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Metabolic Dysfunction in Heart Failure: Diagnostic, Prognostic, and Pathophysiologic Insights From Metabolomic Profiling

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Abstract

Metabolic impairment is an intrinsic component of heart failure (HF) pathophysiology. Although initially conceived as a myocardial defect, metabolic dysfunction is now recognized as a systemic process with complex interplay between the myocardium and peripheral tissues and organs. Specifically, HF-associated metabolic dysfunction includes alterations in substrate utilization, insulin resistance, defects in energy production, and imbalanced anabolic-catabolic signaling leading to cachexia. Each of these metabolic abnormalities is associated with significant morbidity and mortality in patients with HF; however, their detection and therapeutic management remains challenging. Given the difficulty in obtaining human cardiac tissue for research purposes, peripheral blood metabolomic profiling, a well-established approach for characterizing small-molecule metabolite intermediates from canonical biochemical pathways, may be a useful technology for dissecting biomarkers and mechanisms of metabolic impairment in HF. In this review, metabolic abnormalities in HF will be discussed with particular emphasis on the application of metabolomic profiling to detecting, risk stratifying, and identifying novel targets for metabolic therapy in this heterogeneous population.

Keywords

Metabolomics; Metabolism; Heart failure; Bioenergetics; Mitochondrial dysfunction

Introduction

Genome, phenome, proteome, toxome, integrome, variome, exposome... the list goes on. In this era of rapidly expanding 'omes, it can be difficult to identify those ready for primetime

Compliance with Ethical Standards

Conflict of Interest Wynn G. Hunter, Jacob P. Kelly, William E. Kraus, Robert W. McGarrah, and Svati H. Shah declare that they have no conflict of interest.

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clinical or investigative application. The metabolome, now in its third decade of characterization in life sciences research, has solidified its position in the top echelon of well-established ‘omes [1]. Featured in over 13,000 publications since 1998, metabolomic investigations have led to advances in numerous fields ranging from sickle cell disease to autism, to type 2 diabetes, to coronary artery disease [2–7]. Despite the many successes of metabolomic profiling in elucidating molecular mechanisms and developing diagnostic or prognostic models, it has been employed to a limited extent in the setting of heart failure (HF). Given long-standing recognition of metabolic dysfunction as a critical component of HF pathophysiology, we believe the paucity of metabolomic investigation presents an important unmet need. In this review, we will focus on metabolic impairments in HF and will discuss past successes and future promise of metabolomic profiling in advancing the diagnosis, treatment, and mechanistic understanding of these metabolic defects.

Metabolome and Metabolomics: a Primer

Before detailing the contributions of metabolomic profiling to the current understanding of metabolism in HF, it is appropriate to provide a brief introduction to the relevant terminology and methods. The metabolome is the totality of small molecule metabolic intermediates of canonical biochemical pathways that are critical for maintenance of cellular and organism level homeostasis [8]. The metabolome includes the more broadly recognized substrates and products of carbohydrate, amino acid, fatty acid, and ketone metabolism, as well as some of the lesser-known intermediates from nucleoside, phospholipid, and polyamine metabolism [9••]. Presently, over 40,000 metabolite structures have been catalogued and characterized in the Human Metabolome Database, a freely accessible database of small molecules in the human body (<http://hmdb.ca>), with an estimate of 5000–10,000 compounds in the human metabolome.

Rapid expansion in the metabolite catalogue has been facilitated by technical advancements in nuclear magnetic resonance (NMR) and mass spectrometry (MS), the most widely used platforms for metabolomic profiling. Established protocols exist for both NMR and MS to characterize broad arrays of metabolites in a given biological sample. “Nontargeted” or “unbiased” metabolic profiling evaluates the full complement of spectral peaks identifiable in a sample and uses metabolite libraries to identify the relevant peaks. However, the majority of peaks evaluated in nontargeted profiling remain unidentified. Further, these methods rely on relative and not absolute quantification of metabolites. In contrast, targeted metabolomic techniques have been developed to provide absolute quantitation for smaller predefined sets of metabolites through addition of labeled internal standards. Technical advances in NMR and MS have also facilitated significant progress in metabolomic throughput. Most recently, Link et al. measured changes in approximately 300 metabolites in 15–30 s intervals over several hours [10]. Facilitating nearly continuous in-depth monitoring of metabolic shifts, these methods may permit novel investigations of in vivo or in vitro metabolic responses to physiologic changes or therapeutic interventions.

Importantly, greater sophistication in the technology platforms has been matched by increased access to metabolomic profiling over the past decade; there are now hundreds of academic and commercial laboratories worldwide performing in vivo and in vitro

metabolomics on a wide variety of specimens. In fact, almost any biological sample is amenable to metabolomic analysis, but plasma, tissue, and urine are most frequently studied in humans given their easier accessibility. Investigators in a number of disciplines are beginning to realize the potential of metabolomic profiling to enhance understanding of disease pathophysiology, identify new biomarkers for risk stratification, generate novel hypotheses to guide drug or diagnostic development, and integrate with other 'omics data for more robust discovery efforts [11, 12].

Metabolic Dysfunction in Heart Failure: More Than Just “An Engine Running Out of Fuel”

Metabolic perturbation has long been recognized as an intrinsic feature of HF pathophysiology [13, 14•]. Pioneering studies in the 1930s by Herrman and Dechard demonstrated low levels of phosphocreatine in failing myocardium, leading to the “energy depletion hypothesis” [13, 15]. Although no longer viewed as simply “an engine running out of fuel”, metabolism in the failing heart remains the subject of intense investigation [14•]. In decades past, studies probed deeply into metabolism and bioenergetics of the myocardium; however, recent studies have demonstrated that metabolic dysfunction in HF is not just a feature of the myocardium, but rather a systemic issue with important contributions from peripheral tissues and organs [16–18, 19•, 20, 21].

