S
mall biochemicals are the end result of all the regulatory
complexity present in a cell, tissue, or organism, includ-
ing transcriptional regulation, translational regulation, and
posttranslational modifications. Metabolic changes are thus
the most proximal reporters of the body’s response to a
disease process or drug therapy. In 1971, Robinson and
coworkers conceived the core idea that information-rich data
reflecting the functional status of a complex biological
system resides in the quantitative and qualitative pattern of
metabolites in body fluids. In the same year, Horning and
Horning first used the term metabolic profiling to describe
the output of a gas chromatogram from a patient sample. This
new approach to the quantitative metabolic profiling of large
numbers of small molecules in biofluids was ultimately
termed “metabonomics” by Nicholson et al and “metabolo-

mics” by others.

Two core technologies are used to perform metabolic
profiling: nuclear magnetic resonance and tandem mass
spectrometry (MS/MS), as previously reviewed in Circula-
tion: Cardiovascular Genetics. Nuclear magnetic reso-
nance requires relatively little sample preparation and is
nondestructive, allowing for subsequent structural analyses.
However, the method tends to have low sensitivity and
can detect only highly abundant analytes. Tandem mass
spectrometry (MS/MS), coupled with liquid chromatogra-
phy, on the other hand, has much higher sensitivity for
small molecules and is also applicable to a wide range of
biological fluids (including serum, plasma, and urine).
Recent advances in MS technology now enable researchers
to determine analyte masses with such high precision and
accuracy that metabolites can be identified unambiguously
even in complex fluids.

These technologies can be used to characterize biological
samples either in a targeted manner or in a pattern discovery
manner. In the former, the investigator targets a predefined
set of metabolites for analysis. In the latter, the investigator
must analyze a complex pattern of peaks—the molecular
identities of the species giving rise to the peaks are generally
not known. Although the targeted approach is more limited,
the analysis is more straightforward because the biochemicals
giving rise to the signals have already been identified. The
pattern discovery or fingerprint approach is inherently less
biased, but the need for subsequent unambiguous identifica-
tion of the peaks can be difficult.

The vision for human metabolic profiling also extends from
seemant studies of inborn errors of metabolism in
infants. Millington and coworkers pioneered the use of
MS-based methods for monitoring fatty acid oxidation, and
organic and selected amino acids. Rapid identification of
subjects with fatty acid oxidation disorders, organic aci-
demias, and aminoacidopathies through metabolic profiling
has led to dietary modification and amelioration of symptoms
or disease onset. It is anticipated that a global metabolic
analysis of more common diseases such as atherosclerosis
might identify new biomarkers or spotlight pathways for
dietary or drug modulation.

As might be expected, however, the application of
metabolomics to complex cardiovascular diseases has been
more difficult than for Mendelian disorders. Important
progress, however, is reported in this edition of Circula-
tion: Cardiovascular Genetics. Shah et al used a targeted
MS/MS-based platform to profile ~70 metabolites in
subjects from the CATHGEN biorepository who under-
went cardiac catheterization to evaluate suspected ische-
mic heart disease. Particular strengths of this study include
the relatively large number of patients studied, appropriate
corrections for both clinical conditions and medications
that may modulate metabolic profiles, and the use of
derivation and replication groups. They demonstrate that
peripheral blood metabolite profiles, one enriched for
branched-chain amino acid metabolites and another com-
prising urea cycle metabolites, add to the discriminative
capability for coronary artery disease (CAD) compared
with models containing only clinical variables. They spe-
cifically document improvement in the c-statistic with
incorporation of metabolite factors, compared with clinical
characteristics and established circulating biomarkers.
Though the increment in the c-statistic is relatively mod-
est, it underscores the capacity for metabolic profiling to
provide new information on top of known risk factors.
Furthermore, the group reports that a novel metabolite
cluster composed of dicarboxylycycarnitines predicts sub-
sequent cardiovascular events (death and myocardial in-
farction) in individuals with existing CAD.

