Catabolic Defect of Branched-Chain Amino Acids Promotes Heart Failure

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Background—Although metabolic reprogramming is critical in the pathogenesis of heart failure, studies to date have focused principally on fatty acid and glucose metabolism. Contribution of amino acid metabolic regulation in the disease remains understudied.

Methods and Results—Transcriptomic and metabolomic analyses were performed in mouse failing heart induced by pressure overload. Suppression of branched-chain amino acid (BCAA) catabolic gene expression along with concomitant tissue accumulation of branched-chain α-keto acids was identified as a significant signature of metabolic reprogramming in mouse failing hearts and validated to be shared in human cardiomyopathy hearts. Molecular and genetic evidence identified the transcription factor Krüppel-like factor 15 as a key upstream regulator of the BCAA catabolic regulation in the heart. Studies using a genetic mouse model revealed that BCAA catabolic defect promoted heart failure associated with induced oxidative stress and metabolic disturbance in response to mechanical overload. Mechanistically, elevated branched-chain α-keto acids directly suppressed respiration and induced superoxide production in isolated mitochondria. Finally, pharmacological enhancement of branched-chain α-keto acid dehydrogenase activity significantly blunted cardiac dysfunction after pressure overload.

Conclusions—BCAA catabolic defect is a metabolic hallmark of failing heart resulting from Krüppel-like factor 15–mediated transcriptional reprogramming. BCAA catabolic defect imposes a previously unappreciated significant contribution to heart failure. **(*Circulation*. 2016;133:2038-2049. DOI: 10.1161/CIRCULATIONAHA.115.020226.)**

Key Words: amino acids ■ heart failure ■ metabolism ■ oxidant stress ■ pathogenesis ■ remodeling

Alterations in cardiac metabolism are hallmarks of the pathological changes in the failing heart,[1] with studies over the past several decades centered on fatty acid and glucose utilization. Suppression of oxidative phosphorylation with reduced utilization of fatty acid in conjunction with increased glucose consumption is a common feature of heart failure.[2–5] However, little is known about the metabolic changes of amino acid and their functional relevance in the pathogenesis of heart failure.

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Amino acids serve as building blocks for protein synthesis and energy-providing substrates, although the relative importance of a bioenergetic contribution by amino acids in the heart remains unclear under either physiological or pathological conditions.[6] In addition, derivatives of amino acids such as taurine, creatine, carnitine, and glutathione are critical to bioenergenesis and cellular function in the heart.[7] An

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early study by Peterson et al[8] suggested that total free amino acid concentrations were increased in the failing right ventricle. In patients with mitral valve disease, higher glutamine and glutamate concentrations were detected in the dilated left ventricle compared with the right ventricle.[9] A metabolomic study has also demonstrated that intratissue concentrations of several amino acids were changed significantly in the failing rat heart.[10] More recently, 2 reports using multisystems analysis in hypertrophied and early-stage failing mouse hearts after pressure overload or myocardial infarction also revealed profound metabolic derangement, including amino acid metabolism, associated with pathological remodeling.[11,12] These observations indicate that amino acid homeostasis is perturbed in diseased heart tissue.

In this study, we found that the branched-chain amino acid (BCAA) catabolic pathway was the most significantly altered metabolic change in the mouse failing heart and that this coordinated suppression of BCAA catabolic pathway was regulated by Krüppel-like factor 15 (KLF15).[13–15] Furthermore, we found the loss of BCAA catabolic gene expression and the resulting accumulation of intramyocardial levels of BCAA catabolic mediators such as branched-chain α-keto acids (BCKAs) were conserved metabolic signatures in human failing hearts. Impairment of BCAA catabolic pathway impaired heart function and promoted pressure overload–induced heart failure, associated with elevated superoxide production, oxidative injury, and profound metabolic changes in the heart. Finally, pharmacological enhancement of BCAA catabolic activity significantly preserved cardiac function after pressure overload. These findings established that defect of BCAA catabolism is an underappreciated integral part of the metabolic reprogramming in stressed hearts, that amino acid metabolism makes a significant contribution to the progression of heart failure, and that the BCAA catabolic pathway can serve as a potential therapeutic target for the disease.

Methods

Animals and Human Cohorts

PP2Cm germ-line knockout (PP2Cm-KO) mice were generated as previously described.[16] Human cohorts of dilated cardiomyopathy and controls were obtained from Columbia and Duke with Institutional Review Board approval. All animal procedures were carried out in accordance with the guidelines and protocols approved by the University of California at Los Angeles Institutional Animal Care and Use Committee (IACUC). Transaortic constriction (TAC) and cardiac echocardiography were performed as reported earlier[17] on mice from different genotypes between 14 and 1 6 weeks of age. Compound BT2 (3,6-dichlorobenzo[b]thiophene-2-carboxylic acid) was purchased from Sigma-Aldrich and administrated by oral gavage at 40 $mg \cdot kg^{-1} \cdot d^{-1}$ as previously described.[18]

Molecular Methods and Reagents

The details of expression vectors, transfection methods, cell culture, immunoblotting, reverse transcription–polymerase chain reaction (RT-PCR), and chromatin immunoprecipitation methods were provided in the online-only Data Supplement. Superoxide measurement was performed by the electron spin resonance method, and BCKA and BCAA measurements from tissue or plasma were performed following the method published by Olson et al[19] with modifications; details are given in the online-only Data Supplement. The global metabolomic analysis was carried out by Metabolon, Inc (Durham, NC) using heart tissues from PP2Cm-KO and wild-type male mice at 14 to 16 weeks of age. A detailed description of the analysis is given in the online-only Data Supplement**.**

Statistics

Unless otherwise specified, statistical analyses to compare 2 groups were performed with either the Student *t* test or the Wilcoxon rank-sum test (when n<5 or when the variance distributions differed on the basis of the Bartlett test). When >2 groups were analyzed, standard ANOVA followed by the Newman-Keuls test was performed when n>5 for all groups and passed by the Bartlett test of homogeneity of variances. Otherwise, the Kruskal-Wallis test was performed followed by the Dunn multiple-comparison test. Presented values are mean with standard deviation or standard error of the mean. A linear mixed-effect model test was performed for repeated measurements over time. A value of $P<0.05$ was considered statistically significant.

Results

BCAA Catabolic Gene Regulation in Developing and Pathologically Stressed Hearts

It has been well established that postnatal maturation of developing heart results in dynamic shifts from glucose to fatty acid utilization, a phenotype that is reversed in the diseased heart. These changes are orchestrated, at least in part, at the transcriptional level as part of the so-called fetal-like gene expression reprogramming.[20–22] From cardiac transcriptome in pressure overload–induced failing mouse hearts,[23] we performed functional annotation analysis using the Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov) to identify Kyoto Encyclopedia of Genes and Genomes pathways significantly overrepresented in differentially expressed genes.[24] The analysis of downregulated genes in the failing heart revealed >20 specific metabolic pathways that were significantly enriched (Table I in the online-only Data Supplement). Unexpectedly, among them, the valine, leucine, and isoleucine (or BCAAs) catabolic pathway demonstrated the most significant changes associated with heart failure (Figure 1A).

