obesity, insulin resistance, T2D, and cardiovascular diseases. Lowering of BCAA and branched-chain ketoacid (BCKA) levels by feeding BCAA-restricted diet or by the activation of the rate-limiting enzyme in BCAA catabolism, branched-chain ketoacid dehydrogenase (BCKDH), in rodent models of obesity have clear salutary effects on glucose and lipid homeostasis, but BCAA restriction has more modest effects in short-term studies in human T2D subjects. Feeding of rats with diets enriched in sucrose or fructose result in the induction of the ChREBP transcription factor in the liver to increase expression of the BCKDH kinase (BCK) and suppress the expression of its phosphatase (PPM1K) resulting in the inactivation of BCKDH and activation of the key lipogenic enzyme ATP-citrate lyase (ACLY). These and other emergent links between BCAA, glucose, and lipid metabolism motivate ongoing studies of possible causal actions of BCAA and related metabolites in the development of cardiometabolic diseases.

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Keywords Metabolic diseases; Branched-chain amino acids; Lipogenesis; Nutrition; Insulin resistance

1. INTRODUCTION

Many years of research have established critical regulatory roles for the pancreatic islet hormones insulin and glucagon in control of metabolic homeostasis. A major focus of this work has been to define mechanisms by which the hormones control glucose and lipid metabolism, as type 2 diabetes (T2D) is most prominently tied to dysregulated metabolism of these fuels. However, insulin and glucagon also have critical roles in the regulation of amino acid metabolism, and the relevance of these metabolites in the development of insulin resistance, diabetes, and cardiometabolic disease phenotypes has been a topic of renewed interest in recent years. Particular attention has been focused on dysregulated branched-chain amino acid (BCAA— leucine, valine, and isoleucine) metabolism in obesity-related diseases and conditions, including insulin resistance, T2D, and cardiovascular diseases [1,2]. Seminal studies from the late 1960s by George Cahill and associates reported higher levels of BCAA and aromatic amino acids (tyrosine, phenylalanine) and lower glycine levels in obese, insulin-resistant participants compared to lean controls [3]. These findings have been confirmed by many laboratories in the ensuing years, but

whether these analytes play a causal role in the development of cardiometabolic diseases or simply serve as associative biomarkers remains a topic of active investigation and debate. Here, we seek to provide an update on the status of this growing area of research.

1.1. Human metabolomics and genetic studies

With the application of metabolomics technologies, many examples of BCAA associations with insulin sensitivity and related cardiometabolic disease phenotypes have emerged. An understanding of these associations has expanded, this has resulted in inclusion of an array of BCAA-related metabolites such as the branched-chain keto acids (BCKA) [4,5], 3-hydroxyisobutyrate (3-HIB), a valine metabolite [6], and propionyl (C3) and isovaleryl/ α -methylbutyryl (C5) acylcarnitine metabolites in the BCAA-related “signature” [7] (refer to Figure 1 for a schematic summary of BCAA catabolic pathways). Key findings from the studies of circulating metabolites in human cohorts include the following: 1) Demonstration that the association of BCAA and related metabolites with insulin resistance is stronger than that of free fatty acids (FFA) or metabolites derived from FFA oxidation [7,8]; 2) Demonstration that levels of BCAA and aromatic amino acids are

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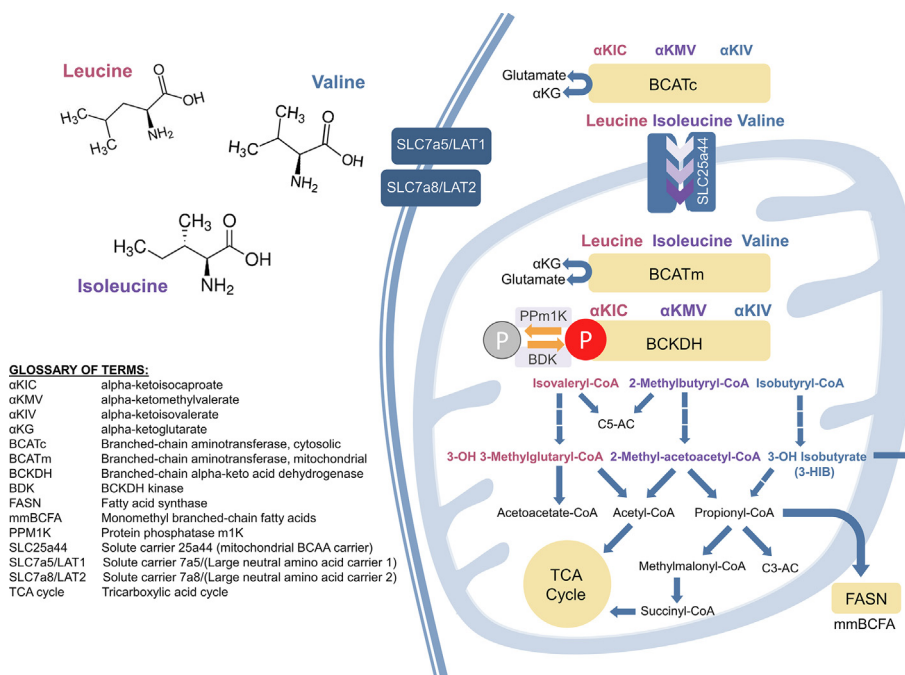


