ESC European Society of Cardiology

Cardiovascular Research (2024) **120**, 2166–2178
https://doi.org/10.1093/cvr/cvae248

REVIEW

Anaplerotic filling in heart failure: a review of mechanism and potential therapeutics

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Received 18 June 2024; revised 8 September 2024; accepted 12 November 2024; online publish-ahead-of-print 21 November 2024

Abstract

Heart failure (HF) is a complex syndrome and a leading cause of mortality worldwide. While current medical treatment is based on known pathophysiology and is effective for many patients, the underlying cellular mechanisms are poorly understood. Energy deficiency is a characteristic of HF, marked by complex alterations in metabolism. Within the tricarboxylic acid cycle, anaplerosis emerges as an essential metabolic process responsible for replenishing lost intermediates, thereby playing a crucial role in sustaining energy metabolism and consequently cardiac function. Alterations in cardiac anaplerosis are commonly observed in HF, demonstrating potential for therapeutic intervention. This review discusses recent advances in understanding the anaplerotic adaptations that occur in HF. We also explore therapeutics that can directly modulate anaplerosis or are likely to confer cardioprotective effects through anaplerosis, which could potentially be implemented to rescue the failing heart.

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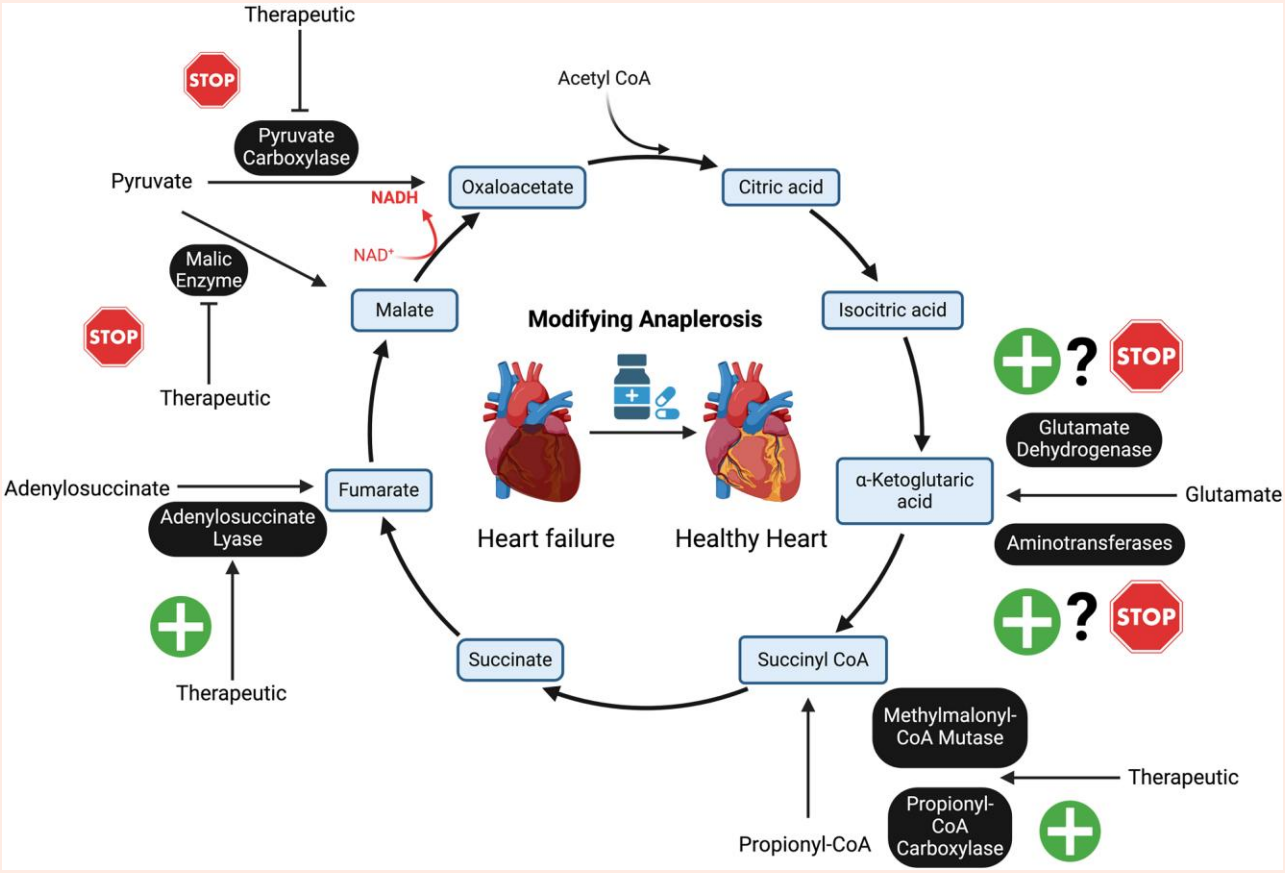
Graphical Abstract



Keywords Anaplerosis • Mitochondria • Cardiac metabolism • Heart failure • Cardiovascular disease

1. Introduction

Heart failure (HF) results from an inability of the heart to pump and/or fill with blood to meet the demands of the body.[1] The clinical syndrome of HF is characterized by structural and/or functional abnormalities corroborated by elevated levels of natriuretic peptides and objective evidence of pulmonary or systemic congestion.[2] Considered a global epidemic, HF currently affects an estimated 64.3 million individuals.[3] As such, it has become a priority to reduce the economic and social burden of this condition.

The heart is an energetically demanding organ that relies heavily on adenosine triphosphate (ATP) production to support its contractile function.[4] To meet its demands, cardiomyocytes rely on multiple energy sources, including fatty acids (FAs), glucose, lactate, amino acids, and ketone bodies, which are ultimately converted into acetyl coenzyme A (acetyl-CoA) prior to entry into the tricarboxylic acid (TCA) cycle (*Figure 1*).[4–8] The TCA cycle is a critical process in the mitochondrial production of ATP through the oxidation of carbon energy substrates. Once acetyl-CoA enters the TCA cycle, it is combined with oxaloacetate (OAA) to form citrate, followed by a series of reactions that produce reducing equivalents which are used by the electron transport chain (ETC) to produce ATP. During this process, two carboxyl groups are lost as CO_2, balancing the two carbon units added by the incorporation of acetyl-CoA into the TCA cycle.

Kornberg first coined the term 'anaplerosis' to describe the replenishment of TCA cycle intermediates in bacteria.[9] This crucial process replaces carbon that has been removed from the TCA cycle, allowing for the continued production of reducing equivalents. In contrast, cataplerosis refers to the removal of intermediates from the TCA cycle for biosynthetic purposes, such as the production of amino acids or glucose. Perturbations in anaplerosis can lead to the depletion of TCA cycle intermediates which can severely impact cellular ATP production with consequences for organs with high energetic demands, including the heart. Current medical treatment of HF is primarily focused on symptom reduction and curative therapy has not yet been established. Improved understanding of the underlying molecular pathology of HF, including how altered mitochondrial energy metabolism and perturbed anaplerosis play a role in disease pathogenesis, could help identify novel defined therapeutic targets.

2. Cardiac anaplerosis: substrates and metabolic pathways

The more specific phrase 'cardiac anaplerosis' was conceived to describe the replenishment of TCA cycle intermediates in the heart.[10] The earliest studies of cardiac anaplerosis confirmed its importance wherein isolated perfused rat hearts exhibited decreased myocardial function when perfusate lacked anaplerotic substrates.[11,12] Further work in the same model and also *in vivo* in swine hearts confirmed the observation that a lack of anaplerotic substrates resulted in contractile dysfunction.[13–19] However, studies investigating cardiac anaplerosis in humans are limited.

