Lipid Signatures of Unstable Atheromas
Fossils or a Step Toward Personalized Lipidomics-Metabolomics?
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Atherosclerosis is a disease of the vasculature, characterized by the retention of low-density lipoprotein and the accumulation of peripheral blood leukocytes in the arterial intima. The role of the innate immune system in the progression of atherosclerosis is now widely recognized, and although several inflammatory cell types infiltrate atherosclerotic plaques, it is undisputed that macrophages accumulate excess lesional lipid and transform into inflammatory foam cells that fail to egress from the lesion. The ensuing inflammatory sequelae is self-perpetuating, perhaps resulting from a failure to resolve the local inflammatory response within the vessel wall, which drives the evolution of fatty streaks to a more complex lesion characterized by extensive lesion remodeling, cellular necrosis in the lesion core, and fibrous cap formation.

Acute myocardial infarction and stroke are the primary clinical manifestations of atherosclerosis, which result primarily from plaque rupture, thrombus formation, and subsequent ischemic injury. For plaque rupture to occur, the protective collagen-rich fibrous cap must be disrupted by matrix-degrading enzymes and other inflammatory mediators. Exposure of the plaque promotes thrombus formation by the release of numerous soluble mediators, such as tissue factor and thromboxane A2. Plaque rupture by means of angioplasty also initiates intracoronary generation of leukotrienes and lipoxins, which are enhanced with aspirin treatment. The intraluminal presence of soluble mediators can affect vascular reactivity and performance.

Because atherosclerosis is one of the most prominent diseases of our time, there is a critical need to identify early markers and local mediators of the dynamic lesion remodeling process so that new therapies can be developed to prevent atherogenesis and plaque rupture. Many research teams have taken on the task to identify such local mediators and assign the role of diverse lipid classes in this pathological process. Indeed, critical roles of cholesterol esters, phospholipids, sphingolipids, nonenzymatic lipid peroxidation products, and lipid mediators in atherogenesis have been described. Nonetheless, a comprehensive global analysis of the lipid composition present in human lesions, and more importantly, how this composition may change in stable versus unstable lesions, is lacking. The time is ripe for this clinically relevant challenge for interrogation with mass spectrometry–based lipidomics and metabolomics.

In this issue of Circulation: Cardiovascular Genetics, Stegemann et al use 2 distinct mass spectrometry–based lipidomic approaches to identify specific lipid signatures of unstable human atherosclerotic plaques in patients undergoing endarterectomies. In the first approach, the authors use a chip-based robotic nanoelectrospray platform to assess the lipid composition directly from tissue specimens. In this system, liquid extraction surface analysis allows for direct tissue sampling without prior sample extraction, using a specialized chip through which solvent is sprayed. Coupling to a triple-quadrupole mass spectrometer allows for the direct analysis of tissue lipids using full scan as well as precursor ion and neutral loss scanning modes to identify specific lipid classes. For comparison, shotgun lipidomics was carried out in parallel using traditional liquid extraction methods. The authors focused their lipidomic analysis on global changes in the levels of individual species within the broad classes of phospholipids, sphingolipids, triacylglycerols, and cholesterol esters (CEs).

As a validation of the liquid extraction surface analysis method for the analysis of plaque lipids, the authors performed an initial comparative analysis with the shotgun lipidomic approach in full-scan mode. This analysis revealed similar signal intensities and lipid species identification between the 2 sample work flows, thus validating the less labor-intensive and time-saving liquid extraction surface analysis approach. Comparing normal regions of the radial artery with atherosclerotic regions of either the carotid or femoral artery revealed 24 (of a total of 150) distinct lipid species that were differentially identified in the atherosclerotic plaques. These included several lysophospholipid and CE species with saturated, monounsaturated, and polyunsaturated fatty acyl groups esterified. Indeed, CE species were the most highly enriched plaque species, which showed a nearly 120-fold increase in plaque localization compared with control arteries. Quantification of specific CE species demonstrated a selective enrichment in cholesteryl-linoleate (18:2) and cholesteryl-oleate (18:1), with much lower levels of esterified polyunsaturated fatty acids such as eicosapenta-
This result is consistent with earlier studies that show a similar enrichment of free fatty acids, such as linoleic and oleic acids, in human atherosclerotic lesions.

Whereas cholesteryl-ara-chidonate (20:4) values were increased above those of control arteries, the levels of this CE were nearly 4-fold lower than that of cholesteryl-linoleate. A useful pie chart in this report clearly illustrates this shift from CEs containing polyunsaturated fatty acids (including 20:4 and 20:5) to linoleic and oleic acids (see Stegemann et al, Figure 4B) from control to diseased arterial segments, respectively. These results suggest that CEs of polyunsaturated fatty acids are associated with healthy vessels and that their levels decline with the progression of disease relative to other fatty acid species.

