Branched-Chain Amino Acids as Critical Switches in Health and Disease

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The aromatic amino acids (AAAs) histidine, phenylalanine, tryptophan, and tyrosine have a side chain with an aromatic ring and are precursors of dopamine, epinephrine, norepinephrine, serotonin, and thyroxine. The branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, share a structurally similar side chain.[1,2] Together with lysine, the AAAs and BCAAs are essential amino acids (EAAs), meaning that mammals, including humans, must meet their metabolic needs via a sufficient dietary intake. This brief review highlights how BCAAs play a key role as gatekeepers feeding into upstream and downstream molecular pathways and in this way are major metabolic regulators involved in the pathophysiology of disease. Maple syrup syndrome, characterized by neurotoxicity, motor disturbances, mental retardation, and premature death, rests on dysregulation of the BCAA catabolism. The disease has a Mendelian inheritance[2] and falls outside the scope of this review.

Metabolism

Gastrointestinal and Cellular Uptake of BCAAs

BCAAs are neutral (nonpolar and hydrophobic) amino acids. System L is the major Na^+-independent system in the intestinal basolateral membrane responsible for the uptake of neutral amino acids.[3] It is expressed as 2 different isoforms: SLC7A5 also known as LAT1 (L-type amino acid transporter 1) and SLC7A8 also known as the LAT2 (L-type amino acid transporter 2). Both belong to the SLC7 (solute carrier family-7) gene family and require SLC3A2 (a heavy chain subunit), which brings the light subunit (SLC7A5 or SLC7A8)—the actual transporter—to the apical cell membrane.[3] LAT1 and LAT2 are obligatory amino acid exchangers with a 1:1 stoichiometry and transport large neutral amino acids.[4] LAT2 has a broader substrate selectivity range than LAT1, also accepting smaller amino acids.[4] SLC7A5 regulates the simultaneous efflux of L-glutamine out of cells and the influx of L-leucine and other EAAs into cells, thereby activating downstream signaling, for instance, via mTOR (mammalian target of rapamycin) kinase.[5,6] The primary BCAA transporter in the gut is LAT2.[3] However, both LAT1 and LAT2 are ubiquitously expressed throughout the human body, in particular, in organs that play an important role in the homeostasis or usage of BCAAs, including the kidney, liver, brain, heart, lung, and muscle.

Recent studies integrating the serum metabolome, the gut microbiome, and clinical phenotypes show that gut microbiota play an important role in regulating the bioavailability of BCAAs in the intestine and their transport through the gut wall.[7] Several observations underpin this concept. First, in humans, the serum concentrations of BCAAs, bacterial BCAA biosynthesis, and BCAA transport through the intestinal wall are functionally interrelated.[7] Second, insulin resistance—a phenotype associated with the BCAA level[5]—is also correlated with circulating bacterial cometabolites.[7] Third, specific microbial species, including *Prevotella copri* and *Bacteroides vulgatus* are the main drivers of the association between the intraintestinal biosynthesis of BCAAs and insulin resistance.[7] Finally, in mice, *P. copri* can augment circulating levels of BCAAs, induce insulin resistance, and aggravate glucose intolerance.[7]

BCAA Catabolism

A large proportion of BCAAs from dietary sources is absorbed from the intestine, but bypasses the liver, and is directly delivered to the peripheral tissues.[8] BCAAs account for >50% of the splanchnic output of amino acids, even if they only constitute 20% of the ingested protein source.[9] Although the enzymatic machinery for BCAA metabolism is active in the liver, BCAA catabolism occurs mainly in other tissues, including skeletal muscle (50%), heart, adipose tissue, renal tubular cells, and neurons.[1]

The initial step in BCAA catabolism (Figure 1) consists of a transamination catalyzed by a BCAT (branched-chain amino acid transferase)—a readily reversible reaction that yields the corresponding branched-chain α-keto acid (BCKA).[1] The human BCAT exists as 2 isoenzymes, encoded by separate genes and, respectively, expressed in the cytosol (BCAT1) and in mitochondria (BCAT2).[10–12] The mitochondrial isoenzyme also functions as the branched-chain α-keto transport protein, which determines the rate of efflux of BCKAs from muscle tissue, when perfused with BCAAs.[11,13] BCKAs are irreversibly catabolized by the BCKA dehydrogenase complex (BCKDC)—a multiprotein enzyme

