Potential and Caveats of Lipidomics for Cardiovascular Disease

Since the seminal publications from the Framingham study in the mid-60s, the measurement of lipid levels, mainly of total cholesterol, total triglycerides, and low-density lipoprotein and high-density lipoprotein cholesterol, is routine clinical practice for cardiovascular disease (CVD) and lipid-lowering therapy. A more detailed assessment of the lipid composition, that is, the molecular species that constitute the lipid classes, is not widely used, mainly because of the caveats of assessing the lipidome. The human lipidome is estimated to include thousands of molecular lipid species with functional diversity. The molecular lipid species within a lipid class share a modular composition with fatty acids being attached to a common backbone. Although a characteristic head group within the backbone defines the lipid class, the diversity of molecular lipid species derives from the conjugated fatty acids. The conjugated fatty acids can differ in their carbon chain length, the number, position, and configuration (cis or trans) of their double bonds, and the position and type (acyl, alkyl, or vinyl) of linkage to the backbone.

Lipidomics refers to the comprehensive profiling of lipids, facilitated by recent advances in mass spectrometry (MS). The identified molecular lipid species are designated by abbreviations that combine the description of the lipid class with the characteristics of the conjugated fatty acids. For example, a diglyceride (DG) with 2 fatty acids of 12 and 22 carbon atoms and 0 and 5 double bonds, respectively, both linked by an acyl linkage, can be described either as DG(12:0/22:5), or as DG(34:5). Most MS studies report the numbers of carbon atoms and double bonds either for the individual fatty acids or in total, but do not assign the precise positions of the fatty acids at the backbone or of the double bonds within the individual fatty acids.

Sample extraction is key for lipidomic analysis. Traditionally, a 2-phase method has been used. The most popular one is the Folch extraction method with chloroform/methanol. Methanol precipitates the proteins, whereas chloroform ensures effective extraction of a broad range of lipid classes from the precipitated lipoproteins. The 2-phase extraction, however, is laborious and not very amenable to automation. Single-phase methods are less time demanding, but concerns have been raised that the lipid extraction might be less uniform and less efficient.

Liquid chromatography is applicable to a broad range of lipid classes and, thus, the principal separation method used in lipidomics. Chromatographic separation of the lipid extract enhances sensitivity and specificity by reducing sample complexity, but can also add technical variability. Before MS analysis, the lipids have to be ionized by electrospray ionization. Then, the mass-to-charge ratio of the lipid species can be determined by MS. Next, fragmentation is commonly induced by tandem MS to enable the structural elucidation of the lipid species. For quantitation, stable isotope-labeled standards are spiked into the samples before lipid extraction to account for technical variation. The addition of authentic standards (targeted MS analysis)
enhances accuracy of analyte identification and quantita-
tion in comparison with untargeted MS analysis without
authentic standards.7 Because of economic consider-
ations and lack of availability, a limited set of standards is
commonly used, and relative quantitation is achieved by
linear extrapolation through response factors.6

This issue of Circulation features 2 studies that break
new ground in relating lipid species to cardiometabolic
outcomes.

Alshehry and coworkers8 investigated 310 lipid species
within 22 lipid classes in almost 4000 subjects with diabe-
tes mellitus and found a signature of 42 lipid species as-
associated with incident CVD, cardiovascular death, or both.
The lipid signature determined by using targeted liquid
chromatography-electrospray ionization-tandem MS was
dominated by glycerophospholipids and sphingolipids. Ad-
dition of select lipid species to conventional risk factors
taIiated moderate improvements in CVD risk prediction,
and replication in independent patient samples was in part
successful. Lipids included in the cardiovascular events
model were: phosphatidylcholine (PC)(O-36:1), choles-
teryl ester (CE)(18:0), phosphatidylethanolamine(O-36:4),
PC(28:0), lyso-PC(20:0), PC(35:4), lyso-PC(18:2). Lipids
included in the cardiovascular death model were: PC(O-
36:1), DG(16:0/22:5), sphingomyelin(34:1), PC(36:5). Alshehry and colleagues integrated their findings for PCs
and phosphatidylethanolamines with underlying enzymatic
pathways.8 Only by considering lipid species in the con-
text of their metabolism can new mechanistic knowledge
be generated.

Syme and coworkers,9 using targeted liquid chro-
matograph-electrospray ionization-MS, report the first
lipidomic study conducted in adolescents. In almost
1000 participants, they measured 69 PCs, ie, members
of the glycerophospholipid class, of which 21 were as-
associated with at least 1 CVD risk factor out of visceral
adiposity, blood pressure, insulin resistance, and athero-
genic dyslipidemia. PC(16:0/2:0), a platelet-activating
factor possessing a 2-carbon acetyl at its sn-2 position,
and PC(14:1/0:0), a lysophosphatidylcholine possessing
only 1 fatty acid of 14 carbon atoms, showed the stron-
gest inverse and direct associations with CVD risk fac-
tors, respectively. The findings by Syme and colleagues
point toward the detrimental effects of CVD risk factors
on biologically active molecular lipid species, such as
PC(16:0/2:0),9 that may impact platelet activation.

At first glance, the difference in the final selection
of molecular lipid species is apparent, also with regard
to previous lipidomics studies on cardiometabolic dis-
 ease.10–15 This is not unexpected given the sources of
variability in lipidomics measurements (Figure). However,
there are also consistencies.

A number of studies targeting CVD and diabetes
mellitus unraveled associations with cholesteryl esters

Figure. Lipidomics for CVD.
Summary of the potential for clinical translation and the current caveats of lipid profiling. Sources of variability include the clinical characteristics, such as medication (heparin administration and lipid-lowering therapy), preanalytical variation (blood sampling and storage), and the different methods for lipid extraction, MS measurements, and statistical analysis of the multidimensional, highly correlated lipid data, as well. Standardization will be required for any future clinical application of lipidomics profiling for
CVD. CVD indicates cardiovascular disease; MS, mass spectrometry; and T2DM, type 2 diabetes mellitus.
reflect associations of apolipoproteins with CVD. Apart from apolipoprotein A1 and apolipoprotein B, other apolipoproteins have not been extensively explored in the context of lipidomics measurements to date. It will be interesting to see which of the 2 postgenomic technologies, lipidomics or proteomics, will deliver new biomarkers with clinical utility for CVD.18,19

**REFERENCES**


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