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STATE-OF-THE-ART REVIEW

Towards Metabolomic-Based Precision Approaches for Classifying and Treating Heart Failure

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HIGHLIGHTS

- **Patients with HF have impaired cardiac and systemic metabolism, and novel therapies to target metabolism are needed. Myocardial and peripheral metabolomics offer comprehensive measurement of small molecule metabolites in biological samples.**
- **Several studies have highlighted the abnormalities in metabolomic profiles of patients with HF with preserved or reduced EF, both from peripheral blood and myocardial tissue. In this review, we synthesize the published reports on the metabolomics of HF and propose future directions.**
- **Future studies are warranted to improve our understanding of single cell metabolomics, cardiac-specific metabolomics, flux through metabolic pathways, subgroup differences in metabolomic profiles, and response to pharmacologic and nonpharmacologic therapies.**

SUMMARY

Both heart failure and cardiometabolic disease are on the rise, and abnormal cardiac and peripheral metabolism are central to the syndrome of heart failure. Advances in metabolomic profiling have improved our understanding of the heart's metabolic flexibility in patients with and without heart failure. Prior studies have noted patients with heart failure display metabolomic profiles associated with marked abnormalities in the metabolism of fatty acids, branched-chain amino acids, ketones, and glucose compared with control subjects. Metabolomics can highlight specific pathways that are dysregulated; however, other metabolites beyond those related to fuel metabolism may also play a role in precision-medicine approaches. Novel approaches include metabolic flux studies, spatial and single-cell analysis, serial monitoring of treatment response, and integration with other -omics data. The goal of these innovative approaches should be to harness metabolomic technologies to affect precision care for patients with heart failure. (JACC Basic Transl Sci. 2024;9:1144-1158) © 2024 Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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The global burden of heart failure (HF) continues to rise, highlighting a need for improved diagnostic, prognostic, and therapeutic tools. Concomitantly, there is a growing burden of metabolic risk factors such as obesity and diabetes fueling this rise, particularly with heart failure with preserved ejection fraction (HFpEF).[1] For example, the prevalence of severe obesity has more than doubled over the last 2 decades.[2] The success of sodium glucose cotransporter-2 (SGLT2) inhibitors in both heart failure with reduced ejection fraction (HFrEF)[3,4] and HFpEF,[5-7] and glucagon-like peptide 1 agonists in HFpEF[8] highlight the potential for new therapies for HF that target cardiometabolic disease. Patients with HF are known to have abnormal cardiac and systemic metabolism compared with control subjects; yet, the ability to modulate metabolism for treatment benefit is debated. Significant knowledge gaps remain regarding the aspects of abnormal cardiac/systemic metabolism that should be targeted. Metabolomic profiling can enable simultaneous evaluation of the biology and biomarkers related to healthy and abnormal cardiac metabolism. In this review, we summarize the current evidence and future directions of metabolomics in HF (**Central Illustration**).

METABOLOMIC PROFILING PLATFORMS AND APPLICATIONS

Metabolites are small molecule substrates, intermediates, and by-products of not only fuel metabolism, but also metabolism of drugs, hormones, and other molecules. While metabolites can be individually assayed, metabolomics refers to the comprehensive measurement of small molecule metabolites in biological samples.[9] Contemporary metabolomic platforms facilitate simultaneous study of many diverse metabolic pathways. Metabolites include both exogenous and endogenous sources, which themselves integrate genetics, diet, microbiome, physiology, and disease pathology. Metabolomics can be performed on a variety of biological samples including cells, tissues, liquids (including blood, urine), and gases. The human metabolome database currently includes >217,000 compounds including endogenous, drug, environmental, microbiome, and dietary metabolites.[10] The most studied classes of metabolites in cardiovascular disease include organic acids, amino acids, lipids (including phospholipids and sphingolipids), free fatty acids (FAs), ketone bodies, acylcarnitines, and acetyl coenzyme As (acetyl-CoAs).

Three main technologies are used to measure metabolites in biological samples: nuclear magnetic resonance spectroscopy, gas chromatography spectrometry, and liquid chromatography-mass spectrometry (LC-MS). Each has advantages and disadvantages, and the use of multiple technologies in a sample can provide broader coverage of metabolites.[11] Nuclear magnetic resonance spectroscopy can be performed in intact samples and is more reproducible[12]; however, it is not as sensitive as mass spectrometry. Mass spectrometry requires extensive sample processing but is more sensitive and quantitative. Metabolomic profiling with these technologies can further be targeted or unbiased. Targeted metabolomics include spiked-in standards for more accurate and absolute quantification, but are limited to known metabolites, whereas unbiased or nontargeted platforms allow a comprehensive overview of the metabolome but are semiquantitative.

Metabolomics technologies provide an excellent tool for studying fundamental cardiac metabolism, but importantly, may affect care of patients with or at risk of HF. For example, measurement of intermediates of specific metabolic pathways will expand our biological understanding of cardiac and noncardiac metabolism in HF. This information can be used to inform novel targeted metabolic therapies as well as the repurposing and personalization of existing pharmacologic and nonpharmacologic therapies. Metabolomics can be used to subclassify heterogeneous HF phenotypes; these subclassifications may have therapeutic implications and inform a more personalized approach to HF diagnosis and management. Finally, circulating metabolic biomarkers can be used as diagnostic and prognostic biomarkers, and for monitoring treatment response.

CARDIAC METABOLISM AND SUBSTRATE UTILIZATION

Changes in cardiac metabolism, in particular fuel (substrate) utilization, play a fundamental role in HF. Metabolomic profiling is a useful platform for studying HF at a biologic and biomarker level. Given the constant systolic and diastolic demands (both energy-requiring processes), the heart accounts for nearly 10% of total body fuel consumption.[13] With limited capacity for energy storage, persistent adenosine triphosphate (ATP) generation is a necessity for normal cardiac function. To meet these demands, the healthy heart demonstrates metabolic flexibility, functioning as a "metabolic omnivore," with the ability to utilize several different fuel substrates while adapting to changing environments.[14,15]