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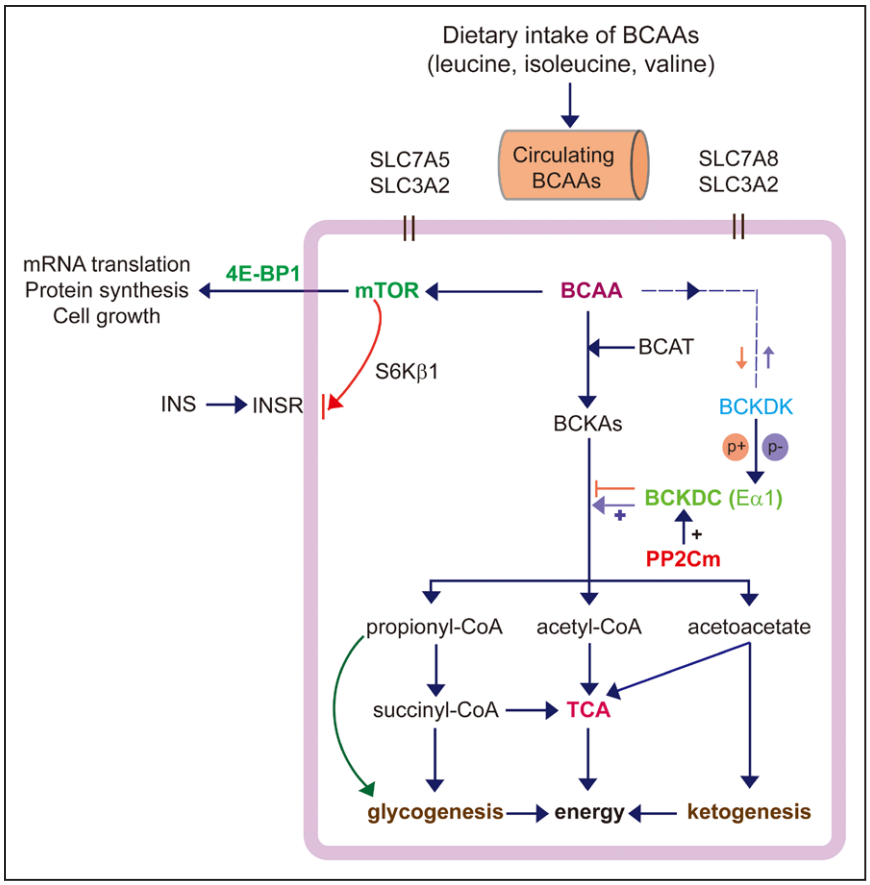

Figure 1. Catabolic pathways of branched-chain amino acids (BCAAs) and downstream signaling initiated by catabolites (for explanation, see BCAA Catabolism section). 4E-BP1 indicates eukaryotic initiation factor 4E-binding protein 1; BCAT, branched-chain amino acid aminotransferase; BCKA, branched-chain keto acid; BCKDC, branched-chain α-keto acid dehydrogenase complex; BCKDK, branched-chain keto acid dehydrogenase kinase; INS, insulin; INSR, insulin receptor; mTOR, mammalian target of rapamycin; p+/p–, phosphorylation/dephosphorylation; PP2Cm, mitochondrial protein phosphatase; SK6β1, ribosomal protein S6 kinase β1; SLC3A2, solute carrier family 3 member 2; SLC7A5, L-type amino acid transporter 1; SLC7A8, L-type amino acid transporter 2; and TCA, tricarboxylic acid cycle.

composed of 3 subunits: E1, E2, and E3.[1,12,14] The E1 subunit of the BCKD complex (BCKDC) is a tetramer composed of 2 α-components and 2 β-components (α2β2).[12] BCKDC has a molecular mass close to 4×10^6 Da and an overall diameter of ≈400 Å.[14] It belongs to the mitochondrial α-keto acid dehydrogenase family, including also pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which are key enzymes that function in the tricarboxylic acid cycle (TCA), also known as the Krebs cycle.[14] Two proteins are attached to the E1 subunit: a PP2Cm (mitochondrial protein phosphatase) and a BCKDK (BCKA dehydrogenase kinase). They function as key regulators of BCKDC activity, which is also allosterically modulated by the availability of substrates (Figure 1). PP2Cm is a required cofactor for the catabolism of BCKAs and activates the BCKDC complex.[12,15] The BCKDK-mediated reaction is the rate-limiting step in the BCAA catabolic pathway. BCKDC expression and activity depend on phosphorylation status of its regulatory subunit E1α (Figure 1), catalyzed by BCKDK.[12,14] When the BCAA level is low, E1α is hyperphosphorylated, leading to inhibition of BCKDC activity and preservation of free BCAAs, whereas when the BCAA level is high, E1α is dephosphorylated, leading to BCKDC activation and reduction of the total BCAA levels.[16] The terminal metabolites of isoleucine catabolism are propionyl-CoA and acetyl-CoA (Figure 1). The isoleucine degradation products are, therefore, both glycogenic and ketogenic.[1] Leucine yields acetoacetate and acetyl-CoA and is ketogenic.[1] Valine yields succinyl-CoA and is glycogenic.[1] Acetyl-CoA can enter the TCA[15] and acetoacetate and succinyl-CoA are tricarboxylic acid intermediates, so that the terminal BCAA catabolites contribute to energy generation (Figure 1).

The activity of BCKDC across organs varies directly with the mitochondrial tissue content and with the subcellular distribution of BCAT between the mitochondrial and cytosolic compartments.[10] The tissue distribution of BCKDK in mice by Western blot[17] and in rats by mRNA detection[18] shows similar patterns. Skeletal muscles contained the highest concentration of BCKDK.[17,18] BCKDK in the liver was below the detection limit.[17,18] Heart,[17,18] kidney,[17,18] lung,[18] and small intestine[18] had intermediate levels with lower levels also being detectable in the brain[17,18] and eye.[18] In humans,[19] kidneys have the highest BCKDC activity, followed by liver, brain, heart, colon, skeletal muscle, and stomach. Subcutaneous adipose tissue and small intestine showed the lowest BCKDC activity.[19] In line with the metabolic role of BCAAs, obese women, compared with nonobese controls, had lower expression and protein levels of BCAA-catabolizing enzymes in visceral adipose tissue.[20]

Function

BCAAs fulfill 3 distinct roles. First, they provide building blocks for protein synthesis and promote protein synthesis. Second, when catabolized via the TCA (described above), they act as a fuel source. Finally, they stimulate cell signaling via activation of mTOR, which uncouples insulin signaling[5] and regulates protein translation, thereby balancing cell growth and autophagy (Figure 1).