Figure 1: Overview of the pathways of branched-chain amino acid (BCAA) catabolism. Following uptake into cells through the LAT1 or LAT2 transporters (SLC7a5 and SLC7a8, respectively), the first common step in the catabolism of the BCAA (leucine, isoleucine, valine) is transamination to yield cognate α -ketoacids (α -ketoisocaproate (α KIC), α -ketomethylvalerate (α KMV), α -ketoisovalerate (α KIV)) and glutamate. Transamination can be catalyzed by a cytosolic form of branched-chain aminotransferase (BCATc), or alternatively, BCAA can be transported into the mitochondria by SLC25a44 to gain access to the mitochondrial isoform of BCAT (BCATm). The branched-chain α -ketoacids then engage with the rate-limiting enzyme of BCAA catabolism, the branched-chain ketoacid dehydrogenase complex (BCKDH), to form CoA-modified intermediates. BCKDH activity is controlled by reversible phosphorylation; phosphorylation by the BCKDH kinase (BDK) inhibits enzyme activity, whereas dephosphorylation of BCKDH by the PPM1K phosphatase activates the enzyme. The CoA-modified metabolites generated by BCKDH are readily converted to carnitine-modified metabolites that serve as convenient biomarkers of BCAA catabolism (e.g. C5-AC and C3-AC), TCA cycle intermediates such as acetyl CoA, succinyl CoA, or a free acid that can leave the cell have been ascribed functions in the regulation of transendothelial fatty acid transport, 3-OH isobutyrate (3-HIB). In white adipose tissue, odd chain metabolites produced from BCAA catabolism such as propionyl CoA can serve as substrates for the synthesis of monomethyl branched-chain fatty acids (mmBCFA) by fatty acid synthase (FASN).

prognostic for future risk of T2D [9]; 3) Demonstration that elevated BCAA in both maternal and fetal circulation associate with neonatal birth weight and adiposity, independent of maternal BMI and blood glucose [10,11]; 4) Demonstration that a principal component factor comprised of BCAA, BCAA-derived metabolites, and aromatic amino acids measured at baseline in overweight subjects is predictive of improvements in insulin sensitivity in response to behavioral weight loss interventions [12]; 5) Demonstration that bariatric surgery in individuals with obesity and T2D causes more robust decreases in BCAA and related metabolites than observed with behavioral intervention when the two groups are matched for the amount of weight loss [13]. Moreover bariatric surgery has a more pronounced effect to improve glycemic control than observed with dietary interventions; 6) Demonstration of elevated levels of BCAA and aromatic amino acids and a decrease in glycine in subjects that converted from normal glucose tolerance to T2D over a 5-year follow-up period in the Insulin Resistance Atherosclerosis Study (IRAS) cohort [14]; and 7) Demonstration that BCAA and related metabolites are correlated with cardiometabolic disease phenotypes other than obesity, insulin resistance, and T2D, including coronary artery disease, heart failure, and “metabolic wellness” [15–17].

In the seminal paper of Cahill and colleagues in 1969 [3], the authors suggested that the elevation of BCAA and aromatic amino acids in individuals with obesity was a consequence rather than a cause of insulin resistance — partly based on results of a glucose infusion study — in which obese and lean participants experienced a similar decrease in a discrete subset of circulating plasma amino acids, including valine,

leucine, isoleucine, phenylalanine, tyrosine, and threonine. However, this decrease occurred in the face of a much larger increase in insulin levels in the obese subjects. From this, they concluded that the rise in BCAA and aromatic amino acids in obese subjects was likely due to diminished insulin-stimulated utilization of these amino acids for protein synthesis. Subsequent studies also reported a diminished effect of insulin to suppress proteolysis in T2D patients [18,19]. Application of hyperinsulinemic, euglycemic, and isoaminoacidemic clamp methods revealed a decreased effect of insulin to stimulate protein synthesis in men and women with obesity relative to lean controls, an effect that was more pronounced in men, but not in women with T2D [18]. The sex-dependent findings of that study align with other studies demonstrating higher levels of BCAA and related metabolites in overweight/obese men compared to overweight/obese women [20]. Other groups have studied links between BCAA levels and insulin sensitivity in non obese participants. A study of Asian-Indian and Chinese men selected to have a BMI of approximately 24 kg/m² found a strong association of a BCAA-related principal component with insulin resistance measured by HOMA-IR, whereas other risk factors such as circulating free fatty acids or inflammatory cytokines were not associated with insulin resistance in this cohort [21]. These data indicate that the association of BCAA with insulin resistance is present even at a relatively low, fixed BMI, although it is recognized that a BMI of 24 — that places Western, Caucasian subjects in the lean category — is considered overweight in Asian subjects. Finally, a recent large study of Finnish men reported a strong association of BCAA and other amino acids with insulin resistance in a baseline cross-sectional

analysis [22]. Those associations were partially replicated in prospective analyses at 7.4 years of follow-up, but at that time point, multiple amino acids were also correlated with a decrease in insulin levels, an increase in fasting glucose levels, and increased risk for incident T2D [22].

Despite the many examples of associations of BCAA with metabolic disease phenotypes, no conclusions can be drawn about a potential causal role of BCAA in disease pathogenesis based on the associative metabolic data alone. Recent human genetic studies also present a complex picture. Three separate groups have reported strong associations of genetic variants affecting insulin sensitivity and lipid traits with BCAA levels [23–25]. However, genetic variants directly linked to circulating BCAA levels, including several variants found in or near the protein phosphatase m1K (*PPM1K*) gene (also known as *PP2Cm*), encoding the enzyme that dephosphorylates and activates the rate-limiting enzyme of BCAA catabolism, branched-chain ketoacid dehydrogenase (BCKDH) do not demonstrate “reverse causality” with insulin resistance in Mendelian randomization analyses [23–25]. Nevertheless, two of the three studies demonstrated that genetic variants associated with increased BCAA levels are linked with increased risk for development of T2D [23,24]. This has led to a proposed unifying model, in which increases in BCAA observed in prediabetic participants with obesity may occur mainly as a consequence of insulin resistance, but once elevated, BCAA could play a causal role in the conversion of prediabetes to full-blown T2D [24]. It should also be noted that several genetic mutations cause inborn errors of BCAA metabolism in humans, including mutations in various components of the BCKDH complex that cause Maple Syrup urine disease, characterized by large elevations in BCAA and BCKA levels. These inborn errors cause a much larger increase of BCAA and related metabolites compared to those observed in obesity-related metabolic diseases (many-fold increases compared to 40–50% increases), and are usually accompanied by a range of complex co-morbidities including developmental disturbances, neurological disorders, and ketoacidosis, making it difficult to parse out the impact of BCAA on core metabolic functions. Detailed reviews of these syndromes have been published elsewhere [26,27].