ABBREVIATIONS AND ACRONYMS

BCAA = branched-chain amino acids

EF = ejection fraction

FA = fatty acid

HF = heart failure

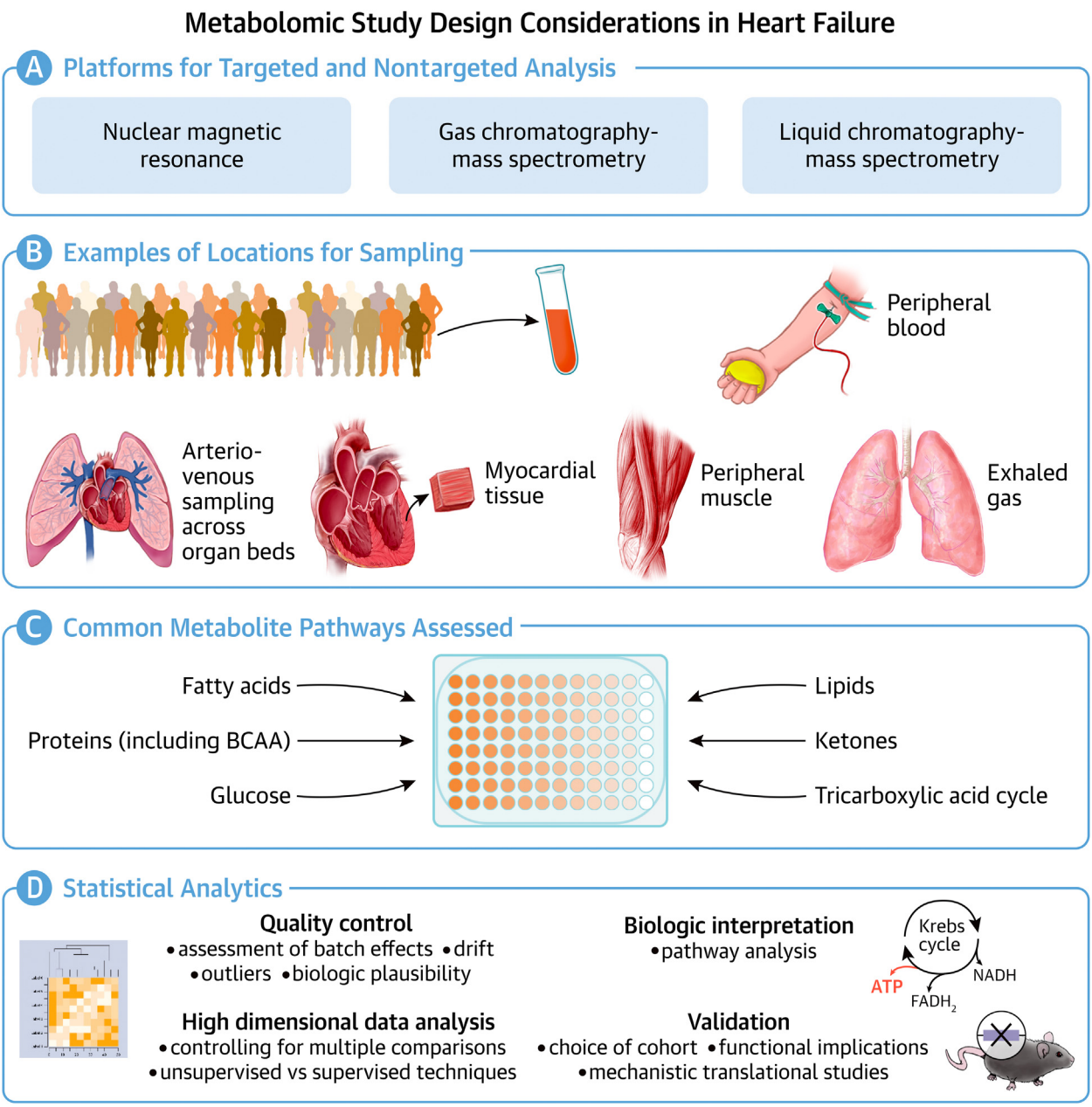
HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

LCAC = long-chain acylcarnitine

SGLT-2 = sodium glucose cotransporter-2

CENTRAL ILLUSTRATION **Overview of Study Considerations for Metabolomics Studies in Heart Failure**



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Examples of common metabolomics platforms (including for both targeted and nontargeted assays), locations of sampling, metabolite pathways interrogated, and statistical analysis considerations are provided. ATP = adenosine triphosphate; BCAA = branched-chain amino acid; $FADH_2$ = falvin adenine dinucleotide; NADH = nicotinamide adenine dinucleotide.

This metabolic flexibility is a defining feature of healthy cardiomyocyte function. Under aerobic, resting conditions, oxidative metabolism of FAs, including those derived from albumin-bound free FA or from lipoprotein metabolism, contributes most to ATP generation,[16-18] with lactate, ketones, amino acids (including the branched-chain amino acids [BCAAs] isoleucine, leucine, and valine) and glucose contributing to a lesser extent.[16] Leveraging substrate arteriovenous gradients measured at the time of

electrophysiology procedures, a recent study comprehensively delineated fuel consumption in failing and nonfailing hearts, as well as in resting skeletal muscle. Confirming previous reports, the myocardium of participants without HF relied heavily on FAs (contributing to roughly 85% of ATP production). Interestingly, glucose metabolism appeared to be minimal, though blood sampling was performed in the fasted state and therefore may have biased away from its combustion.[16] In stark contrast to the heart, the leg obtained the vast majority of carbon balance from glucose and ketones bodies. It is noteworthy that strong metabolite interactions exist (such as that observed with the Randle cycle effect controlling FA vs glucose metabolism),[19] highlighting the importance of comprehensive metabolite evaluation and interpretation.

Substrate utilization by the heart has several steps, any of which can affect substrate metabolism. For example, in the case of FAs, most are stored in circulating lipoproteins or in adipose tissue. Free FAs must be released via lipolysis, transported across the plasma membrane of the cardiomyocyte, then bound to FA binding proteins in the cytosol.[20] FAs are converted to acyl-coAs in the outer mitochondrial membrane, then converted to acylcarnitines by the enzyme carnitine palmitoyltransferase I, an important regulator of FA oxidation. Once converted to acylcarnitines, FAs are committed to oxidation in the mitochondria, which generates acetyl-coA for the Krebs cycle and reducing equivalents for the electron transport chain. However, acylcarnitines can accumulate in the mitochondria because of insufficient acylcarnitine oxidative cleavage, and can be exported out of the cell into the periphery. Given the complexity of this and other metabolic pathways, single measurement of metabolites are insufficient to determine the following: 1) flux through the pathway; and 2) location of a "block" in utilization of a substrate—ie, transport, uptake, oxidation, and so on.