Metabolic Dysfunction in the Myocardium: Shifts in Substrate Selection

In order to contract on average more than one time per second, the myocardium requires tremendous amounts of energy; on a daily basis, it produces and consumes over 6 kg of adenosine triphosphate (ATP) [19•] and only stores enough ATP to support three heartbeats before exhausting supplies. Generating and maintaining adequate ATP supply in the myocardium require continuous provision of carbon-based fuels and efficient energy substrate oxidation. To maintain adequate energy production in the face of dynamic changes in substrate availability and physiologic stresses, the myocardium is able to oxidize a variety of carbon fuels such as fatty acids, glucose, lactate, ketones, and amino acids [22]. Despite its “omnivorous” diet, the myocardium does not utilize all substrates for energy production in equal proportions. Under resting conditions, the healthy myocardium utilizes fatty acids as the predominant fuel source; β -oxidation generates 60–90 % of the total ATP production [23]. The second major fuel source in the healthy resting state is pyruvate; it is derived in nearly equal amounts from glycolysis and lactate oxidation and generates 10–40 % of myocardial ATP [22]. Supplementary contributions are made from amino acid and ketone oxidation [19•, 23].

In contrast, exercise, ischemia, and early stages of mechanical dysfunction or ventricular hypertrophy are marked by a shift in substrate utilization to glucose oxidation as the predominant driver of myocardial energy production [14•]. It is unclear whether this shift is mediated by upregulation of fetal gene programs, hypoxia, increased adrenergic signaling, or a combination thereof; it is clear that in these early stages this metabolic substrate switch is protective, improving myocardial contractile efficiency by increasing the ratio of ATP generation to oxygen consumption and minimizing energetic losses from mitochondrial

uncoupling [19••]. The adaptive increase in glucose oxidation is accompanied by slightly increased or unchanged fatty acid oxidation [22]. With HF progression, this metabolic compensation is lost; New York Heart Association HF class II–III patients have significantly decreased glucose and fatty acid utilization in comparison to healthy controls [19••]. Additional metabolic derangements identified in failing myocardium have been identified and reviewed at length elsewhere [14••, 19••, 24].

Systemic Metabolic Dysfunction: Insights Gained From Metabolomic Profiling

Most metabolomic studies of human HF have analyzed metabolites from serum or plasma of individuals with HF, reflecting a snapshot of the body's global metabolic state. Because HF triggers widespread changes in systemic metabolism, changes in circulating metabolites that occur in HF reflect metabolic changes in peripheral tissues and organs in addition to the changes in myocardial metabolism described above (Fig. 1).

Insulin Resistance—Traditionally recognized as the hallmark metabolic lesion of type II diabetes mellitus, insulin resistance (IR) has also been identified as a key metabolic derangement in HF [14••]. Present in up to 61 % of nondiabetic HF patients [25], IR is inversely correlated with peak VO_2 [26], skeletal muscle contractile function [27], and sympathetic innervation of the myocardium [28]. Additionally, IR is inversely associated with survival time, independent of body composition, and other established cardiovascular risk factors [29]. Given the high prevalence of IR in HF and its well-described associations with adverse outcomes, interest in IR as a potential therapeutic target and phenotypic divisor in HF has increased in recent years [30].

The relationship between IR and HF is complex, resulting from an interplay of pathophysiologic processes including neurohormonal activation, increased circulating inflammatory cytokines, oxidative stress, and tissue hypoperfusion [14••]. In 2009, Newgard and colleagues identified a peripheral blood metabolite signature that correlated significantly with IR [3]. Independent of obese/lean status, age, race, and sex, this IR-associated metabolite signature was mainly comprised of branched chain amino acids (BCAA; leucine, isoleucine, valine) and their catabolites [31]. Subsequently validated in other cohorts [32, 33], this IR-associated metabolite signature has also been identified in human HF cohorts in recent metabolomics investigations.

In 2015, Cheng and colleagues reported findings from a large investigation of peripheral blood metabolite profiles in HF patients (discovery cohorts: $N=183$ HF, $N=51$ healthy controls; validation cohorts: $N=218$ HF, $N=63$ healthy controls). They found that stable, ambulatory American College of Cardiology/American Heart Association (ACC/AHA) stage A HF patients had higher plasma levels of all BCAAs as well as their C3 and C5 acylcarnitine catabolites when compared with healthy controls [9••]. Importantly, this was not the first investigation demonstrating elevated plasma BCAAs and their metabolites in HF patients; indeed, earlier studies by Wang et al. and Tenori et al. found significant elevations in plasma isoleucine in HF cases compared to healthy controls [34, 35]. Additionally, BCAA catabolites hydroxyisoleucine and hydroxyisovalerate were found to predict incident HF in an

epidemiologic cohort of 1744 African American patients without HF enrolled in the Jackson Heart Study [36•].

In addition to supporting the clinical association between IR and HF, these findings have potential mechanistic implications. In murine models, elevated plasma BCAA in conjunction with high free fatty acids has been shown to induce IR via activation of mammalian target of rapamycin, c-Jun N-terminal kinase, and phosphorylation of insulin receptor substrate-1 [3]. This may suggest that elevated BCAA is not just a marker but also a potential precipitant of IR in HF. Further investigation is needed to clarify the relationship of plasma BCAA and IR in HF. Nevertheless, it is clear that the metabolic phenotype of HF as revealed in metabolomics investigations in large part reflects derangements associated with peripheral IR [14••].