As protein building blocks, BCAAs account for ≈35% of the indispensable amino acids in muscle proteins and 40%

of amino acids required for protein synthesis in mammals. BCAAs feed into the aminoacyl-tRNA biosynthesis.[21] The enzyme aminoacyl-tRNA synthetase activates binding of an amino acid to tRNA to form an aminoacyl-tRNA ester. This is the crucial step in protein synthesis by determining how the genetic code is interpreted as an amino acid sequence.[21] BCAAs are among the most hydrophobic of amino acids. Generally, the interior of water-soluble globular proteins consists, largely, of hydrophobic amino acids, principally leucine, isoleucine, valine, phenylalanine, and methionine.[8,22] This configuration is important for the stability of the folded proteins and their function.[8,22,23]

Because of their poor hepatic catabolism, circulating BCAAs, in particular, leucine, act as signaling molecules and nutritional sensors. They promote protein synthesis, cellular metabolism, and cell growth in an mTOR-dependent manner,[24–26] thereby uncoupling insulin signaling (Figure 1). In this way, BCAAs are essential for normal growth and function at cellular and organism level.

BCAAs in Health and Disease

Our literature review highlighted the multifaceted role of BCAAs in various organ systems.

Insulin Resistance

The common feature of metabolic syndrome and type-2 diabetes mellitus (T2DM) is an impaired glucose-insulin homeostasis, commonly on a background of obesity.[20,27] As evidenced by experiments in healthy volunteers,[27,28] EAAs stimulate the insulin secretion without effect on insulin clearance from the blood. Glucose ingestion after an overnight fast triggers an insulin-dependent homeostatic response, which involves the 4 key axes of insulin action: proteolysis, lipolysis, ketogenesis, and glycolysis.[29] Among prediabetic patients, the responses along all 4 axes are blunted,[29] with increases in circulating lactate (marker of glycolysis) and declines in glycerol (lipolysis), leucine/isoleucine (proteolysis) and β-hydroxybutyrate (ketogenesis).[29] Several cross-sectional,[30–39] longitudinal,[40–47] and genetic association[48] studies supported the experimental studies[27,28] showing interference of BCAAs with insulinotropic actions.

Cross-Sectional Studies

In the cross-sectional INTERMAP project (International Study of Macro/Micronutrients and Blood Pressure), the risk of stroke and cardiovascular complications was higher in Northern (Beijing and Shanxi) compared with Southern (Guangxi) China.[49] A further metabolome-wide association study also revealed a higher urinary excretion of BCAAs in Northern China.[30] This led the INTERMAP investigators to speculate that dietary factors and endogenous metabolic influences, partly emanating from the gut flora,[7] might underlie the higher cardiovascular risk in the north.[30]

Several other cross-sectional studies reported positive associations[31–39] of circulating BCAAs with a high cardiovascular risk profile,[34] hypertension,[31,38] or with metabolic dysregulation, including an elevated body mass index,[20,35] central obesity,[20,35,38] impaired fasting glucose,[31,33,39] insulin resistance estimated from the Homeostatic Model Assessment (HOMA-IR) index,[31,32,35] the metabolic syndrome,[33,34,36–38] diabetes mellitus,[38] or dyslipidemia.[35,38] These cross-sectional associations[30–39] were replicated across diverse ethnic groups, women and men, a wide age range, obese and nonobese people, and in population and patients.

Longitudinal Studies

Moving from cross-sectional[30–39] to longitudinal[40–47] studies, in the Framingham Heart Study,[40] 2422 nondiabetic subjects underwent a routine examination between 1991 and 1995, of whom 201 developed new-onset diabetes mellitus during a 12-year follow-up period and of whom 189 could be propensity matched with controls from the same baseline examination who did not develop diabetes mellitus. Cases and controls had a similar sex distribution (42% women), age (56.5 years), body mass index (30.2 kg/m^2), and fasting glucose (105 mg/dL). The risk of developing diabetes mellitus was greater with higher baseline plasma levels of the 3 BCAAs (isoleucine, leucine, and valine) and 2 AAAs (tyrosine and phenylalanine). When compared with single amino acids, combinations of 3 amino acids further improved the prediction of diabetes mellitus with increments in the −2 log likelihood ratio from 6 to 9 ($P \leq 0.035$).[40] In an independent replication sample, from the Malmö Diet and Cancer study consisting of 163 cases and 163 controls (55% women; mean age, 58 years), 4 of the 5 individual amino acids (leucine, valine, tyrosine, and phenylalanine) were significantly associated with incident diabetes mellitus (adjusted odds ratios per 1-SD increment were similar to Framingham, 1.37–2.01; $P \leq 0.04$). The remaining amino acid, isoleucine, had a nonsignificant association in the replication study ($P=0.09$).[40] One potential limitation of these Framingham findings is that they might not be generalizable to a low-risk population sample.[50]