On the path toward mitochondrial oxidative phosphorylation, each substrate is metabolized into metabolic intermediates that can enter the Krebs cycle either as acetyl-CoA or as a cycle intermediate to produce reducing equivalents nicotinamide adenine dinucleotide and flavin adenine dinucleotide. These reducing equivalents are essential electron donors that facilitate the electrochemical gradient that drives ATP synthesis. It is important to note, however, that these intermediaries can be incorporated into biosynthetic pathways and also participate in other nonfuel-generating roles include signaling roles, such as regulation of post-translational modification through acetylation, linking these metabolites with a broad number of processes involved in energy metabolism.[21]

Recent interest in the last decade has arisen in the role of ancillary fuel sources, in particular ketone and BCAA oxidation. Ketone bodies are generally produced in carbohydrate deprived environments with low insulin availability, and are found in low quantities physiologically. Upregulation of ketone metabolism is of great interest across a variety of cardiovascular states (spanning athletes to patients with HF).[22-26] HFrEF has been traditionally characterized by a reversion to the "fetal" state[27] with diminished capacity to oxidize FAs,[28] although this concept has been recently challenged.[29] In this setting, ketone bodies provide a potential alternative substrate that appears to be adaptive,[30-32] although nonoxidative signaling roles of ketones are likely also instrumental.[33] Indeed, intravenous infusions of 3-hydroxybutyrate appear to engender hemodynamic benefits and extend to even non-HF populations.[26,34] Both endogenous (ie, through fasting, medication use) and exogenous (ie, intravenous or oral administration) ketogenic approaches have been studied,[22] and although available data are promising, more studies are needed to determine longer-term effects, tolerability, delivery, off-target effects, and broader efficacy.

Complexities in metabolism of BCAAs and several metabolic intermediates have generated tremendous discussion on the salutary vs detrimental roles.[35-40] BCAA metabolism yields intermediates that participate in numerous physiologic roles, with critical relationships to obesity, insulin resistance, and mTOR activation. Notably, BCAA play a relatively small role in cardiac metabolism compared with other tissue beds such as skeletal muscle, liver and fat.[37] Therefore, changes in circulating BCAA metabolites more often reflect homeostatic patterns in other organs.[41]. Augmentation of BCAA catabolism may be more beneficial than pure supplementation of BCAA.[38,39] However, the level of potential beneficial effects remains controversial, and extramyocardial benefits of enhanced BCAA catabolism may play prominent roles, highlighting the importance of tissue crosstalk with adipose tissue, vasculature, liver, and skeletal muscle.[35,42-44] For example, brown adipose tissue critically regulates BCAA clearance, and inhibition of BCAA catabolism in brown adipose tissue reduces systemic clearance of BCAA.[42] Increased exposure to BCAA may promote myocardial dysfunction.[35,39] Moreover, the liver plays a critical role in regulation of branched-chain ketoacid catabolism and levels, which likewise might impair myocardial health

directly or through cardiac reamination back to BCAA.[38,45-47] Finally, the benefits of augmenting BCAA catabolism may also relate to blood pressure control rather than cardiac specific effects.[43]

METABOLOMIC PROFILING IN HFrEF

METABOLOMIC STUDIES FROM BLOOD IN HFrEF.

Several studies have leveraged metabolomic platforms in patients with HFrEF to advance our biologic understanding of cardiac metabolism. These studies provide an integrated framework of both cardiac and systemic metabolism and identify related circulating biomarkers (**Table 1**). Given greater accessibility, most studies explore metabolomic profiling from peripheral blood.

Targeted and nontargeted metabolomic profiling in blood from human cohorts with (or at risk for) HF have identified circulating metabolites reflecting key substrate metabolic pathways. Circulating long chain acylcarnitine (LCAC) metabolites, markers of mitochondrial FA oxidation, have been associated with HF and HF-related adverse outcomes. One study leveraged targeted profiling from sequential patients undergoing cardiac catheterization. LCACs were significantly higher in HFrEF compared with HFpEF patients, both of which were higher compared with no-HF control subjects.[48] Furthermore, levels of these LCAC metabolites are associated with severity of HF, as patients with end-stage HF have higher levels of LCAC compared with chronic stable HFrEF patients.[49] Importantly, circulating levels of LCACs have been shown to be beneficially modifiable through unloading of the left ventricle[50] and with exercise.[51] Interpretation of circulating LCAC requires caution, because higher circulating LCAC may predominantly reflect liver and skeletal muscle secretion more so than cardiac secretion per se.[52,53] In line with this, LCACs were *lower* in the myocardial tissue of patients with end-stage HF compared with nonfailing hearts [54,55] Therefore the balance between secretion vs uptake of cardiac and noncardiac tissues is unknown.

Peripheral levels of BCAAs have also been associated with HF and are higher in HFrEF patients than those with HFpEF or no HF.[48] Although higher levels of BCAA are linked to diabetes and insulin resistance, these relationships with adverse cardiovascular features appear to be independently linked.[48,49,56] Unraveling the relationships among insulin resistance, diabetes, HF, and BCAA has been a topic of great interest,[35] in part because circulating BCAA are beneficially modifiable by nonpharmacologic therapies including exercise and weight loss.[57,58]

Likewise, metabolites that report on glucose metabolism provide significant insight into HF physiology. Lactate and pyruvate, downstream products of glucose metabolism, levels are associated with worse HF features (functional status).[56] Finally, ketone bodies have emerged as yet another strong metabolite biomarker of adverse HF features. For example, peripheral ketone levels are elevated in HFrEF, and greater levels correlate with more impaired functional status and ejection fraction.[59-63] Indeed, population-based studies have demonstrated their strong prognostic values across the spectrum of cardiovascular disease, including HF.[64,65] This increase in ketone levels is thought to represent an adaptive phenotype in HF, although more studies are needed.[22]

To gain greater clarity into cardiac-specific metabolism, studies have employed sampling from the coronary sinus, a surrogate for cardiac flux of metabolites. In a study leveraging coronary sinus sampling, stark shifts in myocardial substrate use are observed among those with reduced ejection fraction (EF) compared to preserved EF.[16] This includes a nearly 3-fold increase in ketone contribution to ATP, larger contributions from lactate and amino acid combustion, and reduction in FA consumption by the failing heart. Importantly, study of arteriovenous gradients in the fasted state allows for more direct comparability among patients, although is less generalizable to daily physiologic conditions. Recent analysis has shed light into considerable changes in metabolic substrate during intralipid as well as insulin/glucose infusions, demonstrating significant flexibility previously believed to be diminished in the failing heart.[29] Specifically, LCACs were produced by the heart during lipid infusion (but not during insulin/glucose infusion), in keeping with a shift toward FA oxidation. Likewise, insulin/glucose infusion resulted in greater glucose uptake. Although these results could be expected in the healthy heart, they were indeed surprising in setting of HF. BCAA extraction was interestingly not altered with either infusion, whereas ketone uptake was greater during lipid infusion. Indeed, these changes in substrate availability specifically during lipid infusion related to improved myocardial energetics and systolic and diastolic function. Metabolomic analysis, however, highlighted potential notes of caution with the high-dose lipid infusion, where ceremide species production could result in a detrimental long-term effect. These studies highlight the power of arteriovenous

