Metabolic Biomarkers in Heart Failure

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KEYWORDS

• Heart failure metabolomics • Arginine • Nitric oxide • Myocardial energy utilization • Acylcarnitine • TMAO

KEY POINTS

- Metabolomics is the study of small, organic, molecules of biochemical pathways, some of which are strongly implicated in heart failure pathogenesis and progression.
- Modern developments in mass spectrometry and nuclear magnetic resonance (NMR) have enabled identification of approximately 40,000 human metabolites.
- The failing heart exhibits metabolic derangements, particularly in energy utilization and oxidation.
- Creation of metabolomic profiles may aid in the diagnosis, management, and prognosis of heart failure.
- Metabolomics extends to human and microbial products, further adding to the complex gene, protein, and environmental interactions in heart failure.

INTRODUCTION

Heart failure is a complex disease process that affects an increasing number of patients due to advancements in cardiac care and increased longevity of the aging population. From an epidemiologic and macroscopic perspective, timely diagnosis and early interventions in heart failure are likely to have far-reaching impact on health care economics and public health. Many discoveries in pathophysiology and pharmacotherapy have already improved mortality and morbidity in this population of patients in the past few decades. However, for all the progress in the diagnosis and management of this complex disease, there remain many unresolved mysteries, the unlocking of which could lead to profound understanding of heart failure pathogenesis, treatment, and prognosis. From a microscopic perspective, heart failure is a manifestation of metabolic derangements on the cellular, genetic, proteomic, and metabolic levels.[1]

Traditionally, genetic information is translated from DNA into RNA and transcribed into protein. The proteome is made of a variety of proteins that can then undergo posttranscription modification, and through interactions with environmental factors, produce metabolites used for energy production. A metabolite is thus defined as any small organic molecule detectable in the human body with a molecular weight of 50 to 1500 Da. The source of metabolite generation can include any biofluid, such as blood, urine, saliva, and respiratory gases. Thus, a rich collection of metabolites, including peptides, oligonucleotides, sugars, nucleosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids, and small molecule drugs, are included in the study of metabolomics. The metabolite may arise from external sources, such as exposure to medications, toxins, or microbes, or it may be generated by the human host in the homeostatic process of energy utilization (Fig. 1). With growing interest in this field and improved

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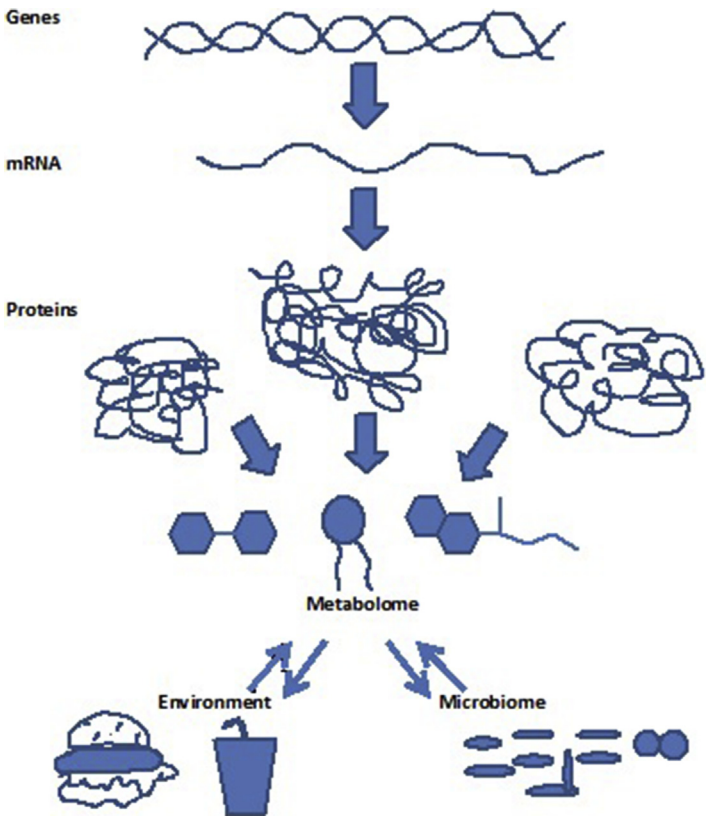

Fig. 1. Scheme of interactions of the metabolome with the genome, proteome, environment, and microbiome.

methods of identification, more than 40,000 unique metabolites have been identified in the Human Metabolome Database.[2] With this expansive list of metabolites, we are now poised with the opportunity to discover metabolomic biomarkers of the failing myocardium to facilitate in the early detection of heart failure,[3] appropriate targeted medical therapy of heart failure,[4] and offer prognostic insights into the progression of this disease. The study of metabolomics, then, represents another example of the spirit of translational research, bridging the gap from bench to bedside. This article will first examine the definition of a clinically useful biomarker, with respect to the biochemical utility of metabolomics, then delve into the methods of metabolomic profiling, examine several metabolomic pathways being pursued in heart failure (**Table 1**), present possible avenues of clinical application, and discuss challenges in the utilization of metabolomics.