The study from Shah et al, which highlights branched-
chain amino acid from ~70 metabolites profiled (Factor 4
in the manuscript), is also noteworthy in the context of
experimental and clinical data suggesting that certain
amino acids may be markers of insulin resistance.
Furthermore, studies of branched-chain amino acid supplementation in both animals and humans, including prior work by the authors of this manuscript, indicate that circulating amino acids may directly promote insulin resistance, possibly via disruption of insulin signaling in skeletal muscle. Shah et al. appropriately adjusted their clinical analyses for diabetes, although their metabolomics platform may be identifying a more subtle metabolic syndrome. Their compelling new findings highlight potential cross talk between insulin signaling and atherosclerosis that merits further investigation both in mechanistic studies in animal models and in clinical populations.

The authors also found an association between a group of urea cycle metabolites (Factor 9) and prevalent CAD, and note that this finding may reflect increased amino acid and ammonia catabolism. Alternatively, 2 of the 4 constituents of this factor (arginine and citrulline) are key substrates in the nitric oxide synthesis pathway, perhaps suggesting altered arginine bioavailability in modulating CAD. Indeed, another recent mass spectrometry-based study found that a low arginine to citrulline plus ornithine ratio in peripheral blood predicts CAD and adverse outcomes.

The study from Shah et al. also highlights an unanticipated metabolite signature, enriched for small- and medium-chain dicarboxylylcarcinintines, that predicts future cardiovascular events. Carnitine and its acyl esters are essential compounds for the metabolism of fatty acids. The main function of carnitine is to assist in the transport and metabolism of fatty acids in the mitochondria, where they are oxidized as a major source of energy. The acyl-CoA dehydrogenases, in turn, are a family of enzymes involved in the mitochondrial β-oxidation of fatty acids. Medium-chain acyl-CoA dehydrogenase acts on fatty acyl-CoA molecules from 4 to 12 carbons in length. Deficiency in this enzyme is the most common defect observed in the process of mitochondrial β-oxidation of fatty acids and is one of the most common inherited disorders of metabolism. Affected individuals can experience severe hypoglycemia and catastrophic nervous system injury in the setting of fasting. How the metabolic findings in adults with CAD might relate in any way to Mendelian disorders remains unknown. Future studies must also address the source of the dicarboxylylcarcinintines, the atherosclerosis-relevant cell types in which they are acting, and the functional effects of their buildup.

The metabolomic studies were performed using a targeted liquid chromatography–MS/MS-based platform. At first glance, the number of metabolites assayed may seem modest in the context of the estimated universe of total human metabolites (∼5000). However, the analytes chosen are key intermediaries in the metabolism of proteins, carbohydrates, and fats. Moreover, use of the targeted approach that incorporates isotope-labeled standards ensures the unambiguous identification of the analytes of interest. The use of standards also allows the investigators to report absolute quantities as opposed to relative quantities of these metabolites. Their rigorous methodology will thus facilitate both animal studies to manipulate levels of these metabolites in physiologically relevant contexts and future biomarker studies in other disease populations.

The relative contributions of diet and genetics to this newly described metabolic disarray also merits further investigation. Indeed, a previous study by this group has documented the heritability of metabolomic profiles in families with premature CAD. Ultimately, the integration of metabolomics findings with genetic data will provide an opportunity to study whether a biomarker also plays a role in disease pathogenesis. For a biomarker that has a causal role, the expected random distribution in a population of a polymorphism that determines high or low biomarker concentrations would be skewed in individuals depending on their disease status. Data from the so-called Mendelian randomization studies are accumulating for several biomarkers.

The study by Shah et al. thus represents important progress in the integration of metabolomics toward our understanding of cardiovascular disease. Although metabolite profiling technologies are still under development, they complement other functional genomic approaches, such as high throughput genome sequencing, RNA expression analysis, and proteomics. Together, they hold great promise to transform our ability to profile samples with the goal of illuminating biology and discovering valuable clinical biomarkers.

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References


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