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What can we learn from metabolomics?

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Abstract

Metabolic signatures have been identified for conditions such as myocardial ischemia as well as for metabolic disorders such as obesity and type 2 diabetes mellitus, which are intrinsically linked to cardiovascular disease. Technological advances in mass spectrometry have allowed the identification of individual molecular entities, such as lipids, branched-chain amino acids, dicarboxylacylcarnitines and trimethylamine N-oxide, purine degradation products, and members of the citric acid pathway as potential biomarkers for cardiometabolic diseases. Recent studies on metabolomics and cardiometabolic diseases collectively highlight the potential of using metabolomics to not only detect disease manifestations earlier than established biomarkers, but, in addition, to monitor metabolic pathways and to identify novel targets for therapeutic intervention. By integrating with genomics information, we envisage that metabolomics will become an essential component of cardiovascular research and personalized medicine. Within this review, we discuss recent advances in metabolomics that shed light on the genesis of atherosclerosis, and provide an indication of plaque instability underlying acute coronary syndromes and myocardial infarction. ■ *Heart Metab*. 2015;67:21-25

Keywords: biomarker; cardiovascular; diabetes; lipidomics; metabolomics

Metabolites are the intermediate and end products of all metabolic processes; a change in metabolic profiles is therefore an integrated read-out of cellular processes in health and disease.[1] Complementary to other "omic" technologies, metabolomics, including the subbranch of lipidomics, aims to capture the vast range of small molecules involved in metabolomic networks. Technological advances in high-resolution mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) have been at the forefront of metabolic research. NMR is widely used, especially for metabolic profiling of large clinical cohorts,[2] owing to high throughput and relatively low costs, but MS has become the analytical platform of choice for metabolite profiling. MS can be performed in a targeted and untargeted manner. Untargeted analysis of metabolites has the potential to reveal previously unknown pathophysiological mechanisms by the simultaneous assessment of multiple metabolic pathways. Targeted analysis, on the other hand, can be used to quantify specific metabolites with greater specificity and sensitivity, with the possibility of determining absolute concentrations using authentic standards.

Cardiovascular diseases (CVDs) are intrinsically linked with metabolic disorders, namely obesity, dyslipidemia, insulin resistance, and type 2 diabetes mellitus (T2DM).[3,4] Thus, the technical advances for metabolic

Abbreviations

ACS: acute coronary syndrome; **BCAA:** branched-chain amino acid; **CAD:** coronary artery disease; **CVD:** cardiovascular disease; **LPC:** lysophosphatidylcholine; **MI:** myocardial infarction; **MS:** mass spectrometry; **mTOR:** mammalian target of rapamycin; **T2DM:** type 2 diabetes mellitus; **TMAO:** trimethylamine N-oxide

profiling will be particularly useful for studying cardiometabolic disorders. Insulin resistance, for example, can exist for years without manifestation of clinical symptoms of T2DM[4] and early detection of insulin resistance may allow effective interventions in order to delay onset and prevent complications like CVD. Here we review key findings of metabolomics studies looking at cardiometabolic diseases (*Figure 1*).