After the 2011 Framingham report,[40] a systemic review[51] identified 7 other prospective studies,[41–47] published from 2012[41,43] until 2015,[42,46,47] which estimated the risk of developing prediabetes mellitus[42,44,46] or T2DM[41–47] from baseline levels of circulating metabolites. The sample size of these 7 additional studies, including the replication sample, if applicable,[41,44,45] ranged from 147[46] to 4678[45] and follow-up from 3[44] to 19[47] years. Study participants were recruited in Germany,[42,45] Finland,[43] Framingham, Massachusetts, and Malmö, Sweden,[41] multiple European countries,[44] the United States,[46] and the United Kingdom.[47] Two studies were multiethnic,[46,47] and 2 enrolled only men.[43,47] Combining the initial Framingham study[40] with the 7 additional prospective reports[41–47] provided summary statistics encompassing 8000 individuals, of whom 1940 had T2DM. Per study-specific SD difference, the pooled relative risk of T2DM amounted to 1.36 (95% CI, 1.24–1.48; $I^2=9.5\%$) for isoleucine, 1.36 (CI, 1.17–1.58; $I^2=37.4\%$) for leucine, 1.35 (CI, 1.19–1.53; $I^2=45.8\%$) for valine, 1.36 (CI, 1.19–1.55; $I^2=51.6\%$) for tyrosine, and 1.26 (CI, 1.10–1.44; $I^2=56\%$) for phenylalanine.[51] Glycine and glutamine were inversely associated with T2DM with relative risks amounting to 0.89 (CI, 0.81–0.96; $I^2=0\%$) and 0.85 (CI, 0.82–0.89; $I^2=0\%$), respectively.[51]

Genetic Association Studies

A polymorphism in the *PPM1K* gene (rs1440581; *C>T*), encoding the mitochondrial protein phosphatase PP2Cm (Figure 1),

affects the ratio of circulating BCAAs to AAAs, which both have been related to insulin resistance and diabetes mellitus in prospective cohort studies.[52] In a 2-year intervention trial of 734 overweight or obese patients randomly assigned to 4 diets with varying content of fat (high versus low) and protein (high versus average), dietary fat significantly modified the genetic effects on changes in weight, fasting insulin, and HOMA-IR at 6 months.[48] In the high-fat group, the *C* allele was related to less weight loss and smaller decreases in serum insulin and HOMA-IR ($P \leq 0.02$), whereas an opposite genotypic effect on changes in insulin and HOMA-IR was observed in the low-fat diet group ($P \leq 0.04$). At 2 years, the gene-diet interactions remained significant for weight loss ($P=0.008$) but became null for changes in serum insulin and HOMA-IR as a result from weight regain.[48] These observations indirectly demonstrated how nutritional and genetic determinants interactively regulate the circulating levels of BCAAs and AAAs and their impact on metabolic traits.[27,28,48]

Synergism Between Lipids and BCAAs in Relation to Insulin Resistance

Decades of research established a strong relation between glucose and lipid metabolism.[53,54] However, accruing evidence suggests that BCAAs synergize with fatty acids and their metabolites in promoting insulin resistance.[25,26,55,56] A plasma metabolomic study of >100 circulating metabolites were grouped by principal component analysis into 18 factors. It was demonstrated that the best factor discriminated between 74 obese insulin resistant patients and 67 lean insulin-sensitive controls (HOMAscore), was not lipid related but comprised the BCAAs valine, leucine/isoleucine, the AAAs phenylalanine and tyrosine, C3 and C5 acylcarnitines and glutamate/glutamine, and alanine.[25] The preferential association of this BCAA-related metabolite cluster with insulin resistance was subsequently confirmed in a cross-sectional study of sedentary patients with a disturbed glucose tolerance test[55] and in cohorts of Chinese and Asian-Indian men in Singapore, matched for body mass index.[56]

Recent publications contributed to a better understanding of the mechanistic link between insulin resistance and BCAA catabolism.[57,58] Insulin resistance in skeletal muscle stems from the excess accumulation of lipids—a process that requires blood-borne lipids to traverse the blood vessel wall.[53,54,57] In mice, 3-hydroxyisobutyrate—a catabolic intermediate of valine—is secreted from muscle cells, activates endothelial fatty acid transport, stimulates muscle fatty acid uptake in vivo, and promotes lipid accumulation in muscle, leading to insulin resistance.[58] Conversely, in a cell model consisting of mouse myoblasts and human umbilical vein endothelial cells, inhibition of the synthesis of 3-hydroxyisobutyrate reduced the endothelial fatty acid uptake.[58] Along similar lines, 3-hydroxyisobutyrate levels were elevated in muscle from db/db mice[58,59] with diabetes mellitus and from human patients with diabetes mellitus,[60] as compared with those without diabetes mellitus. All catabolic products of the BCKAs are trapped inside the cell by covalent linkage to coenzyme A, with the single exception of 3-hydroxyisobutyrate, which is thus ideally suited to act as a secreted reporter of the BCAA catabolic flux in muscle.[58] These findings mechanistically link the catabolic flux of BCAAs to insulin resistance.[58]

Hypertension

The scarce studies that addressed the association of hypertension with dietary,[61,62] urinary,[30] or circulating[63] BCAAs produced inconsistent results. Among 1898 female twins,[61] aged 18 to 75 years, dietary intake of 7 amino acids was assessed from food frequency questionnaires and food composition tables. In multivariable-adjusted cross-sectional analyses comparing the bottom versus the top fifth of the distribution of dietary leucine consumption (1.0% versus 1.6% of energy intake), peripheral (−6/−3 mm Hg) and central (−5/−3 mm Hg) systolic/diastolic blood pressure and aortic pulse wave velocity (−0.5 m/s) were lower (P for trend, ≤0.02) with higher intake.[61] Total protein intake from vegetable and animal sources was not associated with any of the outcomes, but the inverse association between pulse wave velocity and leucine intake was only observed for leucine from animal protein sources.[61] In the Tehran Lipid and Glucose Study,[62] investigators administered a food frequency questionnaire to 4288 normotensive adults, who were followed up for 3 years. Principal component analysis of 8 amino acid groups identified 3 factors, of which one had a high loading on BCAA intake (0.95). In adjusted analyses, contrasting the highest versus the lowest fourth of the distribution of the BCAA-related factor was associated with a 83% higher risk of hypertension (P for trend, 0.002), whereas the 2 other factors were not associated with the hypertension risk.[62] The BCAA-related factor was positively correlated with the dietary intake of animal proteins and dairy products but negatively with the consumption of plant proteins.[62] In the INTERMAP study,[30] blood pressure and the urinary excretion of BCAAs were higher in Northern than Southern China. Finally, in the Project Viva study,[63] 109 girls and 104 boys, aged 6 to 10 years at enrollment, were followed up for 5 years. The factor derived by principal component analysis associated with circulating BCAAs was not associated with the changes in blood pressure in either sex in unadjusted or adjusted analyses.[63]