Catabolic: Anabolic Imbalance—Another overarching pathologic process that significantly impacts the systemic metabolic phenotype in HF is the shift toward hypercatabolism with blunting of anabolic pathways. Excessive lipolysis—driven by insulin resistance, excess catecholamines, and elevated natriuretic peptides—leads to increased circulating free fatty acids and fatty acid intermediates (discussed below in more detail). Additionally, excessive skeletal muscle proteolysis occurs in HF via a complex interplay of hormonal and inflammatory signaling, leading to global imbalance of the skeletal muscle protein pool [14••]. Since by-products of lipolysis and skeletal muscle proteolysis are released into the plasma compartment, metabolite signatures of hypercatabolism may be detected with peripheral blood metabolomic profiling.

Notably, metabolomics investigations have identified hypercatabolic signatures in the plasma and urine of patients with HF and reduced ejection fraction (HFrEF; Table) [37•, 38, 39•]. Specifically, Alexander and colleagues found significantly increased plasma levels of pseudouridine in a sample of 39 HFrEF patients with dilated cardiomyopathy when compared with 31 age-matched healthy controls [37•]. This elevation in pseudouridine, a modified nucleoside incorporated in transfer and ribosomal RNA, likely reflects a marked increase in skeletal muscle turnover and hypercatabolism [40]. Significantly increased plasma pseudouridine levels in HFrEF was also found in an earlier investigation by Dunn et al., which additionally demonstrated that pseudouridine was better than natriuretic peptides in discriminating HFrEF cases from healthy controls (pseudouridine AUC = 0.96; aminoterminal b-type natriuretic peptide AUC = 0.93) [38]. Altogether, these observations may suggest excessive skeletal muscle catabolism as an intrinsic feature of clinical HF, not just limited to severe HF.

Impaired Glucose Oxidation—Impaired glucose oxidation and a switch toward glycolysis are not limited to the myocardium in patients with HF, but also occur in peripheral tissues. The cause of these metabolic shifts is multifactorial; primary contributors are insulin resistance, increased circulating catecholamines, and tissue-level hypoxia [14••]. Regardless of the etiology, impaired glucose oxidation and increased glycolysis in the periphery may be reflected by plasma elevations of pyruvate or lactate. In alignment with this, HF patients demonstrated significantly increased plasma pyruvate and/or lactate levels when compared with healthy controls in several metabolomic investigations [34, 35, 37•].

By comparing plasma metabolite profiles from 39 patients with ischemic HF and NYHA class II–IV symptoms with those from 15 age-matched healthy controls, Wang and colleagues found lactate levels to be 1.29-fold greater ($P < 0.05$) in HF cases [35]. When instead comparing nonischemic HF patients with healthy controls, Alexander et al. found pyruvate levels in the HF group to be 1.31-fold greater in HF ($P < 0.05$) [37•]. HF-associated elevations in plasma pyruvate were also found in an investigation conducted by Tenori and colleagues which featured larger cohorts ($N = 185$ HF, $N = 111$ healthy controls) and greater heterogeneity in HF etiology and disease severity [34•].

Glucose oxidation impairments occurring specifically in skeletal muscle may be evidenced in elevated plasma alanine levels. Such elevations occur in the setting of increased muscle glycolysis, a potential manifestation of decreased glucose oxidation and mitochondrial dysfunction [14••]. In the investigation by Wang et al., HF patients exhibited 2.29-fold greater concentrations of plasma alanine than age-matched healthy controls [35]. Similarly, in the study by Zordoky and colleagues, patients with HF and preserved ejection fraction (HFpEF, $N = 24$) were found to have plasma alanine levels 1.13-fold greater than healthy controls ($N = 38$) [41••].

Furthermore, significantly elevated plasma levels of tricarboxylic acid (TCA) cycle intermediates such as alpha-ketoglutarate, succinate, and citrate also suggest peripheral glucose oxidation impairments and have been identified in several HF cohorts by metabolomic profiling (Table 1) [37•, 38, 41••]. Overall, these systemic changes in glucose metabolism lead to decreased energetic efficiency and could potentially contribute to decreased activity tolerance observed in HF.

Impaired Fatty Acid β -Oxidation—Mitochondrial dysfunction is a common pathophysiologic component of the failing heart [14••]. Evidence suggests that mitochondrial dysfunction also develops in peripheral tissues in the setting of HF [49], leading to impairments in fatty acid oxidation and accumulation of intermediates of fatty acid oxidation in the circulation. Long-chain acylcarnitines (LCAC) are markers of incomplete fatty acid oxidation and are elevated in individuals with HF compared to those without HF [9••, 41••]. Elevated ketone bodies, produced by the liver in states of excess fatty acid delivery, have also been observed in the urine [39•], breath [44], and plasma [37•] of patients with stable or acutely decompensated HF compared with relevant no HF controls. Compounding the impairments in fatty acid oxidation in HF is the globally increased rate of lipolysis due to increased inflammatory cytokines, catecholamines, and natriuretic peptides [14••]. Thus, increased delivery of fatty acids to tissues further overwhelms defective oxidative machinery and leads to worsening accumulation of fatty acid oxidative intermediates. These intermediates, in particular LCAC, may directly contribute to the peripheral manifestations of HF by promoting skeletal muscle inflammation, generating reactive oxygen species, and exacerbating skeletal muscle insulin resistance [21, 50, 51].