1.2. Factors affecting circulating BCAA levels

Elevations of circulating BCAA are driven by diverse effects of obesity within and between different tissues and organs. BCAA are transported into cells by an array of amino acid carriers, most prominently the large neutral amino acid carrier LAT1/LAT2/SLCA5/8 [28]. The first two steps of intracellular BCAA catabolism are shared by all three BCAA (Figure 1). BCAA are first deamidated by branched-chain aminotransferase (BCAT) to form their cognate BCKA, after which they may undergo irreversible oxidative decarboxylation by the mitochondrial enzyme BCKDH. BCKDH activity is determined by phosphorylation state. The BCKDH kinase (BCK) phosphorylates and inhibits BCKDH, whereas the PPM1K phosphatase dephosphorylates and activates BCKDH. In white adipose tissue (WAT) of rodent models of obesity and insulin resistance, obesity impairs BCAA catabolism by coordinating downregulation of multiple enzymes of the BCAA catabolic pathway at the transcriptional level, including BCAT and BCKDH [29–31]. Importantly, a similar transcriptional downregulation of BCAA catabolic enzymes is observed in WAT of human participants with obesity that is reversed by weight loss surgery, accompanied by a decrease in circulating BCAA [29,32]. In the liver, obesity and insulin resistance lead to minimal changes in BCKDH abundance. Instead, BCKDH activity

is mainly impaired by the induction of BCK and repression of PPM1K expression, which results in hyperphosphorylation of BCKDH and inhibition of its enzyme activity [29,33].

Several studies provide partial insight into the extent to which circulating BCAA and BCKA levels are regulated by their usage in different tissues. For example, adenovirus-mediated overexpression of the BCKDH phosphatase PPM1K in the liver of Zucker fatty rats (ZFR) results in a similar lowering of BCAA and BCKA levels as observed with the administration of BT2, the small molecule inhibitor of BCK, the BCKDH kinase [33]. Adenoviruses selectively target the liver when injected with IV, whereas BT2 inhibits BCK in all tissues. This suggests that the liver plays an important role in the regulation of circulating BCAA and BCKA levels, at least in the ZFR model of genetic obesity. Another study demonstrated that the transplantation of adipose tissue from normal mice into mice deficient in BCAA metabolism owing to knockout of BCAT2 — which is required in most peripheral tissues for transamination of BCAA — results in a 30–50% lowering of circulating BCAA levels; demonstrating a capacity of adipose tissue to make a substantive contribution to BCAA consumption [30]. Finally, recent studies suggest that obesity does not cause suppression of BCAA catabolism in skeletal muscle [5,33]. Tracing of stable isotope-labeled BCAA in mice suggests that muscle is a major site of BCAA oxidation, with further increases in BCAA catabolism in muscle and decreases in usage in liver and adipose observed in obese and insulin-resistant rodent models [34]. The obesity-associated increase in circulating BCAA and “spill over” into skeletal muscle have been proposed to contribute to mitochondrial overload and impairment in glucose and fatty acid oxidation [35].

BCAA are essential amino acids, which means that at least a subset of enzymes required for their biosynthesis are lacking in human (and other mammalian) tissues. Thus, their levels in tissues and in the circulation can be regulated by consumption in the diet, their usage rate for protein synthesis or oxidation (which can be influenced by genetic background as mentioned), and the rate of proteolysis and release of free amino acids, but not *de novo* biosynthesis in human tissues. Nonetheless, the enzymatic machinery required for *de novo* BCAA synthesis from simple substrates like pyruvate is present in some bacterial species found in the human gut microbiome. Moreover, recent transcriptomic and metabolomic studies demonstrate concerted upregulation of genes encoding BCAA biosynthetic enzymes and BCAA levels in the bacterial communities present in obese humans compared to lean humans [36]. Colonization of germ-free mice with gut microbial communities from humans participants with obesity increases body weight and levels of circulating BCAA and related metabolites compared to mice colonized with microbiota from lean individuals [36,37]. It is still unclear if the rise in host BCAA levels is caused by their biosynthesis by gut microbes and transport into the circulation, or by the effects of the obese microbiome to regulate host BCAA metabolism.

Finally, recent studies have provided evidence for the involvement of metabolic hormones other than insulin and glucagon in obesity-related dysregulation of BCAA catabolism. Adiponectin, which is low in insulin-resistant states, decreases circulating BCAA and BCKA by activating hepatic BCKDH through downregulation of BCK and upregulation of PPM1K [38]. In addition, insulin signaling in the brain induces expression of hepatic BCKDH, and short-term overfeeding in rats to cause central insulin resistance results in the inhibition of hepatic BCAA metabolism and increased circulating BCAA [39]. This central control is mediated through insulin signaling in agouti-related protein

(AgRP)—expressing neurons in the hypothalamus. Treatment of rats with the orexigenic hormone ghrelin, which stimulates AgRP neurons, is sufficient to raise circulating BCAA [39].