Heart

One of the well-studied metabolic signatures of heart failure is the shift from a fatty acid dominant bioenergetic state into a more glycolytic state, viewed as a compensatory response with the purpose of enhancing the utilization of energy substrate and oxygen.[64] However, a recent paradigm put emphasis on the metabolism of amino acids instead of glucose and fatty acids.[15] Both experimental[65–67] and clinical[68] studies support the implication of BCAAs in modulating left ventricular (LV) function.

A seminal article demonstrated that defective BCAA catabolism was the most important metabolic change in the failing heart of mice subjected to transaortic constriction.[67] Accumulation of BCKAs promoted pressure overload-induced heart failure and was associated with oxidative injury. In mice, pharmacological stimulation of BCAA catabolism by compound BT2 (3,6-dichlorobenzo[b]thiophene-2-carboxylic acid)[69] preserved cardiac function after pressure overload.[67] In a salt-loaded rat model of heart failure[65] and middle-aged mice,[66] BCAA dietary supplements prolonged cardiomyocyte survival,[65] preserved cardiac function,[66] and lengthened life span.[65,66] Expression of PP2Cm in the heart is dynamically

regulated by stressors.[16,69] In hypertrophic and failing hearts, its expression is significantly reduced at both the mRNA and protein level.[16]

KLF15 (Krüppel-like factor 15) is a direct transcriptional activator of BCAT2.[70,71] In cultured cardiomyocytes, overexpression of KLF15 induces mRNA expression of BCAT2, the E1α, E1β, and E2 BCKDC subunits, and PP2Cm, with the exception of BCKDK.[67] The opposite occurs in *KLF15*-deficient hearts. Also similar to what was observed in failing human and mouse heart samples, BCKA levels were elevated in the myocardium of *KLF15*-null hearts.[67] Thus, suppression of BCAA catabolism disrupts glucose metabolism and sensitizes the heart to injury.[72] Two observations emphasize the relevance of these experimental data with respect to the pathophysiology of heart failure in humans. First, the findings in the failing mouse heart were replicated in mRNA expression studies of human hearts with terminal dilated cardiomyopathy.[67] Second, in patients with heart failure treated with cardiac resynchronization therapy, responders had higher pretreatment circulating BCAA levels than nonresponders.[68]

Mitochondrial proteins are almost all downregulated in end-stage diseased hearts, irrespective of the cause of heart failure.[73] As mentioned above, this also applies to the BCAT2,[67,70,71] the E1α, E1β, and E2 components of the BCKDC,[67] and PP2Cm,[67] which seem to share a transcriptional or posttranscriptional regulatory circuit.[67] BCKA oxidation is impaired in heart failure with reduced ejection fraction.[74] However, our literature search did not reveal any data showing association in experimental or human studies between heart failure with preserved ejection fraction and the metabolism of BCAAs.

Experimental findings and observations in symptomatic patients cannot be extrapolated to subclinical disease, in which biomarkers would have the greatest diagnostic or prognostic relevance.[75] Asymptomatic diastolic LV dysfunction affects >25% of the general population.[76,77] It carries a 10% risk of further deterioration during 5 years[78] and is a forerunner of cardiovascular complications.[79] In 570 randomly recruited Flemish,[80] we investigated the associations of echocardiographic diastolic LV function with 43 circulating metabolites measured by nontargeted nuclear magnetic resonance spectroscopy (Figure 2). Echocardiographic LV function was assessed in 2005 to 2010 (baseline) and 2009 to 2013 (follow-up). Median follow-up was 4.7 years. In early diastole, higher peak velocity of the mitral annulus (e′ [early LV relaxation]) and lower ratio of the transmitral blood velocity to mitral annular velocity (E/e′ [LV filling pressure]) likely reflect a more performant diastolic LV function. This interpretation rests on the observation that the ranges of e′ and E/e′ differ widely between people randomly recruited from populations and patients[81] and that in the general population, lower e′ and higher E/e′ predict worse outcome.[79,82] In multimarker analyses of the baseline and follow-up data and in analyses predicting follow-up e′ and E/e′, the BCAAs leucine and valine were associated with more performant diastolic LV function (Figure 2).[80] Valine, leucine, and isoleucine metabolism ($-\log_{10} P$, 12.8) and aminoacyl-tRNA biosynthesis (10.2) were the top metabolic pathways associated with diastolic LV function.[80] These findings among people covering the range from normal diastolic LV function to asymptomatic dysfunction illustrate how BCAAs may act as critical switches in health and disease. Elevated circulating BCAA levels may

