Lipomic profiling, profiled

In functional genomics, toxicology, and drug discovery, knowing the full breadth of lipid metabolism can help.

BY AMY ADAMS AND JAN KINGSBURY

Modern medicine has come to rely on a small suite of single biomarkers, such as plasma cholesterol or triglycerides, to assess the risk of certain diseases. Plasma cholesterol measurements alone are the impetus behind a multibillion-dollar industry for lipid-lowering drugs. A recent study showing the benefits of deep reductions in plasma low-density lipoprotein (LDL) cholesterol has magnified cholesterol's prominence as a key risk indicator for coronary heart disease (*1*). However, such single-biomarker assessments overlook the inherent complexity of metabolic disorders involving hundreds of biochemical processes. Assessing the full breadth of lipid metabolism is what drives the field of lipomic profiling.

Like proteomics, metabolomics, or gene expression profiling, lipomic profiling provides information about a broad swath of biomolecules. However, unlike the other "-omic" sciences, in which only a small portion of the genes or proteins is known, lipid metabolic pathways are well characterized. Relative lipid concentrations yield a complex picture of the regulation or dysregulation of lipid metabolism resulting from genetics, disease, diet, and drugs. Lipomic profiling is a particularly powerful tool for drug discovery and target validation given that many prevalent health conditions in the industrialized world—including cardiovascular disease, obesity, and diabetes—involve changes in lipid metabolism.

"What lipomic profiling gives you is a snapshot of all the products and substrates in lipid metabolism. When you perturb those pathways and watch what changes, you get a lot of information," says Steve Watkins, president and chief scientific officer of Lipomics Technologies.

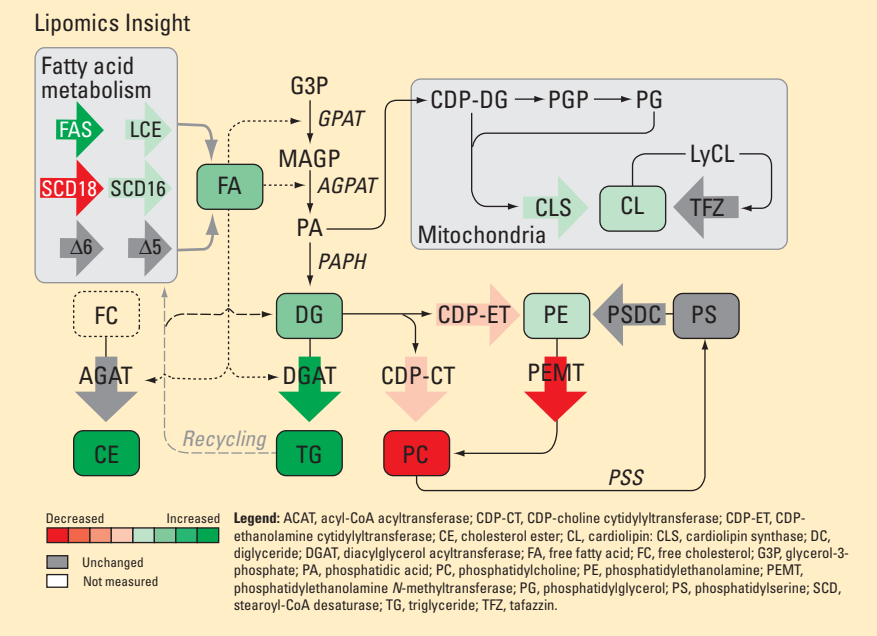
Currently, methods for assaying lipids in a biofluid are not standardized. An ideal technique would provide quantitative data for each lipid in a sample. MS, chromatography, and NMR spectroscopy have been used to varying degrees and with varying success. For example, the NIH is funding the LIPID MAPS Consortium (*www.lipidmaps.org*), a large collaborative effort led by the University of California, San Diego, to identify and quantify all lipids in specific cells using MS. This technique has also been used to identify the lipid composition of high-density lipoprotein (HDL) particles in different states (*2*). MS has the advantage of being conducive to high-throughput studies, but it cannot produce highly quantitative profile data because many lipid metabolites share identical molecular masses. NMR has strong quantitative capabilities but, as with MS, most lipid metabolites produce similar spectra and cannot always be specifically identified.

Chromatography is the only solution providing consistent quantitative and qualitative analytical results. Technology developer Lipomics Technologies uses chromatography as the basis of its TrueMass technique for identifying and quantifying structural, energetic, and nutritional lipids. Although this approach can effectively quantify high-abundance lipids, it is less well suited for low-abundance lipids such as eicosanoids or lipid oxidation products.

Metabolic pathways

Lipomic profiling has already been used to study enzyme-catalyzed metabolic reactions. Although the metabolic pathways themselves are understood, the regulation of the enzymes is still poorly characterized.

One of the first knockout mice to be characterized by lipomic profiling lacked phosphatidylethanolamine *N*-methyltransferase (PEMT), which catalyzes phosphatidylcholine biosynthesis. These mice initially showed no altered phenotype compared with normal mice, indicating that PEMT existed to generate phosphatidylcholine only during periods of choline deficiency.