A total of 25 of 46 genes in the Kyoto Encyclopedia of Genes and Genomes BCAA catabolic pathway showed reduced expression in the failing heart compared with the sham controls (Figure 1A and Table II in the online-only Data Supplement). The reduced expression of these key BCAA catabolic enzymes, including BCAT2, BCKD subunits E1α, E1β and E2, and the BCKD phosphatase PP2Cm, was verified at both the mRNA and protein levels (Figure 1B and 1C). In contrast, no reduction was seen in the expression of BCKD kinase (BCKDK; Figure 1B and 1C and Figure IA in the online-only Data Supplement). However, we observed a coordinated induction of the same set of genes during postnatal maturation from neonatal to adult. This dynamic expression pattern is comparable to what is observed for glucose and fatty acid metabolic genes, including *Glut4* (glucose transporter 4) and *Mcad* (medium-chain acyl-CoA dehydrogenase*),* along with other well established fetal-like marker genes, including *Nppa* and *Myh7* (Figure 1B). Therefore, the rate-limiting and downstream steps of the BCAA catabolic pathway are coordinately downregulated as part of the fetal-like transcriptome remodeling in failing heart.

A significant reduction in *PP2Cm* expression along with an unchanged expression of *BCKDK* led to enhanced

phosphorylation of BCKD regulatory subunit E1α in the failing hearts comparing with controls (Figure IB in the online-only Data Supplement). Phosphorylation level of BCKD E1α subunit inversely correlates with the BCKD enzymatic activity; consequently, the levels of the intramyocardial BCKA were significantly increased in the mouse failing hearts (Figure 1D), whereas the total BCAA levels remained unchanged (Figure II in the online-only Data Supplement).

Defect of BCAA Catabolism in Human Failing Hearts

Human cardiomyopathy hearts demonstrated a striking parallel to the observations in rodents, with coordinated

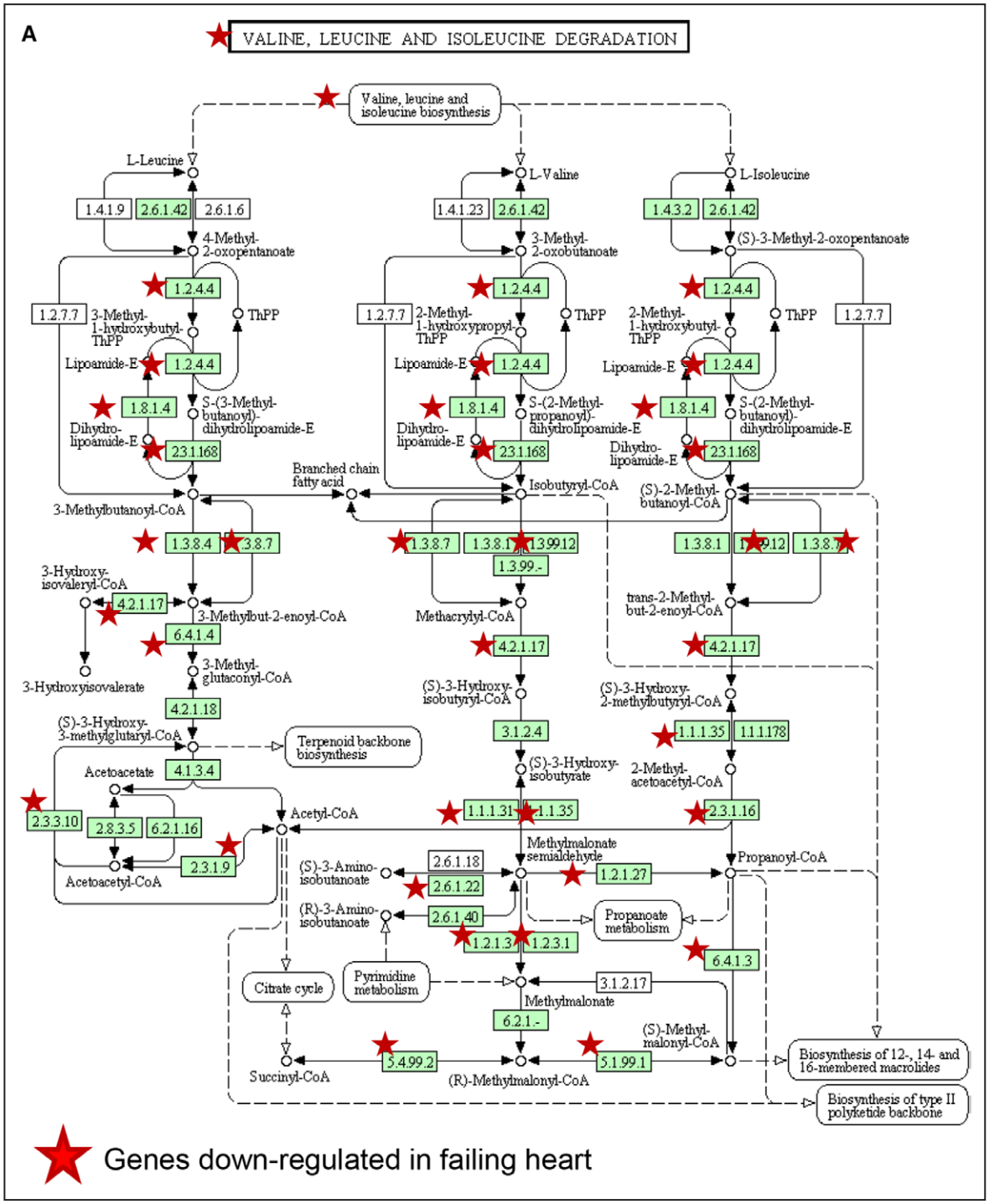

Figure 1. Remodeling of branched-chain amino acid (BCAA) catabolism in murine failing heart. **A**, The downregulated genes in failing heart were mapped into BCAA catabolism pathway by Kyoto Encyclopedia of Genes and Genomes. **B**, Real-time reverse transcription–polymerase chain reaction result of specific genes using mRNA from myocardium of neonatal (n=3), normal (adult sham; n=3), and failing (adult failing; n=3) mouse hearts. The *y* axis represents the relative mRNA level. ANOVA followed by the Newman-Keuls (*Continued*)