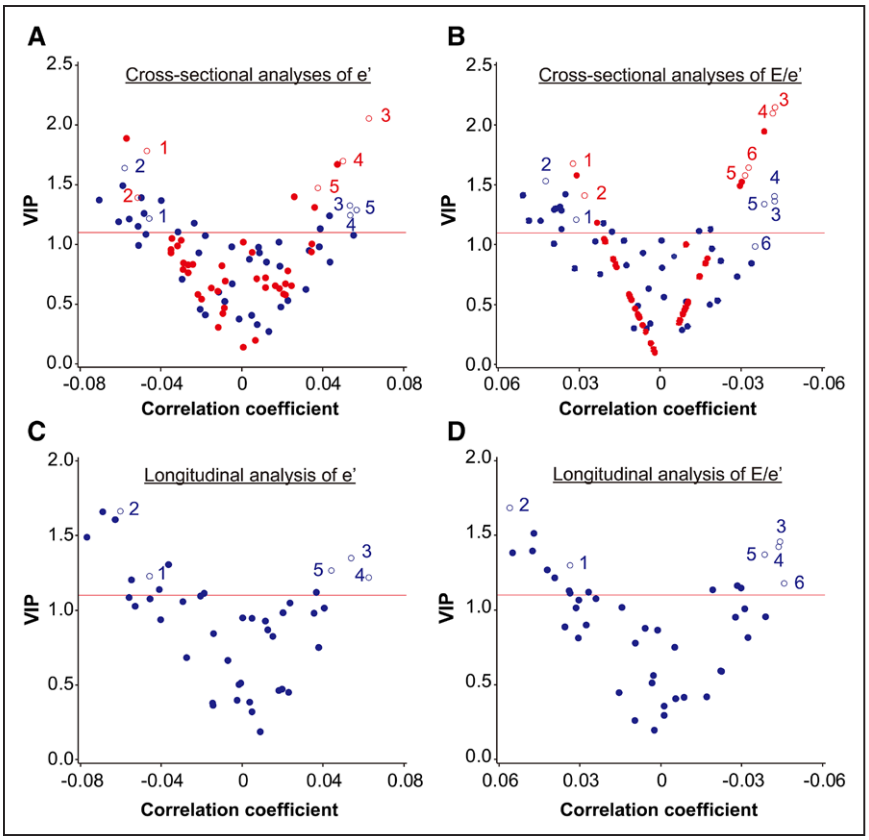

Figure 2. Results from the Flemish Study on Environment Genes and Health Outcomes, relating echocardiographic diastolic left ventricular (LV) function to circulating metabolites using partial least squares analysis. The graphics are overlaid V plots for the cross-sectional associations of e′ (**A**) and E/e′ (**B**) with the metabolites at baseline (2005–2010) and follow-up (2009–2013) and for the longitudinal associations of e′ (**C**) and E/e′ (**D**) at follow-up (2009–2013) with the baseline metabolites (2005–2010). The variable importance in projection (VIP) score indicates the importance of a metabolite in the construction of the partial least squares factors. VIP is plotted against the centered and rescaled correlation coefficients, which reflect the associations of the echocardiographic measurements with the metabolites. For E/e′, the *x* axis was inverted to be directionally similarly associated with diastolic LV function as for e′. Markers in the **top left** quadrant (lower e′ and higher E/e′) and **top right** quadrant (higher e′ and lower E/e′) of the V plots are associated with worse and better diastolic LV function, respectively. Numbers identify metabolites: (1) pentanoate, (2) glucose+glutamine, (3) 4-hydroxybutyrate, (4) 2-aminobutyrate, (5) leucine, and (6) valine. Reprinted from Zhang et al[80] with permission. Copyright © 2018, European Journal of Preventive Cardiology.

reflect a healthy nutritional state and may be beneficial in normal or slightly dysfunctional hearts but become harmful when BCAA catabolism is defective and cardiotoxic BCKAs accumulate in the failing heart (Figure 3).

Skeletal Muscles

Striated skeletal muscle is the largest organ in the human body representing on average 40% of body weight and 20% of protein by mass.[83] About half of the enzymatic machinery to catabolize BCAAs is located in skeletal muscles.[1] Muscular proteins are continuously synthesized to replace protein lost because of breakdown. The 3 BCAAs are among the 9 EAAs that must be present in adequate amounts to replenish muscle proteins. The majority of amino acids show a concentration gradient from the extracellular to the intramuscular compartment, which in healthy human volunteers averages 1.16 for valine, 1.75 for isoleucine, and 1.24 for leucine.[84] In humans, the synthesis of myofibrillar, sarcoplasmic, and mitochondrial proteins is acutely regulated by the concentration of circulating EAAs and becomes saturated at high levels.[85] Thus, anabolism of muscular proteins is not only regulated by hormonal stimuli, such as insulin, insulin-like growth factor, and growth hormone,[83] but is also critically dependent on sensing of the extracellular rather than intracellular concentration of EAAs.[85]

Stimulation of protein synthesis in skeletal muscle in response to a mixed meal is largely because of BCAAs, and of the 3 BCAAs, leucine is the one primarily responsible for the stimulation of muscular protein synthesis.[86] In the postprandial state after a meal containing protein, all of the EAAs precursors required for new muscle protein synthesis can be derived from either the elevated plasma concentrations resulting from digestion of the consumed protein or from recycling amino acids from protein breakdown.[87] In this circumstance of abundant availability of EAAs, the rate of muscle protein synthesis exceeds the rate of breakdown, thereby producing an anabolic state. In the postabsorptive state, the plasma EAA levels fall below the postprandial values.[87] As a result, EAAs are no longer being taken up by muscle but rather released by muscle into plasma.[87,88] This catabolic state of muscle protein in the postabsorptive state enables continued availability of EAAs for other tissues to maintain the rate of protein synthesis at the expense of muscle protein, which can be considered as the reservoir of EAAs for the body to draw on.