Insight metabolic pathway maps developed by Lipomics integrate quantitative TrueMass measurements (shown in boxes) and semiquantitative Signature equations (shown in arrows) to explain differences in lipid metabolism between two sample groups. (Courtesy of Lipomics Technologies.)

However, lipomic profiling of knockout and wild-type mice fed varying levels of choline revealed a previously unsuspected role for PEMT (*3*). The PEMT-deficient mice had lower levels of two important plasma lipid fatty acids, suggesting that PEMT is key for mobilizing essential fatty acids from the liver and plasma.

A lack of this enzyme may now be a suspected factor in diseases related to essential fatty acid metabolism. In these diseases, supplementing the diet with required fatty acids may not entirely reverse the symptoms because of PEMT's additional role.

In another example, lipomic profiling was used to characterize the biological roles of two enzymes—DGAT1 and DGAT2—that catalyze the synthesis of triacylglycerols (*4*). Mouse knockouts of either of these two enzymes have different phenotypes even though the enzymes catalyze the same reaction. DGAT1 knockout mice have reduced triacylglycerols in their tissues, are resistant to diet-induced obesity, and are more sensitive to leptin and insulin. DGAT2 knockout mice, on the other hand, are smaller than normal, don't suckle normally, have dry, cracked skin, and usually die within 24 hours of birth.

Lipomic profiling illustrated DGAT2's critical role in synthesizing triacylglycerols throughout the body. In DGAT2 knockout mice, triacylglycerols were virtually undetectable in tissues, while plasma glucose, triacylglycerol, and free fatty acid levels were reduced 70–90%. DGAT1 activity was not enough to compensate for the loss of DGAT2 activity even though the enzymes are expressed in many of the same tissues.

Because DGAT2 function is critical for survival, DGAT1 appears to be the more likely drug target for treating obesity and diabetes.

Toxicology and discovery

Lipomic profiling has the potential to identify toxic effects of drug compounds. Because toxins can alter lipid levels in serum or tissues, assaying for these changes could point out potential pitfalls early in the discovery process, before much time or money has been spent on a drug.

Lipomic profiling was used to assess the effects of the environmental toxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in both chickens (*5*) and primates (*6*). Assaying plasma lipid concentrations revealed a previously unknown mode of action.

In chickens treated with estrogen, TCDD seemed to antagonize rise in plasma triacylglycerols and phospholipid components. Similarly, when pregnant macaques received TCDD doses, they had altered profiles of plasma cholesterol ester and phospholipids, the primary carriers of essential fatty acids in the blood. Before this study, TCDD was known to interact with estrogen but had no known role in lipid metabolism. This finding opens up new research avenues for understanding TCDD toxicity.

Perhaps lipomic profiling's most important application is in drug target discovery and validation. The lipomic profile of a sample from a diseased individual can reveal broad changes in metabolic pathways that are undetectable with conventional biomarkers. Enzymes involved in these dysregulated pathways might be useful drug targets, whereas key metabolites could be practical disease biomarkers.

Targeting drugs for individual patients could be an important outcome of lipomic profiling.

Throughout the development process, lipomic profiling can be used to evaluate a drug's effect on metabolic pathways and to quickly identify good drug candidates. A lipomic profile can also eliminate seemingly effective drugs that perturb metabolic pathways to cause unwanted side effects.

"You can get a long way into drug development not understanding all of the pathways affected by a drug, then get surprised in the end," Watkins says.

For example, lipomic profiling has been used to assess the effects of the diabetes drug rosiglitazone (Avandia), a PPARγ agonist, on lipid metabolism in a mouse model of Type 2 diabetes (*7*). This drug effectively treats diabetes and reduces plasma triglyceride levels in human diabetics.

The mice used in the study were genetically obese and diabetic. After four weeks on rosiglitazone, the mice had lower plasma concentrations of glucose, insulin, leptin, triacylglycerols, and cholesterol. Despite these positive indicators, the mice stored additional fat in the fat tissues, heart, liver, and pancreas. The mice also had signs of liver toxicity and hepatic lipid deposition in dissections. The lipomic profile of the treated mice showed an increase in lipids associated with fatty acid synthesis, particularly in the liver and fat tissues. These new lipids weren't being exported into the plasma and were instead stored in the fat and liver.

Previous studies of rosiglitazone in diabetic mouse models did not show liver toxicity. However, the lipomic profiling data suggests triacylglycerol accumulation in the liver resulted from a specific genetic trait involving hepatic phospholipid synthesis. By understanding the genetic background of this new mouse model, it might be possible to identify similar risk factors for humans and treat diabetic patients with drugs appropriate to their genetic backgrounds.

Targeting drugs for individual patients could be an important outcome of incorporating lipomic profiling in drug development. Knowing how a drug alters metabolic pathways, researchers might identify people who won't respond well to treatment. And excluding these individuals from trials may improve a drug's profile for a clearly identified patient population who do respond well and with few side effects.

References

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