Table 1
Summary of metabolites in heart failure

Metabolite	Findings in heart failure
Nitric oxide	Improves left ventricular dilation, vasodilation[14–16]
Arginine	Bioavailability and methylation affects nitric oxide synthesis[17–20]
Ketones	Myocardial metabolism switch from lipids to ketogenic state in heart failure[23,26]
Long-chain acylcarnitines	Myocardial metabolism switch in heart failure states[24,25]
Breath analysis of pentane, acetone, nitric oxide	Heart failure patients may expel a unique "breathprint"[28–31]
Trimethylamine *N*-oxide (host-gut microbiome interactions)	Elevated levels implicated in poor prognosis in myocardial infarction and chronic and acute heart failure[38,39,41–44,48]

METABOLITES AS BIOMARKERS: A PROLIFIC PROFILE

With advancements in technology, it is now feasible to generate large databanks of metabolites, and these extensive repositories of metabolites have become hypothesis generating in the pathogenesis

of various diseases. For example, biochemical analysis of the human myocardium has revealed both its remarkable efficiency of energy utilization, as well as the pathologic alterations in biochemical substrate utilization in the failing myocardium.[5] As a metabolically active organ, the heart is in constant need for fuel, which comes in the form of adenosine triphosphate (ATP). Generation of ATP occurs either via catabolism, or breakdown, of exogenous molecules circulating in the blood, such as glucose, fatty acids, amino acids, or of endogenous stores of energy such as triacylglycerols or glycogen (**Fig. 2**).[6] In the healthy human heart, energy production occurs via efficient production of ATP with rapid turnover occurring every 10 seconds such that the healthy heart metabolizes 30 g of fat and 20 g of carbohydrates daily.[7]

By understanding biochemical derangements occurring in heart failure, we can aim to produce metabolomic biomarkers as an indicator of presence of heart failure or gauge of the severity of cardiac metabolic dysregulation. Indeed, the concept of unique metabolomic patterns, or fingerprints, has already been validated in various small cohorts of patients with chronic heart failure.[8] The concept that unique patterns of metabolomic expression and energy utilization occur in different etiologies of heart failure could revolutionize the diagnosis and management of heart failure in this era of personalized medicine.