TABLE 1 Examples and Descriptions of Metabolomics Studies in Human Heart Failure With Reduced Ejection Fraction

First Author, Year	Metabolomics Source	Metabolomics Platform	Sample Size	Key Findings
Chokshi, 2012[72]	Myocardial tissue	LC-MS	61 HFrEF patients who underwent LVAD implantation and 9 control subjects	Mechanical unloading was associated with reversal of diacylglycerol and ceramide accumulation.
Marcondes-Braga, 2012[62]	Exhaled breath	GC-MS	89 HFrEF patients and 20 control patients	Exhaled acetone had similar accuracy to natriuretic peptides for the diagnosis of HF and was associated with HF severity.
Gupte, 2014[76]	Myocardial tissue	Tandem flow-injection MS and GC-MS	6 HFrEF patients before and after LVAD implantation and 6 control subjects	Increased pyruvate and reduced Krebs cycle intermediate and short-chain acylcarnitines demonstrated reversibility with mechanical unloading.
Cheng, 2015[66]	Peripheral blood	LC-MS	515 total participants, including 401 patients with HF (preserved and reduced EF)	A metabolite panel provided equivalent diagnostic accuracy to natriuretic peptides and was superior for prognosis
Nemutlu, 2015[75]	Peripheral blood	GC-MS and nuclear magnetic resonance	24 HFrEF patients undergoing cardiac resynchronization therapy and 10 control patients	Cardiac resynchronization therapy increased the succinate/glutamate ratio and glucose/palmitate ratio. Metabolite profiles predicted response to device therapy.
Ahmad, 2016[49]	Peripheral blood	Tandem flow-injection MS	453 HFrEF patients randomized to exercise training vs. usual care; 41 patients who underwent left ventricular assist device placement	Circulating long-chain acylcarnitines are associated with adverse outcomes and are reversible after mechanical circulatory support.
Bedi Jr, 2016[30]	Myocardial tissue	LC-MS	15 NICM patients and 20 control subjects.	Decreased intramyocardial lipid intermediates with increased ketone body utilization were observed in the nondiabetic, failing heart.
Sun, 2016[38]	Myocardial tissue	GC-MS and LC-MS	15 failing hearts and 4 control subjects	Deficit in branched-chain amino acid catabolism is a hallmark of the failing heart
Ji, 2017[71]	Peripheral blood and myocardial tissue	LC-MS	65 HFrEF patients (including 34 who underwent LVAD implantation) and 22 control subjects.	HF patients have increased circulating and myocardial ceramides, which was partially reversible with mechanical unloading.
Lanfear, 2017[56]	Peripheral blood	LC-MS and GC-MS	1,032 HFrEF patients	Metabolite score profiles offered incremental risk prediction compared with conventional predictors.
Ruiz, 2017[69]	Peripheral blood	GC-MS and LC-MS/MS	68 HFrEF patients and 72 control patients	Dysregulated fatty acid oxidative markers including mitochondrial and extra-mitochondrial acylcarnitine derivatives were signatures of HF and associated with disease severity.
Murashige, 2020[16]	Arterial, femoral vein, and coronary sinus blood	LC-MS	110 patients (23 with HFrEF)	The failing heart disproportionally consumed more ketones and lactate compared with nonfailing hearts, and also demonstrated evidence of greater proteolysis.
Truby, 2021[51]	Peripheral blood	Tandem flow-injection MS	664 HFrEF patients randomized to exercise training vs usual care	Long-chain acylcarnitines associated with exercise status with differential effects in patients with diabetes compared with those without diabetes
Flam, 2022[55]	Peripheral blood and myocardial tissue	LC-MS	39 patients with HFrEF and 48 control subjects	Significant reductions were noted in fatty acids, acylcarnitines, ceramides, tricarboxylic intermediates, with an accompanying accumulation of polyols. In contrast, there was evidence of increased pyruvate, lactate, and ketone metabolism.
Selvaraj, 2022[74]	Peripheral blood	Tandem flow-injection MS	234 HFrEF participants randomized to either dapagliflozin or placebo	Dapagliflozin increased short- and medium-chain acylcarnitines and ketone-related metabolites compared with placebo.
Watson, 2023[29]	Arterial (left main coronary artery) and coronary sinus blood	LC-MS	9 NICM patients with arteriovenous sampling	Nonischemic heart failure myocardium retains significant metabolic flexibility to metabolize fat and glucose sources

Several human heart failure with reduced ejection fraction (HFrEF) metabolomics studies cited in this review are detailed in this table. See reference section for individual publication details.
GC = gas chromatography; HFpEF = heart failure with preserved ejection fraction; LC = liquid chromatography; LVAD = left ventricular assist device; MS = mass spectrometry.

gradients in delineating cardiac-specific metabolism across the nutritional spectrum.

Although much focus has been centered around acylcarnitines, BCAAs and their derivates, as well as ketone bodies, broader groups of metabolites elucidated by more comprehensive platforms also appear to play an important role in HF pathogenesis. For example, compared with control subjects, patients with HF had lower levels of glutamine and citrulline (reporting on arginine metabolism) and several phosphatidylcholines, while levels of aromatic amino acids, glutamine, ornithine, spermine, and spermidine were higher.[66] Further, metabolites that report on the glutamate-ornithine-proline pathway,

polyamine synthesis, and phosphatidylcholines synthesis have greater diagnostic and prognostic value for HF than natriuretic peptides, which to-date have been the clinical workhorse. Interestingly, this metabolic disarray improved in patients whose severity of HF also improved. Other studies support the role of broader metabolite investigation, including Krebs cycle intermediates, to improve current risk stratification approaches.[56] Other studies have focused on single metabolites and their prognostic role in HF. Serum uric acid is associated with worse outcomes in HF (both HFrEF and HFpEF).[67,68] Recent focus on the gut microbiome has highlighted the relevance of trimethylamine-N-oxide (TMAO) in HF as summarized in this recent review.[67] TMAO is generated by gut microbes and correlates with worse renal function. TMAO levels are consistently higher in patients with HF (both HFrEF and HFpEF) and associated with HF severity and poor outcomes, although its prognostic value is somewhat diminished once adjusting for renal function.

