Branched-chain amino acids in cardiovascular disease

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Abstract

Research conducted in the past 15 years has yielded crucial insights that are reshaping our understanding of the systems physiology of branched-chain amino acid (BCAA) metabolism and the molecular mechanisms underlying the close relationship between BCAA homeostasis and cardiovascular health. The rapidly evolving literature paints a complex picture, in which numerous tissue-specific and disease-specific modes of BCAA regulation initiate a diverse set of molecular mechanisms that connect changes in BCAA homeostasis to the pathogenesis of cardiovascular diseases, including myocardial infarction, ischaemia–reperfusion injury, atherosclerosis, hypertension and heart failure. In this Review, we outline the current understanding of the major factors regulating BCAA abundance and metabolic fate, highlight molecular mechanisms connecting impaired BCAA homeostasis to cardiovascular disease, discuss the epidemiological evidence connecting BCAAs with various cardiovascular disease states and identify current knowledge gaps requiring further investigation.

The branched-chain amino acids (BCAAs) — isoleucine, leucine and valine — are essential amino acids that are primarily derived from the diet, although they can also be generated de novo by the gut microbiota[1,2]. Beyond their role as building blocks for protein synthesis and the widely appreciated function of leucine as a nutrient stimulus for the serine/threonine protein kinase mechanistic target of rapamycin (mTOR) complex[3], the biochemical processing of BCAAs yields an array of metabolic intermediates, many of which have unique signalling properties that we are only now beginning to discover.

Increased circulating levels of the BCAAs were first identified >50 years ago in the foundational studies by Cahill and colleagues in individuals with obesity and insulin resistance[4]. With the widespread application of metabolomic profiling over the past decade, increased levels of BCAAs and related metabolites are now widely considered to be a metabolic hallmark of obesity, insulin resistance and type 2 diabetes mellitus (T2DM) in humans[5,6]. Metabolic disturbances are intimately involved in the pathogenesis of cardiovascular disease (CVD), and independent associations have emerged that support a direct role for BCAAs in heart failure (HF)[7–9], vascular disease[10,11], hypertension[12] and arrhythmias[13]. Therefore, the link between BCAAs and CVD has become an area of intense interest in both the clinical and basic science arenas. Mechanistic work in this area is in its infancy, with models and tools requiring development in order to directly test the causal relationship between tissue-level disturbances in BCAAs and CVD. Nonetheless, efforts to expand our understanding of how BCAAs influence cardiovascular physiology have already identified promising new candidate molecular targets for treating CVD. In this Review, we outline the current understanding of the factors regulating BCAA abundance and metabolic fate, provide an in-depth discussion of the emergent mechanistic and epidemiological findings implicating altered metabolism of BCAAs in the aetiology of CVD and highlight important areas for future research.

Systems biology of BCAA metabolism

The circulating pool of BCAAs is determined by the rate of their release from proteolysis and their utilization in protein synthesis and oxidation. The BCAA oxidation pathway can terminate at the tricarboxylic acid (TCA) cycle or earlier in the formation of intermediates that have paracrine signalling activity, such as 3-hydroxyisobutyrate (3-HIB)[14], or that are used for the generation of monomethyl branched-chain fatty acids (mmBCFAs)[15]. How the balance of these outcomes is controlled is currently unclear. BCAA oxidation occurs in the mitochondria, after the BCAAs have been imported by the newly identified solute carrier, SLC25a44 (REF.[16]). The initial enzymatic steps in the BCAA catabolic pathway are shared by isoleucine, leucine and valine. First, BCAAs undergo reversible transamination mediated by mitochondrial BCAA aminotransferase (BCAT2), yielding branched-chain α-keto acids (BCKAs). Second, the BCKAs undergo irreversible decarboxylation by the BCKA dehydrogenase (BCKDH) complex[6]. After multiple additional reactions, the BCAA catabolic pathway terminates in the generation of acetyl-CoA and succinyl-CoA and their oxidation in the TCA cycle or contribution via anaplerotic reactions to cellular biosynthetic pathways.

Important physiological roles are emerging for several intermediates of BCAA oxidation, including BCKA[17], 3-HIB[14] and mmBCFA[15]. Landmark tracing studies in mice have demonstrated that the major sites of BCAA oxidation are skeletal muscle (59%), brown adipose tissue (19%), liver (8%), kidney (5%) and heart (4%), whereas the major sites of BCAA incorporation into protein are the liver (27%), pancreas (24%), skeletal muscle (24%), kidney (9%) and brown adipose tissue (6%)[18] (FIG. 1). Therefore, in addition to the direct influence of BCAAs and related metabolites on the heart, local defects in the BCAA metabolic pathway in the liver, fat and skeletal muscle could have a potent inter-organ effect

on the cardiovascular system elicited by secondary factors such as adipokines, myokines or non-coding RNAs through exosome-mediated transfer.

Much insight has been gleaned from the study of systemic changes in BCAA metabolism in metabolic diseases[6], such as maple syrup urine disease. This congenital syndrome is caused by rare mutations in the genes encoding BCAA metabolic enzymes and results in systemically impaired BCAA metabolism. However, how the various CVD states reshape systemic BCAA homeostasis is unclear. One common observation in the setting of HF is the coordinated downregulation of many genes encoding proteins in the BCAA oxidative pathway in the heart[9]. Consequently, it is tempting to speculate that increased circulating levels of BCAAs in CVD are simply reflective of intrinsic shifts in cardiac BCAA metabolism. However, the level of BCAA oxidation in the TCA cycle in the healthy heart is relatively low compared with the level at other major sites of BCAA oxidation, rendering this explanation unlikely[17–19]. Additionally, in terms of mass, the heart is a minor site of BCAA oxidation relative to other tissues, such as skeletal muscle and fat[18]. Therefore, impairments in non-cardiac BCAA oxidation are likely to be the source of increases in circulating BCAAs in CVD. Low levels of BCAA oxidation in the healthy heart can be attributed to high baseline inhibitory phosphorylation of the cardiac BCKDH complex responsible for the first, irreversible step in BCAA oxidation, and low expression of the newly identified mitochondrial BCAA carrier, SLC25a44 (REF.[17]). Therefore, rather than generating catabolic intermediates, an alternative fate of BCKAs is reamination to their cognate BCAAs, as shown by ^{13}C tracing studies in isolated hearts from healthy rats[17]. Similarly, exposure of isolated cardiomyocyte mitochondria to BCKAs results in a substantial accumulation of BCAAs, indicative of active BCKA reamination in the heart[20]. This process does not occur in liver mitochondria, which lack the BCAT enzyme responsible for reamination[20]. These data suggest that CVD-mediated changes in the other tissue compartments regulating BCAA homeostasis, such as skeletal muscle, liver and adipose tissue (FIG. 1), are responsible for the rise in circulating BCAA levels in CVD. Therefore, a 'spill-over' model of BCAAs in CVD is proposed, in which altered BCAA metabolism in one compartment leads to increased exposure in another, such as the heart, with potential pathogenic consequences. Future studies surveying systemic and cellular changes in BCAA utilization across the various CVD states are needed to determine causal mechanisms. An exciting question still to be addressed is how do alterations in cardiovascular health influence systemic BCAA utilization?

Mechanisms linking BCAAs and CVD

Heart failure.