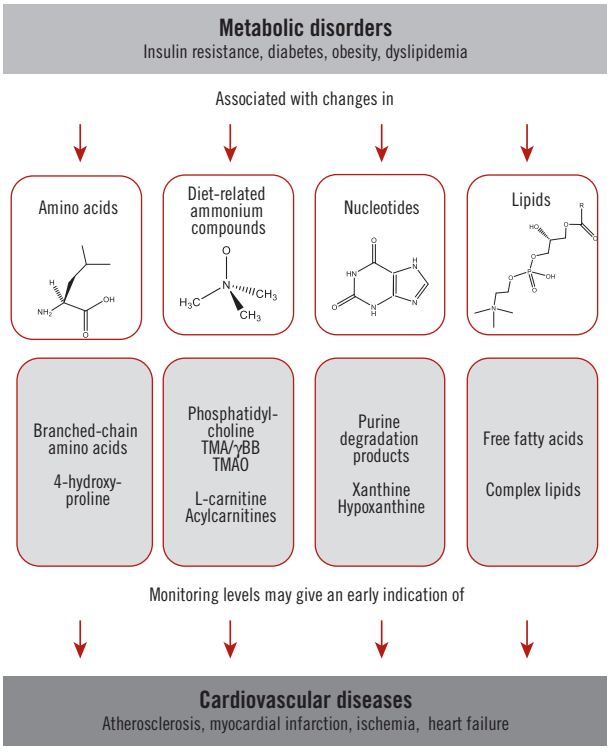

Fig. 1 *Metabolomics and cardiovascular disease (CVD). Overview of metabolites with reported associations to CVD.*
Abbreviations: *BB, butyrobetaine; TMA, trimethylamine; TMAO, trimethylamine N-oxide.*

Branched-chain amino acids and type 2 diabetes mellitus

Increased levels of branched-chain amino acids (BCAAs) contribute to insulin resistance[3] and predict T2DM.[4] In a cross-sectional study, Newgard et al documented differences in blood metabolite profiles of 74 obese and 67 lean individuals, with significant differences found in the abundance of BCAAs (valine, leucine, and isoleucine) as well as acylcarnitines (C3 and C5) and other amino acids.[3] Obese subjects displayed a BCAA "signature" by which dysregulated BCAA metabolism (due to overload) contributes to the development of insulin resistance and glucose intolerance, leading to T2DM.[3] In rats, dietary BCAAs seemed to induce mammalian target of rapamycin (mTOR)–insulin receptor substrate-1 phosphorylation and treatment with rapamycin, an mTOR inhibitor, alleviated insulin resistance only in rats fed a BCAA/high-fat diet compared with rats fed a high-fat diet only.[3] Expanding on these findings by the Newgard group, Wang et al confirmed BCAAs as potential biomarkers for incident T2DM in two large, longitudinal studies.[4] The results showed baseline levels of five branched-chain and aromatic amino acids (isoleucine, leucine, valine, tyrosine, and phenylalanine) to be significantly associated with future T2DM, highlighting the potential of monitoring amino acid metabolism in addition to established markers of glucose metabolism to detect early manifestations of T2DM.[4]

Acylcarnitines and coronary artery disease

Shah et al used targeted liquid chromatography–MS/MS and isotopically labeled standards for absolute quantitation of 69 metabolites in coronary artery disease (CAD) patients (who underwent cardiac catheterization) and controls from the CATHeterization GENetics (CATHGEN) biorepository.[5] As well as changes in glutamate/glutamine, proline, methionine, and urea cycle metabolites, the BCAAs leucine, isoleucine, and valine were also associated with CAD. As expected, T2DM was more prevalent among cases than controls. After statistical adjustment for T2DM, however, a significant association of circulating BCAAs with CAD remained, indicating that changes in BCAAs may either identify patients with insulin resistance before manifestation of T2DM[3] or that in addition, the BCAA pathway is also associated with CAD. A further signature composed of short-chain dicarboxylacylcarnitines was not only associated with the prevalence of CAD, but also with occurrence of death or myocardial infarction (MI) during a median of almost 3 years of follow-up in patients with existing CAD.[5] Carnitine, with its predominant source in red meat, is required for the import of long-chain fatty acids into mitochon-

dria. The transfer of an acyl group on coenzyme A results in the formation of acylcarnitines, which can then be shuttled across the mitochondrial membrane. Shah and colleagues have since reported similar findings in a much larger cohort of more than 2000 patients, in which short-chain dicarboxylacylcarnitines as well as medium-chain acylcarnitines and fatty acid levels in plasma were shown to independently predict cardiovascular events after adjustment for standard risk predictors.[6]