METABOLOMIC STUDIES FROM HUMAN MYOCARDIAL TISSUE IN HFrEF. Although peripheral blood provides global insight into metabolic derangements, a significant limitation is the lack of specificity of pathophysiology at the organ level. Although this limitation is partially overcome by regional blood sampling, tissue sampling allows for study of organ-specific metabolism as well as enabling carbon-labeled techniques to determine metabolite flux and fate. For example, in a study of 87 explanted human hearts (end-stage nonischemic HF vs nonfailing donor control hearts), a global bioenergetic deficit was suggested.[55] This study integrated metabolomics with transcriptomics and protein abundance/post-translational modification to estimate flux through metabolic pathways. Deficits in intermediates of glycolysis and related pathways (pentose phosphate pathway, glycogen synthesis, and serine/glycine synthesis) were observed. Further analysis appeared to indicate impaired glucose influx to glycolytic pathways as well as an increase in pyruvate oxidation (supplied by lactate) that outpaced the ability to replenish related metabolite pools. Reductions in FAs and acylcarnitines were also observed despite peripheral blood elevation that characterizes end-stage HF, along with metabolic enzymes related to FA utilization. Although inferences of the degree of FA metabolism from tissue acylcarnitines are limited,[30,48,69,70] the addition of metabolic enzyme expression and arterial-venous uptake studies (including in response to lipid infusion) provide more support for lower FA utilization in HFrEF. Ceramides (a potentially toxic byproduct of FAs) were correspondingly low, although other studies in end-stage HF demonstrated higher ceramides.[71,72] Additional significant derangements included a suggestion of insufficient cardiac anaplerosis leading to lower tricarboxylic acid cycle intermediates, broadly increased ketone body intermediates (consistent with increased ketone body oxidation), and increased abundance of BCAA and branched-chain ketoacids, yet decreased downstream catabolic intermediates suggesting impaired BCAA oxidation.[55] This intramyocardial surplus of BCAA and branched-chain ketoacids is believed to be a signature of the failing heart,[73] although the excess of BCAA has not been consistently observed.[38] In contrast to a discrepancy between peripheral vs myocardial markers of FA oxidation, there appears to be greater concordance with ketone bodies.[22,30,55] For example, at the time of transplantation or left ventricular assist device placement, increased levels of the ketogenic derivative, β-hydroxybutyryl-CoA, are found in myocardial tissue accompanied by decreased ketone body in myocardial tissue, suggesting increased utilization. Animal models of HF likewise have identified metabolite signatures of increased ketone body oxidation, accompanied by augmentation of metabolic enzymes to support increased ketone uptake.[32] Nucleotide metabolites were also altered in HFrEF vs control subjects,[55] with total ATP levels lower in HFrEF, and consistent changes were suggestive of a specific reduction in adenine-based purines hypothesized to be caused by lower salvage and/or lower synthesis.

IMPACT OF EXERCISE, DRUG, AND DEVICE TREATMENT ON METABOLOMIC SIGNATURES. As highlighted, HFrEF is characterized by key changes in circulating and local metabolites. However, whether these signatures are beneficially modifiable is of significant therapeutic interest. For example, peripheral LCAC metabolites have been shown to decrease with exercise in the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) randomized trial in patients with HFrEF, perhaps reflecting augmented cardiac or peripheral mitochondrial functioning.[51] In addition, metabolite changes have been investigated after pharmacotherapies and device placement (including left ventricular assist devices and cardiac resynchronization therapy). For example, one study investigated targeted metabolomic changes among stable HFrEF patients randomized to 12 weeks of dapagliflozin vs placebo in the DEFINE-HF (Dapagliflozin Effects on Biomarkers, Symptoms and Functional Status in Patients with HF

with Reduced Ejection Fraction) clinical trial.[74] Dapagliflozin was found to increase peripheral metabolite clusters enriched with ketone bodies as well as short- and medium-chain acylcarnitines, the latter potentially implying some degree of metabolic reprogramming with SGLT2 inhibitor therapy. Whether these changes underly the beneficial effects of this drug class remains to be elucidated.

More advanced HF phenotypes seem to metabolically respond to device treatments, such as resynchronization therapy and mechanical unloading. Using paired samples before and after cardiac resynchronization therapy, substantial changes in metabolites were observed, including decreases in BCAA levels as well as changes in metabolites reflecting the Krebs cycle, lipid metabolism, and more.[75] Interestingly, some metabolites returned to levels comparable with control subjects. Highlighting the potential role of metabolomic precision medicine approaches, higher levels of BCAA predicted ejection fraction response to cardiac resynchronization therapy.[75]

Similarly, in end-stage HF, metabolic disturbances appear to be partially reversible after advanced therapies. In a small study of patients with paired myocardial samples obtained at the time of LVAD insertion and subsequent heart transplant, myocardial samples at the time of LVAD placement showed global impairment of substrate oxidation compared with a nonfailing left ventricle comparison group, including higher pyruvate concentration and reduced Krebs cycle intermediates. Mechanical support appeared to reverse numerous metabolic disturbances, including abundances of short-/medium-chain acylcarnitines, Krebs cycle intermediates, pyruvate, and several amino acids.[76] A subsequent study demonstrated reversal of peripheral blood LCAC after mechanical circulatory support.[49] Given that the latter study assayed the periphery, the level of metabolic reversal could include skeletal muscle, which also demonstrates early improvement in sarcopenia post-LVAD insertion.[77] Taken together, these results suggest that metabolomics may serve at the least as a surrogate measure of therapeutic effect, and hint that metabolic reprograming may underly their mechanisms of benefit.

In sum, metabolite signatures are evident in the failing heart across a broad array of substrates. Metabolomics can highlight, and sometimes pinpoint, pathways of particular dysregulation. In addition, analysis of peripheral blood vs cardiac tissue may not always provide concordant insight into disease pathogenesis. However, both are important and provide complementary roles in providing greater clarity in local disease pathogenesis (tissue) vs prognostic value in larger populations (peripheral blood). Studies have also demonstrated the malleability of metabolite profiles with modern treatments in HFrEF patients, which could serve as surrogate endpoints.

METABOLOMIC PROFILING IN HFpEF

METABOLOMICS STUDIES FROM BLOOD IN HFpEF. Although the obesity epidemic has affected all phenotypes of HF, there is a particularly strong association between obesity and HFpEF.[1] Similar to HFrEF, most metabolomic profiling in patients with HFpEF has been done from the circulation (**Table 2**). Heart tissue samples are even more rare in patients with HFpEF than in HFrEF, in whom we have access to LVAD core tissue and explanted hearts before transplantation. However, peripheral blood metabolomics integrate whole body metabolism and are more readily accessible for serial sampling in response to therapy.

Metabolite profiling can predict incidence of HFpEF, as shown in a study of 3,443 participants in the Framingham Heart Study and the Women's Health Initiative.[78] Of the 372 metabolites measured, 11 were associated with incident HFpEF after adjustment for covariates; however, none were significant after adjustment for multiple comparisons. The metabolites were nominally associated with several pathways including nitrogen cycling (ornithine), nitric oxide signaling (L-NMMA, an inhibitor of nitric oxide synthase), and fat metabolism (glycerol). Both asparagine and 2-hydroxyglutarate partially mediated the association between left ventricular wall thickness and HFpEF. Both have been previously associated with cardiac hypertrophy and the associated higher protein synthesis required. Of note, this study population was relatively lean (with a median body mass index of 26-28 kg/m^2).