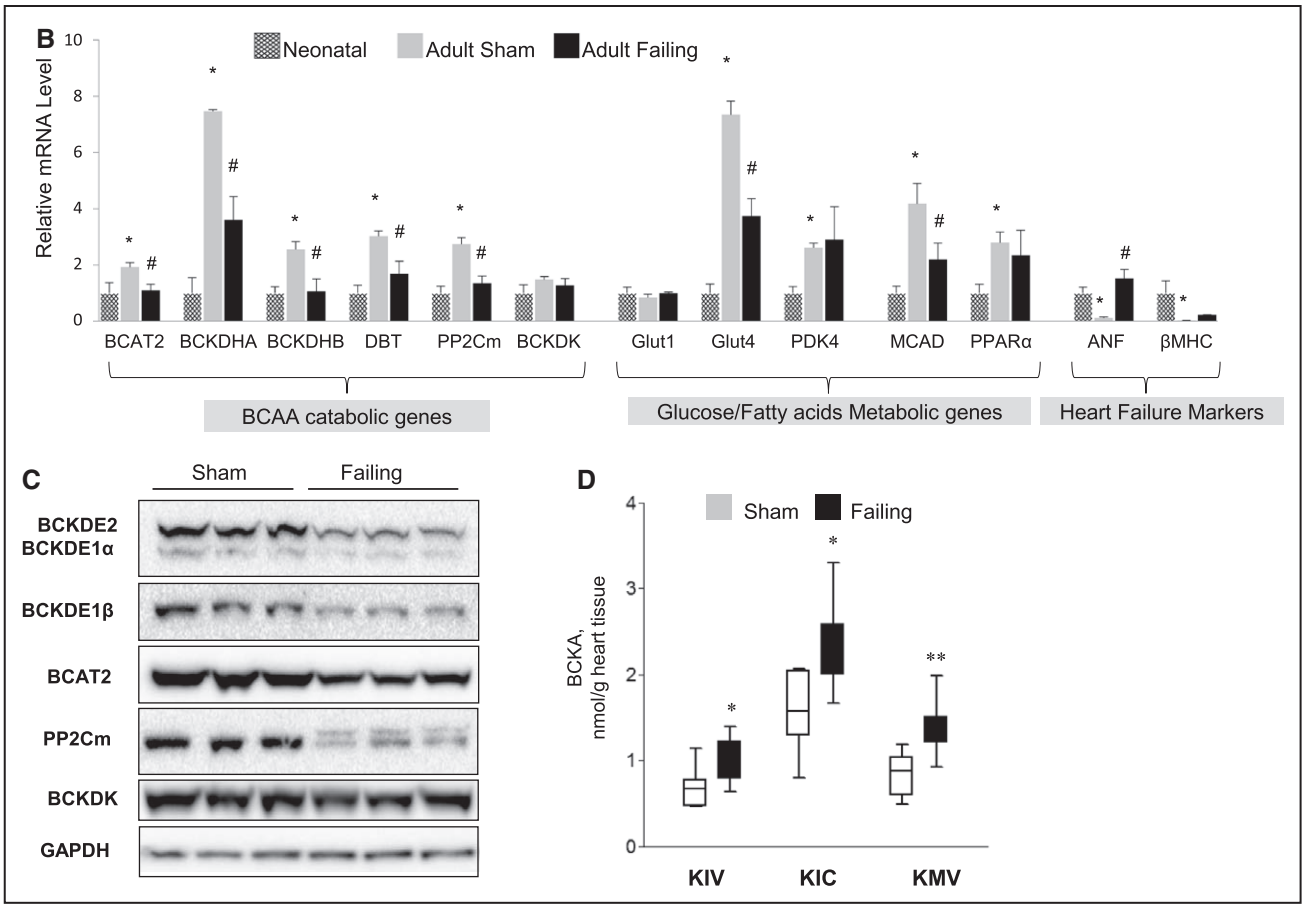

Figure 1 Continued. test was performed. *P<0.05 vs neonatal; #P<0.05 vs adult sham. **C**, Western blotting result of proteins involved in BCAA catabolism (GAPDH as loading control) using tissue lysates from 3 individual normal (sham) or failing mouse hearts (n=3). **D**, Individual branched-chain α-keto acid (BCKA) concentration in tissues from normal (sham, n=9) and failing (n=7) mouse hearts. Error bars represent SD (**B**) or SEM (**D**). *P<0.05, **P<0.01.

reduction of all key BCAA catabolic gene products, including BCAT2, BCKD subunits, and PP2Cm, whereas BCKDK expression was slightly increased (Figure 2A). Importantly, intramyocardial levels of BCKA were also significantly increased in human cardiomyopathy hearts (Figure 2B). A significantly higher level of KMV but not KIC or KIV was also observed in plasma from humans with heart failure (Figure III in the online-only Data Supplement). In contrast, intramyocardial BCAA levels were not significantly altered (Figure IV in the online-only Data Supplement). Therefore, impairment of BCAA catabolic activity and intramyocardial accumulation of BCKA metabolites are conserved metabolic alterations in mouse and human failing hearts.

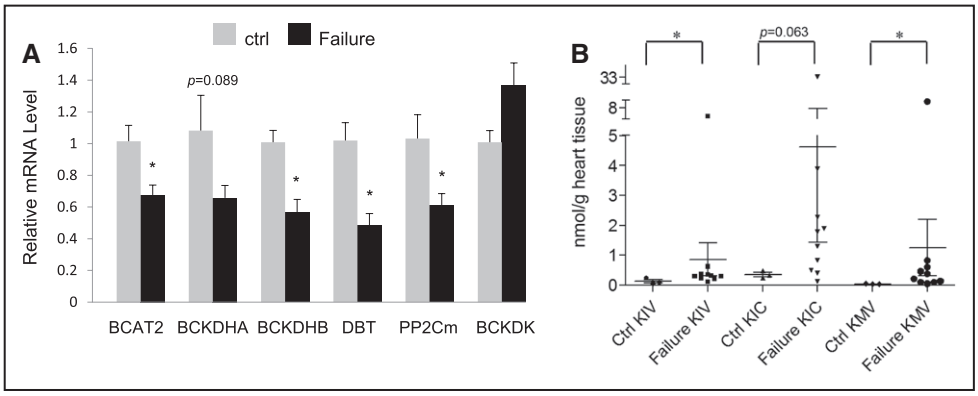

Figure 2. Impaired branched-chain amino acid (BCAA) catabolism in human failing heart. **A**, Real-time reverse transcription–polymerase chain reaction result of specific genes using mRNA from myocardium of control (Ctrl; n=4) and failing (failure; n=11–15) human hearts. The *y* axis shows the relative mRNA level. **B**, Individual branched-chain α-keto acid (BCKA) concentration in tissues from control (n=3) and failing (n=10) human hearts. Error bars represent SEM. BCKDK indicates BCKD kinase. *P<0.05.