As reviewed elsewhere,[87] a multimillion industry of nutritional supplements rests on the concept that dietary BCAA supplements produce an anabolic response in humans driven by stimulation of the synthesis of muscle proteins[87] with potential applications in sports medicine[89–91] and a variety of pathological conditions, such as burns,[91] liver failure,[92–94] end-stage renal disease,[95] or the sarcopenia associated with aging[91,96] or heart failure.[97] In a still ongoing randomized clinical trial with parallel group design, older patients (≥65 years) with systolic or diastolic LV dysfunction enrolled in a cardiac rehabilitation program are being recruited.[97] They are randomly assigned to cardiac rehabilitation with or without dietary BCAA supplementation (1144 mg of L-valine, 1904 mg of L-leucine, and 952 mg of L-isoleucine, BID).[97] After completion of the cardiac rehabilitation at 20 weeks, patients undergo a second randomization to continued BCAA supplementation or not for another 16 weeks.[97] The primary outcome is the rate of change in the anaerobic threshold workload from baseline to postintervention.[97]

Experts in the field question the efficacy of dietary BCAA supplementation.[87] A dietary supplement of BCAAs alone cannot sustain an increased rate of muscle protein synthesis. The availability of the other EAAs will rapidly become rate limiting for accelerated protein synthesis.[98] Consistent with this perspective, studies in human subjects have reported decreases, rather than increases, in muscle protein synthesis after intake of BCAAs. Moreover, downstream signaling

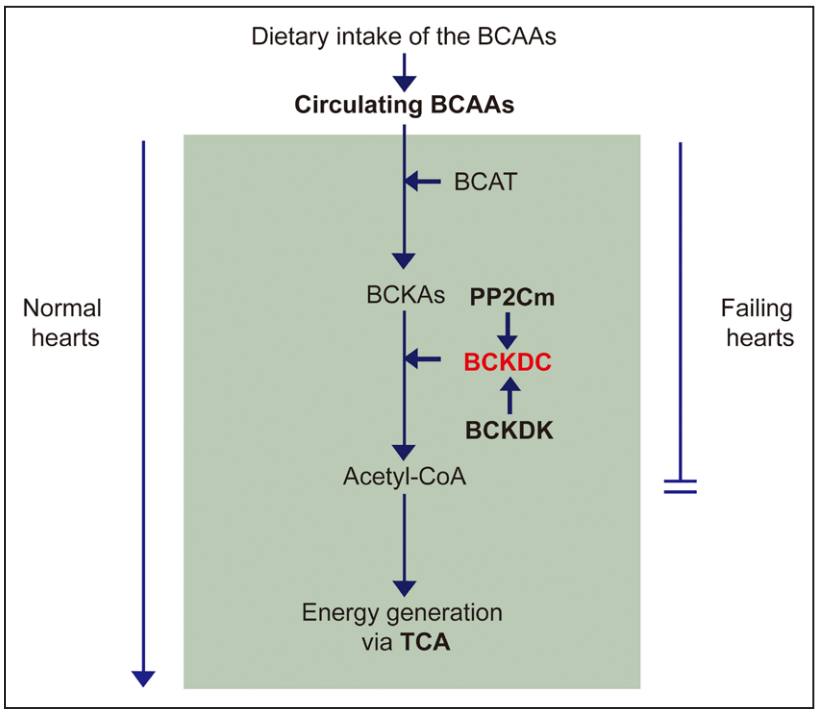

Figure 3. Differential role of the branched-chain amino acid catabolic pathway in normal and failing hearts. The branched-chain amino acids (BCAAs) are essential nutrients. In the myocardium, BCAAs are catabolized to branched-chain keto acids (BCKAs) by BCAT (branched-chain amino acid aminotransferase) and to acetyl-CoA by branched-chain α-keto acid dehydrogenase complex (BCKDC) under control of PP2Cm (mitochondrial protein phosphatase) and BCKDK (branched-chain keto acid dehydrogenase kinase). Acetyl-CoA can enter the tricarboxylic acid (TCA) cycle and contribute to energy generation. Circulating BCAA levels, reflecting the balance between nutritional intake and catabolism, are beneficial in normal hearts. Expression of the mitochondrial machinery to catabolize BCAAs is reduced in failing hearts, so that cardiotoxic BCKAs accumulate.

of BCAA catabolites also limits their clinical application (Figure 1). Indeed, the glucose transport proteins (GLUT1 [glucose transporter 1] and GLUT4 [glucose transporter 4]) facilitate glucose transport into insulin-sensitive cells. GLUT1 is insulin-independent and is widely distributed in different tissues. GLUT4 is insulin-dependent and responsible for the major part of glucose transport into muscle and adipose cells. Activation of the INSR (insulin receptor) activates an intracellular cascade, mainly through the PI3K (phosphatidylinositol 3-kinase)/AKT (a serine/threonine protein kinase also known as PKB [protein kinaseB])/mTOR pathway, which promotes the expression, translocation, and fusion of the cell membranes with GLUT4. As mentioned before (Figure 1), BCAA metabolites interact with mTOR, which via activation of ribosomal protein S6 kinase β1 leads to uncoupling of INSR and, therefore, to less expression of the GLUT4.[99]

Autism and Neurodegenerative Disease

The heterodimeric amino acid transporter LAT1 is the principal carrier transporting BCAAs and the AAAs, phenylalanine, tyrosine, and tryptophan, from the circulating blood into the brain with a smaller contribution of the Y-type transporter family and the Na^+-dependent large neutral amino acid transporter.[3,100,101] LAT1 is almost exclusively expressed at the basolateral and apical membranes of the endothelial cells making up the blood-brain barrier.[102] Its expression changes in response to amino acid availability.[103] At normal plasma amino acid concentrations, the aforementioned amino acid transporters are fully saturated, and circulating amino acids compete with one another for transport across the blood-brain barrier.