The reactions of the TCA cycle allow for a total recovery of cycle intermediates, as the two carbons lost as CO_2 are matched by two carbons entering the TCA cycle from acetyl-CoA (*Figure 2*). However, a portion of TCA cycle intermediates are released from mitochondria to participate in other metabolic or biosynthetic pathways.[20] This process, known as

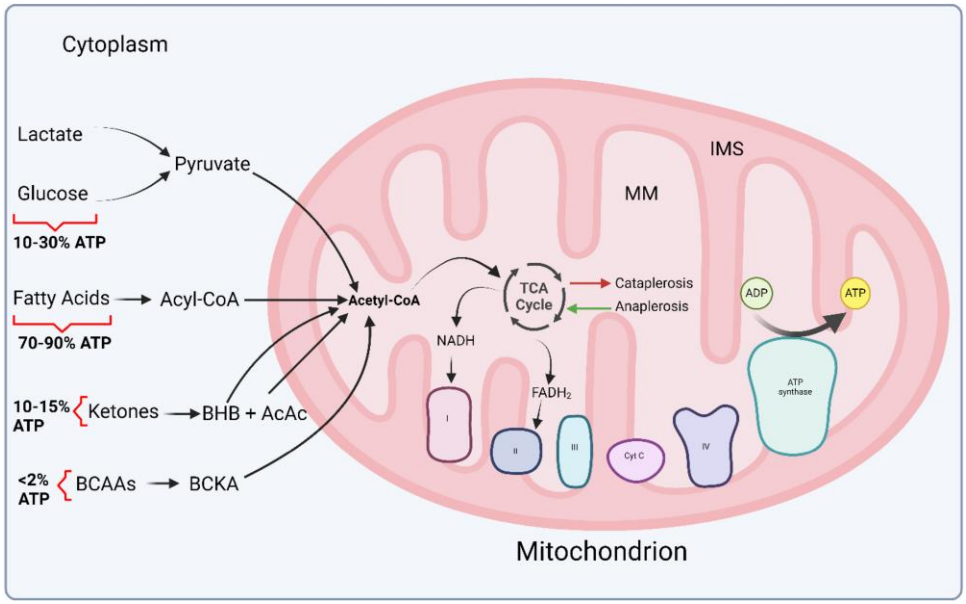


Figure 1 Overview of metabolism in healthy cardiomyocytes. Under aerobic conditions, FAs, glucose, lactate, ketone bodies, and BCAAs are converted to acetyl-CoA which enters the TCA cycle and permits the generation of reducing equivalents NADH and $FADH_2$ which transfer electrons to the mitochondrial ETC for the production of ATP. AcAc, acetoacetate; BHB, β-hydroxybutyrate; BCKA, branched chain keto acids; IMS, intermembrane space; MM, mitochondrial matrix. Figure created using Biorender.

cataplerosis, depletes TCA intermediates, necessitating their replenishment to sustain catabolic function and meet the heart's energy demands.[21,22] To maintain metabolic homeostasis, anaplerosis plays a vital role in counterbalancing this loss, restoring essential intermediates to the TCA cycle, and ensuring continuous energy production.

There are five anaplerotic entry points into the TCA cycle including malate, OAA, α-KG, succinyl-CoA, and fumarate.[10] Direct anaplerotic substrates include pyruvate, aspartate, adenylosuccinate, propionyl-CoA, and glutamate. Indirect anaplerotic substrates are short- or odd-chain FAs (OCFAs) [i.e. propionate (C_3), C_{15}, and C_{17}] and C_5-ketone bodies which are all precursors of propionyl-CoA. In addition, glucose, FAs, lactate, and amino acids [including glutamine and branched chain amino acids (BCAAs)] also contribute to cardiac anaplerosis (*Figure 2*).[23]

3. Anaplerosis in the healthy heart

Pyruvate is the end-product of glycolysis in the cytosol, after which it is transported into the mitochondrial matrix to participate in the TCA cycle. While the majority of pyruvate is destined for acetyl-CoA production for the TCA cycle, some of this pyruvate can be used for anaplerosis through three different pathways. Pyruvate can undergo reversible nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent pyruvate carboxylation reactions, catalyzed by malic enzyme (ME) to produce malate. Alternatively, pyruvate can undergo irreversible pyruvate carboxylation, catalyzed by pyruvate carboxylase (PC), to produce OAA. Studies have shown that ME plays a more prominent role in anaplerosis compared to PC.[13,24] OAA may also be replenished through a transamination reaction catalyzed by aspartate aminotransferase (AST). Lastly, the reverse reaction of lactate dehydrogenase catalyzes the conversion of lactate to pyruvate and thus indirectly contributes to anaplerosis through subsequent PC- and ME-catalyzed reactions.

Mitochondrial OAA levels are influenced by the malate–aspartate shuttle which links cytosolic glycolysis with the TCA cycle for transport of decreased nicotinamide adenine dinucleotide (NADH) molecules in cataplerotic metabolism.[25–27] The malate–aspartate shuttle allows for cataplerosis and export of TCA cycle intermediates such as α-KG and malate, facilitated by the malate-α-KG antiporter (SLC25A11). In the cytosol, α-KG and aspartate undergo transamination by AST to form glutamate and OAA, respectively, and OAA is then converted back to malate by cytosolic malate dehydrogenase. The malate is re-imported into the mitochondria, completing the shuttle. This exchange coordinates anaplerotic and cataplerotic fluxes, allowing the TCA cycle intermediates to participate in biosynthetic capacity, redox balance, and energy production while maintaining TCA cycle homeostasis.

Glutamine is another anaplerotic precursor that enters the mitochondrial matrix to be processed via glutaminolysis.[28,29] First, glutamine is deaminated into glutamate in a rate-limiting reaction catalyzed by glutaminase (GLS). Then, glutamate is converted into α-KG by two different reversible reactions: one catalyzed by aminotransferases, and the other by glutamate dehydrogenase (GDH).[10,30] Succinyl-CoA represents an entry point into the TCA cycle for several molecules including OCFAs, cholesterol, propionate, and the BCAAs (particularly valine and isoleucine) which converge into propionyl-CoA, then succinyl-CoA.[31] Fumarate replenishment occurs through the guanine triphosphate-hydrolyzing reaction of the purine nucleotide cycle, and the deamination of aspartate.[32]

Branched chain amino transferase 2 (BCAT2) also contributes to anaplerosis, converting branched chain ketoacids (BCKAs) to BCAAs, and glutamate to α-KG. However, BCAT2 can also function in the opposite direction, converting α-KG to glutamate in the first step of BCAA oxidation. The directionality of BCAT2 depends largely on the levels of BCKAs and BCAAs, but this enzyme primarily functions in the cataplerotic direction as BCAA levels usually predominate.[33]

4. Alterations in cardiac anaplerosis in HF

Recognizing the critical role of anaplerosis in the healthy heart, it is important to consider how these pathways may be altered in HF. Diminished levels of TCA cycle intermediates contribute to the reported defects of the TCA cycle in HF, and alterations in cardiac anaplerosis are associated with the development or