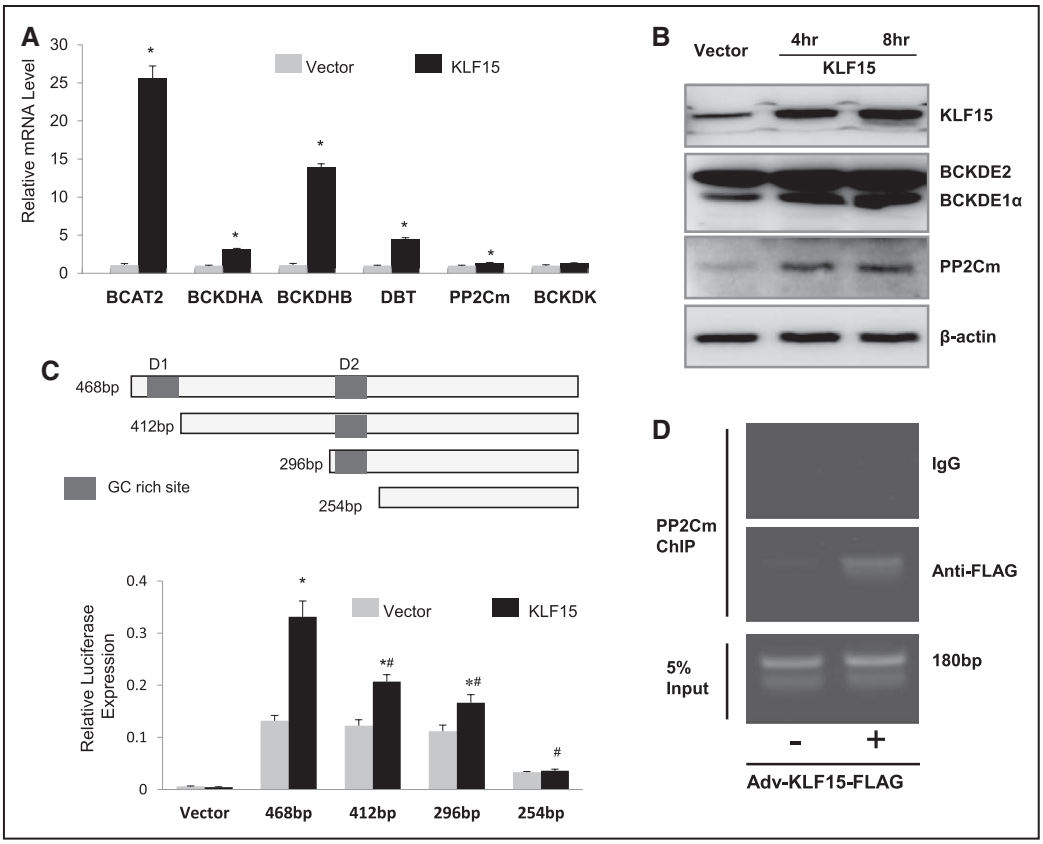

Figure 3. Krüppel-like factor 15 (KLF15) regulates branched-chain amino acid (BCAA) catabolic gene expression. **A**, Real-time reverse transcription–polymerase chain reaction result of specific genes using mRNA from neonatal rat ventricular myocytes with (KLF15) or without (vector) KLF15 overexpression (n=6). **P*<0.05 vs vector control. **B**, Western blotting result of proteins involved in BCAA catabolism (GAPDH as loading control) using cellular lysates from KLF15-overexpressed Hela cells. **C**, Illustration of partial mouse PP2Cm promoter fragments with 2 GC-rich sites and luciferase assay result of PP2Cm promoter–luciferase in HeLa cells cotransfected with either KLF15 or corresponding empty vector. The data represented the average values, with the SD of triplicate samples from 1 experiment representative of 3 independent experiments. **P*<0.05 vs same promoter without KLF15 overexpression; #*P*<0.05 vs 468-bp promoter with KLF15 overexpression (n=3). **D**, Representative result of chromatin immunoprecipitation–polymerase chain reaction validation for KLF15 binding to the PP2Cm gene promoter in neonatal rat ventricular myocytes after KLF15 overexpression. The experiment was repeated twice with similar results.

KLF15 Regulates Cardiac BCAA Catabolic Gene Expression

Coordinated regulation of BCAA gene products suggests a shared regulatory mechanism at the transcriptional level. We performed an upstream regulator analysis using Ingenuity Pathway Analysis software (http://www.ingenuity.com) for the genes showing altered expression in the mouse failing heart.[23] The analysis of downregulated genes in the failing heart predicted numerous factors involved in their regulation (Table III in the online-only Data Supplement). The top 3 candidates were MAP4K4, KLF15, and PPARA. KLF15 was reported to be a direct transcriptional activator of BCAT2.[25–27] In cultured cardiomyocytes, overexpression of KLF15 significantly induced the mRNA expression of BCAT2, BCKD subunits, and *PP2Cm*, with a notable exception of *BCKDK* (Figure 3A). Ectopic expression of KLF15 also induced the expression of these targets in nonmyocytes (Figure 3B and Figure VA in the online-only Data Supplement). Using a PP2Cm promoter luciferase reporter, we showed that KLF15 directly induced the transcriptional activity of the PP2Cm promoter containing putative KLF15 binding motifs (Figure 3C and Figure VB in the online-only Data Supplement). Finally, chromatin immunoprecipitation analysis revealed a significant accumulation of KLF15 binding to the endogenous PP2Cm promoter in cardiomyocytes (Figure 3D). Taken together, these data support a previously unidentified broad regulatory role for KLF15 in myocardial BCAA catabolic gene expression.

We examined the abundance of BCAA catabolic genes in *Klf15*-null hearts. Consistent with in vitro observations, the KLF15-deficient hearts displayed reduced expression of BCAT2, BCKD (E1a, E1b, E2), and PP2Cm, again with the notable exception of BCKDK, at both the mRNA and protein levels (Figure 4A and 4B and Figure IVA in the online-only Data Supplement), phenocopying what was observed in diseased mouse and human hearts (Figures 1 and 2). Also similar to what we observed in failing human and mouse heart samples, elevated intramyocardial BCKA levels were identified in the *Klf15*-null hearts (Figure 4C). Moreover, KLF15 expression was reduced in pressure-overloaded murine hearts (Figure VIB in the online-only Data Supplement) and in human cardiomyopathy, as previously demonstrated.[14,15] Therefore, our data identify KLF15 as a central transcriptional regulator of the BCAA catabolic