Urea Cycle Dysfunction—In addition to their interesting BCAA-related observations described above, Cheng and colleagues also found that ACC/AHA stage C HF was associated with significant elevations in plasma ornithine and its downstream polyamine metabolites (spermidine, spermine), along with decreased arginine, when compared to

normal controls [9••]. Ornithine is a key urea cycle intermediate, and its elevation may indicate impairment in the hepatic urea cycle that can be observed with congestive hepatopathy [9••]. Arginine is also an important urea cycle metabolite, with depressed levels also pointing towards urea cycle dysfunction. Additionally, arginine is a substrate for nitric oxide synthesis with decreased plasma concentrations potentially associated with impaired nitric oxide production and endothelial dysfunction [9••].

Potential Applications of Metabolic Profiling in HF

In addition to elucidating metabolic impairments, an important application of metabolomics is identifying novel biomarkers to aid in HF diagnosis and risk stratification. This has been the focus of several prior studies, which are reviewed below and described in the accompanying Table.

Clinical Diagnostic Value

In general, studies aiming to identify metabolite biomarkers for HF diagnosis have used a case-control design, comparing metabolite profiles of clinical HF cases with one or more control groups comprised of patients without HF. These investigations have highlighted a variety of metabolites that discriminate HF cases from controls that are (a) healthy, without HF; or (b) diagnosed with cardiovascular disease, but not HF (Table).

For example, Cheng et al. identified a panel of four metabolites—histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4—that effectively discriminated ACC/AHA stage B/C HF from healthy controls (area under receiver operator curve (AUC) = 0.99; adjusted odds ratio for HF diagnosis, 4.19; $P = 0.02$) [9••]. Zordoky and colleagues achieved a similar degree of fidelity in discriminating patients with HF and preserved ejection fraction (HFpEF) or HF and reduced ejection fraction (HFrEF) from age-matched healthy controls using metabolites only (AUC, 0.92, 0.96, respectively) [41••]. However, the metabolites discriminating HFpEF and HFrEF from controls differed from those identified in the investigation by Cheng and colleagues. Specifically, HFpEF and controls were discriminated by levels of octanoylcarnitine, arginine, asparagine, lysophosphatidylcholine C18:2, and sphingomyelin C20:2; in contrast, HFrEF and controls were discriminated by levels of creatinine, carnitine, acetoacetate, α -hydroxybutyrate, lysophosphatidylcholine C18:2 (the only common metabolite finding amongst these three studies), and lysophosphatidylcholine C20:4.

Still, other metabolites differentiated HF with severe systolic impairment from control groups in the investigation by Deidda et al.: a panel of glycine, methylmalonate, myoinositol, and α -hydroxybutyrate differentiated healthy controls or HF with mild-moderate systolic impairment from patients with HF and severe systolic impairment (AUC = 0.84) [42]. Several other studies had similar success in identifying metabolites with excellent accuracy discriminating HF from normal controls; however, metabolites identified in these studies were similarly disparate from each other and the aforementioned studies (see Major findings column in Table 1).

The substantial differences across studies in metabolites having greatest discriminative capacity are due in part to differences in case and control definitions. For example, some studies measured metabolite profiles in hospitalized patients, comparing acutely decompensated HF with hospitalized patients having CVD but no HF [44, 45]. In contrast, other studies compared stable HF cases with NYHA Class I–II and healthy ambulatory controls [39•]. Another key difference amongst studies is the inclusion or exclusion of HF patients based on disease etiology or left ventricular ejection fraction (LVEF); whereas some studies enrolled all HF patients regardless of LVEF [35] or etiology [41••], others included only ischemic [39•] or nonischemic HF [37•] with reduced LVEF [38]. Furthermore, studies varied considerably in the extent of adjustment for confounders such as medication use, renal function, and diabetic status.

Despite differences in case–control inclusion criteria and major findings amongst studies, there were similarities with respect to which metabolites were consistently elevated or decreased in HF (Table 1). For example, Cheng et al. and Zordoky et al. observed significant elevations in histidine and phenylalanine in HF groups compared with controls [9••, 41••]. Elevations in phenylalanine in HF groups were also observed in studies by Tenori et al. and Alexander et al., which used markedly different inclusion criteria for HF cases [34, 37•]. Additional patterns included HF-associated elevations in ketones (especially acetone), the BCAA's leucine and isoleucine, TCA intermediates (especially α -ketoglutarate), nucleosides, and acylcarnitines. Individuals with HF were also commonly characterized by decreased levels of glutamine and long-chain free fatty acids compared with controls. The metabolite patterns described are not perfectly consistent across every study but instead are general patterns taking into account relevant cohort characteristics and study details.

Due to the disparate findings from prior metabolomic studies, it is uncertain at present which metabolites have the greatest promise as diagnostic biomarkers in HF. Nevertheless, previous metabolomics studies of human HF have clearly demonstrated the potential utility of metabolite profiles in HF diagnosis. Larger investigations with careful phenotyping, robust adjustment for demographic and clinical confounders, statistical analyses of incremental discriminative value on top of clinical models, validation cohorts, and assessment in changes in metabolites with therapeutic interventions will be required to identify definitive metabolite biomarkers useful in HF diagnosis. Furthermore, the utility of metabolomic profiling in identifying left ventricular dysfunction prior to HF development [48], and characterizing relevant comorbidities (e.g., major depressive disorder, coronary artery disease) in the context of prevalent HF, are just beginning to be explored [43, 47].