Human epidemiological studies primarily show an association between increased plasma BCAA concentrations in HF and adverse outcomes. Similarly, increased plasma levels of BCAAs are observed in some, but not all, animal models of HF. Serum metabolomics performed in rats with HF induced by myocardial infarction (MI) showed that BCAA concentrations discriminate between animals with HF and sham-operated controls and that serum BCAA concentrations increase progressively over time in parallel with worsening cardiac function[21]. These changes in circulating BCAA levels were accompanied by

downregulation of the genes encoding proteins in the BCAA catabolic pathway in the heart and accumulation of cardiac BCAAs[21]. Likewise, in mice with MI-induced HF, cardiac BCAA concentrations were increased, accompanied by decreases in the abundance of BCAA catabolic enzymes in the heart, consistent with decreased BCAA metabolism over time[22]. However, no increases in circulating BCAA levels were observed[22]. HF induced by pressure overload via transaortic constriction (TAC) in mice leads to increases in cardiac BCKA concentrations, with downregulation of genes encoding proteins involved in BCAA catabolism[9]. Although plasma concentrations of BCAAs or BCKAs in these TAC mice were not reported, a human cohort of individuals with HF with reduced ejection fraction (HFrEF) was used to validate the findings in the mouse model, while also demonstrating an increase in circulating levels of α-keto-β-methylvalerate (the BCKA of isoleucine)[9]. In another study in human patients with dilated cardiomyopathy, a marked increase in cardiac BCAA concentrations was found compared with controls, together with evidence of impaired BCAA metabolism demonstrated by decreased levels of mitochondrial protein phosphatase 1K (PPM1K) and BCAT2, with increased inhibitory phosphorylation of BCKDH[23]. Comparative metabolomics in mouse models of both TAC-induced and MI-induced HF also showed an increase in cardiac BCAA concentrations, but with no changes in plasma BCAA levels in either model[24]. In this study, cardiac BCAA levels positively correlated with cardiac insulin resistance and negatively correlated with cardiac function[24]. In 2022, findings were reported from a new 'chemogenetic' mouse model of HF, driven by cardiac delivery of adeno-associated virus (AAV9) expressing the yeast enzyme d-amino acid oxidase[25]. When animals are fed d-alanine, the enzyme generates hydrogen peroxide in the cardiomyocytes, leading to dilated cardiomyopathy. Unbiased transcriptomic and metabolomic profiling in this model of HF induced by oxidative stress found that BCAA catabolic enzyme gene expression was downregulated, with a concomitant increase in cardiac BCAA concentrations, but no change in plasma BCAA concentrations[25]. These animal models consistently demonstrate impaired cardiac BCAA metabolism and increased tissue concentrations of BCAAs as shared features of cardiac dysfunction, while increases in circulating BCAA levels, as is observed in humans with HF, are model-dependent. Interestingly, integrated omics studies comparing changes in cardiac metabolic pathways in mouse models of physiological (endurance training) and pathological (TAC) hypertrophy have found that downregulation of BCAA metabolism (akin to the well-d escribed reversion to the cardiac fetal gene programme[9]) seems to be a unique feature of cardiac injury not seen in physiological hypertrophy[26,27].

A fundamental question relating to the mechanisms linking BCAAs and HF is how much does the increased delivery of BCAAs to the heart contribute to HF pathogenesis compared with perturbations in intrinsic cardiac BCAA metabolism? Using dietary BCAA supplementation, pharmacological manipulation of BCAA metabolism and genetic animal models, several studies have attempted to answer this question. In mice with MI, oral administration of BCAAs in the drinking water increases cardiac BCAA concentrations and worsens cardiac function compared with mice given normal water[22]. Mice lacking *Ppm1k* (also known as *Pp2cm*), encoding the protein phosphatase that activates BCKDH and therefore BCAA catabolism, have an increase in both plasma and cardiac concentrations of BCAAs and BCKAs compared with wild-type mice[9,28]. *Ppm1k*^{−/−} mice also have a

mild decline in cardiac function over time, with no obvious cardiac structural changes[9]. However, when *Ppm1k*^{−/−} mice are exposed to cardiac stress in the form of TAC, they show accelerated progression of HF[9].

The small-molecule allosteric inhibitor of BCKDH kinase (BDK), 3,6-dichlorobenzo[b]thiophene-2-carboxylic acid (BT2), is a powerful tool for studying the role of BCAA metabolism in various pathological conditions[29]. Just 1 week of BT2 administration leads to nearly complete dephosphorylation and maximal activation of BCKDH in the heart, skeletal muscle, kidneys and liver of mice[29] and rats[30], with reductions in plasma BCAA and BCKA concentrations. Likewise, BT2 rescues the BCAA catabolic defect seen in *Ppm1k*^{−/−} mice and normalizes plasma BCAA and BCKA concentrations[9]. Several studies have now shown that BT2 treatment can delay HF progression in mice when administered at the onset of TAC[9,22,23], and can stabilize cardiac function when administered 2 weeks after TAC[31]. The findings from these studies indicate that defective cardiac BCAA catabolism contributes to both HF pathogenesis and progression, given that impaired baseline cardiac BCAA catabolism (for example, in *Ppm1k*^{−/−} mice) predisposes animals to worsened cardiac function under stress, and that restoration of cardiac BCAA catabolism (via BT2 treatment) improves cardiac function under stress. However, because these models use approaches that affect systemic BCAA metabolism, and therefore change both circulating and cardiac BCAA concentrations, the precise contribution of impaired cardiac-specific BCAA metabolism to cardiac dysfunction is unclear.

Several mechanisms have been proposed to connect impaired cardiac BCAA metabolism and accumulation of cardiac BCAAs with cardiac dysfunction (FIG. 2a). BCAAs are well known to be prime activators of mTOR, activation of which has been observed in HF induced by MI or pressure overload[32]. Oral supplementation with BCAAs after MI leads to worsening cardiac function in mice, accompanied by increased mTOR phosphorylation and decreased autophagy[22]. This decline in cardiac function can be prevented by concomitant treatment with the mTOR inhibitor rapamycin, implying mTOR-dependent deleterious effects of cardiac BCAAs[22]. Increased cardiac BCAA concentrations are also associated with mTOR activation — as measured by phosphorylation of mTOR and its downstream targets ribosomal protein S6 kinase-β1 (also known as P70S6K) and insulin receptor substrate 1 — in cardiac tissue from humans with dilated cardiomyopathy[23]. These changes are associated with decreases in phosphorylation of enzymes involved in insulin signalling, namely AKT (also known as protein kinase B) and glycogen synthase kinase 3, suggesting that BCAA-mediated mTOR activation might contribute to cardiac insulin resistance[23]. This finding is consistent with animal models of cardiac insulin resistance (mice fed a high-fat diet) that show decreased cardiac BCAA oxidation and increased cardiac BCAA concentrations[19]. Acute 30-min exposure of rat isolated hearts to concentrations of BCKAs that are the same as those observed in the plasma of individuals with HF is sufficient to activate protein translation, with a corresponding increase in phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1, which activates protein synthesis by inactivating the translation repressor function of this protein[17]. Acute exposure to BCKAs also causes widespread changes in the cardiac phosphoproteome, suggesting activation of the MEK–ERK mitogen-activated protein kinase signalling pathway, which has been implicated in the development of pathological cardiac hypertrophy[17,33].