Several smaller studies in clinical HFpEF have found differences in peripheral blood metabolomics compared with control subjects and HFrEF. However, significant heterogeneity in comorbidity distribution (such as with coronary artery disease, diabetes, and obesity) limits comparability in findings. The largest study to date included 282 patients with HFpEF, 279 patients with HFrEF, and 191 control subjects.[48] Patients were enrolled at the time of coronary angiography, and thus, the patients with HFpEF had a high prevalence of obstructive coronary artery disease (57%). A total of 60 metabolites were included in the

TABLE 2 Examples and Descriptions of Metabolomics Studies in Human Heart Failure With Preserved Ejection Fraction

First Author, Year	Metabolomics Source	Metabolomics Platform	Sample Size	Key Findings
Zordoky, 2015[79]	Peripheral blood	LC-MS and 1H-NMR spectroscopy	24 HFpEF, 20 HFrEF, 38 control subjects	HFpEF vs control subjects: HFpEF had higher ACs, carnitine, betaine, and AAs; lower phosphatidylcholines, lysophosphatidylcholines, and sphingomyelins; HFpEF vs HFrEF: HFpEF had higher LCAC and ketone bodies
Hunter, 2016[48]	Peripheral blood	Tandem flow-injection MS	282 HFpEF, 279 HFrEF, 191 control subjects	HFpEF vs control subjects: HFpEF had higher LCAC; HFpEF vs HFrEF: HFpEF had lower LCAC, lower BCAA and related metabolites, lower glutamine, glutamate
Wang, 2017[82]	Peripheral blood	Tandem flow-injection MS	160 HFpEF (baseline and 24 weeks after sildenafil)	Placebo: no change in metabolites; Sildenafil: Sildenafil treatment decreased alanine, proline and lactate; increased SCDA and 1 LCAC, which correlated with increases in endothelin-1 and worsening renal function. Baseline SCDA and AA were associated with worse outcomes in HFpEF.
Hage, 2020[80]	Peripheral blood	LC-MS	46 HFpEF, 75 HFrEF	HFpEF vs HFrEF: HFpEF had higher hydroxyproline, symmetric dimethyl arginine, alanine, cystine, and kynurenine; HFpEF had lower serine, cAMP, cGMP, L-carnitine, lysophosphatidylcholine, lactate, and arginine
Bekfani, 2022[81]	Peripheral blood	LC-MS	17 HFpEF, 18 HFrEF, 20 control subjects	Kynurenine was higher in HF than control subjects and was associated with reduced muscle endurance.
Hahn, 2023[54]	Myocardial tissue and peripheral blood	LC-MS	38 HFpEF, 30 HFrEF, 20 control subjects	HFpEF vs control subjects: HFpEF had lower myocardial LCAC, unchanged or higher plasma LCAC, lower myocardial TCA intermediates; higher myocardial BCAA and lower myocardial BCAA catabolites, generally unchanged plasma BCAA/catabolites (other than Valine), higher myocardial and plasma AA, unchanged ketone bodies; HFpEF vs HFrEF: HFpEF had higher myocardial LCAC and lower plasma LCAC; HFpEF had higher myocardial nonbranched-chain amino acids and similar plasma AA (both HF groups higher than control subjects)
Hundertmark, 2023[92]	Peripheral blood	MS	36 HFpEF, 36 HFrEF, baseline and 12 wks after empagliflozin vs placebo	No changes in 19 metabolites measured, ketones, or free fatty acids

Human HFpEF metabolomics studies cited in this review are detailed in this table. See reference section for individual publication details.

AA = amino acid; AC = acylcarnitine; BCAA = branched-chain amino acid; GC = gas chromatography; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; LC = liquid chromatography; LCAC = long-chain acylcarnitine; LVAD = left ventricular assist device; MS = mass spectrometry; NMR = nuclear magnetic resonance; SCDA = short-chain dicarboxyacylcarnitines; TCA = tricarboxylic acid.

final analysis. As in HFrEF, LCACs were higher in HFpEF than control subjects,[48] a finding supported by other studies. Data comparing peripheral LCAC in HFrEF vs HFpEF are mixed; studies have reported higher LCAC in HFpEF vs HFrEF,[79] no difference in HFpEF vs HFrEF,[80,81] or lower LCAC in HFpEF vs HFrEF.[48,54] The disparate results may be caused by the differences in LCACs measured, obesity and comorbid conditions, control group, or stage/severity of HF, as has been shown in HFrEF.[49] As noted in the previous text, circulating LCAC levels reflect an amalgam of FA utilization/acylcarnitine secretion from multiple organs, and their interpretation in the context of cardiac FA metabolism requires caution.

Several studies have found differences in peripheral blood measurements of BCAA and nonbranched-chain amino acids in HFpEF compared with control subjects or HFrEF. A study of 38 patients with HFpEF, 30 patients with HFrEF, and 20 nonfailing organ donor control subjects, found that BCAA and nonbranched-chain amino acids in the plasma were broadly increased in HFpEF vs control subjects, and several were higher in HFpEF vs HFrEF (alanine, arginine, lysine, and proline).[54] Higher arginine has been associated with worse prognosis in HFpEF.[82] Arginine is related to NO synthesis by nitric oxide synthase, and there is some heterogeneity in the conclusions about its relative levels in HFpEF vs HFrEF.[80] Alanine is highly concentrated in muscle tissue and has been consistently reported to be higher in HFpEF vs HFrEF in prior studies.[80] In a study of 46 patients with HFpEF compared with HFrEF, patients with HFpEF had higher cystine and kynurenine (both markers of systemic inflammation, while kynurenine is a marker of renal function).[80] Kynurenine was found to be higher in both forms of HF vs control subjects in another study,[81] and was associated with reduced muscle endurance. Kynurenine is associated with atherosclerosis progression, potentially through oxidative stress and repression of anti-inflammatory signaling.[83,84]

Metabolites from other metabolic pathways have also demonstrated differences in HFpEF. Compared with HFrEF, patients with HFpEF had higher