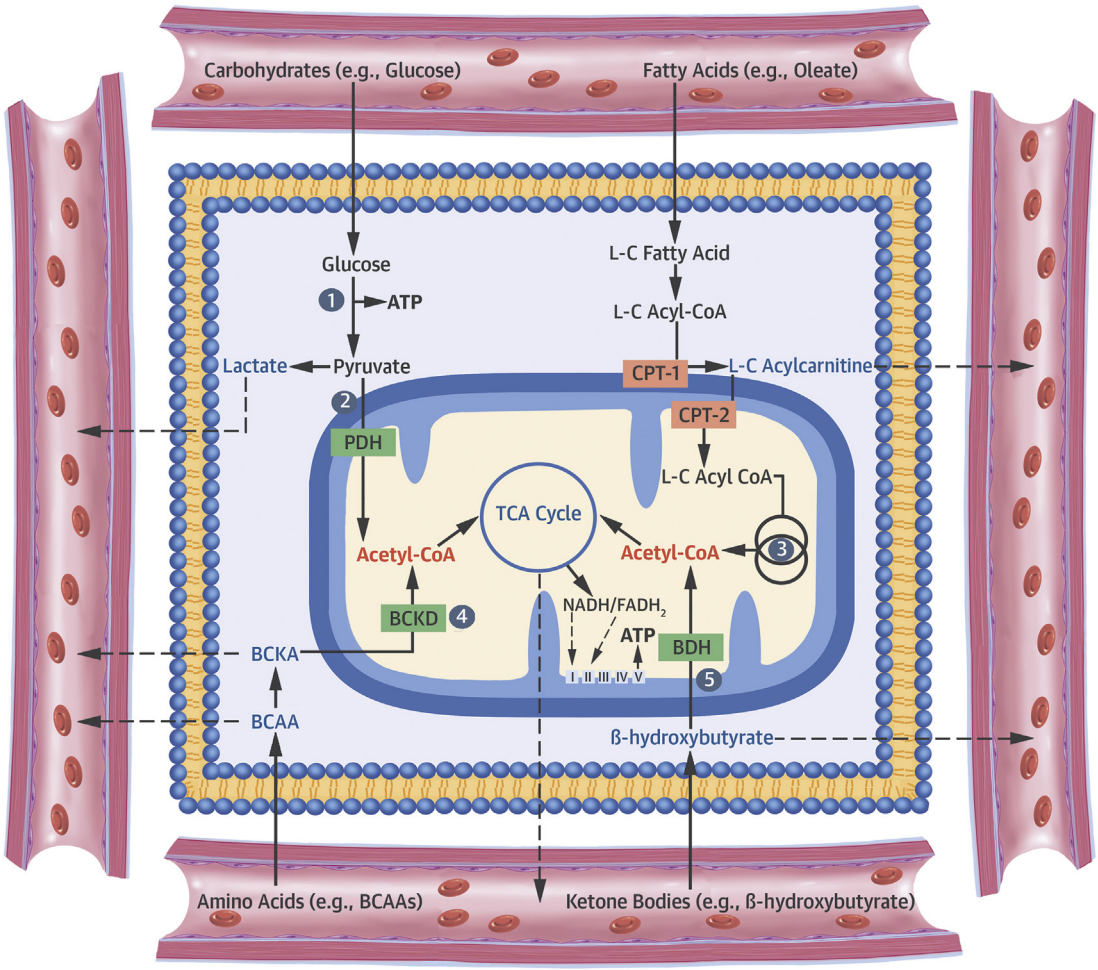

Fig. 2. Cellular metabolism and implications of metabolomic profiling. ATP, adenosine triphosphate; BCAA, branched-chain amino acid; BCKA, branched-chain α-keto-acid; BDH, β-hydroxybutyrate dehydrogenase; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; $FADH_2$, flavin adenine dinucleotide; L-C, long-chain; NADH, nicotinamide adenine dinucleotide; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid. (*From* Ussher JR, Elmariah S, Gerszten RE, et al. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. J Am Coll Cardiol 2016;68:2853; with permission.)

METABOLOMIC DISCOVERY

With the application of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), it is now feasible to efficiently analyze large volumes of metabolites derived from human samples, such as blood, urine, and gut contents. NMR allows for detection and quantification of metabolites based on chemical shifts in resonance frequency when subject to an electromagnetic field.[9,10] On the other hand, MS, identifies metabolites based on their unique mass/charge (m/z) ratio, and MS is often preceded by the use of gas chromatography, which separates metabolites based on solubility through a stationary media.[11,12] MS is more sensitive than NMR, and can detect a large quantity of metabolites. MS can also be used either in a "targeted" manner to detect prespecified molecules or in an "untargeted" manner without pre-specification for discovery of a large cohort of molecules.[13] The development and application of these techniques to the study of human myocardial metabolism has led to the creation of metabolomic patterns in healthy and pathologic cardiac states. The next section will address some of the examples of metabolomic studies in heart failure patients.

METABOLOMIC BIOMARKERS AND PROFILING

Nitric Oxide Production and Arginine Methylation

Nitric oxide (NO) is an important molecule that regulates vasodilation, left ventricular relaxation, and diastolic relaxation.[14–16] A series of animal and human studies have implicated alterations in NO synthesis in the pathogenesis of heart failure. NO is synthesized from its precursor L-arginine and oxygen by various NO synthases, and this process may be altered by arginine methylation in an epigenetic phenomenon. These alterations on NO synthesis are theorized to be a response to inflammation and oxidative stress. Alterations in the arginine regulation implicate its role in heart failure pathogenesis (**Fig. 3**).

In a single-center study of patients with chronic systolic heart failure with left ventricular (LV) ejection fraction $\leq$35%, plasma samples taken from these patients were analyzed for concentration of plasma aminoterminal pro-B-type natriuretic peptide (NT-proBNP) and endogenous arginine metabolites including asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and *N*-mono-methylarginine (MMA).[17] ADMA inhibits nitric oxide synthase (NOS) activity, whereas SDMA does not. This study found that both ADMA and SDMA plasma levels were positively correlated with echocardiographic estimates of LV filling pressures. In addition, the level of NT-proBNP was also correlated to all 3 arginine methylation products (MMA, ADMA, and SDMA), whereas no correlation was found with methyl-lysine, a metabolite with no NO inhibition, which was used as the internal control. Using Cox proportional hazard analysis, ADMA levels also increased the hazard ratio for death along, death or cardiac transplantation, and the combine endpoint of death, cardiac transplantation, or heart failure hospitalization. In addition, ADMA and MMA levels were lower in patients treated with beta-blocker, indicating response to therapy.