Cardiac metabolism has a circadian pattern, such that ATP production is maximized during the wake cycle and remodelling and repair processes occur during the sleep cycle[34]. Perturbations in this circadian rhythmicity can lead to a host of cardiometabolic diseases, including HF[35]. Like other metabolic pathways, cardiac BCAA metabolism is under circadian regulation. In the heart, mTOR activity is at its peak during the awake-to-sleep transition, during which protein translation is increased[36]. Feeding mice a BCAA-rich diet at the end of the awake period augments this mTOR-mediated increase in protein translation, resulting in increased cardiac mass and cardiomyocyte size[37]. This BCAA effect is not observed when mice are concurrently treated with rapamycin or fed a BCAA-rich diet at the beginning of the awake period, whereas deletion of a crucial cardiomyocyte circadian gene — *Arntl* (also known as *Bmal1*), encoding aryl hydrocarbon receptor nuclear translocator-like protein 1 — renders the heart insensitive to time-o f-day fluctuations in BCAA responsiveness[37]. Moreover, although chronic feeding of a BCAA-rich diet at the end of the awake period does not result in cardiac dysfunction per se, it exacerbates HF following TAC compared with mice fed a BCAA-rich diet at the beginning of the awake period[37]. How cardiac injury disrupts intrinsic circadian metabolism is an area of active investigation.

The transcription factor Krüppel-like factor 15 (KLF15) is a central regulator of circadian BCAA metabolism in the heart[38]. Mice lacking cardiac KLF15 have altered cardiac conduction and are susceptible to arrhythmias[39]. Moreover, HF induced by pressure overload in mice reduces *Klf15* expression, with coordinated downregulation of gene expression of multiple BCAA metabolic enzymes and a corresponding increase in intramyocardial BCKA concentrations[9]. Similar decreases in cardiac *KLF15* expression have also been observed in human cardiomyopathy[40,41]. Overexpression of *Klf15* in cultured cardiomyocytes induces the expression of BCAA metabolic enzymes[9]. Therefore, given that KLF15 is a translational regulator of BCAA catabolism, the downregulation of KLF15 is a potential upstream event in cardiac injury that results in the accumulation of cardiac BCAAs and BCKAs.

Other studies have suggested that the accumulation of BCAAs and BCKAs, from either the downregulation of cardiac BCAA metabolism or the increased delivery of BCAAs and BCKAs from the plasma, might lead to mitochondrial dysfunction and affect redox homeostasis. Mitochondria isolated from normal mouse cardiomyocytes and treated with BCKAs have dose-dependent inhibition of complex I-mediated respiration and an increase in superoxide production[9]. Mitochondria isolated from cardiomyocytes from *Ppm1k*$^{-/-}$ mice also show an increase in superoxide production, together with evidence of increased oxidative modification (carbonylation) of proteins from *Ppm1k*$^{-/-}$ cardiac tissue lysates[9]. Knockdown of *Ppm1k* in cultured cardiomyocytes leads to the induction of cell death associated with loss of mitochondrial membrane potential and possible opening of the mitochondrial permeability transition pore[42].

Changes in cardiac fuel selection are a hallmark of most cardiac diseases[43]. BCAAs can be oxidized to the level of the TCA cycle to function as anaplerotic substrates for ATP production. Therefore, the decrease in BCAA metabolism seen in HF could result in an energetic deficit and contribute to pathogenesis and disease progression. However, metabolic

tracing studies using both ^{14}C-labelled and ^{13}C-labelled BCAAs and BCKAs consistently show that the absolute amount of BCAA oxidation in the heart is quite low, with minimal contribution to ATP production when compared with fatty acids and glucose[17–19]. This finding has been validated in a study of humans with HF, in which changes in metabolites were compared across the myocardium in failing hearts[44]. Moreover, cardiac BCAA oxidation is only slightly augmented by BT2 treatment, despite clear increases in BCKDH activity[17–19]. These findings of minimal cardiac BCAA oxidation could be related to the low level of expression of the newly described mitochondrial BCAA carrier, SLC25a44, in the heart[17].

Decreased cardiac BCAA metabolism is unlikely to contribute to an energy deficit per se; however, several studies hint at an interplay between BCAA metabolism and the metabolism of other fuel substrates. Dietary restriction of BCAAs in Zucker obese rats normalized circulating BCAAs to levels seen in lean control rats[45,46]. Isolated heart perfusion studies with ^{13}C stable isotope tracing showed that dietary BCAA restriction decreases glucose oxidation and increases cardiac fatty acid oxidation coupled with a decrease in intracardiac triglyceride stores[46] (FIG. 2c). The mechanisms underlying this shift in fuel selection are not completely understood, but rats receiving the BCAA-restricted diet had decreased cardiac concentrations of hexokinase 2 and solute carrier family 2, facilitated glucose transporter member 4. Interestingly, dietary BCAA restriction had no effect on cardiac BCKDH activity or the abundance of downstream metabolites, suggesting that the observed metabolic changes are independent of flux through BCKDH[46]. In cultured cardiomyocytes, either overexpression of solute carrier family 2, facilitated glucose transporter member 1 (which increases glucose uptake) or provision of high-glucose media decreased expression of the transcription factor KLF15, which leads to coordinated downregulation of enzymes in the BCAA metabolic pathway and accumulation of intracellular BCAAs. This glucose–BCAA regulatory network is necessary for sustained mTOR activation and growth signalling in the setting of hypertrophic stimuli both in vitro and in vivo[47]. In cultured cardiomyocytes treated with phenylephrine, which stimulates hypertrophy, and in mice undergoing TAC, BT2 treatment alters the expression of genes involved in fuel utilization. Expression of the gene encoding pyruvate dehydrogenase (PDH) kinase, isoenzyme 4 (*Pdk4*) is increased, which would be expected to decrease glucose oxidation via inhibitory phosphorylation of PDH. In addition, expression of the gene encoding the plasma membrane fatty acid transporter, platelet glycoprotein 4 (*Cd36*), as well as the genes encoding enzymes involved in fatty acid β-oxidation (acyl-coenzyme A oxidase 1, palmitoyl (*Acox1*); acyl-coenzyme A dehydrogenase, long-chain (*Acadl*); and acyl-coenzyme A dehydrogenase, medium-chain (*Acadm*)) were increased[31].

In most studies, BCAAs and BCKAs are assumed to exert equivalent physiological effects; however, evidence suggests that these metabolites might influence distinct intracellular processes. For instance, studies in isolated pancreatic islets from mice lacking *Bcat2*, which catalyses the reversible transamination of BCAA to BCKA (FIG. 1), showed that conversion of α-ketoisocaproate (KIC) and glutamate to leucine and α-ketoglutarate is necessary for KIC-stimulated insulin secretion[48]. By contrast, *Bcat2* is not needed for leucine to stimulate insulin secretion[48]. Mice lacking *Bcat2* in the heart accumulate cardiac BCAAs, with a corresponding decrease in cardiac BCKAs and, interestingly, have improved

insulin-stimulated glucose oxidation compared with wild-type mice[49]. Exposure of cardiac *Bcat2*-knockout hearts to increased concentrations of BCKAs suppressed insulin-stimulated glucose oxidation rates, suggesting that the accumulation of BCKAs, but not BCAAs, results in cardiac insulin resistance[49]. In the heart, reverse transamination of BCKA to BCAA can occur when mitochondrial BCAA transport is limited or when baseline activity of cardiac BCKDH is low[17]. Whether BCAT enzymes predominantly function in the forward or reverse direction not only helps to determine the fate of BCKAs, but also contributes to the cellular levels of other important metabolites, such as glutamate and α-ketoglutarate. A study of cells from patients with acute myeloid leukaemia showed that BCAT activity is crucial to facilitate α-ketoglutarate-mediated DNA methylation and leads to widespread changes in gene expression that drive leukaemic phenotypes[50]. Whether similar changes in DNA modification result from BCKA metabolism in the heart is an exciting area for future investigation.