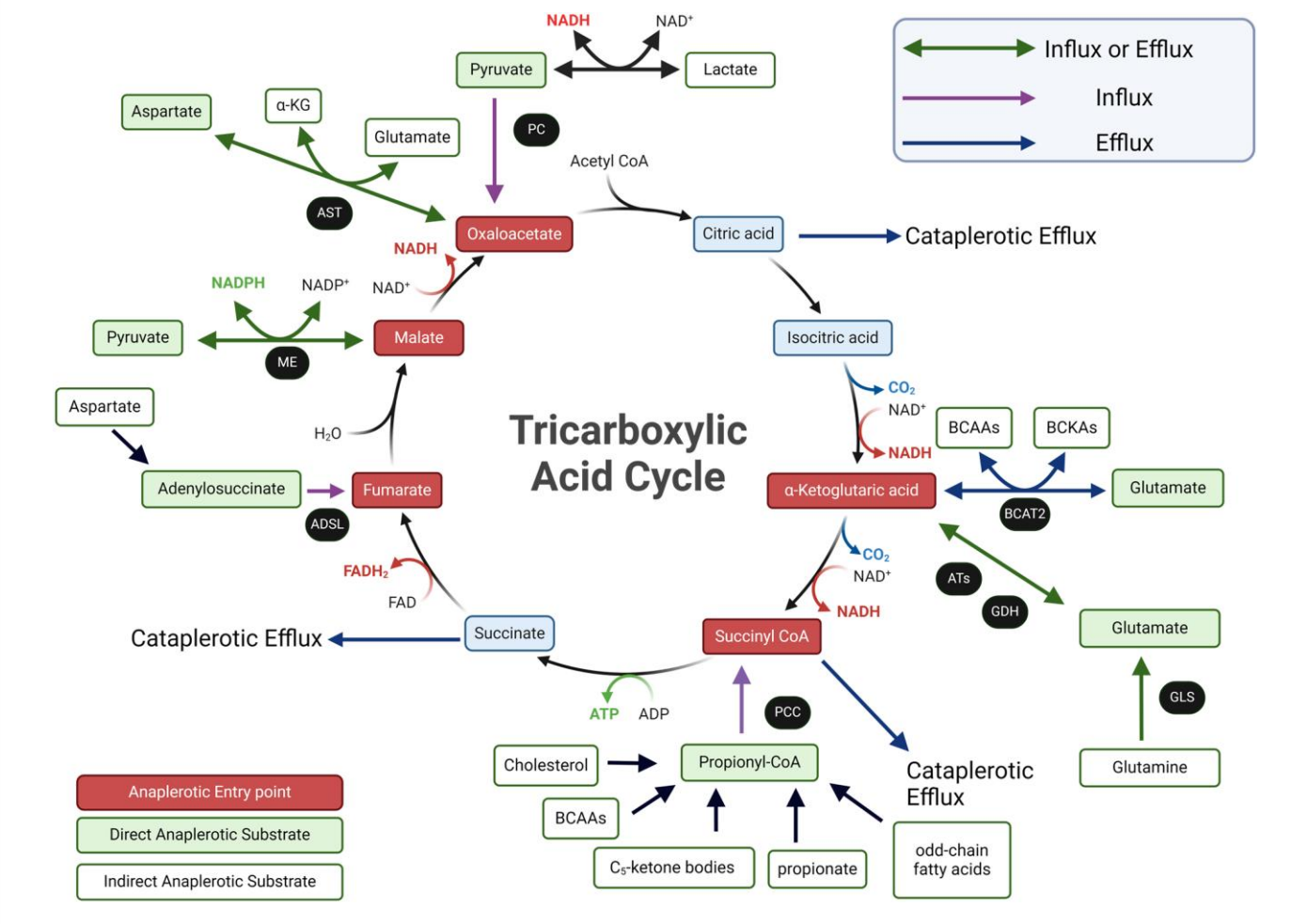


Figure 2 Anaplerotic and cataplerotic pathways with entry points, anaplerotic enzymes, and the molecules directly or indirectly involved in anaplerosis in the TCA cycle. Double-headed arrows represent reversible reactions involved in anaplerosis and cataplerosis. Single-headed arrows indicating non-reversible anaplerotic reactions. Blue single-headed arrows indicate cataplerotic efflux of TCA cycle intermediates. PC, pyruvate carboxylase; ME, malic enzyme; AT, aminotransferases; GLS, glutaminase; GDH, glutamate dehydrogenase; PCC, propionyl-CoA carboxylase; ADSL, adenylosuccinate lyase. Figure created in Biorender.

worsening severity of HF in animals and humans.[31,34–36] Multiple studies have suggested that abnormalities of cardiac anaplerosis exist across varying HF stages and in varying pathological states associated with HF including hypertension, ischaemia, toxin-mediated cardiomyopathy, and hypertrophy.[22,37,38]

Anaplerosis enables the heart to balance carbon between ATP generation and the replenishment of TCA cycle intermediates as needed. Disruptions in anaplerosis can destabilize cellular energy balance, redox homeostasis, and the availability of precursors necessary for biosynthetic processes.[39–41] In HF, protein synthesis is upregulated, often in conjunction with by decreased amino acid degradation, which increases the demand for anaplerotic inputs like pyruvate to provide biosynthetic precursors. Furthermore, diminished anaplerosis can exacerbate oxidative stress by lowering decreased NADP (NADPH), NADH, and flavin adenine dinucleotide ($FADH_2$) levels, leading to redox imbalance, increased electron leakage, and the generation of ROS.[40,41]

5. Pyruvate anaplerosis in HF

Cardiac hypertrophy is a physiological adaptation, but can also precede the onset of HF with decreased ejection fraction (HFrEF) which develops as a consequence of pressure overload.[42] Hypertrophy initially helps to maintain cardiac function but eventually becomes maladaptive leading to decompensated hypertrophy and HF.[42,43] Myocardial hypertrophy is commonly associated with increased rates of glycolysis, decreased mitochondrial oxidative metabolism, and altered anaplerosis.[44–49] In response to diminished glucose oxidation, there is increased conversion of pyruvate to lactate and increased anaplerotic pyruvate flux bypassing pyruvate dehydrogenase (PDH), at the expense of energy efficiency (see Supplementary material online, *Table S1*).[22,37,50,51] These changes are driven by the required upregulation of biosynthetic pathways to support protein synthesis in hypertrophy.[52]

Sorokina *et al.* induced hypertrophy in rat hearts by subjecting them to 10 weeks of transaortic constriction (TAC) and assessed cardiac metabolism using dynamic ^{13}C-nuclear magnetic resonance (NMR). The isolated hearts were perfused with ^{13}C-palmitate and glucose.[22] The study detected diminished FA oxidation alongside increased glycolysis, without significant changes in glucose oxidation via PDH.[22] Flux measurements indicated an enhanced anaplerotic flow into the TCA cycle, accompanied by increased ME mRNA expression, suggesting that this anaplerotic pathway compensates for the diminished contribution of glycolysis-derived pyruvate. The energetic state was monitored using ^{31}P-NMR spectroscopy to assess phosphocreatine and ATP content. Despite the activation of anaplerosis and the preservation of TCA cycle flux, cardiac ATP generation in hypertrophy was inefficient, as evidenced by diminished ATP levels compared to control rat hearts.[22]

Pound *et al.*[50] later validated these findings in TAC-subjected isolated rat hearts perfused with ^{13}C-labeled palmitate or glucose. They detected an increase in anaplerotic flux through cytosolic ME, and increased levels of ME protein in TAC-treated rat hearts compared to sham-operated animals.[50] Aside from increased anaplerotic flux via ME, levels of myocardial triacylglycerol were decreased in TAC-treated rats compared to sham-operated animals, suggesting lower rates of lipogenesis. This was reversed with PDH activation which competitively decreased anaplerosis, resulting in a significant increase in triacylglycerol content and enhanced cardiac contractility. Overall, this suggests that anaplerosis of pyruvate by ME could be depleting NADPH, a biosynthetic factor and electron donor required for lipogenesis and protection against oxidative stress.

Lahey *et al.*[51] studied TAC-induced hypertrophic rat hearts and exposed them to an adenovirus carrying miRNA targeting cytoplasmic rat ME1 which inhibits the enzyme. Next, the hearts were isolated, perfused with ^{13}C-labelled palmitate, glucose, and lactate and divided into three groups, TAC, sham, and miRNA-ME1. To measure cardiac anaplerosis, the authors used high-resolution ^{13}C-NMR spectroscopy and applied isotopomer analysis. Increased anaplerosis was seen in the TAC group but was lowered in the miRNA-ME1 group. Moreover, inhibiting ME1 restored increased NADPH levels and as a result increased levels of antioxidant glutathione.[51] Overall these findings indicate that failing hearts rely on anaplerosis at the expense of decreasing the rate of biosynthesis. Supporting these animal observations, quantitative polymerase chain reaction analysis confirmed increased expression of ME1 in failing human myocardium.[51]

Turer *et al.*[37] subjected mice to severe TAC (sTAC) for 6–8 weeks, to quickly transition the animals from compensated hypertrophy to decompensated hypertrophy and severe HF. To study cardiac substrate preference, the authors used a perfusate containing ^{13}C-labelled glucose, ethylacetoacetate, and enriched FAs and performed ^{13}C flux measurements using liquid chromatography-mass spectrometry (LC-MS). Cardiac anaplerosis was assessed using ^{13}C-NMR spectroscopy. Surprisingly, substrate utilization studies showed that glucose was the only energy substrate that showed significantly increased contribution to acetyl-CoA in overt HF, suggestive of increased glucose oxidation. This reveals an important difference between the models of sTAC and TAC-induced hypertrophy, wherein a decrease in glucose oxidation is observed.[44–49] Furthermore, in sTAC-treated animals, succinyl-CoA anaplerosis was elevated, while pyruvate anaplerosis remained unchanged, underscoring another key difference between the sTAC and TAC models of HF. However, Turer *et al.* did not identify the specific anaplerotic substrate responsible for replenishing succinyl-CoA, leaving it unclear whether this metabolic alteration has a beneficial or detrimental effect on cardiac function.

Other groups have studied pyruvate anaplerosis by genetically modulating FA oxidation in animal models of HF. Deletion of acetyl-CoA carboxylase 2 (*ACC2*) increases the entry of long-chain FAs into the mitochondria, thereby preserving substrate utilization and FA oxidation in the heart. Kolwicz *et al.*[53] performed a cardiac-specific Cre-induced deletion of *ACC2* in mice and subjected them to 8 weeks of TAC. The isolated mouse hearts were then perfused with ^{13}C-labeled glucose and FAs, and ventricular tissue metabolites were analysed using LC-MS and gas chromatography-mass spectrometry (GC-MS). In addition, myocardial energetics were assessed via ^{31}P-NMR spectroscopy. This study identified increased glycolysis and increased anaplerosis as a response to TAC, representing a metabolic signature of cardiac hypertrophy in mice.[53] However, in TAC-treated mice with a cardiac-specific deletion of *ACC2*, substrate utilization did not differ from the profile observed in sham controls. Moreover, these mice had preserved FA oxidation, and no increases in glycolysis or anaplerosis.[53] This genetic modification was also associated with decreased cardiac fibrosis and hypertrophy and preserved systolic function. These data suggest that inhibiting anaplerosis leads to improved metabolic and cardiac function.