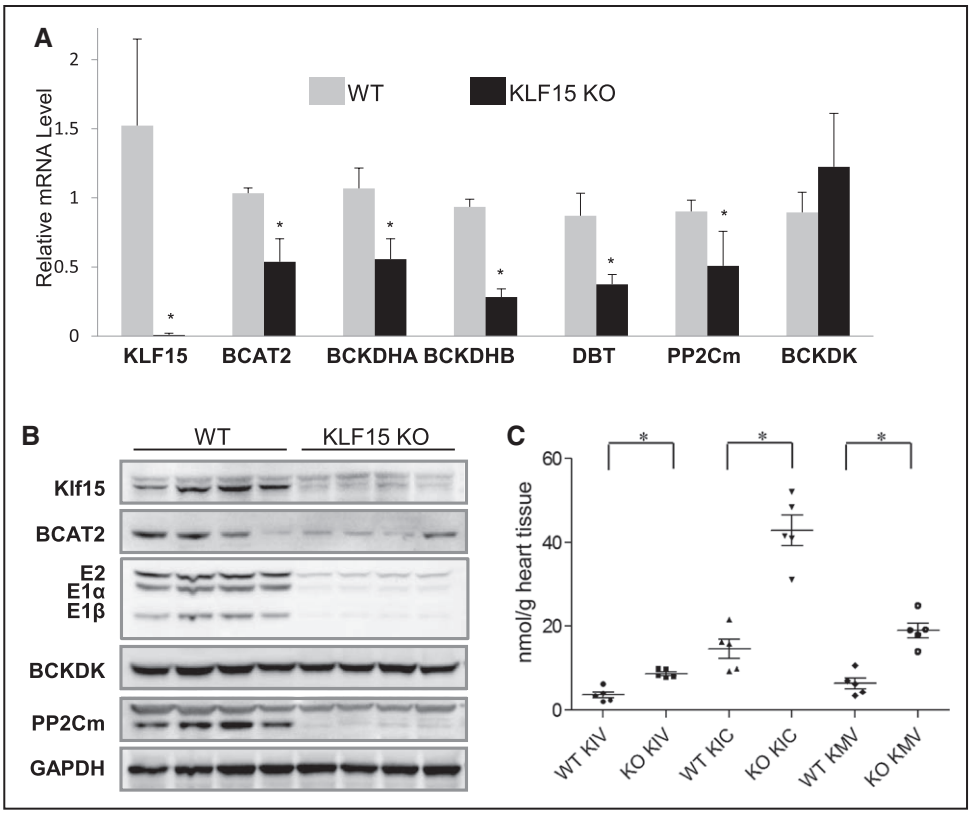

Figure 4. Ablation of cardiac Krüppel-like factor 15 (KLF15) downregulates branched-chain amino acid (BCAA) catabolism. **A** and **B**, Real-time reverse transcription–polymerase chain reaction (**A**) and Western blotting (**B**) result of specific genes in wild-type (WT; n=4) and KLF15-deficient (KLF15 KO; n=4) hearts. **C**, Level of branched-chain α-keto acids (BCKAs) in WT (n=4) and KLF15 deficient (n=5) heart. Error bars represent SD (**A**) or SEM (**C**). *P<0.05 vs wild-type.

pathway and show that loss of KLF15 is a potential molecular mechanism underlying stress-induced BCAA catabolic defects in the diseased heart.

BCAA Catabolic Defect Impaired Cardiac Contractile Function

To directly assess the effect of BCAA catabolic defect on heart function, we used a mouse model carrying the genetically inactivated PP2Cm coding gene *ppm1k* (PP2Cm-KO) in which BCKD activity is significantly inhibited because of the constantly elevated E1α phosphorylation.[16,28] Indeed, compared with wild-type controls, intramyocardial BCKA and BCAA levels were significantly increased in the PP2Cm-deficient hearts from mice fasted for 6 hours (Figure 5A) at levels (<5 nmol·L^{-1}·g^{-1}) comparable to what was observed in mouse and human failing hearts (Figures 1D and 2B). However, cardiac BCKA concentrations became much higher (15–45 nmol·L^{-1}·g^{-1}) in the PP2Cm-KO heart under feeding conditions (Figure 5B and Figure VIIA in the online-only Data Supplement), highlighting the potential dietary influence on BCKA accumulation in the diseased heart when BCAA catabolic activity is compromised. Echocardiogram measurements showed a modest but statistically significant reduction in cardiac systolic function in the PP2Cm-deficient mice at 3 months of age (Figure 5C). By 18 months of age, their cardiac function was further reduced compared with the age-matched wild-type controls (Figure 5D). However, young PP2Cm-deficient mice exhibited no major changes in cardiac morphology, histology, and ultrastructure, as well as molecular markers of myocardial remodeling (Figure 5E–5G and Figure VIIB in the online-only Data Supplement). Therefore, abnormal BCAA catabolism is sufficient to promote contractile dysfunction over time in the absence of any external pathological stressor.

BCAA Catabolic Defect Enhances Susceptibility to Heart Failure in Response to Pathological Stress

We then subjected wild-type and PP2Cm-KO mice (3–4 months of age) to pressure overload. From the second week of TAC, PP2Cm-deficient mice exhibited a marked reduction in contractile function (Figure 6A and Figure VIII in the online-only Data Supplement). A repeated-measures linear model analysis demonstrated that changes in cardiac echocardiogram parameters such as left ventricular internal dimension in systole for group×day (P=3.54e−6) and left ventricular fractional shortening (P=1.27e−3) were significant between the PP2Cm-deficient and wild-type mice. At 8 weeks after TAC, PP2Cm-deficient mice displayed signs of heart failure, as evidenced by significantly reduced left ventricular ejection fraction, chamber dilation, and elevated wet lung weights, an indicator of severe pulmonary congestion resulting from heart failure (Figure 6). Collectively, these data indicate that deficient BCAA catabolism can directly impair cardiac function and accelerates pressure overload–induced cardiomyopathy.

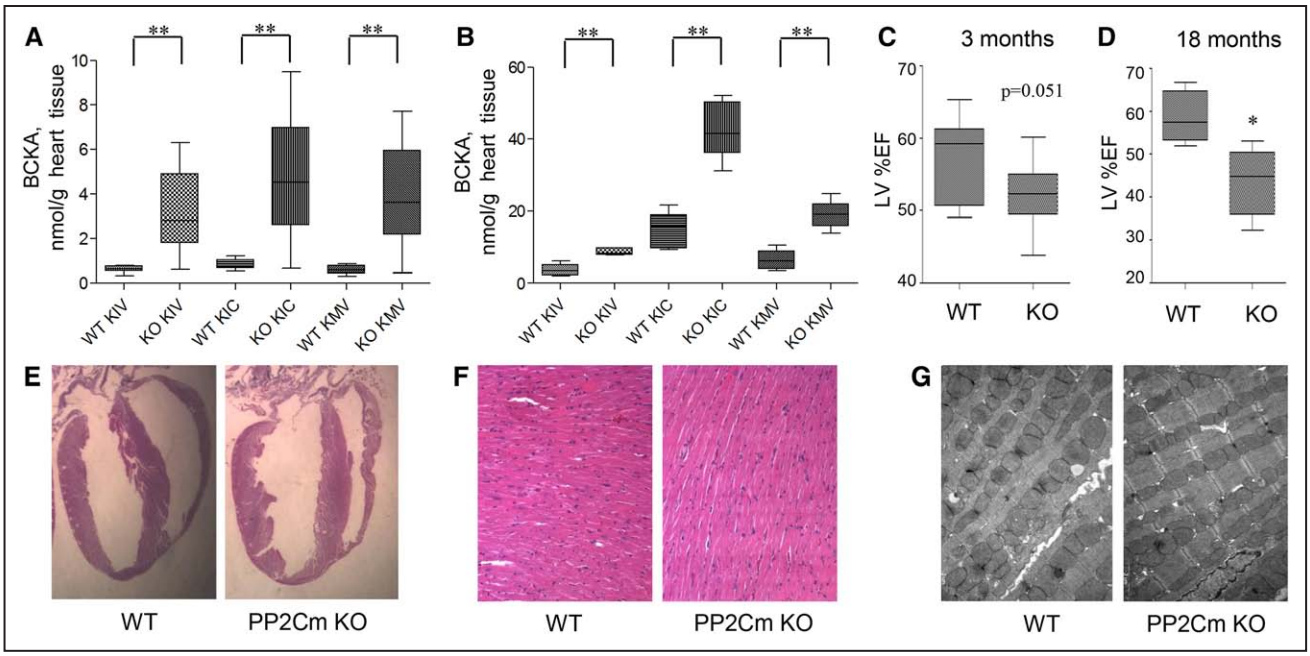

Figure 5. Branched-chain amino acid (BCAA) catabolic defect impairs cardiac function but not structure. **A** and **B**, Individual branched-chain α-keto acid (BCKA) concentrations in cardiac tissue of PP2Cm germ-line knockout mice (KO) and wild-type (WT) mice. **A**, Mice on a normal chow (20% protein) were fasted for 6 hours (WT, n=9; KO, n=10). **B**, Mice were fasted overnight and fed with a high-protein diet (40% protein) for 2 hours (WT, n=5; KO, n=5). Error bars represent SEM. $^{**}P<0.01$ vs WT. **C** and **D**, Left ventricular ejection fraction (LV%EF) from WT and PP2Cm-KO mice at 3 months (**C**; WT, n=12; KO, n=15) or 18 months (**D**; n=5 in each group) of age. **E**, Morphology of hearts from WT and PP2Cm-KO mice. **F**, Longitudinally sectioned heart was stained with hematoxylin and eosin. Magnification ×200. **G**, Transmission electron microscopy was used in hearts from PP2Cm-KO and WT mice. Magnification ×7400.