Choline/trimethylamine N-oxide and cardiovascular risk

Another known diet-related metabolite is choline. In a study by Wang et al, metabolism of dietary phosphatidylcholine by the gut flora was shown to increase risk of atherosclerosis and CVD by generating the catabolites choline, trimethylamine N-oxide (TMAO), and betaine in both humans and mice.[7] Dietary choline promoted the formation of atherosclerotic plaques in mice, and this could be prevented by antibiotic treatment.[7] A distinct second pathway for TMAO formation was highlighted by Koeth et al: supplementation of γ-butyrobetaine, a gut microbial intermediate in the metabolism of L-carnitine to trimethylamine and TMAO, also increases atherosclerotic plaque area significantly (50%) in mice.[8] Again, no increase was observed in γ-butyrobetaine–fed mice when given an antibiotic cocktail to suppress gut microbes.[8] More recent findings, however, would suggest that the association between TMAO and CVD could, at least partially, be explained by impaired kidney dysfunction. TMAO levels are greatly influenced by the glomerular filtration rate and are elevated in chronic kidney diseases.[9] Renal impairment is a well-established cardiovascular risk factor, putting into question the causal relationship of dietary choline and CVD.[9] Similarly, impaired renal clearance could contribute to increased systemic TMAO levels in patients with stable heart failure; elevated TMAO was associated with more than a 3-fold increase in mortality.[10]

Metabolomics in myocardial ischemia and infarction

Ischemia leads to impaired adenosine triphosphate (ATP) metabolism and causes an accumulation of sequential purine degradation products (adenosine diphosphate [ADP], adenosine monophosphate [AMP], inosine, hypoxanthine, and xanthine). Using targeted MS metabolomics and plasma samples from 36 patients before and after exercise testing, Sabatine et al reported elevated lactic acid and metabolites involved in skeletal muscle AMP catabolism as well as significant changes in six members of the citric acid pathway in response to myocardial ischemia after exercise.[11] Lactate and glucose have also been found to predict exercise-induced ischemia in patients with suspected CAD in blood samples obtained before exercise.[12] In patients undergoing alcohol septal ablation for the treatment of hypertrophic obstructive cardiomyopathy, Lewis et al analyzed serial blood samples from the coronary sinus and periphery.[13] Metabolite profiles by targeted MS were compared to identify markers associated with induced MI. Changes in metabolites were detected as early as 10 minutes after planned MI producing a metabolic signature consisting of aconitic acid, TMAO, threonine, and hypoxanthine, all of which differentiated patients with spontaneous MI from those undergoing diagnostic coronary angiography. Purine degradation products, namely hypoxanthine and xanthine, have been proposed to be potentially useful markers of ischemia. They not only increase in the circulation after induced MI,[13] but the urinary excretion of hypoxanthine and xanthine is also elevated in acute coronary syndrome (ACS) patients.[14] A point to note is that alterations in these metabolites were seen when no significant rises in the clinically available biomarkers, myocardial creatine kinase (CK-MB) and troponin T, were detectable in the plasma,[13] illustrating the potential of metabolites to detect the presence of very early myocardial injury; no currently used biomarkers are elevated within a time frame of 10 minutes. A caveat of using metabolites as biomarkers for cardiac ischemia is the lack of tissue specificity. Unlike cardiac troponins, these metabolites are ubiquitously present.