More recently, Choi *et al.*[54] created a tamoxifen-inducible cardiac-specific knockout of *ACC2* in 12- to 14-week-old mice and infused these animals with angiotensin-II. The mice hearts were then isolated and perfused with ^{13}C-labelled glucose and ^{13}C-labelled FAs.^{13}C-NMR spectroscopy and isotopomer analysis were used to measure and quantify substrate utilization and anaplerosis. Deletion of *ACC2* sustained FA oxidation, enhanced glycolytic-oxidative metabolism, and decreased anaplerosis, leading to decreased cardiac hypertrophy and fibrosis while preserving diastolic function in TAC-treated mice.[54] Overall, these studies suggest that modifying FA oxidation reduces anaplerosis and protects cardiac function, and that anaplerosis may not always be beneficial.

Anaplerotic changes are not unique to surgically induced cardiac hypertrophy, as similar findings have been found in alternative models. In rat hearts with triiodothyronine-induced cardiac hypertrophy, Atherton *et al.*[55] performed an *in vivo* evaluation of TCA cycle metabolites using 2-^{13}C pyruvate and magnetic resonance spectroscopy. A unique pattern was identified in which the incorporation of label into [1-^{13}C]citrate, [5-^{13}C]glutamate, and [1-^{13}C]acetylcarnitine was significantly decreased in hypertrophied rat hearts compared to controls. A significant lowering in PDH flux, diminished glucose oxidation, and increased anaplerosis through PC were evident in this model of cardiac hypertrophy. Interestingly, maintaining PDH activity with dichloroacetic acid treatment decreased hypertrophy but did not significantly impact anaplerosis through PC or alter cardiac output.[55] This illustrates that metabolic abnormalities can persist despite augmenting glucose oxidation. Therefore, inhibiting anaplerosis could be a potential alternative strategy to enhance cardiac output.

In summary, the evidence suggests that an activation of pyruvate anaplerosis plays a significant role in driving pathological cardiac hypertrophy by diverting glucose metabolism away from efficient energy production and depleting NADPH reserves, which are crucial for antioxidant defense. This shift in metabolism not only reduces the heart's energetic efficiency but may also exacerbate oxidative stress and promote detrimental remodelling of the heart muscle, ultimately impairing cardiac contractility and function. It is also clear that PC activity in the heart is minimal, indicating that ME is the predominant enzyme mediating pyruvate anaplerosis in HF.[13,24] Moreover, while pyruvate is a major source for anaplerosis via ME and PC, aspartate can contribute via transamination reactions that generate OAA. However, aspartate remains relatively understudied in the context of HF. Investigating how aspartate metabolism impacts cardiac function could yield new insights, particularly in terms of its contributions to energy homeostasis and redox balance. Based on the current evidence, the inhibition of ME may represent a multifaceted therapeutic strategy to not only improve cardiac energy efficiency but also to reduce cardiac hypertrophy, protect cardiomyocytes from oxidative stress, and thereby improve heart function.

6. Targeting pyruvate anaplerosis in HF

Pyruvate is a significant source of acetyl-CoA fuelling the TCA cycle, as well as a substrate for cardiac anaplerosis. If the failing heart is energetically inefficient, modifying pyruvate anaplerosis represents a potential therapeutic option. Many animal and human studies have tested various interventions to modify pyruvate anaplerosis in HF.

Bøgh *et al.*[56] performed pulmonary valve suturing in porcine hearts to induce volume overload and eventually right-sided HF. A combination of magnetic resonance imaging and hyperpolarized [1-^{13}C] pyruvate magnetic resonance spectroscopy were used to assess cardiac contractility, hypertrophy and metabolism. Consistent with the findings of Pound *et al.*,[50] administering dichloroacetate, a PDH kinase inhibitor which increases PDH flux, improved pyruvate oxidation and contractile function and decreased hypertrophy.[56] These results demonstrate that recoupling glycolysis to mitochondrial metabolism can reduce inefficient pyruvate anaplerosis, contributing to enhanced energy efficiency and cardiac performance.

Some studies have attempted to stimulate pyruvate metabolism in HF by altering the mitochondrial pyruvate carrier (*MPC*), a transporter composed of two subunits (*MPC1* and *MPC2*) that is responsible for the transport of pyruvate across the mitochondrial membrane.[57] Mice with a *MPC2* deletion spontaneously developed cardiac dilation and contractile dysfunction at 6 weeks.[58] Targeted metabolomics analysis of *MPC2*-deficient mice hearts revealed an accumulation of pyruvate, unaltered lactate levels, a decrease in acetyl-CoA, and an accumulation of fumarate, malate, and OAA suggesting that anaplerosis is unaffected by *MPC2* deletion. This study also found decreased protein levels and mRNA expression of both *MPC1* and

MPC2 in heart tissue samples from HF patients undergoing left ventricular assist device (LVAD) implantation. This finding is consistent with the observation that myocardial recovery in HF patients after LVAD implantation is associated with increased myocardial expression of *MPC*.[59] These findings were further validated in adult mice with Cre-induced cardiac-specific deletion of *MPC1*, which led to cardiac hypertrophy and HF.[59] The impact of *MPC* downregulation was further analysed using ^{13}C-glucose tracing and MS in H9c2 cells with an inducible shRNA-mediated knockdown of *MPC1*.[59] An increase in extracellular and intracellular ^{13}C-lactate was observed as well as an overall decrease in labelled citrate, malate, and fumarate, suggesting a reduction in mitochondrial pyruvate oxidation and anaplerosis. Increased lactate export was also supported by increased protein expression of monocarboxylate transporter 4 (*MCT4*) in hypertrophied H9c2 cells. The overexpression of *MPC* and inhibition of *MCT4* attenuated the drug-induced hypertrophy in cultured cardiomyocytes.[59] Overall, the evidence on the impact of modifying MPC on pyruvate anaplerosis is limited and somewhat conflicting. McCommis *et al.*[58] suggest that anaplerosis remains partially preserved despite diminished pyruvate oxidation. In contrast, findings from Cluntun *et al.*[59] indicate that decreased pyruvate oxidation is accompanied by a decline in pyruvate-driven anaplerosis. The implications of these pre-clinical findings for optimal cardiac energy metabolism and the role of pyruvate anaplerosis remain unclear.

When investigating the therapeutic potential of pyruvate anaplerosis in human HF it is crucial to consider the type and stage of HF that this intervention aims to target. For example, considering that increased pyruvate anaplerosis is associated with cardiac hypertrophy in animal models of HF, it may be beneficial to stimulate pyruvate decarboxylation through the activation of PDH.[50,51] However, this approach may not be reasonable in later stages of HF because studies in patients with end-stage systolic HF or ischaemic cardiomyopathy have reported increased PDH expression.[60,61] To further complicate this, findings in end-stage non-ischaemic dilated cardiomyopathy (DCM) reveal that PDH subunit levels remain largely unchanged.[36] Instead, PDH activity is notably enhanced in DCM, primarily due to the decreased expression of PDH kinase 4, an inhibitor of PDH.

The majority of clinical studies to-date have explored supplementation with pyruvate in HF, exploiting pyruvate's role both as a source of acetyl-CoA for the TCA cycle and as an antioxidant. Early studies attempted to elevate physiological pyruvate levels in HF patients to elicit cardioprotection.[23] Hasenfuss and colleagues showed marked improvements in cardiac function following administration of pyruvate to patients with HF, without inducing adverse side effects.[62,63] Years later, this finding was validated in a study that showed improved systolic and diastolic function in HF patients who received intracoronary infusions of pyruvate.[64] Although the utilization of pyruvate as an energetic fuel is necessary for enhancement of cardiac function, it is not entirely clear if cardioprotection with pyruvate supplementation was conferred due to increases in pyruvate oxidation, increased anaplerosis, or its antioxidant properties.[65–68] Furthermore, at least for humans, limited data exist regarding the optimal dose of pyruvate needed to rescue the failing heart. Herman *et al.*[62] observed that two 15-min intracoronary doses of 3 or 6 mmol/L significantly improved cardiac stroke-volume indices and decreased pulmonary capillary wedge pressure.