1.3. Findings from dietary BCAA supplementation studies in preclinical models

Some, but not all studies of BCAA supplementation in preclinical models provide evidence for a causal role of BCAA in the development of insulin resistance and other cardiometabolic disease phenotypes (reviewed in 1). For example, supplementation of BCAA to high fat (HF) diet fed to Wistar rats results in the full development of insulin resistance despite lower body weight gain than that experienced in rats fed with a control, non-BCAA supplemented HF diet, whereas rats pair fed with HF diet to the lower rate of intake observed in HF-BCAA supplemented rats are normally glucose tolerant [7]. However, feeding of rats with a standard chow diet supplemented with BCAA had no impact on body weight or insulin sensitivity, suggesting a diet context-dependent effect of excess BCAA on insulin action [7]. A long-term study (12–15 months) involving feeding of mice with low fat, BCAA supplemented diet resulted in hyperphagia, body weight gain, and shorter life-span; the hyperphagia was reversible by supplementation with tryptophan [40]. Similarly, tryptophan reversed the effects of BCAA supplementation of high energy diets to elicit anxiety-like behaviors in rats [41]. In both cases, BCAA supplementation reduced levels of tryptophan in the cerebral cortex, an effect reversed by dietary tryptophan supplementation. One explanation for these findings is that BCAA and aromatic amino acids such as tryptophan are transported in common by LAT1, both peripherally and across the blood–brain barrier, with the two groups of amino acids serving as competitive substrates [42]. Once in the brain, tryptophan can be metabolized through the kynurenine pathway by indoleamine 2,3-dioxygenase (IDO) or through the serotonin pathway by tryptophan hydroxylase. Interestingly, inhibition of serotonin re-uptake with fluoxetine blocked the hyperphagic response to BCAA supplementation [40], but not the effect of excess BCAA to promote anxiety-like behaviors [41]. Consistent with the latter result, BCAA supplementation lowered levels of metabolites in the kynurenine pathway, an effect reversed by tryptophan supplementation, suggesting that the anxiety-like behavior is mediated by metabolite(s) downstream of IDO rather than the serotonin pathway [42].

It should be noted that several studies that reported no effect of BCAA supplementation on disease phenotypes have been conducted with leucine alone, whereas studies consistent with causality have usually involved manipulation of all three BCAA, simultaneously. Supplementation with Leu alone causes the concentrations of the other two BCAA to decrease in the circulation, and thus does not mimic human disease states, in which all three BCAA are elevated [1]. Taken together, BCAA supplementation has complex effects on physiologic and behavioral phenotypes that require further study for complete understanding, including further analysis of species-specific differences, as discussed below.

1.4. Effects of dietary BCAA restriction

Preclinical studies on the effects of dietary BCAA restriction on physiological phenotypes are less complex and more consistent. BCAA restriction in obese rats, or in lean or obese mice, results in improved insulin sensitivity [5,43,44], accompanied in obese rats by improvements in insulin-stimulated glucose uptake and glycogen storage in skeletal muscle [5]. Moreover, feeding of a BCAA-restricted diet to Zucker fatty rats (ZFR) causes healthy changes in cardiac metabolism, including lowering of myocardial triglyceride levels and enhanced use

of fatty acids relative to glucose for oxidation and energy production [45]. Consistent with these effects, feeding of a BCAA restricted diet to ZFR also causes a decrease in whole animal respiratory exchange ratio (RER), which indicates an enhanced propensity to oxidize fat relative to glucose for energy production [5]. Suggesting that these results may be at least partially translatable to humans, a recent short-term (four week) trial of dietary BCAA restriction in human participants with well-controlled T2D demonstrated improvement in oral glucose sensitivity index and reduced postprandial insulin secretion; although insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp was not improved [46]. One salient difference between the BCAA restriction studies performed in obese rodent models and those in human T2D participants was the duration of the dietary intervention; 10–15 weeks in the rodent studies and 4 weeks in the human studies. Further investigation of responses to longer duration of BCAA restriction in obese humans could be informative.

1.5. Therapeutic impact of pharmacologic or genetic activation of BCKDH

Another approach for investigating the causality of BCAA in metabolic disease phenotypes is to alter their systemic metabolism with molecular or pharmacologic tools. Such studies have been greatly aided by the development of small molecule BDK inhibitors by David Chuang and associates [47,48]. Administration of the most potent of these inhibitors, 3,6-dichlorobenzo(b)thiophene-2-carboxylic acid (BT2), to obese, insulin-resistant rodents (both genetically obese ZFR and DIO mice) resulted in reduced phosphorylation and increased enzymatic activity of BCKDH in multiple tissues, a lowering of circulating BCAA and BCKA levels, and a clear improvement in glucose tolerance accompanied by lowering of insulin concentrations, signifying improved insulin sensitivity [33,49]. A similar phenotype was obtained in response to adenovirus-mediated overexpression of PPM1K in the liver of ZFR [33]. Moreover, one week of BT2 administration or hepatic PPM1K overexpression caused an approximate 50% decrease in liver triglyceride levels in ZFR, an extreme model of hepatic steatosis. BT2 treatment also caused a decrease in RER, indicative of an effect of the drug to enhance propensity for fatty acid oxidation [33]. Thus, manipulation of the BDK:PPM1K ratio to favor dephosphorylation and activation of BCKDH increases the disposal of BCAA and BCKA and improves both glucose and lipid homeostasis.

As a cautionary note, it can be pointed out that although BT2 was developed as a BDK allosteric site binding ligand based on the crystal structure of the enzyme [47,48], its specificity for BDK relative to other kinases has not been extensively evaluated or reported. Thus, the effects of BT2 on metabolism and disease phenotypes could possibly be mediated by effects on kinases other than BDK. Tempering this concern, phosphoproteomics studies demonstrated that the number of liver proteins exhibiting a significant change in phosphorylation in response to the treatment of ZFR with BT2 for one week was quite limited relative to vehicle-treated controls (a total of 12 phosphopeptides from 8 proteins). Also, whereas the E1 component of BCKDH was one of the proteins with reduced phosphorylation, BT2 did not change the phosphorylation status of the structurally similar E1 component of the pyruvate dehydrogenase complex, which is regulated by kinases similar to BDK [33]. Also, BDK itself has been reported to undergo phosphorylation at Ser31 hepatocellular carcinoma to alter its function [50], but no changes in phosphorylation of this site were detected in the liver samples of ZFR treated with BT2 relative to vehicle-treated [33]. In addition, the effects of BT2 administration to lower BCAA and BCKA levels, improve glucose metabolism, and lower liver fat in ZFR were recapitulated by overexpression of the BCKDH

phosphatase and PPM1K — demonstrating that an orthogonal approach for altering the BDK:PPM1K ratio led to similar changes in physiologic phenotypes. Nevertheless, the impact of off-target effects of BT2 on physiologic outcomes remains a formal possibility and should be considered in interpreting available data.