Another study by Vallejo et al used a gas chromatography–MS platform to compare the "metabolomic fingerprint" of plasma samples from patients with non–ST-segment elevation ACS, stable atherosclerosis, and healthy patients (n=9-10 per group).[15] Among other changes, 4-hydroxyproline was found to decrease in ACS patients compared with controls. Change in 4-hydroxyproline is of interest as it is a component of collagens. The vascular extracellular matrix stabilizes atherosclerotic plaques to help

prevent rupture. Circulating levels of 4-hydroxyproline are also thought to prevent the binding of low-density lipoprotein (LDL) to lipoproteins previously deposited in the vascular wall and to release already deposited LDL from atherosclerotic lesions.[15]

Lipidomics and cardiovascular risk

Traditionally, cardiovascular research focused mainly on the role of lipid classes rather than individual molecular species. Recent studies, however, highlight the importance of recognizing subtypes of lipids. Detailed knowledge of how individual lipid species contribute to pathophysiology of CVD may provide better biomarkers and novel therapeutic targets.[16] We have performed MS-based lipidomics in the prospective, population-based Bruneck study to identify molecular lipid signatures for cardiovascular risk; triacylglycerols and cholesterol esters with low carbon number and double-bond content were associated with predicting CVD events over a 10-year period,[17] including triacylglycerol 54:2 and cholesterol ester 16:1. The observed shift in fatty acid composition in complex lipids would be consistent with fatty acids derived from hepatic de novo lipogenesis (14:0 [myristic acid], 16:0 [palmitic acid], 18:0 [stearic acid], 16:1 [palmitoleic acid], and 18:1 [oleic acid]) being associated with a higher CVD risk than essential fatty acids. Subsets of triacylglycerols with the same nonessential fatty acids were also associated with increased risk of T2DM.[18] In the EPIC (European Prospective Investigation into Cancer and nutrition)-InterAct case-cohort study, the even-chain saturated fatty acids (14:0, 16:0, and 18:0) were positively associated with incident T2DM.[19] The ratio of 16:1 (n-7) to 16:0 was significantly positively associated with T2DM, but the ratio of 18:1 (n-9) to 18:0 was not. Unlike even-chain saturated fatty acids, odd-chain saturated fatty acids displayed an inverse relationship with the risk of T2DM, showing that not all saturated fatty acids have an adverse effect as conventionally classified.[19]

As expected, elevated levels of most lipid classes are associated with increased cardiovascular risk.[17] Lysophosphatidylcholines (LPCs), however, showed an inverse relationship with incident CVD in the Bruneck study.[17] A similar inverse association between LPCs and CVD were demonstrated by Ganna and colleagues investigating metabolic profiles of more than 3600 individuals from three population-based studies.[20] Using both an untargeted and targeted MS approach, the authors identified four metabolites to be associated with incident coronary heart disease, independently of main cardiovascular risk factors; LPCs 18:1 and 18:2, monoglyceride 18:2, and sphingomyelin 28:1. LPCs were negatively associated with body mass index, markers of inflammation, and subclinical CVD, whereas the opposite was the case for monoglyceride 18:2. When added to a model for risk prediction, monoglyceride 18:2 was a better predictor of coronary heart disease compared with triacylglycerol levels. It was associated with higher levels of cardiovascular risk factors and markers of subclinical CVD and oxidative stress.[20] Notably, LPC 18:2 has also been found to be inversely associated with incident T2DM and impaired glucose tolerance.[21]

Conclusions and future outlook

Both untargeted and targeted metabolomics will improve the identification of pathophysiological disturbances in metabolic networks. Enzymatic activities are the determinants of metabolite levels, thus to understand biological systems, small molecule metabolite data should be integrated with other "omics" analysis.[1] Although targeted methods can provide absolute measurements with the use of labeled standards, this inevitably means that one is limited to changes in metabolites whose retention times and MS parameters have been incorporated into the MS method. Knowledge of the human metabolome is anticipated to grow, hence untargeted methods are useful for hypothesis-free discovery analysis. As the use of single metabolites may lack sensitivity or specificity for risk stratification,[22] risk assessment can be improved by using multiple biomarkers to create a systems-level integration of "metabolomics" data.[23] To aid in this complex analysis, the use of computational algorithms is a requirement. Further understanding of metabolic networks may advance our understanding of the underlying mechanisms of CVD, as well as improve risk prediction and aid the development of novel therapeutic interventions. ■

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