Another study compared the plasma metabolites of patients admitted to the intensive care unit with advanced, acute decompensated heart failure to patients with stable chronic heart failure, to elucidate the role of arginine regulation in heart failure. ADMA levels were significantly higher in the acute decompensated population.[18] This study again suggests that arginine metabolism via ADMA, an NO synthase inhibitor, plays an important role in the endothelial dysfunction in patients with acute decompensated heart failure. Furthermore, Shao and colleagues[18] examined the global arginine bioavailability ratio (GABR), defined as the quotient between substrates (arginine) and products (ornithine + citrulline) of NOS detected by MS, between the 2 heart failure populations. They found that GABR is significant lower in the acutely decompensated heart failure patients. Low GABR also has been implicated in major adverse cardiovascular events of death, myocardial infarction, and stroke.[19] In the heart failure population, low GABR has been associated with more severe LV and right ventricular dysfunction as well as higher levels of plasma natriuretic peptide levels. Treatment with beta-blocker therapy improves GABR levels.[20] In summary, NO regulation, arginine bioavailability, and methylation are important metabolomic pathways in the pathophysiology of cardiac dysfunction.

Ketones and Long-Chain Acylcarnitine

Energy utilization by the heart and alterations in cellular metabolism has been a topic of interest in heart failure research. Normally, fatty acids are the predominant source of energy for the myocardium. Acylcarnitines are the primary lipid substrate involved in fatty acid oxidation. Under normal metabolic conditions, β-oxidation produces 50% to 70% of ATP used by the myocardium.[21] Long-chain fatty acids, such as oleic and palmitic