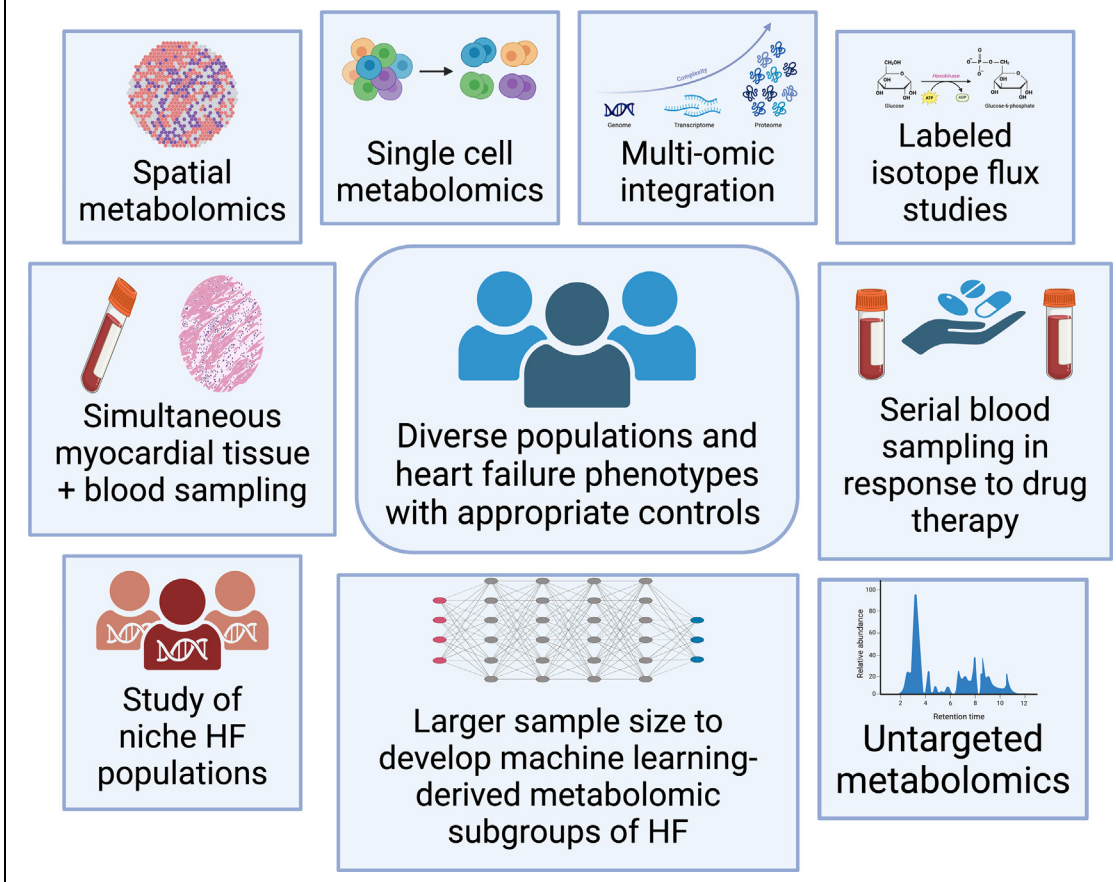
hydroxyproline (a component of collagen potentially linked to fibrosis), symmetric dimethylarginine (inhibitor of NOS that is also a marker of renal function), and amino acids discussed in the previous text.[80] Patients with HFpEF had lower cyclic nucleotides cAMP and cGMP (second messengers important in cardiovascular pathophysiology) and L-carnitine,[80] whereas other studies have noted higher carnitine in HFpEF vs control subjects.[79] Prior studies have noted that relative carnitine deficiency is a prognostic predictor in HFpEF.[85] Phosphatidylcholines and sphingomyelins have been reported to be lower in HFpEF vs control subjects.[79,80] Both are major components of biological membranes, are associated with dietary intake of fish, meat, and animal products,[86] and are associated with cholesterol levels[87] and atherosclerosis.[88,89] The significance of lower levels in HFpEF is unknown.

METABOLOMICS STUDIES FROM MYOCARDIAL TISSUE IN HFpEF. Prior studies have highlighted the significant contributions of other organs, particularly skeletal muscle, to the circulating metabolome.[16] Tissue sampling is necessary to study cardiac metabolism in HFpEF; however, this has been limited caused by the rarity of tissue sampling in HFpEF. In the previously mentioned study of metabolomics in HFpEF,[54] both plasma and myocardial metabolomics were performed using research endomyocardial biopsies in patients with HFpEF. The HFpEF patients had a median body mass index of 40 kg/m^2, the highest compared with other published metabolomic studies in HFpEF. Metabolomic profiles from myocardium vs plasma were at times disparate—particularly for LCAC, which were higher in HFpEF vs control subjects in the plasma, and lower in HFpEF vs control subjects in the myocardium. These data were integrated with prior RNAseq data, and together suggested a deficit in FA oxidation in the HFpEF heart. This is despite the prevailing hypothesis that patients with HFpEF and obesity would have higher cardiac FA oxidation, as has been reported in obese/diabetic subjects without heart failure.[90,91] As reviewed in the previous text, peripheral blood and tissue LCAC levels alone have limitations in their ability to estimate FA oxidation. However, the pattern of lower tissue LCAC, higher plasma LCAC, and lower genes related to FA metabolism matched the pattern seen in HFrEF, where the totality of the data support a defect in FA metabolism in HFrEF.

In addition, BCAA were higher in the HFpEF myocardium vs control subjects, whereas BCAA catabolites were lower, suggesting impaired oxidation of BCAA as fuel and accumulation of BCAA that may worsen insulin resistance and mTOR signaling. Tricarboxylic acid cycle intermediates were also lower in HFpEF vs control subjects, suggesting insufficient anaplerosis as found in HFrEF.[55] Ketones were unchanged in HFpEF vs control subjects, in contrast to HFrEF, suggesting that ketone oxidation is not up-regulated in HFpEF to compensate for potentially lower FA oxidation. Amino acids were generally higher in HFpEF vs control subjects, potentially related to higher protein turnover. Metabolomic profiles were generally not associated with clinical comorbidities, including diabetes and obesity. Taken together, these data suggest HFpEF myocardium may have impairments in FA oxidation and usage of alternative fuel sources, and that study of cardiac metabolism in HFpEF will require analysis of cardiac tissue. Future studies are needed to confirm these findings in other HFpEF populations.