Impaired Metabolic and Redox Homeostasis by BCAA Catabolic Deficiency in Heart

The impact of BCAA catabolic defects on cardiac function is consistently correlated with elevated BCKA metabolites in the diseased heart tissue. We found that in isolated cardiac mitochondria, BCKAs directly inhibited complex I– but not complex II–mediated respiration (Figure 7A and Figure IXA and IXB in the online-only Data Supplement). The inhibition was dose dependent with a marked decrease observed at concentrations as low as 20 μmol/L BCKAs (Figure 7B). In the meantime, BCKAs also promoted superoxide production in isolated cardiac mitochondria in a dose-dependent manner (Figure 7C). A significant increase in superoxide production was detected from the PP2Cm-deficient mitochondria (Figure 7D) and myocardium (Figure 7E), associated with the enhanced oxidative injury to cardiac proteins (Figure 7F). These data suggest that accumulated BCKAs resulting from BCKD inactivation may directly affect cardiac mitochondrial activity and redox homeostasis.

We performed additional targeted metabolomic analysis on >300 metabolic intermediates in hearts from wild-type and PP2Cm-KO mice. Principal component analysis revealed a divergent separation between wild-type and PP2Cm-KO hearts (Figure 7G), suggesting global metabolic changes associated with BCKD inhibition. When random forest analysis was applied to cluster all samples, changes in intracardiac metabolites separated the wild-type and PP2Cm-KO mice with 100% predictive accuracy (Figure XA in the online-only Data Supplement). In addition to BCAA and their metabolites, the top 30 most significantly changed metabolites in the PP2Cm-KO hearts include lipids and carbohydrates (Figure 7H). Specifically, the levels of glucose, glycolytic intermediates, and glucose-derived sugars such as fructose and mannose 6-phosphate were markedly elevated in the PP2Cm-deficient heart (Figure XB in the online-only Data Supplement). These results support the notion that BCAA catabolic deficiency and elevated BCKA can result in impaired mitochondrial function, reactive oxygen species induction, and global perturbations in the myocardial metabolic profile.

Inhibition of BCKDK Promoted BCKA Degradation and Preserved Heart Function

Given the significant contribution of BCAA catabolic defect to cardiac dysfunction, we investigated the impact of enhancing BCAA catabolic activity on pressure overload–induced heart failure in mice. 3,6-Dichlorobenzo[b] thiophene-2-carboxylic acid (BT2) is a highly specific and potent inhibitor of BCKDK.[18] Administration of BT2 in mice significantly reduced the phosphorylation of BCKD subunit E1α in heart (Figure 8A and 8B) and dramatically enhanced cardiac BCKD activity in both wild-type (≈7-fold) and the PP2Cm-KO mice (≈9-fold; Figure 8C). Consequently, the plasma BCKA level in both wild-type and PP2Cm-KO mice was markedly reduced (Figure 8D) but with modest impact on plasma BCAA level (Figure XI in the online-only Data Supplement). More important, at 4 weeks after TAC, BT2-treated mice displayed significantly preserved left ventricular ejection fraction and reduced chamber dilation (Figure 8D–8F and Figure XII in the online-only Data Supplement). These results suggested that enhancing BCKA degradation

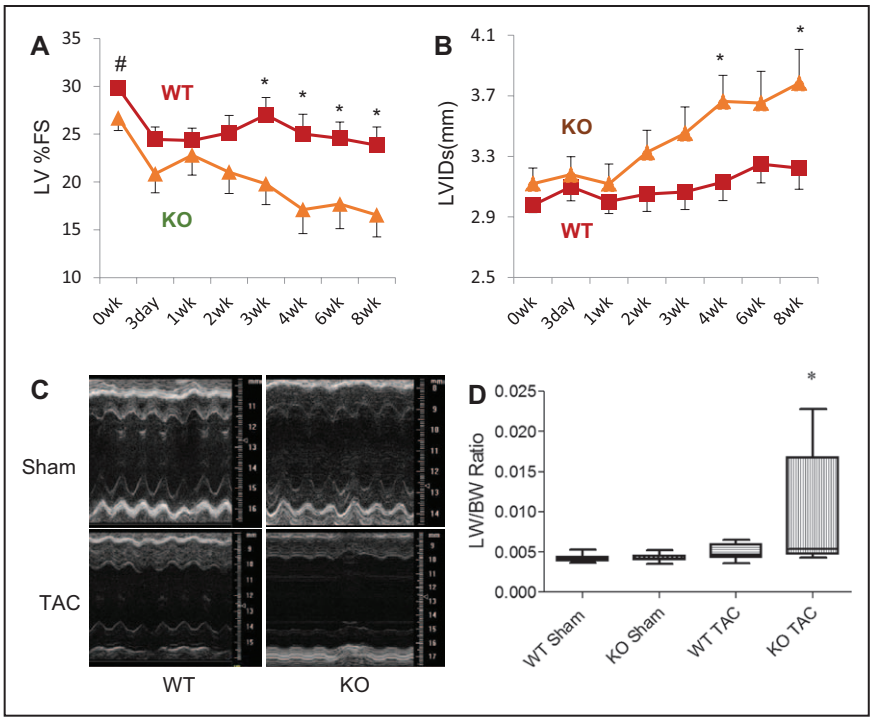

Figure 6. Branched-chain amino acid (BCAA) catabolic defect promotes heart failure progression. **A** and **B**, Time course for left ventricular fractional shortening (LV%FS; **A**) and left ventricular internal dimension (in millimeters) at systole (LVIDs; **B**) from wild-type (WT; n=11–15) and PP2Cm germ-line knockout (PP2Cm-KO) mice (n=13–19) with transaortic constriction (TAC) surgery. The *x* axis shows the time in weeks after surgery. **C** and **D**, Representative M-mode echocardiographs (**C**) or ratio of lung weight to body weight (LW/BW; **D**; WT sham, n=9; KO sham, n=10; WT TAC, n=8; KO sham, n=8) from WT and PP2Cm-KO mice at 8 weeks after surgery. Error bars represent SEM. Statistical analyses were performed with the Student *t* test (**A** and **B**) to compare the values of WT and PP2Cm-KO at the same time point (#*P*=0.05, **P*<0.05) or the Kruskal-Wallis test followed by the Dunn multiple-comparison test (**D**; **P*<0.05 vs KO sham). A repeated-measures linear model was also fitted for LVIDs (**A**) and LV%FS (**B**).

by targeted inhibition of BCKDK significantly preserved cardiac function in response to pathological stress.