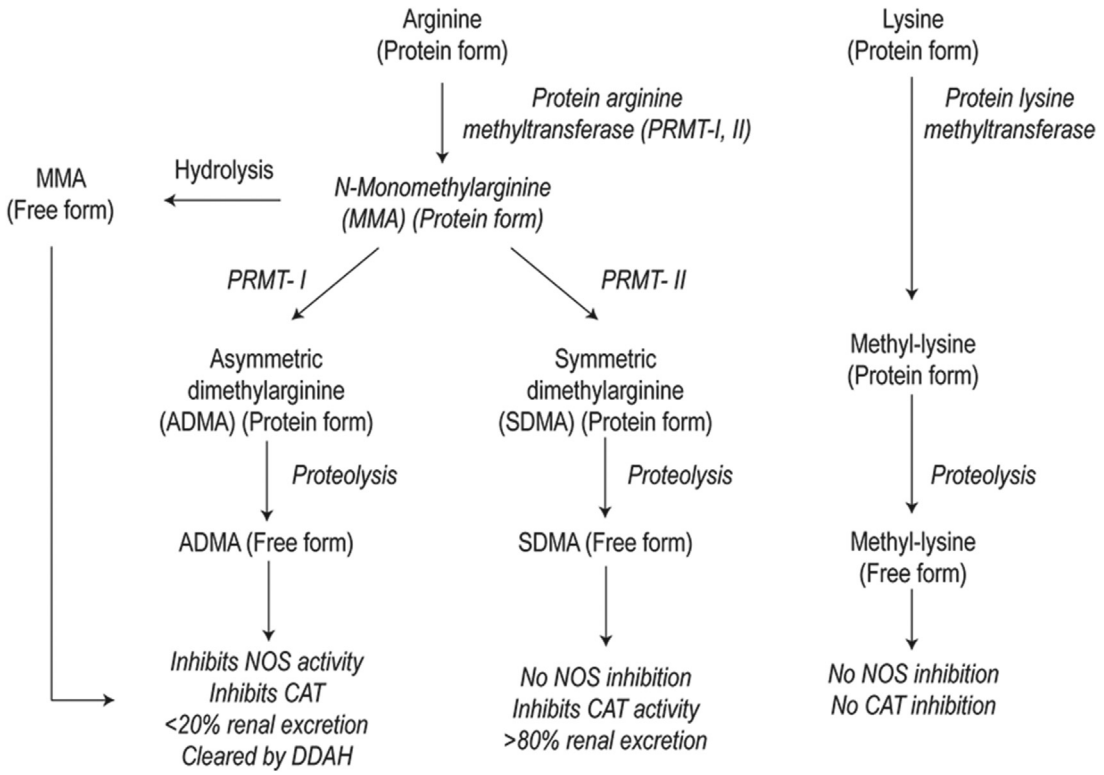

Fig. 3. Scheme of arginine metabolic pathways in high throughput MS arrays. ADMA, asymmetric dimethylarginine; CAT, cationic amino acid transport; DDAH, dimethylarginine dimethylaminohydrolase; MMA, N-mono-methylarginine; NOS, nitric oxide synthase isoforms; PRMT, protein arginine methyltransferases; SDMA, symmetric dimethylarginine. (*From* Tang WHW, Tong W, Shrestha K, et al. Differential effects of arginine methylation on diastolic dysfunction and disease progression in patients with chronic systolic heart failure. Eur Heart J 2008;29:2507; with permission.)

acids, are esterified into long-chain acylcarnitines (LCACs). LCACs then serve as lipid intermediates that transport carbon atoms into the mitochondrial for β-oxidation of fatty acids into triglycerides. In the failing myocardium, a metabolic switch from fatty acid utilization to oxygen-sparing carbohydrate metabolism has been observed.[22] This metabolic switch leads to accumulation of LCACs, which are postulated have pleotropic adverse effects on myocytes by way of promoting inflammation, increasing apoptotic signals, and generating ion channel dysregulation (Fig. 4).

Using liquid chromatography–MS in a nondiabetic advanced heart failure population of patients at time of heart transplantation or LV assist device implantation, Bedi and colleagues[23] evaluated the energy utilization in this group of patients with severe cardiac dysfunction. They found that failing myocardium increasingly used ketogenic β-hydroxybutyryl-CoA and β-hydroxybutyrate, while there was a decrease in the availability of lipid energetic substrates such as medium-chain and LCACs.

Other studies have assessed the metabolic derangements in heart failure with regard to energy utilization. In support of the metabolic switch theory by which the failing myocyte preferentially uses glucose rather than fatty acids, metabolic profiles of patients with chronic systolic heart failure reveal alterations in fatty acid metabolism. Ahmad and colleagues[24] evaluated a cohort of 453 patients with chronic systolic heart failure along with 41 end-stage heart failure patients undergoing left ventricular assist device (LVAD) implantation. Patients were randomized to exercise training versus usual care, and frozen plasma samples were collected and analyzed using MS. LCAC (C16 and C18) were associated with lower peak Vo_2 as well as increased risk of all-cause mortality, all-cause hospitalization, and cardiovascular death or hospitalization. Additionally, levels of long-chain acylcarnitine also decreased after placement of LVAD, suggesting response to therapy.

In a study that included both patients with heart failure with preserved ejection