IMPACT OF DRUG TREATMENT ON METABOLOMIC SIGNATURES IN HFpEF. Peripheral blood metabolomics allow for serial sampling in response to an intervention. In a substudy of the RELAX (PhosphodiesteRasE-5 Inhibition to Improve CLinical Status And EXercise Capacity in Diastolic Heart Failure) trial of sildenafil treatment in HFpEF, investigators measured 65 metabolites at baseline and 24 weeks of placebo vs sildenafil therapy.[82] None of the metabolites changed in the placebo group, highlighting the potential stability of metabolites. In total, 7 metabolites changed with sildenafil treatment: amino acids alanine and proline, 3 short-chain dicarboxyacylcarnitines, 1 LCAC, and lactate. Some of these metabolite changes correlated with higher neurohormones and worse renal function in the sildenafil treatment group. Higher baseline short-chain dicarboxyacylcarnitines and amino acids were associated with worse outcomes in HFpEF, although this relationship was attenuated after adjusting for renal function. Despite the often hypothesized effect on systemic metabolism of SGLT2 inhibitors, a recent study found no difference in metabolomic profile in 36 patients randomized to placebo or empagliflozin.[92] This included no difference in the ketone body beta-hydroxybutyrate or free FAs. However, the metabolome was a secondary endpoint, and only 19 metabolites were measured. The study may have been underpowered to find a difference, and did not assay many metabolites that have shown a difference in HFpEF vs control subjects. Larger metabolomic

FIGURE 1 Future Proposed Personalized Approach to Metabolomic Phenotyping In Heart Failure



Advances in metabolomics technology will improve biological interpretation of metabolomics studies, test the metabolic effect of pharmacologic therapies, identify metabolomic subgroups of heart failure (HF), and contribute understanding of rare etiologies of heart failure. Created with BioRender.com.

studies from recent clinical trials in HFpEF are needed to potentially identify effects of novel therapies on the peripheral metabolome in HFpEF, and to identify metabolomic signatures of subgroups who benefitted from pharmacotherapies.

METABOLOMICS IN THE FUTURE

Advances in metabolomics technologies have garnered excitement because they have the potential to improve our understanding of cardiac metabolism in HF (**Figure 1**). Novel studies utilizing imaging mass spectrometry allow for spatial analysis of metabolites in tissue. Matrix-assisted laser desorption/ionization imaging mass spectrometry utilizing trapped ion mobility spectrometry has allowed improved spatial resolution without compromising sensitivity.[93] Single-cell metabolomics allows for even higher resolution of heterogenous metabolite levels by cell type.[94] Future studies will incorporate labeled isotopes (ie, ^{13}carbon and ^{15}nitrogen) into metabolic substrates to estimate their flux through metabolic pathways as measured by their incorporation into downstream metabolites.[95] Although these studies can be performed from myocardial tissue, noninvasive molecular imaging has the added benefit of measuring metabolism of the entire heart and assessing regionality of metabolic changes, and has the ability to obtain serial measurements in response to interventions. Recent studies have highlighted the promise of hyperpolarized ^{13}C-pyruvate cardiac magnetic resonance imaging to estimate flux through glycolysis and the Krebs cycle.[96]

Although metabolomics offer a snapshot of metabolite levels, interpretation of overall activity of a metabolic pathway can be limited. Integration of

metabolomics with other -omics data (transcriptomics, proteomics) can mitigate this limitation. Prior studies have integrated metabolomics with gene/protein expression and post-translational modifications to infer overall metabolic pathway changes in HFrEF[55] and animal models of HFpEF.[97] Further multi-omic metabolism studies are needed, particularly from human myocardial tissue, to confirm hypothesized changes in fuel source. Furthermore, metabolomic studies are restricted to the upper and lower limits of quantification and degree of precision depending on the method used. For example, one study of coronary sinus sampling could not detect glucose uptake from the heart,[16] whereas glucose can clearly be measured in myocardial tissue in HFrEF.[55] It is possible that the difference between glucose concentration in arterial and coronary sinus samples was too small to detect a difference, highlighting the limits of metabolomics studies vs measurement of individual metabolites.

Studies from myocardial tissue should include concomitant peripheral blood analysis to determine which metabolite changes are concordant vs discordant between peripheral blood vs myocardial tissue. Serial metabolomic profiling in response to interventions can identify metabolic effects of diet,[98] exercise,[99] and pharmacologic interventions as previously done with sildenafil[82] and SGLT2i inhibitors.[74,92] Metabolomics can also be used to monitor drug metabolism and ascertain therapeutic levels,[100,101] and future studies are needed to determine the relevance in HF, particularly as new metabolic therapies are developed.

Larger-scale metabolomic studies in HF are necessary to discover metabolomic phenogroups in HF, particularly given the heterogeneity in comorbidities, underlying pathophysiology, and clinical outcomes across the spectrum of HF. High-throughput metabolomic technologies allow for simultaneous measurement of more metabolites per sample, generating larger data sets, and novel bioinformatic approaches are needed for metabolite identification, data normalization and imputation, analysis of high-dimensional data sets, hypothesis-driven analysis, biomarker development and validation, fluxomic interpretation, and biological interpretation. At the same time, further mechanistic studies are needed to understand the biological significance of novel metabolomic signatures, particularly those that are outside the scope of fuel metabolism. Future metabolomics studies will inform development of targeted metabolic therapies for HF. Several therapeutic targets have been identified: 1) increasing FA oxidation[29]; 2) increasing ketone body oxidation[22,26,31,102]; 3) increasing glucose oxidation; and 4) increasing BCAA oxidation.[38,43,103] Future studies will lead to targeted therapeutic trials in HF subgroups.

CONCLUSIONS

Cardiac and noncardiac metabolism are central to the healthy myocardium; dysregulation of these central metabolic pathways has been found in many different types of HF and HF-related risk factors. Advances in high-throughput metabolomic profiling technologies have expanded our understanding simultaneously around the biology and related biomarkers underlying metabolism in HF. These advances have included a refined knowledge of substrate utilization pathways but also identification of novel metabolic pathways. With our growing knowledge of the complex interplay among the liver, adipose tissue, vasculature, and skeletal muscle with myocardium in promoting or mitigating HF, more studies are needed that leverage metabolomic profiling including emerging techniques for in vivo evaluation of flux and fate in multiple tissues.

Circulating markers reflecting cardiac and noncardiac dysregulation of these pathways show great promise as diagnostic, prognostic, and subclassification biomarkers. Importantly, many of these pathways and related biomarkers are beneficially modifiable to known and emerging HF therapies suggesting their potential for use in more personalized and precise approaches to HF management. One could imagine a day when patients undergo measurement of multiple metabolites in blood to understand their future HF and HF-related adverse risk, to have more precise subclassification for personalized therapeutics as is currently the paradigm in precision oncology, and for monitoring of precision therapeutic strategies. However, despite the incredible advances detailed in this review, much work is needed to bridge the gap between discovery and patient care. Development of point-of-care testing, biomarker-guided trials, implementation studies building patient-facing protocols evaluating efficacy and effectiveness of biomarker-guided strategies, and dissemination efforts are necessary to realize the full potential of metabolomics in a more personalized approach to HF care. Regardless, metabolomic profiling as a tool has significantly changed our understanding of HF over the past decade and holds great potential in improving the care of patients with, and at risk of, HF.

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