In conclusion, research on pyruvate as a therapeutic option for HF has shown promising potential, especially regarding its roles in energy metabolism, anaplerosis, and antioxidant effects. Although studies indicate that enhancing pyruvate metabolism could be cardioprotective, the precise mechanisms, whether through anaplerosis, oxidative benefits, or other pathways, remain unclear. Pyruvate's cardioprotective effects likely extend beyond anaplerosis, suggesting intricate interactions with oxidative stress reduction, mitochondrial efficiency, and the broader metabolic adaptations of failing cardiomyocytes. These pathways, including pyruvate's role in modulating redox balance and NADPH availability, offer crucial insights into how metabolic dysfunction contributes to HF progression. Despite the promising results, it remains unclear whether pyruvate exerts its protective effects predominantly through energy replenishment, antioxidant capacity, or a combination of pathways that affect overall cardiac function. Addressing this gap requires a deeper understanding of how pyruvate metabolism intersects with broader metabolic networks, including the underexplored roles of metabolites such as aspartate and OAA. Therefore, further pre-clinical and clinical studies are necessary—not only to elucidate these mechanisms but also to identify the most effective methods of delivering pyruvate therapeutically, optimizing its benefits for patients with HF. By unravelling these connections, we can potentially harness pyruvate's role in both stabilizing cardiac function and mitigating the metabolic disturbances associated with HF.[69]

7. Glutamine anaplerosis in HF

Being the most prevalent amino acid in the bloodstream, glutamine serves as a major carbon source for replenishing TCA cycle intermediates through mitochondrial glutaminolysis.[70] Ultimately, glutamine is converted to glutamate and then α-KG (*Figure 2*). Multiple studies highlight the cardioprotective effects of glutamine supplementation, but the precise mechanism of action is unknown.[71–74] Fewer studies have been conducted to study glutamine anaplerosis in HF, although current knowledge creates a foundation for further investigation and potential clinical translation.

Lauzier *et al.*[75] perfused *ex vivo* rat hearts with ^{13}C-labeled carbohydrates and oleate in the control group, and restricted pyruvate anaplerosis by removing insulin and pyruvate in the experimental group. Using GC-MS, this study showed that the addition of physiologically relevant levels of glutamine significantly improved cardiac function in *ex vivo* rat hearts under conditions of restricted pyruvate anaplerosis. In agreement with previous work, the isotopic labelling patterns of TCA cycle intermediates derived from 5-^{13}C glutamine suggest that anaplerotic mechanisms may not be the reason for the cardioprotective effects of glutamine. Instead, glutamine activates the hexosamine biosynthetic pathway and increases the recruitment of FA transporter CD36, leading to a rewiring of cardiac metabolism. More specifically, Lauzier *et al.* suggest that glutamine shifts cardiac substrate utilization from carbohydrates to FAs and promotes FA oxidation.[10,75,76]

However, more recently, Watanabe et al. treated rat neonatal cardiomyocytes with H_2O_2 to create an *in vitro* model of oxidative stress-induced HF.[70] With LC-MS analysis they showed decreased intracellular levels of glutamine, glutamate and α-KG in H_2O_2-treated cells. Stable isotope tracing with [U-^{13}C]-glutamine and GC-MS were used to assess glutamine anaplerosis. They found increased relative amounts of α-KG, glutamate, glutamine, fumarate, and malate derived from ^{13}C-glutamine, indicating increased anaplerotic flux of glutamine. Supporting this observation, the enzymatic activity of GLS increased over time following H_2O_2 treatment. However, ideally, CO_2 production should be measured to validate the anaplerosis results. Lastly, the authors also tested the impact of the GLS inhibitor compound '968' and dimethyl α-ketoglutarate (DMKG), a membrane-permeable ester of α-KG. Inhibition of GLS decreased cardiac cell viability, ATP production, and glutathione in rat neonatal cardiomyocytes exposed to H_2O_2 which was reversed with DMKG-treatment. Overall, Watanabe *et al.*[70] suggest that modulation of glutamine anaplerosis could prevent metabolic remodelling, improve energetic status and ameliorate the oxidative stress associated with HF.

Another group showcased the cardioprotective effects of α-KG supplementation in pressure overload-induced HF.[77] In TAC-subjected mice, α-KG supplementation decreased myocardial hypertrophy and fibrosis and improved cardiac function and LV strain.[77] These data aligned with *in vitro* work where myocardial hypertrophy and injury were induced by angiotensin-II.[77] Neonatal rat ventricular myocytes treated with α-KG in the presence of angiotensin-II maintained their mitochondrial membrane potential, displayed enhanced myocardial mitophagy, and showed decreased levels of hypertrophy, injury, apoptosis, and oxidative damage.[77]

Conversely, Yoshikawa *et al.*[29] saw increases in glutamine anaplerosis in angiotensin-II treated hypertrophic murine hearts, rat neonatal cardiomyocytes, and rat neonatal cardiac fibroblasts. Contrary to their previous *in vitro* findings, this study suggested that glutamine anaplerosis is a reason for pathological cardiac hypertrophy and fibrosis, and that its inhibition could be therapeutic in HF.[29,70]

Recently, Li *et al.* found that α-KG has regenerative effects in the heart. More specifically, an accumulation of α-KG in cardiomyocytes activates

KDM5 demethylases, leading to the demethylation of H3K4me3 histone protein, and a reprogramming of cells to a less mature, more proliferative state. This process promotes heart regeneration by enhancing cellular de-differentiation and tissue repair following injury.[78]

In summary, recent data underscore the ambiguity of glutamine anaplerosis in HF. Although glutamine serves as a crucial source of carbon for replenishing TCA cycle intermediates, its anaplerotic effects and therapeutic potential remain poorly understood. Some studies suggest that glutamine supplementation could protect from HF, but there is conflicting evidence for benefit in the context of cardiac hypertrophy.[29,70,75] These discrepancies raise important questions about the differential regulation of glutamine metabolism in normal vs. failing hearts and how these variations influence both energy efficiency and the progression of hypertrophic remodelling. Understanding these distinctions could be key to determining whether glutamine acts as a metabolic buffer or exacerbates maladaptive remodelling under certain conditions. Therefore, additional pre-clinical investigation of glutamine anaplerosis is warranted, especially in cellular and animal models of HF.

8. The role of glutamine anaplerosis in dilated cardiomyopathy with ataxia syndrome fibroblasts

The dilated cardiomyopathy with ataxia syndrome (DCMA) is a rare mitochondrial disease that is associated with DCM and early mortality due to intractable HF.[79–81] Deficiency of *DNAJC19*, a poorly characterized and understudied mitochondrial protein, underlies DCMA.[81–83] Previous work revealed mitochondrial fragmentation in DCMA patient-derived fibroblasts and in cardiomyocytes differentiated from patient-derived induced pluripotent stem cells.[84,85] Mitochondrial diseases such as DCMA are commonly associated with alterations in oxidative phosphorylation and glutamine metabolism.[86] Recent work in DCMA fibroblasts has demonstrated that DCMA is a disorder of mitochondrial glutamine metabolism with suspected defects in glutamine anaplerosis.[87] King *et al.*[87] demonstrated normal TCA cycle functioning in fibroblasts from patients with both mild and severe cardiac dysfunction. However, DCMA fibroblasts illustrated a greater reliance on glutamine metabolism concurrent with increased glutamate secretion and diminished glutamine recycling. Given the heart's reliance on mitochondrial metabolism for ATP production, altered glutamine metabolism could impact cardiac function by disrupting the balance between energy demand and supply in cardiomyocytes. A greater dependence on glutamine metabolism may indicate an adaptive response to mitochondrial dysfunction, but it could also contribute to metabolic inefficiency and redox imbalance, exacerbating HF in DCMA patients. Future research should focus on how these metabolic shifts in glutamine anaplerosis directly affect cardiomyocyte function, remodelling, and progression toward HF.

9. Propionyl-CoA anaplerosis in HF

Propionyl-CoA carboxylation is an important source of anaplerotic succinyl-CoA for the heart. There are several molecules that provide substrates for this reaction including BCAAs, short-chain FAs (i.e. propionate), OCFAs (particularly C_{15} and C_{17}) and C_5-ketone bodies. Propionyl-CoA carboxylase (PCC) and methylmalonic CoA mutase (MCM) play a role in replenishing succinyl-CoA and deficiency of PCC or MCM are associated with diminished anaplerosis.[88,89] Abnormalities in propionyl-CoA anaplerosis may contribute to cardiomyopathy and HF by disrupting the replenishment of succinyl-CoA and causing a build-up of propionic acid.[88–91] He *et al.*[92] subjected *ex vivo* rat hearts to ischaemia by stopping the perfusate flow and measured the metabolite profile using GC-MS. Hearts exposed to prolonged ischaemia (30 min) exhibited an accumulation of propionyl-CoA and lowered cardiac levels of succinyl-CoA. Takada *et al.*[31] have shown that mice with coronary artery ligation (CAL)-induced ischaemic chronic HF exhibit lowered cardiac levels of succinyl-CoA, compared to sham controls. Flam *et al.*[36] employed plasma and cardiac tissue metabolomics to analyse 87 explanted human hearts, comparing 39 patients with end-stage HF to 48 non-failing donor hearts. Failing human cardiac tissue had a diminished relative abundance of propionyl-CoA. In summary, propionyl-CoA originates from various sources that all contribute to replenishing succinyl-CoA. This process appears to be essential for normal cardiac function but is poorly understood in the context of cardiac pathology and HF.