Competition between BCAAs and AAAs may impact on the biosynthesis of neurotransmitters, including dopamine, norepinephrine, and serotonin.[94,104] Elevation of circulating BCAAs may influence neurotransmitter levels in the brain with effects on central fatigue,[90] behavior, and brain function. This phenomenon is the rationale underpinning the use of BCAAs in patients with liver failure, in which a decreased ratio of BCAAs to AAAs plays a role in the pathogenesis of hepatic encephalopathy.[92,93] In a pilot study involving patients with motor neuron disease and matched controls, circulating metabolites that discriminated between cases and controls included the BCAAs valine and isoleucine and the BCAA metabolites 2-oxoisovaleric acid, isobutyric acid, and 2-oxo-3-methyl isovaleric acid.[105]

In consanguineous families, inactivating mutations in the *BCKDK* gene are associated with autism, epilepsy, and intellectual disability.[106,107] Patients with homozygous *BCKDK* mutations display reductions in BCKDK mRNA and protein and E1α phosphorylation,[106] explaining a higher rate of BCAA catabolism and lower plasma BCAA levels (Figure 1). BCKDK knockout mice show abnormal brain amino acid profiles and neurobehavioral deficits that respond to dietary BCAA supplementation.[106]

Hypothalamic insulin signaling controls food intake, hepatic glucose production, adipose tissue lipolysis, de novo lipogenesis, and BCAAs catabolism. In a mouse model of dementia (APP/PS1), amyloid plaque deposition starts approximately at 6 weeks of age in the neocortex. These genetically engineered mice exhibit impaired hypothalamic insulin signaling and are more susceptible to high-fat feeding and aging-induced metabolic dysregulation, including disrupted systemic BCAA homeostasis.[108] However, genetic[106] and experimental[106–108] studies pointing to potential applications of BCAA supplementation in Alzheimer disease,[108] autism,[106,107] and epilepsy,[106] to our knowledge, have not yet been carried through into human disease.

Cancer

The PI3K/AKT signaling pathway is a key regulator of normal cellular processes involved in cell growth, proliferation, metabolism, motility, survival, and apoptosis. Aberrant activation of the PI3K/AKT pathway promotes the survival and proliferation of human cancer cells. PI3K, AKT and mTOR are 3 major nodes in the pathway.[109] BCKAs interact with mTOR (Figure 1). Furthermore, tumor cells abundantly express LAT1[110] and use BCAAs in various biosynthetic pathways and as an energy source,[111] so that LAT1 is now considered as a novel pharmacological target.[110]

In human breast cancer, plasma and tissue levels of BCAAs are increased, which is accompanied by an elevated expression of the catabolic enzymes, including the cytosolic BCAT.[112] Knockdown of *BCAT* represses the growth rate and colony formation capacity of breast cancer cells, whereas opposing results were observed when BCAT was overexpressed.[112] In mechanistic studies, BCAT expression activated mTOR signaling to promote mitochondrial biogenesis and function and to facilitate growth and colony formation of breast cancer cells.[112] The role of BCKDK as regulator of BCAA catabolism in tumorigenesis of colorectal cancer remains equivocal.[113,114] In 117 patients with colorectal cancer, expression levels of BCKDK and BCKDC-E1α were higher in tumor tissue than in adjacent tissue, but there was no association with survival time.[113] In a second cohort of 163 patients with colorectal cancer, survival 24 months after diagnosis was also unrelated to the BCAA levels in tumor tissue.[114]

Perspectives

Metabolic dysregulation associated with the flux of BCAAs across the human body is an expanding but challenging research field. Major questions still need to be addressed. The interorgan interaction and tissue-specific contributions to BCAA-mediated metabolic dysregulation remain unclear. The mechanism of BCAA-mediated signaling should be extended to their metabolites, as recently demonstrated.[58] There is also a need to develop tissue-specific and temporally controlled genetically engineered mouse models to demonstrate the in vivo function of BCAA catabolic activities in organ physiology and disease. Notwithstanding these knowledge gaps, this brief review highlights that the BCAA catabolic pathway can serve as a potential therapeutic target. However, the first approach of providing dietary BCAA supplements is not supported by most of the currently available evidence.[87] BCAAs have to be combined with other EAAs. Whether the desired effects of full dietary EAA supplementation remain sustained over time is uncertain.[87] Another critical issue is that the effects of BCAA supplementation in our view might be opposite in health and disease, as demonstrated in studies of

asymptomatic LV dysfunction (Figure 3) and advanced heart failure.[67] The alternative approach to develop novel drugs interacting with the PP2Cm-mediated BCKA degradation has not yet been explored in humans. Such agents are available,[115] but only 1 (BT2) has been investigated in experimental studies.[67] Furthermore, several epidemiological studies implementing metabolomic approaches focused on longevity,[116] renal dysfunction,[117–120] coronary heart disease,[121,122] new-onset atrial fibrillation,[123] and heart failure.[124–126] Probably because of the varying metabolomic platforms used and differences in the definition of the study end points, there was little consistency among the metabolites reported to be associated with the outcomes of interest, and BCAAs were not among them. Further standardization in the evolving metabolomic technology will open new horizons for scientists working in this field.

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