1.6. Emergence of a regulatory node linking BCAA, carbohydrate, and lipid metabolism

Another recent study has revealed a regulatory node that links carbohydrate, lipid, and BCAA metabolism that may help to explain the strong associations of BCAA levels with cardiometabolic disease phenotypes [33]. Feeding of diets that combine high levels of fat and carbohydrate, particularly when the dietary sugar is sucrose (a dimer of glucose and fructose) or fructose alone, promotes obesity, insulin resistance, and type 2 diabetes in rodent models [51]. A key transcription factor regulating sucrose- or fructose-mediated metabolic responses is the Carbohydrate Response Element Binding Protein (ChREBP), particularly a constitutively active product of the gene expressed from an alternative promoter termed ChREBP- β [51]. A recent study identified a ChREBP binding element in the enhancer region of the BDK gene that is present in humans, nonhuman primates, and rats, but not in mice [33]. Refeeding of fasted rats with a fructose-supplemented diet compared to a standard chow/low fructose diet caused a large induction of ChREBP- β and several of its classical lipogenic and glycolytic target genes in the liver, including pyruvate kinase (PKLR), ATP-citrate lyase (ACLY), and fatty acid synthase (FASN). Importantly, refeeding of the high fructose diet also caused a clear increase in the expression of BDK and a reciprocal decrease in the expression of PPM1K relative to standard chow feeding [33]. Supporting a role for ChREBP- β in mediating these effects, adenovirus-mediated overexpression of ChREBP- β in liver of normal rats increased BDK and suppressed PPM1K mRNA levels. Also, ChREBP- β and BDK mRNA levels were strongly correlated in liver biopsies from human subjects with nonalcoholic fatty liver disease (NAFLD) [33]. These studies demonstrate that feeding of diets enriched in sucrose or fructose drives ChREBP- β -mediated changes in the BDK:PPM1K ratio to favor increased phosphorylation and inactivation of BCKDH, thus potentially contributing to obesity-linked increases in BCAA and BCKA levels.

Further insight was gained from phosphoproteomics analysis of liver samples from the aforementioned ZFR studies involving one week of daily injections of BT2 or vehicle, or treatment with adenoviruses expressing PPM1K or green fluorescent protein (GFP) as a control. TMT labeling/LC-MS/MS phosphoproteomics was used to demonstrate reduced phosphorylation of Ser454 in a peptide from the ACLY protein in the livers of ZFR from both the BT2 versus vehicle and the PPM1K versus GFP adenovirus studies; findings confirmed by immunoblot analysis with a p-ACLY-specific antibody [33]. Phosphorylation of ACLY on Ser454 has been reported to increase the activity of the enzyme [52], suggesting that a decrease in phosphorylation mediated by the inhibition of BDK with BT2 or overexpression of PPM1K would lower enzyme activity. ACLY catalyzes the cleavage of citrate to form acetyl CoA and oxaloacetate in the cytoplasm, with the former contributing to the formation of malonyl CoA, which inhibits carnitine palmitoyltransferase-1 (CPT-1) to slow fatty acid oxidation, but also serving as a substrate for *de novo* lipogenesis. The findings of reduced liver triglyceride levels and a decrease in whole animal RER in response to BT2 treatment are consistent with these effects.

Demonstrating that increased expression of BDK is sufficient to impact lipid metabolism, adenovirus-mediated overexpression of BDK in liver of normal rats resulted in increased phosphorylation of ACLY accompanied by a 2.5-fold enhancement in *de novo* lipogenesis as measured

by the incorporation of deuterium from $^2\text{H}_2\text{O}$ into newly synthesized palmitate in the liver [33]. Overall, these recent findings outline a regulatory node through which BDK and PPM1K — thought of primarily as a kinase/phosphatase pair dedicated to the regulation of BCAA metabolism — also contribute to the control of lipid homeostasis by regulating the activity of the key lipogenic enzyme ACLY. These findings suggest that feeding of diets enriched in lipids and sucrose/fructose not only drives ChREBP-mediated activation of genes directly involved in *de novo* lipogenesis — such as ACLY and FASN to contribute to hepatic steatosis and hyperlipidemia — but also promotes post-translational modification and activation of one of the key enzymes in the pathway, ACLY. Through this mechanism, elevated circulating BCAA and BCKA as observed in obesity may be reporting on underlying alterations of lipid homeostasis.

It is noted that some of the prior inconsistency in preclinical studies from different laboratories investigating links between BCAA and physiologic phenotypes, such as insulin sensitivity, may relate to the finding that the ChREBP binding element in the enhancer region of the BDK gene is present in humans, nonhuman primates, and rats, but not in mice. This suggests that mice may lack a key regulatory node that links BCAA, lipid, and carbohydrate metabolism, complicating interpretation of BCAA effects in this background. Consistent with this idea, a recent study reported that feeding of mice with a high fat, high sucrose diet [53] resulted in a different pattern of responses than observed in rats fed with a high fructose diet [33]; with the mice exhibiting no change rather than an increase in hepatic expression of BDK, an increase rather than a decrease in PPM1K expression, no change in phospho-BCKDH, and an increase in phospho-ACLY relative to mice fed with a standard chow diet. Also in the mouse study, supplementation of all three BCAA in drinking water consumed by animals fed with high fat diets caused no additional impairment in insulin action relative to the feeding on HF diets alone. In other studies, supplementation of BCAA in high fat diet in rats [7] or supplementation of high fat diet with leucine alone in the drinking water in mice [54] caused a chronic increase in phosphorylation of the mTOR substrate p70S6K in both cases. However, in the rat study, BCAA supplementation exacerbated glucose intolerance, an effect ameliorated by administration of the mTOR inhibitor rapamycin [7], whereas leucine supplementation in mice actually improved glucose homeostasis despite the activation of mTOR [54]. Further studies on the species-specific effects of sucrose/fructose-enriched and BCAA-supplemented diets on physiologic phenotypes and metabolic homeostasis are required.