10. Branched chain amino acid anaplerosis in HF

BCAAs play a key role in cardiac metabolism. Aside from providing acetyl-CoA for mitochondrial oxidative metabolism, they may also be an important anaplerotic source of propionyl-CoA and succinyl-CoA (*Figure 2*). Cardiac BCAAs are reversibly transaminated into their respective BCKAs by mitochondrial branched-chain amino-transaminase (BCATm). Subsequently, BCAA dehydrogenase (BCKDH) converts BCKAs to either acetyl-CoA or succinyl-CoA. Protein phosphatase 2Cm (PP2Cm) activates BCKDH through dephosphorylation and mitochondrial branched-chain α-keto acid dehydrogenase kinase (BCKDK) inactivates it via phosphorylation.

Decreased BCAA oxidation occurs in HF, usually resulting in the accumulation of BCAAs and BCKAs.[93–95] Elevated levels of cardiac BCAAs have been noted in metabolomic studies of CAL- and TAC-treated murine hearts. In conducting metabolomic profiling of both CAL- and TAC-treated murine hearts, previous groups observed accumulation of cardiac BCAAs, suggesting impairments in BCAA oxidation.[94,95] In agreement with these findings, a transcriptomic and metabolomic analysis by Sun *et al.*[93] identified a downregulation of genes responsible for BCAA catabolism and accumulation of BCKAs in TAC-treated mice. Their group validated the animal findings by observing the same gene expression findings in human myocardial samples from humans with dilated cardiomyopathy.[93]

Despite evidence of defective BCAA catabolism in HF, Murashige *et al.*[96] proposed that BCAA oxidation is unchanged in failing human myocardium and enhanced in a CAL-treated murine model of HF. However, this study did not actually directly measure BCAA oxidation, but rather assessed enrichment of ^{13}C-labelled TCA cycle intermediates originating from ^{13}C-BCAAs. These levels of enrichment are primarily dependent on the levels of the TCA cycle intermediates, as opposed to the actual rates of BCAA oxidation. Therefore, differences in anaplerosis or cataplerosis in HF could provide an alternative explanation for these results. Most studies continue to show suppressed BCAA oxidation in HF.[97] Yang *et al.*[97] subjected 12-week-old mice to TAC and provided them with either a BCAA-containing diet or a BCAA-free diet for 4 days. After 1–2 weeks of TAC, they measured histone propionylation by assessing the enrichment of H3K23Pr through chromatin immunoprecipitation and ChIP-Seq. This study found that diminished BCAA oxidation in TAC-subjected mice may lead to lower propionyl-CoA production, indicating that diminished propionyl-CoA anaplerosis could result from decreased BCAA oxidation. Moreover, diminished BCAA oxidation slowed the progression of cardiac hypertrophy. However, the impact of removing dietary BCAAs (potentially decreasing anaplerosis) on cardiac metabolism and energy status was not measured. Overall, pre-clinical studies suggest impaired BCAA oxidation which may lead to decreased anaplerosis. However, direct evidence for this effect has not been demonstrated.

Findings in human HF studies align with those from animal HF models, both demonstrating impaired BCAA metabolism and potential reduction of BCAA anaplerosis.[34,36,98,99] Diakos *et al.*[99] also showed diminished levels of BCAAs in failing hearts compared to non-failing hearts in humans. A down regulation of genes associated with BCAA metabolism and an accumulation of BCKAs has been shown in myocardial samples from humans with DCM.[93] Uddin *et al.*[98] used NMR spectroscopy to validate this finding, showing a significant elevation of BCAAs concomitant with a downregulation of BCAA catabolic enzymes in LV tissue of patients with DCM. Flam *et al.*[36] have also shown that BCAAs and BCKAs levels are elevated in failing

human cardiac tissue along with decreased gene and protein expression of BCAA catabolism enzymes. Most recently, Hahn *et al.*[34] performed a metabolomics analysis of human myocardium samples from patients with HFpEF and HFrEF. This study identified a significant elevation of myocardial BCAAs in HFpEF myocardium and a similar but non-significant trend in HFrEF compared to non-HF controls. In addition, a lowering in in BCAA-derived catabolites were found both in HFrEF and HFpEF samples, further confirming defects in BCAA catabolism. Overall, these human studies suggest impairments of BCAA metabolism in HF. As a result, anaplerosis from BCAA metabolism could be decreased. However, this was not directly assessed in these studies.

Apart from reducing anaplerosis, decreased BCAA catabolism could also lead to the preservation of TCA cycle intermediates by reducing the flux of α-KG to glutamate (a cataplerotic process) catalyzed by BCAT2. Increased extracellular BCAAs and decreased expression of BCKDH could promote cataplerosis. However, pre-clinical studies indicate that BCAT2 expression is downregulated in the failing heart, suggesting a reduction in cataplerosis.[93,98,100]

In summary, the accumulation of BCAAs and/or downregulation of BCAA-metabolizing enzymes is a common finding in HF. Impaired BCAA oxidation may result in lowered anaplerosis or increased cataplerosis, disrupting the balance of intermediates within the TCA cycle. As such, the interplay between anaplerosis and cataplerosis of BCAAs in the TCA cycle should be further studied using dual stable isotope labelled BCAAs and measurement of CO_2 production.[101] The data illustrating impairments of BCAA oxidation in HF are convincing and suggest a limitation in propionyl-CoA and succinyl-CoA anaplerosis, which are critical for maintaining the function of the TCA cycle. Given the relatively small contribution of BCAAs to ATP production in healthy and failing hearts, an anaplerotic deficiency due to BCAA oxidation defects may not significantly impact overall myocardial energy metabolism.[102–105] While BCAAs are not a major source of ATP, their role in anaplerosis and cataplerosis could still have important implications for metabolic flexibility and efficiency in the failing heart. Enhancing BCAA oxidation might alleviate anaplerotic stress, as has been observed in other metabolic diseases.[106–108] However, further studies are needed to determine whether restoring BCAA metabolism can significantly improve cardiac function or mitigate progression of HF.

11. Targeting BCAA anaplerosis in HF

Several groups have studied the therapeutic effects of BCAAs in HF through dietary and pharmacological interventions. Tanada *et al.* fed Dahl salt-sensitive rats a high-salt diet and supplemented their drinking water with BCAAs starting at 11 weeks of age, a point when cardiac hypertrophy was present but cardiac function remained preserved. In rats with HF and cardiac cachexia, the addition of BCAAs in drinking water preserved cardiac metabolism and function and prolonged survival.[109] More recently, a study demonstrated promising cardioprotective effects of essential amino acids in HFrEF.[100] Ragni *et al.* subjected adult wild-type and PP2Cm knockout mice to TAC for 4 weeks and provided a saturated fatty acid-essential amino acid (SFA-EAA) diet either before or after TAC surgery. Providing a SFA-EAA diet before TAC surgery preserved cardiac function and prevented cardiac enlargement and fibrosis. Similarly, providing a SFA-EAA diet after TAC surgery prevented the progression of HF. LC-MS metabolomics showed that the SFA-EAA diet lowered the accumulation of BCAA catabolites. Gene expression data showed that SFA-EAA reversed the decreased expression of BCAA oxidation genes and decreased protein expression of PP2Cm. The cardioprotective effects of stimulating BCAA catabolism was confirmed, as the SFA-EAA diet did not provide cardioprotection in PP2Cm-null mice, a model characterized by impaired BCAA oxidation.

Despite the promising results of dietary BCAAs, it is not entirely clear if the strategy is exclusively beneficial. Clinical and basic science studies suggest that increasing serum BCAAs and an accumulation of their metabolites (BCKAs) may exacerbate HF.[98,110–117] One explanation for this negative effect is decreased insulin-stimulated glucose oxidation in the heart in response to an accumulation of BCKAs.[98] Considering that HF is associated with BCAA oxidation defects, dietary supplementation with BCAAs may lead to further accumulation of BCAA metabolites and potentially intensify HF severity.