The upstream events that trigger downregulation of cardiac BCAA metabolism in response to stress are unclear. However, two separate studies — one in mice using TAC and one using heart tissue from patients with dilated cardiomyopathy — have shown a decrease in the gene expression and protein abundance, respectively, of KLF15, which acts upstream of several BCAA catabolic enzymes, including PPM1K and BCAT2 (REFS.[9,23]). In addition, an increase in phosphorylation of mitogen-activated protein kinase kinase 7 (MAP3K7; also known as transforming growth factor-β activating kinase 1) and of p38 mitogen-activated protein kinase (p38 MAPK) was observed in the human study[23]. Because transforming growth factor-β-mediated activation of p38 MAPK inhibits *KLF15* expression[51], MAP3K7 could be an early mediator of stress-induced changes in cardiac BCAA metabolism[23].

Atherosclerosis and endothelial dysfunction.

Data supporting the role of BCAAs in the pathogenesis of atherosclerosis are conflicting[52] and depend on the animal models used, the presence or absence of other atherosclerotic and metabolic risk factors, and the specific BCAA studied. In support of an anti-atherogenic role for BCAAs, adding leucine to the drinking water of *Apoe*$^{-/-}$ mice decreases serum LDL-cholesterol levels through increasing hepatic cholesterol efflux to bile acids and results in a >50% reduction in aortic atherosclerotic plaque[53]. In both cell culture systems and in macrophages derived from *Apoe*$^{-/-}$ mice, leucine supplementation decreases macrophage lipid content and foam cell formation[54].

In the setting of insulin resistance, elevated plasma leucine concentration is associated with impaired endothelium-dependent vascular relaxation in Zucker obese rats with T2DM, suggesting that increased BCAA levels might be linked to impaired nitric oxide (NO) metabolism[55]. This association is supported by data showing that infusion of leucine in the rat isolated kidney increases renal vascular resistance in a NO-dependent manner[56]. Conversely, in studies using isolated aortic rings from rats, inhibition of leucine catabolism leads to increased NO-independent contractility[57]. The mechanisms linking elevated leucine concentration with endothelial dysfunction are not fully delineated, but could involve activation of mTOR. Incubation of mouse aortic rings with leucine increases phosphorylation of 40S ribosomal protein S6, a marker of mTOR activation, and reduces

endothelium-dependent relaxation evoked by acetylcholine[58]. Similarly, overexpression of ribosomal protein S6 kinase (S6K) in mouse aortic rings is sufficient to reproduce this phenotype, and overexpression of an S6K dominant-negative construct abrogates impairment of endothelial relaxation by leucine[58]. Mechanistically, the increase in leucine-driven mTOR signalling induces a pro-oxidant gene programme, leading to increased generation of reactive oxygen species and subsequent vascular endothelial dysfunction[58,59] (FIG. 2d).

The role of the valine metabolite 3-HIB is under-studied among the potential mechanisms connecting BCAA metabolism to atherosclerosis (FIG. 2d). In a study of how the transcriptional co-activator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) coordinates angiogenesis and fuel delivery to skeletal muscle, 3-HIB was identified as a crucial regulator of transendothelial fatty acid uptake and transport[14]. Overexpression of skeletal muscle PGC1α led to excessive 3-HIB production and accumulation of lipid in the muscle, resulting in insulin resistance[14]. Although these findings provide insight into the association between BCAAs and insulin resistance, whether 3-HIB-mediated transendothelial flux of fatty acids affects the development of atherosclerosis is unknown.

Deposition of lipids and cholesterol in the vascular endothelium and the infiltration of inflammatory cells are early processes in atherosclerotic plaque formation. Following plaque rupture, activation of platelets and the coagulation cascade ultimately lead to intravascular thrombus with subsequent myocardial or cerebrovascular infarction. The effects of BCAAs on platelet activity have been investigated in mouse models and humans. In healthy human volunteers, acute ingestion of BCAAs increased platelet BCAA concentrations accompanied by collagen-induced platelet activation and aggregation[60]. Interestingly, whereas downregulation of BCAA catabolism tends to be associated with adverse cardiovascular outcomes, platelets from *Ppm1k*$^{-/-}$ mice in this study had reduced activation and were less prone to arterial thrombosis than platelets from wild-type mice. Instead, the abundance of downstream BCAA catabolic intermediates, especially α-ketoisovalerate (the BCKA of valine), was associated with increased platelet activation, possibly due to increased propionylation of key proteins involved in thrombosis[60]. These findings suggest that platelets are sensitive to BCAA exposure, consistent with a metabolic overflow model, in which decreased oxidation elsewhere results in a higher BCAA load in these cells and, subsequently, increased BCAA-dependent activation of thrombotic pathways.

Myocardial ischaemia and reperfusion.

BCAAs also have a role in the cardiac response to ischaemia and reperfusion (FIG. 2b). A study using *Ppm1k*$^{-/-}$ mice showed that accumulation of cardiac BCAAs downregulates the hexosamine biosynthetic pathway, thereby decreasing O-linked *N*-acetylglucosamine (O-GlcNAc) modification of PDH and inhibiting glucose oxidation. This BCAA-related inhibition of glucose oxidation worsens cardiac function after ischaemia and reperfusion[61]. Conversely, in mice with T2DM (induced by a high-fat diet and low-dose streptozotocin), activation of cardiac BCAA metabolism via PPM1K overexpression or BDK inhibition

via BT2 administration ameliorated ischaemia–reperfusion injury by decreasing oxidative stress[62].

Arrhythmias.

Sudden, lethal cardiac arrhythmias are the most common cause of sudden cardiac death[63], but aside from genetic determinants, ischaemic heart disease and HF, the risk factors that predispose individuals to these events are poorly understood. Using an *N*-ethyl-*N*-nitrosourea mutagenesis screen, a variant in the *Bcat2* gene was identified that predisposes mice to sudden cardiac arrhythmias[13]. These mice experience sudden death at a young age, associated with an increase in plasma BCAA levels, without any overt cardiac dysfunction. Cardiomyocytes isolated from these mice have impaired electrophysiological properties, including prolonged QTc and PR intervals, and evidence of inducible pro-arrhythmic characteristics. Similar electrophysiological properties were seen in human induced pluripotent stem cell-derived cardiomyocytes incubated in the same concentrations of BCAA as observed in the mouse model. These findings were reversed when the cardiomyocytes were treated with rapamycin, indicating that the pro-arrhythmic effects of BCAAs are mTOR-dependent [13].

Ageing.

Ageing and CVD are intricately linked. Ageing is a major risk factor for the development of CVD, whereas freedom from CVD is a major determinant of longevity and healthspan. Therefore, the interplay between BCAA metabolism and lifespan has implications for the future development of CVD.