Clinical Prognostic Value

In addition to its potential utility in HF diagnosis, metabolic profiling may be useful in identifying patients at increased risk for HF development. By performing plasma metabolic profiling in $N=1744$ African American patients enrolled in the epidemiologic Atherosclerosis Risk in Communities/Jackson Heart Study cohort, Zheng et al. identified a plasma signature of three metabolites that predicted incident HF: elevated hydroxyleucine or hydroxyisoleucine (BCAA derivatives) along with decreased dihydroxydocosatrenoic acid (very long chain polyunsaturated fatty acid) [36•].

For those with previously diagnosed HF, metabolomics may also provide helpful prognostic information, identifying patients at increased risk for adverse events (Table). By performing nontargeted plasma metabolic profiling in French cohorts of $N=126$ (training) and $N=74$ (validation) patients hospitalized with acute decompensated HF, Desmoulin and colleagues showed that admission lactate/cholesterol (L/C) ratios predicted increased 30 day mortality with greater accuracy (AUC = 0.82) than the Acute Physiologic and Chronic Health Evaluation II (APACHE II) risk assessment score (AUC = 0.76) [45]. Moreover, L/C ratio 0.4 on admission was associated with a hazard ratio of 3.26 for 30-day mortality compared with admission L/C ratio of <0.4 . Powerful prognostic capacity of metabolomics was also demonstrated in the investigation by Cheng et al., in which a panel of asymmetric dimethylarginine/arginine ratio, butyrylcarnitine (C4), spermidine, and the total amount of essential amino acids predicted all-cause death or HF readmission with greater accuracy than b-type natriuretic peptide levels (AUC, 0.85 and 0.74, respectively; hazard ratio for all-cause death or readmission for metabolite panel, 3.08; $P < 0.0001$) [9••].

An alternate option for providing prognostic information is analyzing associations between metabolite levels and clinical or pathologic features with known impact on prognosis. Using this approach, Du and colleagues identified significant associations of plasma β -hydroxybutyrate, acetone, and succinate with myocardial energy expenditure, a known predictor of mortality in HF [46].

Potential Application to Clinical Practice

From a clinical perspective, HF is a complex syndrome with significant phenotypic heterogeneity. Evidence is mounting that more granular classification of HF phenotypes is needed to guide decision-making at the bedside and experimental initiatives at the bench. Metabolomics is well-suited to aid in such phenotyping efforts given its capacity for dense, integrated molecular characterization, and responsiveness to short-term changes in (patho) physiology [52, 53]. Data-driven, unbiased approaches using cluster analysis have shown promise in identifying latent HF phenotypes; [54, 55] similar methodology using metabolomic data in addition to established clinical measures or biomarkers could reveal novel HF phenotypes with unique sets of metabolic and systemic pathologies. With substantial evidence that HF subgroups respond to therapies differently (e.g., HFpEF vs. HFrEF), elucidation of metabolic phenotypes in HF may inform trial design and help identify particular patient clusters most amenable to targeted metabolic and/or mitochondrial therapeutics [14••, 19••, 56].

Identification of Targets for Drug Development

Although metabolic dysfunction has been well characterized in HF, there are currently no metabolic or mitochondrial therapies proven effective in reducing morbidity or mortality for patients with HF. The landscape and experimental history of these therapies are complex and beyond the scope of this review (excellent summaries provided by Doehner et al., and Ardehali et al.) [14••, 19••]. However, a handful of metabolic modulators that impact substrate selection and flux through various oxidative pathways remain promising. In particular, agents increasing glucose oxidation via enhanced flux (e.g., dichloroacetate increasing activity of pyruvate dehydrogenase) or via decreased inhibition (e.g., etomoxir or

perhexiline decreasing fatty acid oxidation product inhibition of glucose oxidation) have shown benefit in early phase clinical investigations, improving exercise tolerance and echocardiographic endpoints such as ejection fraction [57–61]. Unfortunately, their widespread use has been limited by on- and off-target adverse effects. It is likely that continued metabolomics studies in HF will lead to new potential therapeutic targets.

Integration With Genomics and Other ‘Omic Technologies to Uncover Mechanistic Pathways

While metabolomic profiling can provide an integrated downstream “snapshot” of genetic, genomic, and proteomic variation, its power to identify mechanisms of health and disease pathogenesis will likely be enhanced through integration with other ‘omic technologies. In this “systems biology” approach, a more comprehensive and accurate picture of biological pathways can be identified through analysis of upstream (e.g., DNA) and downstream (e.g., proteins and metabolites) reporters of those pathways. For example, a recent study by our group integrating genetics, epigenetics, transcriptomics, and metabolomics identified a novel pathway of endoplasmic reticulum stress mediating risk of incident cardiovascular events that would not have been identified from any single platform [11]. The potential for integrated ‘omics remains untapped in the setting of HF and may lead to similar discoveries of novel pathways mediating risk in patients with HF.

Perspectives, Considerations, and Future Directions

Extracting Signal From Noise

The above studies are provocative and serve as an important proof of principle for the application of metabolomics to HF. However, there are important methodologic considerations that investigators need to explicitly address to optimize the ability to extract the metabolic signal from the background noise. As with any biomarker study, one cannot underemphasize the importance of accurate and dense clinical phenotyping. This is especially true with HF where “lumping” of HF phenotypes may be particularly detrimental given the heterogeneous clinical and molecular etiologic pathways leading to disease. Careful, accurate, and adjudicated phenotyping should be addressed early in study design discussions and should include consideration not just of HF specific phenotypes but also of potential confounders of the relationship between metabolite and disease outcome such as renal function and medication use. Relatedly, analyses should address these potential confounders through, for example, adjustment in statistical models, stratification, or matching. Metabolic biomarkers identified from discovery projects that are moved into subsequent validation, and expansion studies should not only address association between metabolite and disease outcome independent of these clinical variables but also incremental discriminative/predictive capabilities.