Precise pharmacological modulation of BCAA oxidation may be an alternative therapeutic strategy. Stimulating BCATm, may reduce BCAA levels and reduce mammalian target of rapamycin (mTOR) signalling and cardiac hypertrophy.[98,118] However, this stimulation would also lead to elevated levels of the BCKAs, reducing insulin-mediated glucose oxidation which may aggravate the maladaptive glycolysis-glucose oxidation mismatch and increased pyruvate anaplerosis seen in HF.[98,117,118] Conversely, inhibition of BCATm may improve insulin-mediated glucose oxidation in HF and reduce pyruvate anaplerosis, while decreasing the loss of α-KG. However, the secondary accumulation of BCAAs would likely increase cardiac hypertrophy via activation of the mTOR signalling pathway.[98] Modifying BCATm is therefore a nuanced therapeutic strategy for HF. It is plausible that modulating BCATm will alter anaplerosis in the failing heart, but this remains to be investigated.

BT2 (3,6-dichlorobenzo[b]thiophene-2-carboxylic acid) is an inhibitor of BCKDK and has demonstrated positive effects in animal models of HF.[93,96,98,119] BT2 has also been shown to increase gene expression of enzymes responsible for glucose and FA oxidation which would provide the failing heart with additional fuel.[6,93,98] Sun *et al.*[93] tested the impact of BT2 on wild-type and PP2Cm knockout mice subjected to TAC for 8 weeks. Treatment with BT2 increased BCKDK activity, decreased plasma BCKA levels, and preserved cardiac function. In a proof-of-concept study, Chen *et al.*[119] demonstrated the therapeutic effectiveness of BT2 in 6- to 8-week-old mice subjected to TAC for 2 weeks. Administration of BT2 for 6 weeks after TAC decreased cardiac levels of BCAAs and BCKAs and prevented deterioration of systolic function. Uddin *et al.*[98] subjected 10-week-old mice to TAC and administered BT2 starting 1-week post-surgery for a duration of 3 weeks. The production of $^{14}CO_2$ released by metabolism of U-^{14}C valine/leucine/isoleucine was measured to quantify BCAA oxidation. Isolated working hearts from TAC-subjected mice showed increased BCAA oxidation, decreased accumulation of BCAAs in hearts in response to BT2 and improved systolic function. Voronova *et al.*[120] validated the findings of these animal studies *in silico* by studying a quantitative systemics pharmacology model simulating BCAA metabolism. This study showed that BT2 decreased BCAA levels in cardiac tissues and improved heart function in HF models, leading to a 12% improvement in left ventricular ejection fraction.

Murashige *et al.*[96] recently conducted a metabolomics analysis after infusing U-^{13}C-labelled BCAAs into BT2-treated CAL/TAC-treated mice. In response to BT2 treatment, TAC-treated mice were protected from HF, with increased vascular relaxation and decreased blood pressure.[96] Cardiac-specific, skeletal muscle-specific and whole body-knockouts of BCKDK prevented the cardioprotective effects typically induced by BT2.[96] However, BCAA oxidation measurements by Murashige *et al.* were indirect and only illustrated the enrichment of TCA cycle intermediates as opposed to BCAA oxidation rates. While this is informative, TCA cycle flux must be measured to truly assess BCAA oxidation.

PP2Cm is a protein that can stimulate BCAA oxidation, and thereby potentially stimulate anaplerosis, through the dephosphorylation and activation of BCKDH.[118] Deficiency of PP2Cm was shown to render TAC-treated mice more susceptible to contractile dysfunction.[93] Stimulating PP2Cm is a potential therapeutic approach for HF, though it remains relatively unexplored.

In summary, modifying BCAA metabolism may represent a viable potential therapeutic strategy for HF. Pharmacological interventions targeting BCATm and PP2Cm may be more precise in targeting BCAA anaplerosis compared to dietary interventions.[101] Enhancing BCAA oxidation in the heart is a strategy that could potentially increase anaplerosis or cataplerosis and warrants further study. Although this strategy may theoretically support anaplerosis, excessive accumulation of metabolites such as BCKAs poses a risk of exacerbating metabolic dysfunction, particularly in glucose oxidation. Further research is required to clarify whether increasing BCAA oxidation can enhance anaplerosis or cataplerosis without promoting maladaptive metabolic consequences, such as cardiac hypertrophy through mTOR signalling. This underscores the importance of a more refined understanding of BCAA metabolism in HF, particularly through direct measurements of TCA cycle flux and BCAA oxidation rates, to optimize therapeutic strategies

12. OCFA anaplerosis in HF

Altered FA metabolism is a characteristic of HF but may be increased or decreased depending on the specific type of HF. FAs serve as the primary source of energy for cardiomyocytes and they can be categorized into three main types based on their length: short-chain fatty acids (SCFAs, <6-carbons), medium-chain fatty acids (6–12 carbons) and long-chain fatty acids (LCFAs, >12 carbons). FAs can also be further classified based on whether they contain an odd or even number of carbon atoms. OCFAs, such as propionate (C3), heptadecanoate (C17), and pentadecanoate (C15), are important sources for anaplerosis. Medium-length OCFAs (C_{17} and C_{15}) are metabolized into acetyl-CoA and propionyl-CoA in the final spiral of β-oxidation. Therefore, medium and long OCFAs are also a source of gluconeogenic OAA. Conversely, short OCFAs (propionate) are direct precursors of propionyl-CoA, an anaplerotic source of succinyl-CoA.

Evidence from animal and human studies demonstrate increased short chain fatty acid (SCFA) metabolism in HF.[92,105,121–123] Lewandowski *et al.*[122] subjected pig hearts to 5 h of CAL, after which the *in vivo* hearts were perfused with [2-^{13}C] butyrate and [2-^{13}C] glucose. NMR spectroscopy data indicated that SCFAs contributed more significantly to acetyl-CoA production, suggesting increased and preferential oxidation of SCFAs in pig hearts subjected to CAL. Carley *et al.*[121] subjected rat hearts to 14 weeks of TAC, after which hearts were isolated and perfused with either 4-^{13}C-labelled ketone (D3-hydroxybutyrate) or 4-^{13}C-labelled butyrate in the presence of glucose and palmitate. NMR spectroscopy, along with mRNA and protein expression data, indicated that failing hearts preferentially use ketones and exhibit increased butyrate oxidation. He *et al.*[92] perfused isolated rat hearts using the Langendorff model, followed by exposure to varying durations of stop-flow global ischaemia (0, 5, 15, and 30 min). The metabolic profiles were analysed using LC-MS to understand the relationship between ischaemia and metabolism. Cardiac ischaemia led to a build-up of propionyl-CoA and a decrease in methylmalonyl-CoA levels, suggesting decreased propionate anaplerosis.

Murashige *et al.*[105] used metabolomics to quantify nutrient uptake and release in failing and non-failing human hearts by measuring the arteriovenous gradient of 277 metabolites in 110 patients. This study suggested that SCFAs, particularly acetate, are actively taken up by the heart in both failing and non-failing conditions.[105] The uptake of SCFAs was found to be directly proportional to their circulating levels, suggesting that substrate availability drives consumption without requiring regulation. However, a recent study by Kirschner *et al.*[123] found that patients with HF had significantly lower plasma concentrations of key SCFAs, including propionate, butyrate, and isovalerate, compared to healthy controls. decreased SCFA levels were associated with poorer muscle quality, higher emotional distress, and impaired cognitive function in the HF group.

In summary, discrepancies exist between animal models of HF and human HF studies regarding OCFA metabolism. Overall, most studies suggest increased SCFA utilization. However, findings could be related to the decreased microbial diversity seen in HF patients, leading to decreased propionate production through fermentation and lower absorption.[124] Lower levels of propionate utilization may contribute to decreased energy metabolism in HF by reducing succinyl-CoA replenishment. Furthermore, the reliance on SCFA metabolism in failing hearts may reflect an adaptive mechanism to maintain ATP production under metabolic stress. However, whether this shift compensates for impaired anaplerotic pathways or exacerbates metabolic dysfunction remains to be clarified. Propionate anaplerosis and its role in HF remain poorly understood, and additional research is needed to clarify its contribution to TCA cycle replenishment and overall cardiac metabolism.