Dietary and caloric restriction have long been reported to prolong lifespan across numerous species, and similar results are observed with the inhibition of nutrient-sensing pathways such as mTOR, insulin-like growth factor 1 and AMP/ATP-binding subunit of AMP-activated protein kinase[64]. Many of these beneficial effects have been attributed to protein restriction, and several studies have uncovered how dietary BCAA restriction, in particular, might influence longevity and healthspan. However, it should be noted that associations between BCAA intake and ageing or age-related disorders remain controversial, and the underlying mechanisms are complex[65] and beyond the scope of this Review.

Observationally, calorie restriction in mice is associated with a decrease in circulating BCAA concentrations that inversely correlate with lifespan[66]. Mice fed a long-term diet of excess BCAAs had hyperphagia, obesity and decreased lifespan compared with mice fed an isocaloric and macronutrient content-controlled diet with normal BCAA concentrations[66]. Interestingly, this effect was not the result of increased BCAA-mediated signalling through mTOR, which might have been predicted, but instead was related to appetite control through the imbalance of BCAAs and other essential amino acids. Rebalancing the BCAA–essential amino acid ratio in BCAA-excess mice eliminated hyperphagia and promoted longevity[67]. Dietary BCAA restriction in genetic mouse models of accelerated ageing extends lifespan, reduces frailty and improves metabolic health, with certain sex-specific effects on these outcomes, depending on the age at which the BCAA-restricted diet is started[68]. Despite improvements in age-r elated phenotypes, dietary BCAA restriction does not delay the

cardiomyopathy that is characteristic in these genetic models[68], highlighting the complex interplay between dietary BCAA intake, systemic BCAA metabolism and cardiac function.

Epidemiology of BCAAs in CVD

Metabolomic studies reporting on the association between BCAAs and CVD have been conducted in numerous epidemiological cohorts (TABLE 1). In the sections that follow, we describe the findings of these studies across a broad range of CVD states.

Heart failure.

As we have discussed, elevations in plasma BCAA levels are associated with impaired cardiac function. In a cross-sectional study of individuals with HF with preserved ejection fraction (HFpEF), HFrEF or no HF, plasma levels of BCAAs were significantly higher in those with HFrEF than in those who had HFpEF or no HF[7]. Importantly, beyond simply being associated with the presence of HF, plasma BCAA levels are predictive of clinical outcomes. In a single-centre study in >1,000 individuals with HFrEF, leucine, valine and BCAA-derived acylcarnitines were identified as components of a plasma metabolite profile that incrementally predicted survival, independent of established HFrEF risk prediction tools and biomarkers[8]. Moreover, in 138 individuals with ST-segment elevation MI and acute HF, baseline BCAA level outperformed amino-terminal pro-B-type natriuretic peptide (NT-proBNP; an established risk biomarker) level in predicting adverse cardiovascular events over a 3-year follow-up period[69]. Plasma BCAA levels were also associated with maximal oxygen consumption in individuals with HFrEF in the HF-A CTION clinical trial[70]. These studies all showed associations between BCAAs and HF, independent of T2DM. However, an investigation using the Hong Kong Diabetes Register found that baseline plasma BCAA levels in a cohort of ~2,100 individuals with T2DM predicted incident HF after adjustment for other established HF risk factors. This finding suggests that elevated plasma BCAA levels could act as metabolic mediators of HF pathogenesis in the setting of T2DM[71].

Interestingly, in a study of 24 individuals with HFrEF undergoing cardiac resynchronization therapy, increased plasma BCAA levels at baseline were associated with improvement in left ventricular systolic function[72]. Moreover, in individuals without structural heart disease who were followed up over time with serial echocardiography, increased baseline plasma BCAA levels were associated with a decreased risk of developing left ventricular diastolic dysfunction, which is a marker of future adverse cardiovascular events[73]. These findings, which seem to contradict the results of other studies discussed above, suggest that in certain populations and conditions, increased plasma BCAA concentrations might be beneficial.

Coronary artery disease.

Plasma BCAA levels have also emerged as a biomarker of several other CVDs. A BCAA-related signature is not only associated with the presence of coronary artery disease (CAD) but also with CAD severity in individuals undergoing coronary angiography. Of note, this association is independent of BMI, T2DM status and glycated albumin level (a marker of insulin resistance)[10,11]. Additionally, the plasma BCAA signature independently predicted MI and adverse cardiovascular events in this cohort[10,74]. In patients with ST-

segment elevation MI requiring percutaneous coronary intervention, baseline plasma BCAA levels were associated with an increased risk of death from cardiovascular causes and the development of acute HF[75].

In the primary prevention PREDIMED study[76], baseline plasma leucine and isoleucine concentrations were independently associated with an increased risk of incident MI and death from cardiovascular causes. Similarly, in a European cohort of individuals with no CVD, plasma concentrations of isoleucine and the aromatic amino acids tyrosine and phenylalanine independently predicted the development of atherosclerosis as measured by carotid intima–media thickness, as well as inducible myocardial ischaemia seen on exercise stress testing[77]. Therefore, plasma BCAA level can predict future atherosclerotic disease in those with a wide range of baseline cardiovascular risks.

In >27,000 women enrolled in the Women's Health Study[78], elevated baseline BCAA levels were associated with incident CVD over almost 19 years of follow-up, with predictive power similar to that of baseline LDL-cholesterol levels. Interestingly, the association between BCAAs and CVD was stronger in women who developed T2DM before developing CVD than in those without T2DM[78], again implicating BCAAs in the pathogenesis of CVD in patients with T2DM.

The association between BCAAs and CAD might depend on race. In an analysis from the Diabetes Heart Study[79], in which untargeted metabolomics was performed in a racially diverse cohort, BCAA levels were associated with coronary artery calcium (an indicator of subclinical atherosclerosis) in European Americans after adjusting for age, sex, BMI, diabetes duration, LDL-cholesterol level and statin use. By contrast, this association was not seen in African Americans[79]. Moreover, elevated leucine levels were associated with a decreased risk of incident CAD in a metabolomics analysis from the Jackson Heart Study[80], in which the study population is composed of African American individuals.

Hypertension, stroke and arrhythmia.

Compared with HF and CAD, few studies have been conducted on the metabolic phenotypes of hypertension, stroke and arrhythmias in humans. Nonetheless, evidence is beginning to emerge that supports an association between BCAAs and these conditions. In a study in >4,100 participants of the PREVEND study[12], baseline BCAA concentration predicted incident hypertension over ~9 years of follow-up, independent of hypertension risk factors (including age, sex, history of T2DM, tobacco and alcohol consumption, BMI, systolic blood pressure, estimated glomerular filtration rate, urinary albumin excretion, and levels of glucose, total cholesterol, HDL cholesterol, triglycerides and insulin). Similar associations have been observed in cross-sectional analyses of two separate cohorts of Asian individuals (8,589 Japanese[81] and 472 Chinese[82] individuals), in which plasma BCAA levels positively correlated with blood pressure. Consistent with these findings, in a study examining dietary protein intake in 6,493 Iranian individuals without hypertension, a dietary amino acid pattern rich in BCAAs was associated with an increased risk of hypertension over 3 years of follow-up[83]. Of note, however, plasma BCAA levels were not measured in this study. By contrast, in a study in 1,898 female twins from the TwinsUK registry, increased dietary intake of BCAAs was associated with a reduced risk of arterial stiffness and incident hypertension[84].

However, plasma BCAA concentrations were not correlated with dietary BCAA intake in this study, indicating that both tissue-level perturbations in BCAA metabolism and dietary intake of BCAA are necessary to affect plasma BCAA concentrations.