Noise in metabolomic studies can arise from several sources; while not all sources can be identified and/or explicitly addressed, it is important to understand the potential sources. Analytical variability can relate to batch, length of storage of samples, lack of consistency in sample collection protocols, and differences between metabolic profiling platforms. Batch effects can be minimal to strong depending on the metabolite and metabolic platform

utilized. Spiked in samples across batches can be used to normalize batches and minimize such effects. Biological variability can arise from heterogeneity in fasting status (and even length of time of fasting), chronic and recent diet, time of day of sampling, and medications.

Challenges and Opportunities in Metabolic Profiling

While our group and many others have shown the power of quantitative, targeted metabolic profiling [5, 6, 11, 62–64], these approaches are biased towards the standards chosen for inclusion. More comprehensive metabolome-wide approaches using nontargeted metabolomics are compelling and show great promise for expanding our repertoire of metabolic pathways in health and disease, but the relative quantification and difficulties with metabolite annotation remain obstacles to a full realization of that promise. Ongoing efforts for better libraries and standards for more unambiguous metabolite identification will no doubt improve this field of science.

Another challenge in metabolic profiling across diseases that is highlighted in HF is that of power to detect effects. Power is certainly improved by optimizing the signal/noise ratio as detailed earlier. Genetic studies in complex disease have moved to consortia efforts, combining genetic data across cohorts, ethnicities, geographical regions, epidemiologic study designs, and diseases to improve power of identification of variants with lower effect sizes. While similar efforts are lagging in metabolomics and markedly so in HF, they are being initiated across academic institutions, foundations (i.e., AHA), and government agencies including the NIH. A challenge unique to these efforts is harmonization of metabolite data across different analytic platforms, compounded by difficulties in a common nomenclature for metabolites.

Conclusions

HF is a clinical entity that at its mechanistic core is related to metabolic derangement. As such, it lends itself well to application of metabolomic profiling, which has great promise to identify novel mechanisms, biomarkers, and subtypes of HF. As reviewed herein, the current literature has several examples of the utility of this approach in HF; however, the potential for even more scientific investigation is great. Attention to careful study design, quality control, integration with genomics and other ‘omics data in a systems biology approach, and dense and adjudicated phenotyping will enhance the potential for important, impactful, and clinically relevant output from such studies. Metabolomic profiling may be a surrogate for tissue availability in cardiovascular disease, serving as a “liquid biopsy” of metabolic derangement, useful for diagnosis, prognosis, mechanism, and drug development in HF.

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Fig. 1.

Major metabolic impairments identified in metabolomic investigations of human heart failure. Metabolite abnormalities are listed below their associated impairments. *Red* color indicates increased peripheral blood concentrations whereas *blue* indicates decreased concentrations. *Green* boxes show potential therapeutic interventions spatially organized proximate to associated metabolic impairments

Table 1

Investigations using metabolomic profiling in human heart failure

Author, year, source	Study population	Sample size	Metabolic profiling method	Major findings	Summary of key metabolites ^a
Cheng, et al. <i>J Am Coll Cardiol.</i> 2015. ⁹	Derivative: Cases: HF, stage B/C (ambulatory and w/o severe CAD) Controls: healthy, no HF Validation: Cases: stage C HF (hospitalized w/AHF) Controls: healthy, no HF	Derivative: N= 183 HF N=51 controls Validation: N= 218 HF N=63 controls	LC -MS of plasma	Discriminated HF from healthy controls using metabolite panel of histidine, phenylalanine, spermidine, and phosphatidylcholine (PC) C34:4. Values of this panel significantly improved at 6 and 12 months. Metabolite panel consisting of asymmetric dimethylarginine/arginine ratio, acylcarnitine (AC) C4, spermidine, and total amount of essential amino acids (AA) predicted adverse events in HF independent of traditional risk factors.	HF vs. Healthy - Histidine - Phenylalanine - Spermidine - PC: C34:4 Adverse events in HF - Dimethylarginine - AC: C4 - Essential AA - Arginine
Deidda, et al. <i>J Transl Med.</i> 2015. ⁴²	Cases, group no. 1 HF with LVEF = 42 ± 4%, NYHA I Cases, group no. 2 HF with LVEF = 25 ± 10%; NYHA III Controls: healthy, no HF, age, sex, BMI matched. *Excluded cachectic patients	N=9 HF mildly reduced LVEF N=15 HF severely reduced LVEF N=9 healthy controls	¹ H-NMR of plasma	Discriminated between HF patients and controls with glycine, methylmalonate, and myoinositol, which were higher in HF, and α-hydroxybutyrate, which was lower in HF. These metabolites also discriminated HF subjects with severe systolic dysfunction from those with mild -to-moderate dysfunction and healthy controls (AUC=0.84).	HF vs. healthy - Glycine - Methylmalonate - Myoinositol - α-hydroxybutyrate Worse systolic impairment in HF - Glycine - Myoinositol - α-hydroxybutyrate - Methylmalonate
Zordoky, et al. <i>PLoS One.</i> 2015. ⁴¹	Cases: stable, ambulatory HFpEF, NYHA I-III Controls no. 1: stable, ambulatory HFrEF, NYHA II-III.	N=24 HFpEF N=20 HFrEF	LC -MS and ¹ H-NMR of plasma	Discriminated HFpEF from healthy, no HF controls with a panel consisting of C8 acylcarnitine (AC), arginine, sphingomyelin (SM) C20:2, and NT-proBNP (AUC = 0.94). HFpEF had significantly higher acylcarnitines (C2-C18) and amino acids. Discriminated HFpEF from HFrEF with acetate, α-hydroxybutyrate, C7-DC and C18:1 acylcarnitines, PC 40:4, and NT-proBNP (AUC = 0.98). Acylcarnitines and ketones were higher in HFpEF than HFrEF, while SM(C24:1), some PCs and LysoPCs were lower in HFpEF than HFrEF.	HFpEF vs. non-HF - Histidine, - Phenylalanine - AC: C2, C3, C5, C8, C10, C12, C14, C18, - Arginine - Alanine - Citrate - SM: C14-26 - PC: C26-42 HFpEF vs. HFrEF - Acetate - AC: C7-C18