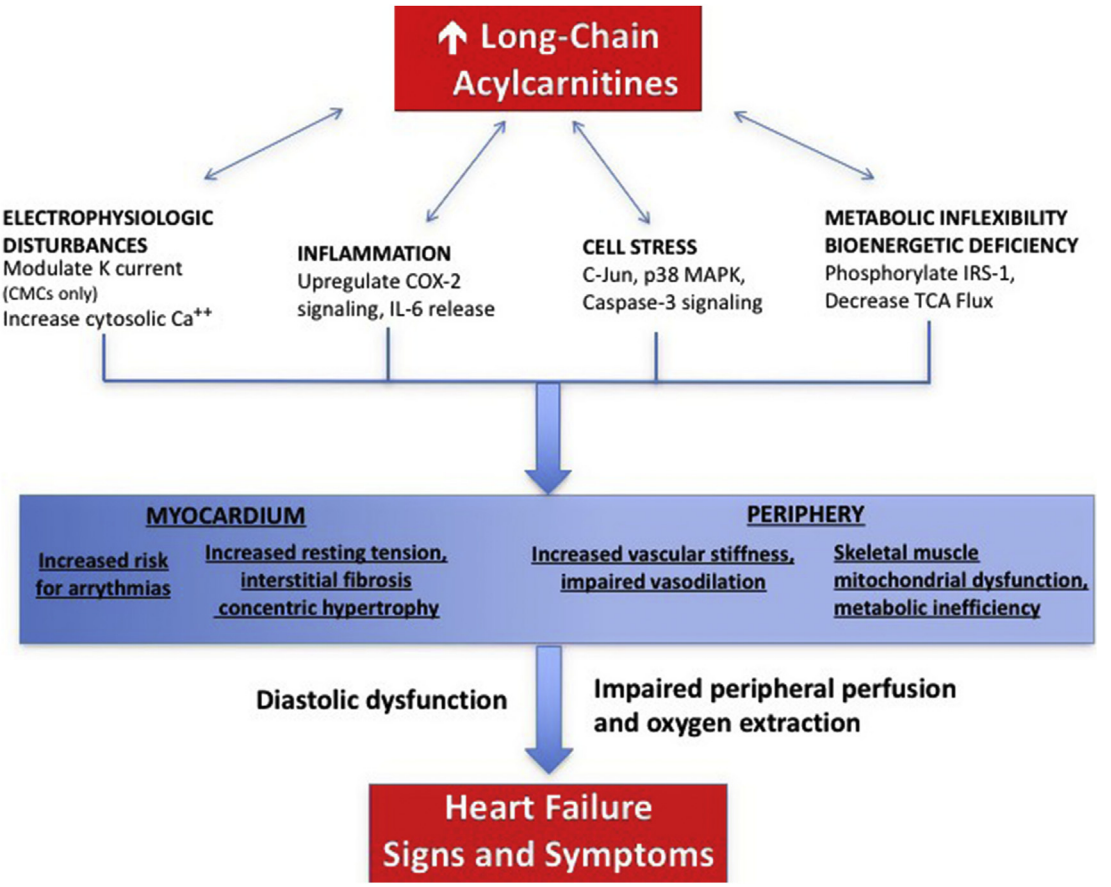

Fig. 4. Proposed model for plasma long-chain acylcarnitine contributions to the heart failure phenotype. Ca^{++}, calcium; CMC, cardiomyocyte; COX, cyclooxygenase; IL, interleukin; IRS, insulin receptor substrate; K, potassium; MAPK, mitogen-associated protein kinase; TCA, tricarboxylic acid. (*From* Hunter WG, Kelly JP, McGarrah RW 3rd, et al. Metabolomic profiling identifies novel circulating biomarkers of mitochondrial dysfunction differentially elevated in heart failure with preserved vs reduced ejection fraction: evidence for shared metabolic impairments in clinical heart failure. J Am Heart Assoc 2016;5(8):7; with permission.)

fraction ≥45% (HFpEF) and heart failure with reduced ejection fraction less than 45% (HFrEF), Hunter and colleagues[25] created metabolomic profiles of these patients to assess for differential alterations energy utilization. They quantified levels of 60 metabolites consisting of 45 acylcarnitines and 15 amino acids, expanding on an early study by Zordoky and colleagues.[8] These investigators find that elevated levels of LCAC were independently associated with worse functional status and higher mortality in both patients with HFpEF and HFrEF. Additionally, the level of LCAC was significantly higher in patients with HFrEF compared with HFpEF, and both heart failure phenotypes had elevated levels of LCAC compared with healthy controls. These studies further support the role of LCAC as a prognostic marker of disease severity in chronic systolic heart failure, as well as present potential targets for new therapeutic interventions in heart failure.[26]

Human and Microbial Byproducts

Beyond analysis of plasma metabolites, the study of metabolomics also includes the analysis of human and microbial products and byproducts. A few tantalizing studies have evaluated the exhaled gases of patients with heart failure with respect to acetone, pentane, and other molecular excretion in the creation of a "breathprint" in chronic heart failure. The measurement of molecular concentrations via exhaled breath offers a major advantage in the noninvasive nature of the test, and several breath analysis test have already been approved by the Food and Drug Administration for the diagnosis of noncardiac conditions such as *Helicobacter pylori* infection, asthma inflammation, and

carbon monoxide poisoning.[27] Within the cardiovascular realm, breath analysis studies have implicated that patients with heart failure have elevated levels of expired pentane, which is generated from the perioxidation reaction of free radicals with cellular membrane lipids.[28] In a similar manner, the concentration of exhaled acetone has also been implicated as a biomarker of heart failure severity.[29] Exhaled NO is another potential prognostic biomarker that has correlated to higher pulmonary venous hypertension in patients with stable chronic systolic heart failure.[30] Drawing from the previously mentioned studies, the feasibility of collecting exhaled breath samples in patients admitted with decompensated heart failure was validated in a recent proof of concept study.[31] Although breath analysis is subject to sampling discrepancy and requires careful collection methods, the analysis of exhaled breath gases and small molecules offers a noninvasive assessment of heart failure severity. A unique "breathprint" raises the possibility that unique metabolomic breath profiles can be used to identify and prognosticate patients with systolic heart failure.