Among the CVD outcomes investigated in the primary prevention PREDIMED study[76], incident stroke was most strongly associated with increased baseline BCAA concentrations. By contrast, in another study, plasma BCAA levels were decreased in patients with acute ischaemic stroke compared with control individuals, and a lower BCAA concentration predicted poor neurological outcomes[85]. These disparate findings are likely to be due to the disease state — prediction of stroke versus response to acute stroke, respectively — but also reflect the dynamic changes that can occur in systemic BCAA metabolism depending on the pathophysiological condition.

Epidemiological data linking BCAA and cardiac arrhythmias are limited; however, plasma valine and leucine levels were shown to be correlated with several electrocardiographic parameters — such as PR interval, QRS duration and QTc interval — in 2,304 individuals enrolled in the German, community-based KORA F4 study[13]. These findings suggest that cardiac exposure to increased BCAA concentrations can affect cardiac conduction and repolarization[13].

Ageing.

In humans, ageing is characterized by increased skeletal muscle metabolism, decreased protein intake and subsequent decreased muscle mass, all of which contribute to frailty[65]. As a result, decreased plasma BCAA concentrations are often seen in older individuals[86]. Therefore, in aged populations, increased plasma BCAA levels might reflect better systemic and cardiometabolic health. For example, in the CHAMP study[86] of ~900 older men in Australia (mean age 81 years), individuals with low concentrations of BCAA were more likely to be categorized as frail and had an increased risk of death and major adverse cardiac events (stroke, MI, HF and revascularization). Low plasma BCAA levels could also be a marker of severe systemic cardiac disease and, therefore, an indicator of poor prognosis. For instance, in a biomarker substudy from the FIGHT trial[87], increased plasma BCAA concentrations predicted 90-day improvements in 6-min walking distance and NT-proBNP level, both of which are important clinical outcomes in HF. Individuals in this trial had advanced HFrEF and had been hospitalized in the previous 14 days. As HF progresses, an imbalance between catabolic and anabolic activity develops, leading to sarcopenia[43]. As seen with ageing, plasma BCAA level was positively correlated with skeletal muscle mass in individuals with HFrEF[88]. Therefore, higher plasma BCAA levels in individuals with advanced HFrEF could reflect better systemic health (that is, less sarcopenia) and predict better functional outcomes.

Therapeutic targets

Dietary and pharmacological manipulation of BCAA metabolism offers an exciting prospect for future CVD therapeutics. In maple syrup urine disease, the mainstay of therapy is dietary restriction of BCAAs[89]. Although dietary restriction of BCAAs has not been investigated in patients with CVD, in the setting of obesity this strategy leads to improvements in markers

of metabolic health after ~40 days[90]. Additionally, sodium phenylbutyrate, a drug used to treat urea cycle disorders, has also been reported to reduce circulating BCAA and BCKA levels[91]. This finding was subsequently replicated in patients with late-onset, intermediate maple syrup urine disease and in healthy control individuals[92]. Sodium phenylbutyrate activates BCKDH by binding to and inhibiting BDK in a similar manner to BT2 (REF.[92]). Because BDK inhibition with BT2 has been shown to improve outcomes in animal models of CVD, the repurposing of sodium phenylbutyrate to treat patients with CVD should be considered in future clinical trials.

Conclusions

The link between BCAAs and a variety of CVD states is well-established. In humans and animals with HF, the BCAA metabolic pathway is consistently downregulated in cardiac tissue, leading to an accumulation of BCAAs and BCKAs, which contributes to HF pathogenesis through a variety of potential mechanisms. Epidemiologically, increased plasma BCAA concentrations are biomarkers for HF, CAD and hypertension and can predict adverse outcomes in individuals with CAD and HF, and predict stroke, MI, coronary revascularization and death from cardiovascular causes in individuals without CVD. The mechanisms underlying dysregulated BCAA metabolism and other CVDs, such as atherosclerosis, hypertension and cardiac arrhythmias, are less well-defined and are active areas of investigation. Several unanswered questions on the role of BCAA metabolism in CVD are areas for future research:

- Does impaired BCAA metabolism influence vascular tone and endothelial function, and are the effects mediated by endothelium-dependent or endothelium-independent relaxation?

- Do impairments in intrinsic BCAA metabolism in vascular smooth muscle cells contribute to hypertension?

- Does BCAA metabolism affect atherosclerotic plaque progression and, if so, through which vascular compartment?

- What is the role of BCAA metabolism in monocyte and macrophage recruitment to atherosclerotic plaque and transition to foam cells?

- Does impaired BCAA metabolism lead to a pro-atherogenic inflammatory state?

- Does endothelial cell BCAA metabolism contribute to the expression of cell adhesion molecules?

The answers to many of these questions call for the generation of cell-specific and tissue-specific animal models to precisely manipulate BCAA metabolism in relevant vascular compartments. Single-cell omics studies in models of vascular disease could also provide insights into the contribution of impaired BCAA metabolism among the cell populations involved in vascular pathology.

Acknowledgements

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Key points

- branched-chain amino acids (BCAAs) — isoleucine, leucine and valine — are essential amino acids that have a crucial role in metabolic homeostasis through nutrient signalling.

- High circulating concentrations of BCAAs are a hallmark of metabolic disorders, including obesity, insulin resistance and type 2 diabetes mellitus.

- mechanisms underlying the association between BCAAs and cardiovascular disease (CvD) are still being defined, but include activation of the serine/threonine protein kinase mTor, mitochondrial dysfunction, shifts in cardiac substrate utilization and platelet activation.

- epidemiological studies have also shown that high plasma BCAA concentrations identify individuals with heart failure, coronary artery disease or hypertension and predict adverse events in these populations.

- In some other populations (such as african american individuals without CvD and frail elderly individuals), high plasma BCAA concentrations predict improved cardiovascular outcomes.