One of the prominent strengths of the study from Stegemann et al is the differential lipidic analysis of stable versus unstable regions of the same plaque. Principal component analysis showed a clear pattern of separation between stable and unstable plaque regions when considering multiple lipid classes. This set of results highlights that cholesteryl-stearate (18:0), a saturated fatty acid, is the CE species that is the most selectively enriched in unstable versus stable plaques. Again, CEs of linoleate and olate were the most abundant in the plaque tissue, but these particular species were not selectively enriched in stable versus unstable areas. In sharp contrast to the relative enrichment of cholesteryl-stearate in unstable plaques, the cholesterol ester of polyunsaturated dihomo-gamma linolenic acid (20:3) was significantly lower in unstable versus stable regions of the plaques. Thus, a similar pattern of selective depletion of polyunsaturated species is observed when comparing control arteries with those with plaques as well as stable versus unstable regions within the plaque. These results raise important new questions: Where do these polyunsaturated fatty acids go? Are there less to begin with in the diets of these individuals? Or is this simply the saturation or solidification of the molecular fossilized plaques that may occur over decades during lesion development?

The study by Stegemann et al goes far beyond the survey of traditional lipid classes by identifying specific molecular species within each broad lipid class to elucidate global changes in lipid composition in human atherosclerotic lesions and uses network analysis to help visualize the magnitude and relationships for these changes in individual molecular species. Analysis of the specific side chains in the CE and phospholipid pools yields important new insights into the potential contribution of each of these species to atherogenesis. The changes uncovered in this study are in accordance with results from others showing that the typical Western-type diet changes tissue lipid composition, and this has important implications for the biosynthesis of local proinflammatory mediators generated from specific fatty acid precursors. For example, it is established in laboratory animals that feeding a diet rich in omega-3 fatty acids increases tissue levels of eicosapentaenoic acid, and docosahexaenoic acid shifts lipid mediator profiles from arachidonate-derived lipid mediators to novel anti-inflammatory-proresolving lipid mediators. Recent evidence from genetically engineered mice enforce this concept by demonstrating that feeding mice a Western-type diet determines whether genetic predetermined metabolic pathways and enzymes, such as the murine 12/15-lipoxygenase, are protective or detrimental in atherogenesis through the biosynthesis of local anti-inflammatory–proresolving mediators or initiation of proinflammatory atherogenic events in vivo.

As noted, the critical role of diverse classes of lipids in atherosclerosis has been long appreciated. Within the last 2 decades, the important role of specific cyclooxygenase-derived and lipoxygenase (LOX)-derived lipid mediators in atherogenesis has emerged. These enzymes use polyunsaturated fatty acids as substrates. In humans, angioplasty promotes the intracoronary generation of both leukotrienes and lipoxins, and leukotriene biosynthesis correlates with plaque instability.

The contribution of these classic mediators of inflammatory responses derived from arachidonic acid are now of wide interest because of the importance of vascular inflammation in the progression of atherosclerosis. In patients undergoing carotid endarterectomy, the expression of thromboxane synthase and 15 LOX type II increases in lesions compared with control arteries. Moreover, recent results indicate that levels of the anti-inflammatory eicosanoid lipoxin A4 inversely correlate with the progression of peripheral artery disease in humans. Several studies with murine models of atherosclerosis have expanded on these findings by demonstrating that proinflammatory leukotrienes promote atherosclerotic lesion formation and vascular smooth muscle cell proliferation and migration.

Similarly, 5-LOX, 12-LOX, and 15-LOX each play critical roles in atherogenesis, with both proatherogenic and antiatherogenic effects reported that appear to depend on the metabolic substrate flux and local mediators rather than on the genetics of the enzymatic pathway itself. Thus, how changes in the phospholipid pools of fatty acids relate to the production of local bioactive lipid mediators during different phases of plaque progression and rupture, and how this changes with diet in humans, is likely to yield important new therapeutic targets and approaches for treating atherosclerosis and its development.

At this juncture, the limitations of the study from Stegemann et al include the relatively small sample size, the heterogeneity of the sample population, and the impact of pharmacological agents known to alter lipids (ie, aspirin and statins). Such limitations are appreciated with this labor-intensive sample analysis. Independent confirmation in larger and more diverse sample populations will be important. In addition, their report raises several key questions. First, can plaque lipid signatures be used to predict future cardiovascular events? What lipid molecules are potential early markers of the progress of the plaque? What are the similarities, if any, between rodent models of atherosclerosis and humans in terms of plaque lipid composition? What can we learn from murine atherosclerosis that translates to human vascular disease? How do the human lipid signatures temporally change in different phases of plaque progression and/or regression? Importantly, lipidomics-metabolomics can also provide a read-out about which pathways are operative at the gene and protein level. Namely, combining lipidomic-based profiling of tissues with genomics and proteomics approaches.
for a systems-wide analysis of unstable atherosclerotic lesions is likely to be a fruitful area of future investigation. It is intriguing to speculate that lipidomics may provide a new avenue for personalized medicine in which specific lipid signatures would be indicative of relative risk as well as dictate appropriate intervention strategies and lifestyle modifications.

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**References**


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