13. Targeting OCFA anaplerosis in HF

Propionate has been investigated as a potential HF therapeutic in preclinical studies. In rat hearts perfused with acetoacetate, cardiac function was rescued by adding propionate and lactate.[14] Dietary supplementation with propionate has cardioprotective effects in animal models of HF.[125–129] Bartolomaeus *et al.*[126] explored the impact of propionate on cardiovascular damage induced by hypertension in mouse models. This study involved administering propionate to wild-type and ApoE-knockout mice, with atherosclerosis and hypertension induced by infusion with Ang-II. Propionate significantly decreased cardiac hypertrophy, fibrosis, vascular dysfunction, and atherosclerosis. Additionally, propionate was found to moderately decrease blood pressure and lower the risk of arrhythmia. Tang *et al.*[128] explored the influence of gut microbiota and SCFAs, including propionate, on cardiac healing after CAL-induced myocardial infarction in mice. By depleting gut microbiota with antibiotics, they observed worsened cardiac repair, increased mortality, and decreased immune cell response. Supplementation with SCFAs, including propionate, partially reversed these effects by enhancing survival and promoting immune cell infiltration in the heart. Kaye *et al.*[127] used wild-type mice, germ-free mice, and SCFA receptor knockout strains to study the effects of a low-fibre diet and SCFA supplementation on hypertension and cardiac remodelling. Mice were fed either a low-fibre or high-fibre diet while hypertension was induced using Ang-II. Propionate supplementation in mice fed a low-fibre diet significantly decreased blood pressure, cardiac hypertrophy, and fibrosis, even in the presence of a hypertensive stimulus. Zhou *et al.* investigated the effects of propionate on ventricular arrhythmias and cardiac electrophysiological remodelling using a rat CAL model. Male Sprague-Dawley rats were divided into four groups: a sham control group, a sham group treated with propionate, a CAL control group, and a CAL group treated with propionate. Propionate was administered in drinking water for 7 days before myocardial infarction induction. The study revealed that propionate significantly enhanced heart rate variability, decreased ventricular arrhythmias, and improved cardiac electrical stability in CAL-treated rats, primarily through promoting parasympathetic activity via the gut-brain axis. Deng *et al.*[125] employed a myocardial ischaemia-reperfusion (I/R) injury model in male mice, inducing ischaemia via CAL followed by reperfusion. Ang-II was administered during reperfusion to worsen cardiac injury, allowing the investigation of propionate's protective effects under these conditions. Mice were pretreated with sodium propionate in their drinking water for 15 days prior to I/R induction. The study demonstrated that propionate decreased myocardial infarct size, lowered oxidative stress, and enhanced cardiac function, even with Ang-II present. Overall, studies in animal models of HF indicate that propionate supplementation could serve as a nonpharmacological approach for managing HF. However, it remains unclear whether enhanced propionate anaplerosis directly contributes to its cardioprotective effects.

Since administration of propionate in humans is hindered by its toxicity, researchers have employed propionyl-L-carnitine (PLC) as an alternative to increasing propionyl-CoA levels and enhancing anaplerosis.[130,131] Early studies highlighted the therapeutic potential of PLC in acetoacetate perfused rat hearts.[15] In pre-clinical HF studies, PLC has shown cardioprotective effects such as improving exercise tolerance and cardiac function.[132–134] PLC has also been tested in HF clinical trials, showing improved exercise tolerance and cardiac function.[135–138] Zamani *et al.* are currently recruiting HFpEF patients to study the effects of PLC-treated perfusate on exercise tolerance and oxidative phosphorylation in skeletal muscle (NCT04913805).

Triheptanoin (Dojolvi®) is a source of medium (C_7) OCFAs and is an approved therapeutic for human FA oxidation disorders.[139] Triheptanoin is catabolized into glycerol and three heptanoate molecules, the latter of which is a source of acetyl-CoA and anaplerotic molecules including propionyl-CoA and C_5-ketone bodies (β-ketopentanoate and β-hydroxypentanoate).[140] Triheptanoin enhances anaplerosis by providing propionyl-CoA for PCC-catalyzed carboxylation into succinyl-CoA. The therapeutic efficacy of triheptanoin has been demonstrated in animal and human studies.[141–145] Studies of patients with long chain fatty acid oxidation disorders and HF have demonstrated that treatment with triheptanoin may be more effective compared to supplementation with even-chain FAs.[146,147] Clinical studies demonstrate that plasma levels of long (C_{15} and C_{17}) OCFAs are inversely associated with cardiovascular disease.[148] Humans with low levels of pentadecanoic acid (C_{15}) are more prone to developing cardiovascular disease including HF.[149–153]

C_5 ketones are exported from the liver as a byproduct of OCFA metabolism.[154] They are metabolized into acetyl-CoA and propionyl-CoA by

cardiomyocytes, thereby making them an important anaplerotic substrate.[155] In both animals and humans, HF, especially in its advanced stages, is often associated with elevated levels of plasma ketones, concurrent with increased ketone oxidation. However, the precise role of C_5 ketones in HF is unknown and understudied. The effectiveness of triheptanoin may be in part due to its effect of providing anaplerotic C_5 ketones.[156]

In summary, studies suggest that altered OCFA metabolism is a potential therapeutic target for HF. Considering their distinct role in cardiac metabolism, the utilization and oxidation of OCFAs and C5 ketones should be further studied in HF. Quantifying the metabolism of C5 ketones in earlier stages of HF and identifying their potential anaplerotic role in end-stage HF are important research goals. While studies highlight the cardioprotective effects of propionate supplementation in animal models, the precise mechanisms, whether through direct anaplerosis or indirect metabolic modulation, remain unclear. In particular, the contribution of propionyl-CoA to succinyl-CoA formation via OCFA metabolism requires further exploration to determine its full therapeutic potential in HF. PLC and triheptanoin hold therapeutic potential for HF patients, but their effectiveness across different HF types and stages remains to be fully elucidated.

14. Adenylosuccinate

The anaplerosis of adenylosuccinate is a part of the purine nucleotide cycle, which functions to maintain a balance of adenine nucleotides in the heart. A deficiency of ATP in the failing heart leads to an imbalance of flux through the purine nucleotide synthesis pathways (*de novo* synthesis, degradation, and salvage). Increased purine nucleotide degradation is a compensatory response to increased ATP demand or chronic ischaemia in HF.[157] This degradation depletes cardiac adenine nucleotides which is associated with mechanical dysfunction of the heart.[158–161] Lopez *et al.*[159] subjected male rats to TAC-induced pressure overload, resulting in cardiac hypertrophy and decompensated HF. This study found that elevated adenosine monophosphate deaminase activity and depletion of adenine nucleotides were associated with mechanical dysfunction. The increase in purine nucleotide degradation observed in HF could also lead to reductions of adenylosuccinate anaplerosis, further contributing to energy deficiency. However, the activity of adenylosuccinate lyase and anaplerosis of adenylosuccinate were not measured. Pre-clinical investigation of this pathway could identify novel therapeutic targets. For example, adenylosuccinic acid, an orphan drug previously investigated in Duchenne muscular dystrophy, may have potential to improve adenylosuccinate anaplerosis in HF.[162]

15. Conclusions

Alterations in cardiac energy metabolism are a hallmark of HF with specific defects identified in TCA cycle intermediates and anaplerosis. However, the relationship between HF and cardiac anaplerosis remains complex and poorly understood. While the molecular pathways governing anaplerosis are known, what remains elusive is a functional understanding of how these metabolic shifts contribute to the overall energy deficit in HF. Collectively these studies show that there is a complex interplay between metabolic function and HF. Despite this, multiple lines of evidence suggest that metabolism can contribute to the progression of HF or potentially its rescue. Understanding these complex metabolic-physiology dynamics will require a comprehensive understanding of metabolism in the context of cardiac function.

Furthermore, the heterogeneity of HF pathophysiology underscores the need for tailored metabolic interventions. For example, understanding how defects in anaplerosis differ between HFpEF and HFrEF could inform more precise therapeutic strategies. Similarly, the role of specific metabolic substrates, like OCFAs or BCAA-derived metabolites, must be further explored to determine whether they offer cardioprotective benefits or contribute to maladaptive remodelling.

To achieve meaningful progress, additional pre-clinical, translational, and clinical studies must integrate accurate measurements of cardiac TCA cycle flux, intermediates, and metabolic outcomes in the context of myocardial function. Only through this integrative approach will we be able to identify novel, metabolism-based therapeutic targets that are both effective and specific to the underlying cause and stage of HF.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Author contributions

K.A.A., M.A.K., and B.S.B.P. wrote the manuscript. S.C.G. and G.D.L. reviewed the draft manuscript. All authors have approved the final version of the manuscript.

Acknowledgements

S.C.G. acknowledges funding from the Heart and Stroke Foundation. I.A.L. and G.D.L. acknowledge funding from the Canadian Institutes of Health Research.

Conflict of interest: S.C.G. and G.D.L. have served in an advisory capacity for Ultragenyx Pharmaceutical Inc. S.C.G. has received research funding from Ultragenyx Pharmaceutical Inc.

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