- areas for future research include the roles of tissue-specific BCAA metabolism and inter-organ BCAA metabolic crosstalk in various CvD states and the roles of impaired BCAA metabolism in vascular function and atherosclerosis.
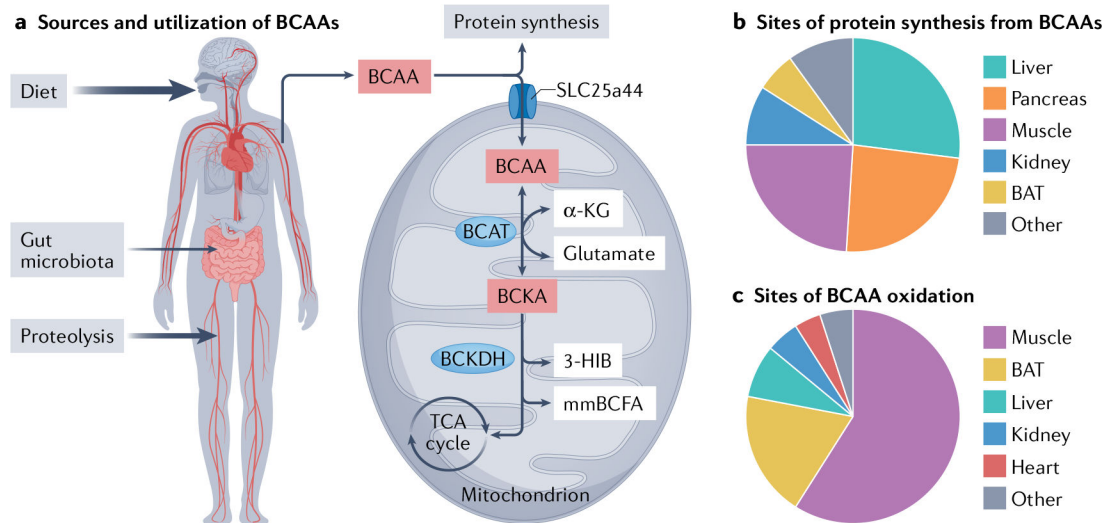
Fig. 1 |. Major sources and sites of BCAA supply and utilization.
a | Branched-chain amino acids (BCAAs) — isoleucine, leucine and valine — are essential amino acids. The circulating pool of BCAAs is derived from the diet and proteolysis, with a smaller contribution from de novo synthesis by the gut microbiota. Circulating BCAAs are used for protein synthesis or are metabolized. BCAA are imported into the mitochondria by the mitochondrial BCAA carrier, SLC25a44. The first two steps in the BCAA catabolic pathway, mediated by the branched-chain amino transferase (BCAT) and the branched-chain α-keto acid dehydrogenase (BCKDH) enzymes, are shared for all three BCAAs. The catabolic pathway terminates at the tricarboxylic acid (TCA) cycle, but also generates important metabolic intermediates, such as the valine metabolite 3-hydroxyisobutyrate (3-HIB) and the monomethyl branched-chain fatty acids (mmBCFAs), which contribute to intracellular and paracrine signalling. **b** | Major sites of BCAA incorporation into protein[34]. **c** | Major sites of BCAA metabolism[34]. α-KG, α-ketoglutarate; BAT, brown adipose tissue; BCKA, branched-chain α-keto acid.
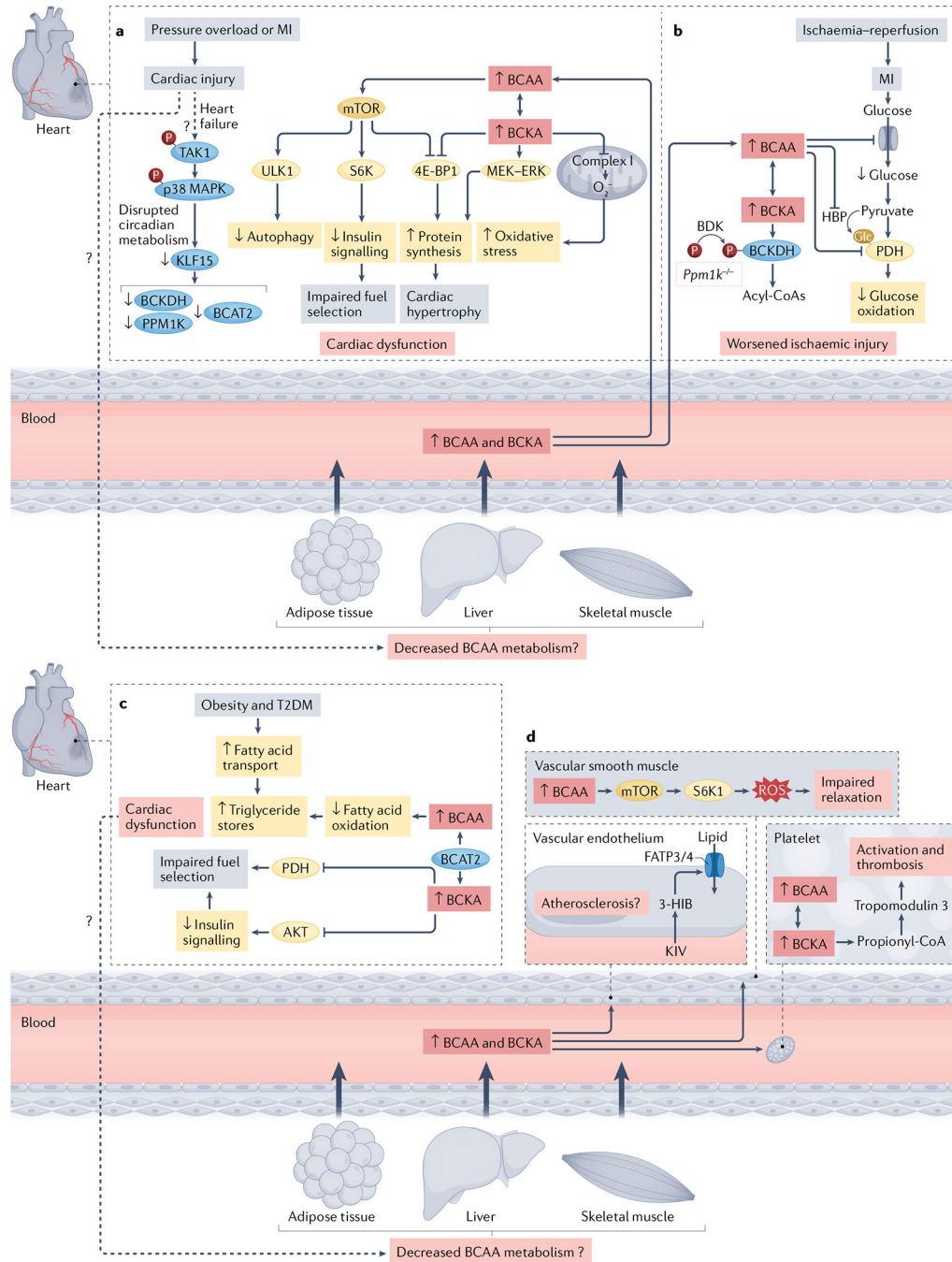
Fig. 2 |. Potential mechanisms connecting dysregulated BCAA metabolism with CVD.
a | In heart failure, the central circadian regulator of branched-chain amino acid (BCAA) metabolism, Krüppel-like factor 15 (KLF15), is downregulated via a mechanism involving transforming growth factor-β-activated kinase 1 (TAK1) and p38 mitogen-activated protein kinase (MAPK) signalling. This leads to decreased expression of BCAA metabolic enzymes, including mitochondrial BCAA aminotransferase (BCAT2), branched-chain α-keto acid dehydrogenase (BCKDH) and mitochondrial protein phosphatase 1K (PPM1K) and accumulation of BCAAs and branched-chain α-keto acids (BCKAs) in the heart.