1.7. Mechanisms by which elevations in BCAA can alter metabolic disease phenotypes

Further studies are required in order to fully understand how BCAA or BCKA levels could play a causal role in the development of insulin resistance and other cardiometabolic disease phenotypes. One promising area of investigation is focused on connections between the content of BCAA in the diet and lipid metabolism [5,33,35]. For example, the high levels of a broad array of fatty acid-derived acyl CoA species found in skeletal muscle of ZFR fed on standard chow diet are lowered to levels found in lean rats by the feeding of a BCAA-restricted standard chow diet [5]. This is relevant because elevations in fatty acid-derived acylcarnitine and acyl CoA species in muscle have been reported in multiple animal models of obesity, and decreasing the levels of these metabolites by molecular manipulation of lipid metabolizing enzymes such as malonyl CoA decarboxylase and carnitine acyltransferase (CrAT) is sufficient to ameliorate insulin resistance [55,56]. The accumulation of acyl CoA and acylcarnitine species has been ascribed to incomplete fatty acid oxidation and mitochondrial fuel

overload [57]. Lowering of muscle acyl CoA levels in response to dietary BCAA restriction is consistent with the idea that elevated BCAA contribute to “clogging” of mitochondria with excess substrate, leading to impaired oxidation of lipid substrates [35]. Possibly contributing to these salutary effects on lipid homeostasis, BCAA restriction is known to activate general control non-derepressable 2 (GCN2) to suppress protein synthesis and conserve amino acids by inhibiting the protein translation factor eIF2 α , and GCN2 is also reported to suppress expression of lipogenic genes [28,58]. Another mechanism that may facilitate muscle lipid clearance in response to feeding of a BCAA-restricted diet is normalization of muscle glycine levels, which promotes formation of acyl-glycine adducts that can leave the cell to be excreted in the urine, thus lowering tissue acyl CoA levels. Consistent with this idea, muscle acyl CoA levels are lowered, and urine acyl-glycine adducts are increased in response to feeding of BCAA restricted diets to ZFR [5].

A recent study has provided an explanation for the consistent observations of an inverse relationship between BCAA and glycine levels in humans and animal models of obesity [59], as summarized schematically in Figure 2. The first step in BCAA catabolism is transamination catalyzed by BCAT to yield the three corresponding BCKA. In this reaction, the amino group

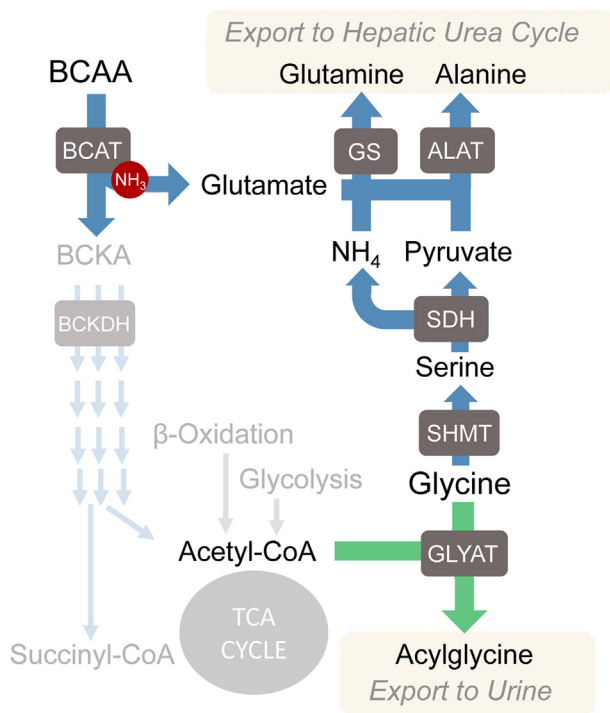


Figure 2: Mechanism by which chronic elevations in BCAA drive glycine depletion. Increases in BCAA are associated with glycine depletion in humans and preclinical models of obesity and insulin resistance. A mechanism contributing to this reciprocal relationship is demonstrated where increased transamination of BCAA by branched-chain aminotransferase (BCAT) generates glutamate as a nitrogen sink. To dissipate the nitrogen load, the glutamate nitrogen is transferred to substrates that can exit the skeletal muscle to engage in hepatic urea cycle and gluconeogenic pathways, including glutamine formed by glutamine synthase (GS), and alanine produced by alanine transaminase (ALAT). Increased flux through these pathways caused by chronic elevations of BCAA depletes pyruvate, which is replenished by the conversion of glycine to serine and pyruvate by the actions of serine hydroxymethyltransferase (SHMT) and serine dehydratase (SDH), ultimately resulting in decreased tissue and serum glycine levels. Low glycine levels may contribute to mitochondrial lipid overload by limiting acyl-CoA export as acyl-glycine adducts. Experimental evidence for this model is provided in references 5 and 53.