Discussion

In the present study, we reveal that BCAA catabolic gene expression is coordinately suppressed in both murine and human failing hearts as part of fetal-like gene expression and metabolic reprogramming. KLF15-mediated transcriptional regulation is central for this coordinated reduction of BCAA catabolism. Genetic and cellular analyses suggest that BCAA catabolic defects and the resulting accumulation of BCKA metabolites cause cardiac reactive oxygen species injury and global metabolic alteration and significantly contribute to the progression of heart failure.

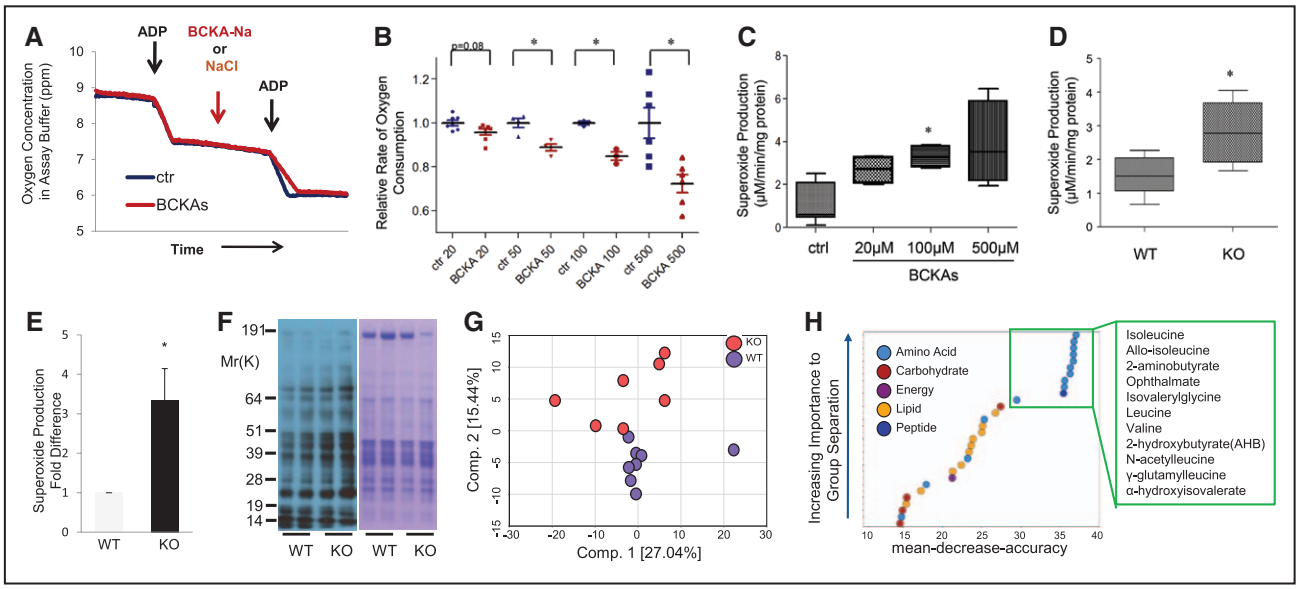

Figure 7. Disturbed metabolic and redox homeostasis by branched-chain α-keto acids (BCKAs). **A**, Oxygen consumption in mitochondria isolated from wild-type (WT) hearts in the absence or presence of 500 μmol/L BCKAs. **B**, Relative oxygen consumption rate in the absence or presence of BCKAs at different concentrations (n=3–8 in each group; **P*<0.05 vs control). **C**, Superoxide production in isolated cardiac mitochondria (n=4–7 in each group; **P*<0.05 vs control). **D** and **E**, Superoxide production in isolated mitochondria (**D**, n=5–6 in each group) and myocardium (**E**, n=3 in each group) from WT and PP2Cm germ-line–deficient (KO) mice. **F**, Immunoblotting of total protein oxidation detected by carbonyl groups (**left**) from tissue lysates of WT and PP2Cm-KO mouse hearts. **G**, Principal component analysis of metabolomic profiles revealed a distinct genotype-based separation for the heart samples (WT, n=8; KO, n=7). **H**, List of the top 30 biochemicals that separated different genotypes based on their importance. Error bars represent SEM (**B**–**E**).

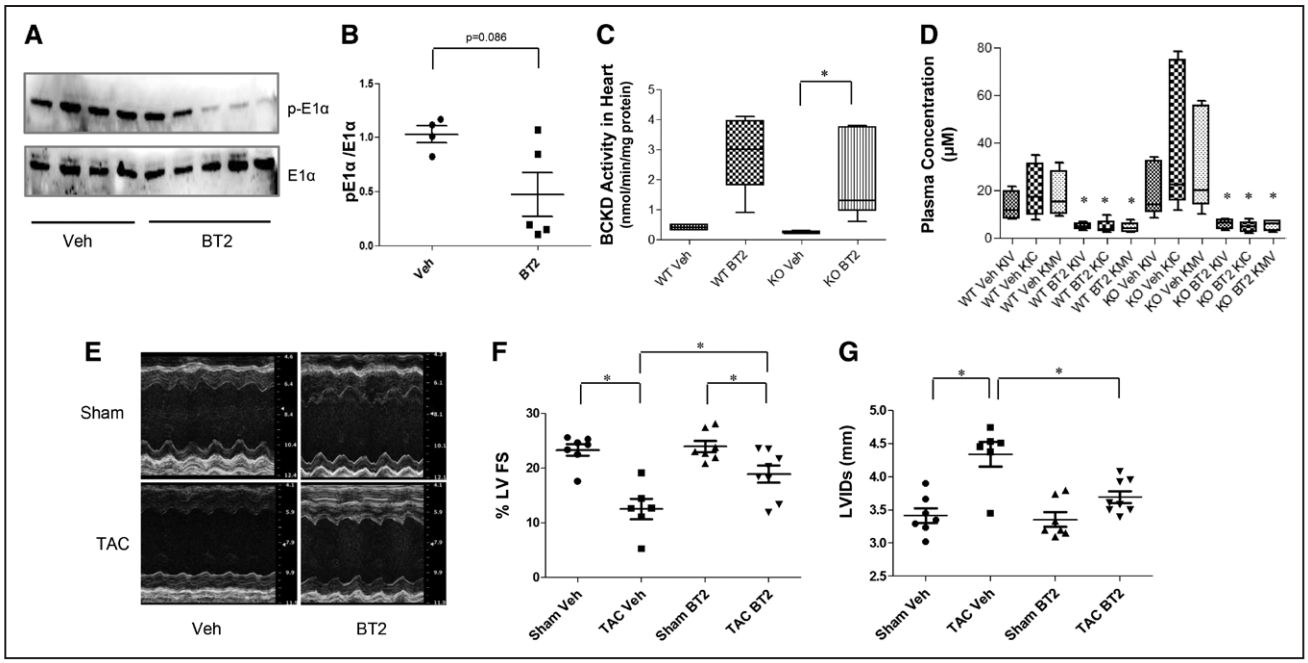

Figure 8. Inhibition of BCKD kinase (BCKDK) by BT2 (3,6-dichlorobenzo[b]thiophene-2-carboxylic acid) promotes branched-chain α-keto acid (BCKA) degradation and preserves cardiac function in the pressure-overloaded heart. **A**, Immunoblot for total and phosphorylated BCKD subunit E1α in heart from wild-type (WT) mice treated with vehicle (veh; n=4) or BT2 (n=5). **B**, The average phosphorylation level of E1α vs total E1α is presented with the SEM. Error bars represent SEM. **P*<0.05 between vehicle- and BT2-treated samples. **C**, BCKD activity in cardiac tissues from WT or PP2Cm germ-line knockout mice (KO) mice treated with vehicle or BT2 (n=4–5 in each group). **P*<0.05, vehicle- vs BT2-treated groups. **D**, Individual BCKA concentration in plasma from WT and PP2Cm-KO (n=4–6) mice treated with vehicle or BT2.**P*<0.05, vehicle- vs BT2-treated groups of the same genotype. **E,** Representative M-mode echocardiographs of mouse hearts after sham surgery or transaortic constriction (TAC) treated with vehicle or BT2. **F**, Left ventricular ejection fraction (%LVEF; n=6–8) and (**G**) left ventricular internal dimension at systole (LVIDs; n=6–8) from mice with sham or TAC surgery for 4 weeks, treated with or without BT2 as indicated. Error bars represent SEM. **P*<0.05 between designated groups.