Cardiac injury also potentially impairs BCAA metabolism in peripheral tissues, resulting in increased circulating BCAA and BCKA levels, and therefore increased delivery of BCAAs and BCKAs to the heart. The BCAA leucine promotes activation of serine/threonine-protein kinase mechanistic target of rapamycin (mTOR) in the heart, which blunts autophagy via serine/threonine protein kinase ULK1 (ULK1), promotes insulin resistance via serine phosphorylation of the insulin receptor substrate 1 mediated by ribosomal protein S6 kinase (S6K) and stimulates protein synthesis by phosphorylation of the translational repressor 4E-B P1. BCKAs also lead to increased phosphorylation of 4E-B P1 and induce the MEK–ERK mitogen-activated protein kinase signalling pathway, which promotes protein synthesis. Moreover, exposure to BCKAs impairs mitochondrial complex I activity in the heart, resulting in superoxide generation and oxidative stress. **b** | In ischaemia–reperfusion injury, whole-body deletion of *Ppm1k* in mice results in the accumulation of circulating and cardiac BCAAs and BCKAs. This accumulation leads to inhibition of glucose transport and oxidation via decreased O-linked *N*-acetylglucosaminylation (Glc) of pyruvate dehydrogenase (PDH), and worsened ischaemic injury. **c** | In animal models of obesity and insulin resistance, BCAA accumulation decreases fatty acid oxidation and increases triglyceride stores. Exposure of the heart to concentrations of BCKAs observed in obesity and type 2 diabetes mellitus (T2DM) inhibits AKT and PDH, resulting in impaired fuel selection. Whether these changes in substrate utilization result in cardiac dysfunction remains to be determined. It is also uncertain whether cardiac-intrinsic changes elicit alterations in BCAA metabolism in other tissues, such as the liver, skeletal muscle or adipose tissue. **d** | BCAAs impair vascular relaxation in an mTOR-dependent manner that involves reactive oxygen species (ROS) generation. BCAA oxidation in platelets promotes thrombosis by stimulating the propionylation of tropomodulin 3, which leads to platelet activation. The valine-derived metabolite 3-hydroxybutyrate (3-H IB), has been shown to increase transendothelial lipid transport dependent on solute carrier family 27 member 3 (FATP3) and long-chain fatty acid transport protein 4 (FATP4). Whether this BCAA–lipid interplay contributes to atherosclerosis remains to be determined. Dashed arrows in the figure depict pathways that are uncertain. BDK, branched-chain keto acid dehydrogenase kinase; HBP, hexosamine biosynthetic pathway; KIV, α-ketoisovalerate; MI, myocardial infarction.

Table 1 |

Human epidemiological studies showing associations between BCAAs and CVD

End point	Population	Findings	Ref.
HF			
Prevalent HFrEF	Single-centre registry of individuals presenting for coronary angiography (HFrEF, $n = 279$; HFpEF, $n = 282$; no HF, $n = 191$)	Plasma BCAA levels higher in HFrEF than in HFpEF and no HF controls	7
Death	Single-centre registry of individuals with HFrEF ($n = 1,032$)	Plasma leucine and valine were part of a prognostic metabolite profile that predicts survival on top of established clinical models and biomarkers	8
MACE (death and HF hospitalization)	Single-centre registry of individuals presenting with STEMI ($n = 138$)	Higher plasma BCAA levels predicted MACE independently and better than NT-proBNP level	69
Peak VO$_2$	HF-ACTION clinical trial ($n = 453$)	Metabolite factor containing BCAAs, phenylalanine, tyrosine, methionine and ornithine associated with increased peak VO$_2$	70
Incident HF hospitalization	Individuals from the Hong Kong Diabetes Register with T2DM and no HFrEF ($n = 2,139$)	Increased plasma BCAA levels associated with increased risk of incident HFrEF	71
Response to CRT	Single-centre, prospective study of individuals with HFrEF receiving CRT ($n = 24$) and controls without HFrEF ($n = 10$)	Higher plasma BCAA levels predicted response (improvement in ejection fraction) to CRT	72
Incident left ventricular diastolic dysfunction	Prospective, family-based population study of individuals without HF ($n = 570$)	Higher plasma leucine and valine levels associated with maintenance of diastolic left ventricular function	73
90-day change in 6MWD and NT-proBNP level	FIGHT clinical trial ($n = 254$)	Higher plasma BCAA levels predicted improvements in 6MWD and NT-proBNP level	87
CAD			
Prevalent CAD	Single-centre registry of individuals with ($n = 214$) or without ($n = 214$) CAD presenting for coronary angiography	Plasma BCAA levels associated with presence of CAD	10
CAD severity	Single-centre registry of individuals presenting for coronary angiography ($n = 1,873$)	Plasma BCAA levels associated with severity of CAD	11
Incident death and MI	Single-centre registry of individuals presenting for coronary angiography ($n = 2,023$)	Plasma BCAA levels predicted death	74
Incident HF hospitalization or death from cardiovascular causes	Single-centre registry of individuals presenting with STEMI who did ($n = 96$) or did not ($n = 96$) undergo HF hospitalization or die from cardiovascular causes	Higher plasma BCAA levels associated with increased risk of HF hospitalization or death from cardiovascular causes	75
Incident stroke, MI or death from cardiovascular causes	PREDIMED clinical trial of individuals without CVD who did ($n = 226$) or did not ($n = 744$) experience stroke or MI, or die from cardiovascular causes	Higher plasma BCAA levels associated with increased risk of stroke, MI and death from cardiovascular causes	76
Incident stroke, MI or death from cardiovascular causes; carotid IMT; inducible ischaemia on cardiac stress testing	Population-based cohort study of individuals without baseline CVD who did ($n = 253$) or did not ($n = 253$) develop incident CVD	Higher plasma BCAA levels associated with increased risk of stroke, MI and death from cardiovascular causes, as well as carotid IMT and ischaemia on cardiac stress testing	77
Incident stroke, MI or coronary revascularization	Individuals from the prospective Women's Health Study without CVD ($n = 27,041$)	Higher plasma BCAA levels associated with increased risk of MI and coronary revascularization	78

End point	Population	Findings	Ref.
Prevalent coronary artery calcium (subclinical atherosclerosis)	Individuals from the population-based Diabetes Heart Study (*n* = 700)	Higher plasma BCAA levels associated with coronary artery calcium only in European Americans	79
Incident MI or death from cardiovascular causes	African American individuals free from CVD in a population-based cohort study (Jackson Heart Study) (*n* = 2,346)	Higher plasma leucine level associated with decreased risk of incident MI or death from cardiovascular causes	80
MACE (stroke, MI, HF and revascularization) or death	Older men from the community-dwelling Australian CHAMP cohort (*n* = 918)	Lower BCAA levels associated with increased risk of frailty, MACE and death	86
Hypertension			
Incident hypertension	Individuals without hypertension from the prospective, population-based PREVEND study (*n* = 4,169)	Higher plasma BCAA levels associated with increased incidence of hypertension	12
Prevalent hypertension	Population-based cohort study in Japan (*n* = 8,589)	Higher plasma BCAA levels associated with prevalent hypertension	81
Incident hypertension	Individuals without hypertension from a prospective, population-based cohort study in Iran (*n* = 4,288)	Increased dietary intake of BCAAs associated with increased incidence of hypertension	83
Prevalent hypertension, pulse wave velocity	Female twins from the TwinsUK registry (*n* = 1,898)	Increased dietary intake of leucine associated with decreased pulse wave velocity and lower central systolic blood pressure	84
Other CVD			
Acute stroke	Single-centre, prospective study of individuals with acute stroke (*n* = 84)	Plasma BCAA levels decreased in acute stroke, and lower plasma BCAA levels associated with poor neurological outcomes	85
Arrhythmia	Individuals from the population-based German KORA study (*n* = 2,304)	Higher plasma BCAA levels associated with altered cardiac conduction and repolarization	13

6MWD, 6-min walk distance; BCAA, branched-chain amino acid; CAD, coronary artery disease; CVD, cardiovascular disease; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IMT, intima-media thickness; MACE, major adverse cardiovascular events; MI, myocardial infarction; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; STEMI, ST-segment elevation myocardial infarction; T2DM, type 2 diabetes mellitus; VO2, oxygen consumption.