Another area of interest has been intestinal flora metabolism in the pathogenesis of cardio-renal disease and heart failure. The intestinal microbiome is composed of trillions of commensal bacteria that populate gut and aid in digestion and absorption of nutrients. The interaction between microbacterial metabolism and different human disease states have recently been studied in the pathogenesis of insulin resistance, obesity, and cardiovascular disease.[32–37] Wang and colleagues[38] initially conducted hypothesis-generating studies to generate untargeted metabolomic maps to identify potential novel metabolites associated with cardiovascular risk. Through this work, 3 novel metabolites of the phosphatidylcholine (PC; lecithin) metabolism: choline (*m/z* 104), betaine (*m/z* 116), and trimethylamine N-oxide, TMAO, (*m/z* 76) were implicated in the pathogenesis of cardiovascular disease. Phosphatidylcholine is the major dietary source of choline in omnivores. Betaine is a direct oxidation product of choline, and TMAO is hypothesized to arise from bacterial metabolism of choline via the intermediate trimethylamine (TMA), and subsequent hepatic oxidation via flavin monooxygenase 3 (FMO3), forming TMAO. Of these 3 metabolites, TMAO demonstrated the strongest correlation to cardiovascular disease, which further implicates the role of the human and gut microbiome interactions that influence cardiovascular risk. TMAO has been linked to accelerated atherosclerosis and major adverse cardiac events (death, myocardial infarction, and stroke, **Fig. 5**).[39,40] TMAO also has been associated with poorer prognosis (death and myocardial infarction) at 2 years after myocardial infarction compared with GRACE (Global Registry of Acute Coronary Events) score or other biomarkers in coronary artery disease including

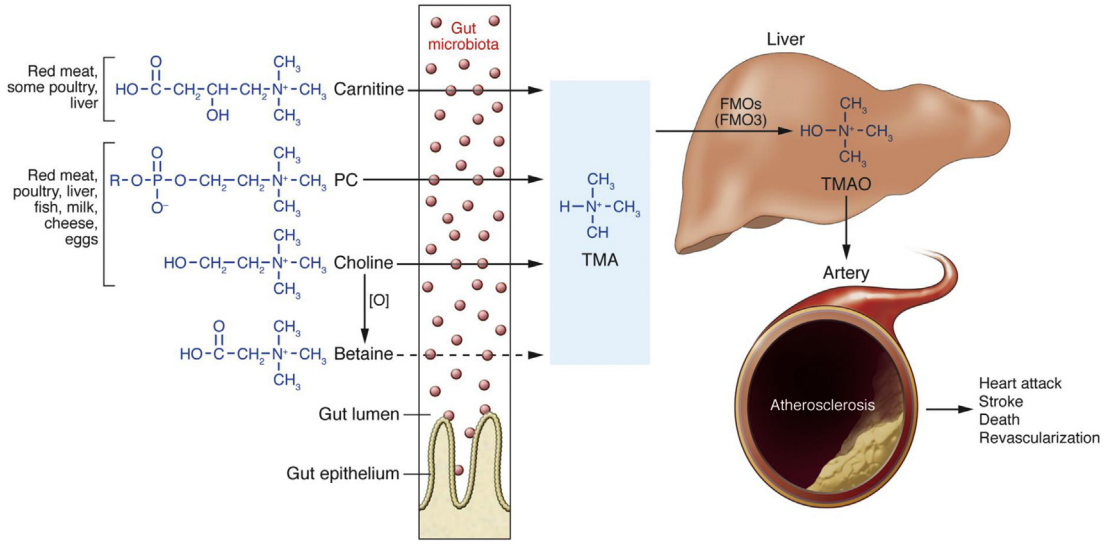

Fig. 5. Nutrient/meta-organismal pathway associated with atherosclerosis and major adverse cardiovascular events. FMO, flavin monooxygenase; O, oxidation; PC, phosphatidylcholine; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide. (*From* Tang WHW, Hazen S. The contributory role of gut microbiota in cardiovascular disease. J Clin Investv 2014;124(10):4205; with permission.)

copeptin and natriuretic peptide, proenkephalin, mid-regional proadrenomedullin, and pro-substance P.[41]

In addition to atherosclerotic heart disease, elevated levels of TMAO have also been identified as a prognostic biomarker in patients with systolic heart failure. Mice fed high-choline diets have more severe pulmonary edema, cardiac enlargement, and LV ejection fraction.[42] In a large single-center study, Tang and colleagues[43] explored the incremental prognostic value of measuring TMAO levels in patients with stable chronic systolic heart failure. They found that TMAO correlates with BNP ($R = 0.23$; $P<.001$) and strong inverse correlation between TMAO and estimated glomerular filtration rate (eGFR, $r = -0.55$; $P<.001$). Furthermore, elevated TMAO levels portended higher long-term mortality that is independent of traditional biomarkers of risk in the heart failure population, such as BNP, eGFR, and markers of inflammation (hsCRP). Suzuki and colleagues[44] analyzed the predictive value of TMAO in patients admitted with acute decompensated heart failure. They found that the level of TMAO correlates with in-hospital mortality in patients admitted with acute heart failure when combined with clinical risk scores that include adjustment for renal function. Although the precise pathophysiologic contributions of TMAO in heart failure remain to be elucidated, the microbacterial-human gut metabolomic interaction presents a potential novel target for heart failure therapeutics both for chronic stable heart failure and in patients with acute heart failure admissions.