removed from BCAA is transferred to a ketoacid acceptor, α -ketoglutarate, to yield glutamate. In obesity, chronic expansion of the BCAA pool increases flux of these substrates through the BCAT reaction, especially in tissues such as muscle that express high levels of the enzyme [60]. This results in an increased tissue nitrogen load, which is dissipated by using glutamate to drive other transamination reactions to yield products that exit muscle cells, including alanine formed from pyruvate by alanine transaminase, and aspartate formed from oxaloacetate by aspartate transaminase. Because of chronic elevations in BCAA levels, active flux through these pathways depletes pyruvate, which can be replenished from glycine by the actions of serine dehydratase and serine hydroxymethyl transferase (Figure 2). Moreover, the free NH_4 liberated by deamination of serine in the serine dehydratase reaction can be used to amidate glutamate to form glutamine, which can readily exit muscle cells. Thus, by providing both the pyruvate carbon skeleton that can be transaminated to alanine and free NH_4 that can be used to convert glutamate to glutamine, each molecule of glycine can be used to export two molecules of NH_3 from skeletal muscle. Consistent with the idea that elevated BCAA exert a “pull” on glycine levels to replenish pyruvate for use in the alanine transaminase reaction, treatment with a specific BCAT inhibitor restores muscle and plasma glycine levels in ZFR [59]. Also, stable isotope tracer studies confirm efficient labeling of skeletal muscle alanine, aspartate, and glutamate from a bolus of ^{15}N -labeled valine. Finally, infusion of U- ^{13}C glycine labels the plasma serine, pyruvate, and alanine pools, and co-infusion of unlabeled valine induces a sharp increase in the labeling of alanine, providing a direct demonstration that glycine serves as a carbon donor for the pyruvate/alanine cycle in a BCAA-regulated manner [59]. Through this mechanism, high levels of BCAA contribute to the lowering of glycine levels in obesity-related conditions.

In addition to these effects on glycine and acyl-glycine excretion, excess BCAA exposure can promote excessive lipid storage and impaired insulin action through the generation of the valine metabolite 3-hydroxyisobutyrate (3-HIB), which has been reported to activate trans-endothelial fatty acid transport to contribute to tissue lipotoxicity and impairment of insulin action [6]. However, in studies using BT2 to activate BCKDH, a treatment that raises 3-HIB levels [34], insulin resistance was attenuated rather than exacerbated, with no changes in lipid-derived metabolites in skeletal muscle [33]. Studies of longer duration will be required to fully investigate this mechanism.

In contrast to the proposed pathogenic effects of 3-HIB on insulin sensitivity and fatty acid metabolism, other BCAA-derived metabolites have beneficial effects. Levels of the valine-derived metabolite, β -aminoisobutyric acid (BAIBA), are elevated in response to exercise or overexpression of PGC-1 α in skeletal muscle, and BAIBA has been reported to serve as an activator of thermogenesis that is negatively correlated with HOMA-IR, circulating triglycerides, and cholesterol [61]. The extent to which valine is metabolized to 3-HIB or BAIBA under different physiologic and pathophysiologic conditions remains to be fully characterized [1].

1.8. Emergent regulatory roles of BCAA catabolism in adipose tissue biology

BCAA have recently been ascribed a role in differentiation of adipocytes, and as substrates for biosynthesis of odd chain and monomethyl branched-chain fatty acids (mmBCFA) in white adipose tissue (WAT) [62]. Catabolism of BCAA to their branched-chain acyl CoA-modified intermediates by mitochondrial BCKDH creates substrates for the synthesis of mmBCFA, in a process requiring interconversion of CoA- and carnitine-modified acyl intermediates by CrAT to facilitate their egress from the mitochondrial matrix to engage with cytosolic FAS. Interestingly, levels of mmBCFA are significantly decreased in WAT of

mice with diet-induced obesity [62], and this decrease occurs in concert with global suppression of transcripts encoding BCAA catabolic enzymes [29–31]. Moreover, opposite to BCAA and BCKA, but consistent with their lower levels in WAT of obese animals, circulating levels of mmBCFA are negatively correlated with insulin resistance and other cardiometabolic disease biomarkers such as C-reactive protein in human participants [62]. The potential roles of mmBCFA in the maintenance of normal WAT function and metabolic health remain to be explored.

Beyond its functions in WAT, critical roles for BCAA catabolism in brown adipose tissue (BAT) have recently emerged. Cold exposure in mice activates BCAA usage in BAT, and specific knockout of BCKDH in mouse BAT results in impaired thermogenesis and cold acclimation [63]. The knockout of BCKDH in BAT also increases circulating BCAA levels, and consistent with its effect to impair thermogenesis, results in increased weight gain, hepatic steatosis, glucose intolerance, and insulin resistance. The acute induction of BCAA usage in BAT in response to cold exposure provided an opportunity to identify new mediators of BCAA catabolism, including the long-hypothesized mitochondrial BCAA transporter that allows BCAA to gain access to the mitochondrial isoform of BCAT (BCAT2, the predominant form in most tissues), and mitochondrial BCKDH. The solute carrier transporter protein, SLC25A44, was found to be induced in BAT in response to cold exposure, and subsequent loss of function and overexpression studies validated its role as a mitochondrial BCAA transporter [63]. Although the role of BAT in metabolic disease pathogenesis is not as well established in humans as in mice, it is noteworthy that metabolomic analyses in cold-exposed humans with high versus low BAT activity revealed an inverse correlation of BCAA levels with BAT [63]. These studies introduce BCAA metabolism in BAT and a new molecular regulator of BCAA metabolism, SLC25A44, as factors to be considered in future studies about the role of BCAA in metabolic disease pathogenesis.