Amino acids serve as both important nutrients and potent signaling molecules.[29] However, compared with the extensive knowledge of fatty acid and glucose metabolism, current understanding of amino acid metabolic regulation under normal development or pathological conditions is very limited. BCAAs, including leucine, isoleucine, and valine, are essential amino acids with a shared catabolic pathway. In addition to participating in de novo protein synthesis, BCAAs function as potent nutrient signal molecules for cellular metabolism and growth. Through the mechanistic target of rapamycin pathway, BCAAs (particularly leucine) can regulate vital cellular processes, including protein translation, autophagy, and insulin signaling,[30] affecting glucose and fatty acid metabolism,[31] muscle anabolism,[32] and life span.[33] Genetic defect of BCAA catabolism leads to maple syrup urine disease.[34] Recently, abnormal plasma BCAA levels have been associated with neurological, cardiovascular, metabolic diseases, and cancer in numerous studies.[35–40] These findings highlight the importance of BCAA metabolism in normal physiology and a broad spectrum of human diseases. Suppressed BCAA catabolic activity appears to be a common feature in the stressed heart. Earlier reports by Kato et al[10] using Dahl salt-sensitive rats demonstrate that cardiac valine, isoleucine, and leucine levels are elevated after a high-salt diet. Several other studies, including our present study in both rodents and humans, have now linked high levels of BCAAs with cardiac diseases.[10,36,41–43] Therefore, BCAA catabolic defect is another metabolic hallmark of heart diseases that may be exploited as additional metabolic biomarkers for cardiac pathology.

The coordinated loss of BCAA catabolic gene expression suggests a common regulatory machinery for the pathway. This notion is consistent with 2 recent studies reported in hypertrophied and early-stage failing heart.[11,12] From both bioinformatic and genetic approaches, we identify KLF15 as a master transcription factor responsible for BCAA catabolic gene expression in heart. The functional role of KLF15 is well documented in cardiac hypertrophy,[13,44] heart failure,[14] and cardiac fibrosis.[45] In addition to hypertrophic genes, KLF15 serves as a key regulator of glucose, fatty acid, and amino acid metabolism.[25,26,46–51] KLF15 is reported to directly modulate the expression of BCAT2 as a mechanism to modulate mechanistic target of rapamycin signaling in skeletal muscle.[25,26] KLF15 has previously been shown to be regulated by diverse pathological stimuli. Human and murine forms of pressure-overload cardiomyopathy have been shown to reduce KLF15 levels, a result in humans that is reversed by mechanical unloading.[15,47,52,53] Moreover, hypertrophic stimuli (including angiotensin II, phenylephrine, and endothelin-1) have been shown to reduce KLF15 levels both in vivo and in vitro.[52,53] Our data reinforce the notion that KLF15 is an important regulator for metabolic reprogramming in heart by modulating several important branches of macronutrient metabolism, including fatty acid, glucose, and amino acids.

It is intriguing that metabolic profiling in hypertrophic (1 week after TAC) or post–myocardial infarction hearts revealed elevated BCAA concentrations[11,12] in cardiac tissue,

in contrast to what we observed in both end-stage human cardiomyopathy hearts and mouse failing hearts 8 weeks after TAC. It is plausible to speculate that BCAA catabolic reprogramming is a compensatory mechanism at least at the initial stage of the response of the myocardium to stress, given that BCAA preservation would redirect amino acids from catabolic consumption to protein synthesis and cell growth during cardiac hypertrophy. Perturbation of BCAA catabolic activity may have a significant impact on mechanistic target of rapamycin signaling, leading to potential changes in cardiac growth, metabolism, and survival. However, defective BCKD activity also causes accumulation of BCKA in hearts, which may lead to a detrimental effect resulting from cytotoxic effects on mitochondrial function and reactive oxygen species homeostasis.[16,54] Indeed, a direct and dose-dependent impact of BCKA treatment on mitochondrial function and reactive oxygen species production as demonstrated in this study highlights the potential contribution of BCKA as the true pathogenic culprit underlying BCAA catabolic defects in the progression of heart failure. BCKA and BCKA-mediated mitochondrial and cellular defects should be further explored as both metabolic biomarkers and therapeutic targets for heart failure.

Our genetic data from this report clearly implicate BCAA catabolic defect as a significant contributor to the pathogenesis of heart failure. The results from our study using BCKDK inhibitor (Figure 8) clearly demonstrate the translational value of targeting BCAA catabolism as a therapy for heart failure.[17,55] They also raise the question of the potential impact of dietary influence on disease progression. Tanada et al[56] showed that BCAA supplement ameliorated the progression of heart failure in rats associated with preserved skeletal muscle weight and mitochondrial function. It is not clear if the benefit of BCAA supplement at the early stage of heart failure is derived from enhanced BCAA flux and preserved BCAA catabolic activities. Clearly, more preclinical and clinical studies are needed to fully establish the therapeutic window and approaches to manipulate BCAA catabolism in heart failure.

Conclusions

We have elucidated a previously unappreciated role for BCAA catabolism in the cardiac metabolic adaptation to stress. This insight can be further exploited for future diagnostic and therapeutic development.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Heart failure is a leading cause of mortality and hospitalization, and effective therapies remain elusive. The present study uncovers that branched-chain amino acid (BCAA; including leucine, isoleucine, and valine) catabolic defect is a hallmark of metabolic changes in murine failing heart and human dilated cardiomyopathy hearts. Accumulation of branched-chain α-keto acids resulting from BCAA catabolic defect directly impairs mitochondrial activity, induces oxidative stress, and promotes cardiac dysfunction. More importantly, restoration of BCAA catabolism by pharmacological agents blunt disease progression in pressure overload–induced heart failure. Therefore, defects in BCAA/branched-chain α-keto acids catabolism are not only a new metabolic biomarker for heart failure, but also a significant contributor to heart failure. Promoting BCAA catabolic activity with a pharmacological agent can be a potentially effective therapeutic strategy to ameliorate the pathogenic progression of heart failure. Finally, considering the dietary source of BCAA, the present study indicates a role of protein intake in the disease progression of heart failure and thus serves as a preclinical basis to develop a more appropriate nutritional intervention approach for this disease.