CLINICAL APPLICATIONS IN HEART FAILURE

With improvements in technology and discoveries in new metabolomics pathways, our ever-expanding knowledge of metabolomics is paving the way for clinical application in the heart failure population. Several studies have already demonstrated a unique metabolomic profile in patients with heart failure.[9,10,12,45] Although not yet in wide clinical use, it is feasible to design a metabolomic panel based on patient serum or breath analysis that can be used to gauge disease severity and prognosticate disease progression. Such a panel would aid in targeted and personalized treatment. A heart failure metabolomics panel could yield incremental benefit to traditional biomarkers used in heart failure such as NT-proBNP. In a study of novel metabolomics biomarkers analyzed by MS profiling, Cheng and colleagues[12] demonstrated that a metabolomic panel of novel biomarkers including histidine, phenylalanine, spermidine, arginine, and phosphatidylcholine C34:4 have similar diagnostic value to BNP but have higher prognostic value for combined endpoints of death and heart failure–related hospitalization than BNP.

Metabolomic profiles can also be used to predict response to heart failure therapy. In small studies of patients undergoing ventricular assist device, trends in metabolomic biomarkers have indicated improvement in metabolomic profiles after LV unloading.[24] In patients undergoing cardiac resynchronization therapy (CRT), the assessment of metabolomic profiles have confirmed similar metabolomic derangements in patients with both ischemic and nonischemic systolic heart failure.[46] Although data appear controversial on baseline differences in metabolomic profiles between CRT-responders and CRT-nonresponders, some small prospective studies have indicated that CRT-responders have baseline differences in plasma concentrations of isoleucine, phenylalanine, leucine, glucose, valine, and glutamate.[47] This suggests that baseline metabolomic differences may be able to predict response to CRT and further prognosticate progression of heart failure in patients with severe LV dysfunction.

CHALLENGES AND POTENTIALS

Although advancements in technology have propelled the study of metabolomics in heart failure, many challenges and questions remain. Due to the diversity and complexity of molecular pathways and human-microbiome interactions encompassed by the field of metabolomics, we are only beginning to tease out the intricate relationships between these biochemical pathways. Because of vast variations in biological diversity and assay variability, the field of metabolomics is subject to sampling error, and the discrimination of true signals from noise remains challenging. Standardization of data collection methods would be needed to improve sampling accuracy, and alterations in medication history, dietary patterns, and environmental exposures may influence metabolomic sampling.[13] Furthermore, current methods of biomolecule profiling and discoveries are based on the assumption that the molecules implicated in heart failure pathogenesis are causative, but these molecules and pathways could simply be a downstream effect of the metabolomic derangements in heart failure. Additionally, systemic versus local production of metabolites remains unclear. Metabolomic assays are currently being used in other disease states such as mitochondrial diseases and inborn errors of metabolism; however, the analytical variability of samples, the

instability of sample collection, and errors in collection have proved challenging in large-scale clinical use. Thus, the clinical application of metabolomics in heart failure remains limited at present time.

As the field of biomedical research has begun to discover the intricate biochemical pathways involved in disease pathogenesis, the field of medicine is moving toward more personalization of diagnostics, prognostics, and therapeutics. Metabolomics provides a more discrete dataset that is likely functionally impactful for human health and disease. Some studies have delved deeper into myocardial metabolism such as studies on ketone and acylcarnitines, whereas other discoveries, such as TMAO, have given us insights onto the host and microbial interactions that are implicated in heart failure pathogenesis. The study of small molecular compounds and pathways involved in heart failure represents an area of active clinical investigation. The creation of individualized metabolomic profiles in patients with heart failure could reveal novel pathways of heart failure pathogenesis, better define patients with a biochemical vulnerability for heart failure, and identify new targets of intervention. The study of metabolomics can also shed light onto the complex gene-environment interactions involved in heart failure onset and progression. We stand on the precipice of molecular discoveries that hold the potential to revolutionize the management of heart failure.

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