1.9. Possible roles of BCAA and BCKA in a broader array of cardiometabolic diseases

There are also indications that changes in BCAA/BCKA availability and metabolism could influence a broader array of cardiometabolic disease

phenotypes. Thus, feeding of ZFR with a BCAA-restricted diet induces a shift from glucose to fatty acid catabolism, as measured by stable isotope flux measurements in isolated perfused hearts [45]. In addition, the elevated levels of triglyceride found in the hearts of ZFR are reduced to the levels found in lean animals by feeding BCAA-restricted diet. Other studies have revealed that mice with whole body knockout of PPM1K develop a more severe heart failure phenotype in response to pressure overload compared to wild-type mice, whereas suppression of BDK by BT2 administration improves cardiac function [64]. It is still to be determined if these effects of whole body knockout of PPM1K or global suppression of BDK activity with BT2 are influencing cardiac functions — by altering cardiac BCAA metabolism or working indirectly through their potent effects to lower circulating BCAA and BCKA levels. Consistent with the latter model, a recent study reports that branched-chain keto acids (BCKA) are preferentially reaminated to BCAA in the heart, and perfusion of the working heart with levels of BCKA found in obesity causes cardiac phosphoproteomic remodeling to activate total protein synthesis [65]. These findings suggest that elevations of BCKA as found in obesity could contribute to unhealthy cardiac hypertrophy by chronic activation of protein synthesis and cardiac remodeling. More generally, the study provides a clear example of direct effects of BCAA-derived metabolites on tissue function. Finally, another connection between emergent diabetes, BCAA metabolism, and cardiac function resides in the finding that glucose suppresses BCAA catabolism in cardiomyocytes by reducing expression of the KLF15 transcription factor, a global activator of genes encoding BCAA catabolic enzymes [66]. The resultant accumulation of BCAA is suggested to activate mTORC1 to drive protein synthesis and cardiac hypertrophy, thus potentially contributing to diabetes-related cardiomyopathy.

2. SUMMARY AND CONCLUSIONS

BCAA and related metabolites are now well established as among the strongest biomarkers of an array of cardiometabolic diseases and related conditions, including obesity, insulin resistance, T2D, and coronary artery disease [1,2]. There is evidence to suggest that changes in body mass and function in the early stages of development of T2D and other cardiometabolic diseases contributes to the rise in

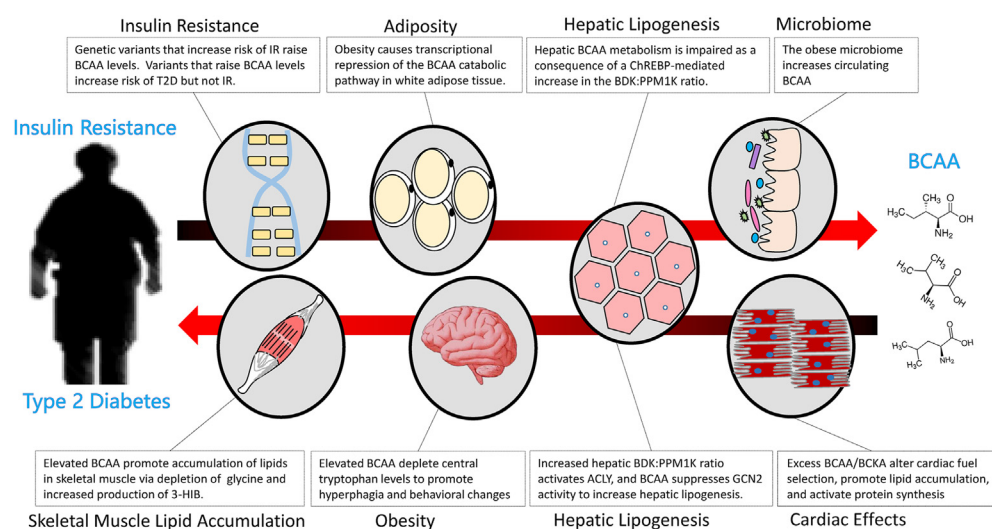


Figure 3: BCAA, insulin resistance, and type 2 diabetes: a two-way street. In the early stages of development of type 2 diabetes (T2D), obesity and insulin resistance contribute to elevations in BCAA levels through the mechanisms summarized by panels in the top half of the figure, reading left to right. Once elevated, BCAA can in turn contribute to the development of disease phenotypes through the mechanisms summarized by panels in the bottom half of the figure, reading right to left.

BCAA and their catabolic products (see Figure 3 top panels, left to right for summary). Thus, genetic variants that predispose to insulin resistance are associated with an increase in BCAA levels, whereas variants that specifically raise BCAA levels increase the risk for T2D, but not insulin resistance. Obesity also results in a general repression of the BCAA catabolic pathway in adipose tissue at the transcriptional level, while suppressing the use of hepatic BCAA via an increase in the BDK/PPM1K ratio, resulting in phosphorylation and inactivation of BCKDH. A further increase in BCAA in obesity appears to be driven by the microbiome of obese individuals. Once BCAA are elevated through the foregoing effects, BCAA metabolism contributes to the development of disease phenotypes to create a “two way street” (see Figure 3, bottom panels, right to left for summary). Increases in BCAA and BCAA alter cardiac fuel selection, promote lipid accumulation, and cause phosphoproteome remodeling and activation of protein synthesis. Similarly, increased BCAA levels impair lipid oxidation and enhance lipid accumulation in skeletal muscle, mediated by BCAA-regulated lowering of muscle glycine levels, and increases in the valine metabolite 3-HIB, which stimulates trans-endothelial fatty acid uptake. Supporting these mechanisms, feeding of a BCAA-restricted diet or manipulation of the BDK/PPM1K ratio to activate BCKDH has clear salutary effects on glucose and lipid homeostasis in obesity and insulin-resistant rodent models, although the effects of BCAA restriction in short-term human studies are more modest. Feeding of obesogenic diets that include sucrose or fructose cause induction of transcription factors such as ChREBP and SREBP to upregulate lipogenic pathways, and ChREBP- β also increases the BDK/PPM1K ratio to suppress BCKDH and activate ACLY, thus contributing to hepatic steatosis. An increase in BCAA also inhibits GCN2 to block its effect to induce lipogenic gene expression. Finally, long-term supplementation of BCAA in chow or high energy diets regulates food intake, life-span, and anxiety-like behaviors. Further study will be required to fully understand the impact of elevated BCAA levels on disease phenotypes in preclinical models and human subjects. This has become an active area of research, and new insights are likely to emerge soon.

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CONFLICT OF INTEREST

None declared.

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