	Controls no. 2: healthy, ambulatory, no HF	N=38 non-HF controls		Discriminated HFrEF from healthy, no -HF controls with acetoacetate and NT-proBNP (AUC=0.997). Metabolite only panel of acetoacetate, α -hydroxybutyrate, creatinine, carnitine, and some lysoPC's, discriminated HFrEF from health controls with AUC = 0.959.	<ul style="list-style-type: none"> - Ketones - PC: C40:4 <p>HFrEF vs. non-HF</p> <ul style="list-style-type: none"> - AC: C3, C5, C18 - Trimethylamine - Ketones
Luan, et al. Biomarkers. 2013. ⁴³	<p>Derivative: Cases: HF Control 1: CAD w/o HF Control 2: healthy</p> <p>Validation: Cases: HF Controls: CAD</p>	<p>Derivative: N=23 HF N=23 CAD w/o HF N=23 healthy</p> <p>Validation: N=15 HF N=15 CAD</p>	LC-MS of plasma	Discriminated HF from CAD w/o HF : HF had LOWER levels of the following metabolites - free fatty acids (FFA) C12, C14, C16, and C20; sphingolipids: sphinganine and phytosphingosine; and cysteine derivatives. All were significantly lower in HF than CAD w/o HF in univariate analysis.	<p>HF vs. CAD w/o HF</p> <ul style="list-style-type: none"> - FFA: C12, C14, C16, C20 <p>(+) Correlation w/LVEF</p> <ul style="list-style-type: none"> - FFA: C20 - Cysteine derivatives
Tenori, et al. Int J Cardiol. 2013. ⁴³	<p>Cases: stable HF (NYHA I-IV)</p> <p>Controls: age and sex matched healthy controls</p>	<p>N=185 HF</p> <p>N=111 controls</p>	¹ H-NMR of plasma	Metabolite "fingerprint" identified that discriminated stable HF from healthy controls with 86% accuracy, but did not discriminate between NYHA classes or NT-proBNP quartiles.	<p>HF vs. healthy</p> <ul style="list-style-type: none"> - Phenylalanine - Tyrosine - Pyruvate - Isoleucine - Citrate - Lactate - Lysine
Wang, et al. Mol Bio Sys. 2013 ³⁵	<p>Cases: ischemic HF, NYHA class II-IV (median class, III) with mean LVEF 54 ± 14.</p> <p>Controls: age-matched healthy controls</p>	<p>N=39 HF</p> <p>N=15 controls</p>	¹ H-NMR of plasma	Discriminated ischemic HF from healthy with sensitivity of 92% and a specificity of 87%; HF had higher lactate, alanine, creatine, proline, isoleucine, leucine, LDL, VLDL, and lower HDL, valine, glycoprotein, glutamine, glutamate, unsaturated lipid, choline, glycine, glucose, tyrosine, histidine	<p>HF vs. healthy</p> <ul style="list-style-type: none"> - Lactate - Alanine - Proline - Isoleucine - Leucine - Citrate - Lysine - Valine - Tyrosine - Histidine
Alexander, et al. Eur J Clin Invest. 2011. ²⁸	<p>Cases: primary dilated cardiomyopathy (DCM; mean LVEF $23\% \pm 7\%$)</p> <p>Controls: age, sex, BMI, race-matched healthy controls</p>	<p>N=39 DCM</p> <p>N=31 controls</p>	GC-MS and LC-MS of plasma	Primary DCM characterized by significantly increased α -ketoglutarate, malate, pyruvate, phenylalanine, tyrosine, 3-methylhistidine and prolylhydroxyproline (products of collagen and myofibril degradation), α -hydroxybutyrate, pseudouridine, and glutamate when compared with healthy controls, as well as significantly decreased levels of glutamine, histidine, and long chain FFA.	<p>HF w/DCM vs. healthy controls</p> <ul style="list-style-type: none"> - Phenylalanine - Tyrosine - Pyruvate - TCA intermediates - Ketones - Pseudouridine - Glutamate - Glutamine - Histidine - FFA: C18:0, C19:0

Dunn, et al. <i>Metabolomics</i> . 2007. ³⁸	Cases: HFrEF (LVEF <40%) Controls: age matched healthy without HF	N=52 HFrEF N=57 controls	GC-MS of plasma	Discriminated HFrEF from healthy using pseudouridine (AUC = 0.96) or α-ketoglutarate (AUC=0.93). Discrimination as accurate or better than NT-proBNP (AUC = 0.93). Both metabolites significantly higher than healthy.	HFrEF vs. Healthy - Pseudouridine - α-ketoglutarate
Kang, et al. <i>Clin Biochem</i> . 2010. ³⁹	Derivative : Cases: stable NYHA I-II ischemic HFrEF Controls: age and sex matched healthy controls. Validation: Same as derivative	Derivative : N=15 ischemic HFrEF N=15 controls Validation: N=27 ischemic HF N=29 controls	¹ H-NMR of urine	Discriminated stable HFrEF from healthy controls; HFrEF had higher urine concentrations of acetate, acetone, methylmalonate, cytosine, phenylacetylglutamine, methylamines; and lower 1-methylnicotinamide, as well as the TCA cycle intermediates succinate, citrate, α-ketoglutarate, and cis-aconitate.	HFrEF vs. Healthy - Acetone - Methylmalonate - Cytosine - Methylamines - TCA intermediates
Samara, et al. <i>J Am Coll Cardiol</i> . 2013. ⁴⁴	Derivative : Cases: acute decompensated HF (ADHF) Controls: hospitalized w/o HF (n=16) Validation : ADHF (n=36)	Derivative : N=25 ADHF N=16 CVD w/o HF Validation : N=36 ADHF	SIFT-MS of single exhaled breath	Discriminated ADHF from hospitalized patients w/ CVD but no HF ; ADHF had higher acetone, pentane, H ₃ O ⁺ , O ₂ ⁺ , NO ⁺ .	ADHF vs. hospitalized, CVD no HF - Acetone
Zheng, et al. <i>Am J Epidemiol</i> . 2013. ⁴⁹	African-Americans w/o HF, enrolled in ARIC-Jackson Heart Study (n=1,744)	N=1,744	Tandem GC/LC-MS of plasma	Identified metabolites predicting incident HF including hydroxyisoleucine and hydroxyisoleucine (branched chain amino acid derivatives) which were associated with higher risk for incident HF; dihydroxydocosatrienoic acid (a polyunsaturated fatty acid, [PUFA]) was inversely associated with risk of incident HF.	Incident HF - Hydroxyisoleucine - Hydroxyisoleucine - PUFA: C22:3 - (OH) ₂
Desmoulin, et al. <i>PLoS One</i> . 2013 ⁴⁵	Derivative : Acute HF (n=126) Validation : Acute HF (n=84)	Derivative : N=126 AHF Validation : N=84 AHF	¹ H-NMR of plasma	30 day mortality predicted by lactate/cholesterol ratio on admission (AUC = 0.82; HR for lactate/cholesterol ratio ≥ 0.4 compared with <0.4.	HF increased mortality - Lactate/ - Cholesterol ratio
Du, et al. <i>PLoS One</i> . 2014. ⁴⁶	Cases: stable NYHA II-IV HF Controls: Age-matched healthy controls	N=46 HF N=15 Controls	¹ H-NMR of plasma	Increased myocardial energy expenditure (MEE), a predictor of mortality in HF, was associated with elevated β-hydroxybutyrate, acetone and succinate, independent of medication use. Alanine was inversely associated with MEE.	Increased MEE - Ketones - Succinate - Alanine - Glutamine - Valine - Methionine
Steffens, et al. J Geriatr Psychiatry Neurol. 2010.⁴⁷	Cases: stable HF with major depressive disorder (MDD) Controls: stable HF w/o MDD	N=40 HF w/MDD N=40 HF w/o MDD	Tandem GC/LC MS of plasma	Discriminated HF w/MDD from HF w/o MDD with 73% success rate; HF w/MDD had higher levels of alanine, arginine, aspartate, glutamate, serine, phenylalanine, dicarboxylic fatty acids, and lower levels of ketones.	HF w/MDD - Phenylalanine - Arginine - Alanine - Ketones
Turer, et al. Circulation. 2009.⁴⁸	All undergoing cardiac surgery. Cases: CAD or LV dysfunction (LVD) Controls: cardiac surgery w/o CAD or LVD	N=22 CAD or LVD N=17 w/o CAD/LVD	GC: MS of plasma from peripheral artery AND coronary sinus	Discriminated LVD from non -LVD in setting of ischemia/reperfusion. LVD associated with lower myocardial uptake of glucose, fatty acid, amino acids. After reperfusion, LVD associated with significantly lower extraction ratios of most substrates, as well as significant elution of 2 short-chain acylcarnitines: acetylcarnitine and 3-hydroxybutyrylcarnitine.	LVD vs. no LVD - AC: C2, C3-OH

^a Colors reflect metabolite levels: *red* indicates elevated, *blue* indicates decreased levels

1H-NMR indicates proton nuclear magnetic resonance, *AC* acylcarnitine, *ADHF* acute decompensated heart failure, *ARIC* Atherosclerosis Risk in Communities, *AUC* area under the curve, *C* carbon chain length, *CAD* coronary artery disease, *CVD* cardiovascular disease, *EF* ejection fraction, *FFA* free fatty acid, *GC* gas chromatography, *HDL-C* high-density lipoprotein cholesterol content, *HF* heart failure, *HFpEF* heart failure with preserved ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *LC* liquid chromatography, *LDL-C* low-density lipoprotein cholesterol content, *LV* left ventricular, *LVD* left ventricular dysfunction, *MDD* major depressive disorder, *MEE* myocardial energy expenditure, *MS* mass spectrometry, *NO* nitric oxide, *NYHA* New York Heart Association, *O₂* oxygen, *PC* phosphatidylcholine, *PUFA* polyunsaturated fatty acid, *SIFT* selected ion-flow tube, *SM* sphingomyelin, *VLDL-C* very low-density lipoprotein