

# Lipidomics and lipid profiling in metabolomics

J. Bruce German<sup>a</sup>, Laura A. Gillies<sup>a</sup>, Jennifer T. Smilowitz<sup>a</sup>, Angela M. Zivkovic<sup>a</sup> and Steven M. Watkins<sup>b</sup>

## Purpose of review

The field of metabolomics is extending the principles of genomics into cellular and organism metabolism and driving a revolution in lipid biochemistry, physiology and nutrition. Lipids studied using metabolomic approaches – lipidomics – are an ideal subject for metabolomic measurements.

## Recent findings

Lipids are small molecules that share common physical and chemical properties as a class, whose presence and abundance are key to much of metabolic regulation, from subcellular compartments to whole body energy control and signaling. Furthermore, by measuring changes in lipid concentrations, scientists are gaining a more detailed understanding of biochemistry and thus annotating genomes, but also understanding genetic polymorphisms and the postgenetic effects induced by drugs, foods and toxins.

## Summary

Although in its infancy – there are fewer than 200 total articles on lipidomics and metabolomics focusing on lipids – the field of metabolomics is beginning to deliver on its promise to revolutionize lipid and metabolic disease research.

## Keywords

drug development, lipidomics, metabolites, metabolomics, personalized medicine, profiling

## Introduction

This review highlights recent advances in metabolomics of interest to drug discovery and development and personalized medicine. Lipids are among the least understood of the cellular biomolecules. Even in the era of genomics, lipids continue to be an underappreciated subset of the metabolome. Fundamental questions about lipids, whose conceptual counterparts were answered for proteins decades ago, remain. Lipids within membranes exist as complex structural aggregates whose integrity provides multiple functions. This new view of the membrane as a functional network of lipid aggregates poses many questions. Which lipids exist within specific membranes and in what aggregate structures? How do these aggregate structures assemble and dissociate? What are the functions of these structural aggregates and what are the consequences to cells and organisms when they fail to function normally? How do lipids contribute to the signaling within cells and between cells and organelles? How do cells sort lipids to their multiple roles as structures, signals and fuel molecules? Answering these questions will fundamentally change our understanding of basic biology, of intervening in the health of humans and guiding biotechnology to improve the human condition.

The technical strategies for metabolomics, and especially lipidomics, are surprisingly undeveloped in the ‘omics’ era. Why is this surprising? In retrospect, metabolomics should have been the highest priority of systems biology. The knowledge of the biochemical pathways that use and produce metabolites is extensive. There is a long history of successful application of metabolites as measured biomarkers (blood cholesterol, glucose, triglycerides), and the technologies to measure metabolites have been in place for decades, certainly long before sequencing and immobilized nucleotide arrays. Furthermore, variations in human health and disease as well as exogenous influences on health (age, pathogens, toxins, diet and drugs) are reflected in metabolism. The new strategy of measuring all, or subsets of, metabolites provides considerable advantage over focused single metabolite measurements for basic research, health research and assessment, and even discovery of the functions of genes and genomes [1–5]. Now the strategies and toolsets of genomics are being applied to lipids, and the field of lipidomics is emerging. High-resolution chromatography, mass spectrometry, nuclear magnetic resonance are making lipomics possible.

Curr Opin Lipidol 18:66–71. © 2007 Lippincott Williams & Wilkins.

<sup>a</sup>Department of Food Science and Technology, University of California, Davis and  
<sup>b</sup>Lipomics Technologies, 3410 Industrial Boulevard, West Sacramento, California, USA

Correspondence to J. Bruce German, Department of Food Science and Technology, University of California, Davis, CA 95616, USA  
Tel: +1 530 752 1486; fax: +1 530 752 4759; e-mail: jbgerman@ucdavis.edu

**Current Opinion in Lipidology** 2007, 18:66–71

© 2007 Lippincott Williams & Wilkins  
0957-9672

Lipids remain a poorly defined class of biological molecules. Lipids as biological molecules are responsible for much of cellular structure, for multiple, complex signaling systems, and for providing life's most dynamic and efficient fueling and energetic schemes. Why are we still so ignorant of the details of these functions? Lipids provide remarkable capabilities to the processes of cellular life, but the properties that make them so vital make them difficult to study.

### Membranes

The unique properties of complex lipids to self-assemble in water to form the bilayer membrane as the barrier between cells and the outside world were critical to the emergence of cellular life. Equally importantly, the lipid bilayer's ability to maintain an electrochemical gradient has led to biology's ubiquitous and efficient systems of energy transduction and signal transmission. Throughout evolution, the increasing diversity of lipids and their unique structures became an apparently irresistible molecular scaffold for cellular structures, intracellular and extracellular surfaces, vesicular and clustered transporters, and trans-membrane channels. The nature of the forces driving the spontaneous assembly of lipids into 'soft' structures is largely incompatible with the tools of biochemistry. The tools that worked so well on the proteins, polysaccharides and polynucleotides disassemble the delicate structural lipid aggregates found in membranes.

### Signals

The signaling functions of lipids are similarly difficult to study. Lipids evolved into their critical roles as precursors for highly diverse signaling systems within and between cells. The ready availability of labile lipids as signaling molecules led to much of biology's alarm communication system, triggered by various forms of stress and mediating appropriate cellular responses. Again, the chemical fragility of lipid structures that made them candidates for complex signaling systems, makes them difficult to study experimentally. They can readily breakdown via simple, thermodynamically favorable chemical reactions (hydrolysis, oxidation) into products that are frustratingly similar to the enzymatic products that constitute signal molecules.

### Fuel

The roles of lipids as fuel and energy storage are the best understood functions of lipids, yet even these properties are difficult to study. When is a lipid intermediate a precursor for complex lipids, for signals and for simple fuel? The interconnectedness of lipid biochemistry frustrates simple pathway modeling. The insolubility of neutral lipids that make them valuable for storage led to the evolution of self contained aggregates (e.g., lipoproteins) as transport systems, yet the lipid compositions

and structures of these transport systems, so critical for their proper functions, are still poorly understood.

Into the challenging fields of lipid biology have now emerged the tools, strategies, informatics and insights of genomics, in the form of metabolomics. The physical and chemical diversity and the dynamic range of the entire metabolome have created genuine analytical problems. A logical approach, though heretical to a true 'omic' strategy, has been to subdivide the metabolome into functional units of metabolism, including lipids (lipidome) and carbohydrates (glycome). The term lipidomics was coined by Wilson [6] and by general consensus is defined as 'the study of the composition, metabolism, and biological role of lipids in cells at the levels of molecular species' [7]. In this review, we will examine recent research studies using lipidomics, their insights into lipid biology and suggest how these tools will propel the field of lipid research into the future.

### Nomenclature and classification of the lipidome

A critical step in lipidomics has been the ambitious endeavor by the LIPIDMAPS Project to build a classification system of structures, groupings and nomenclature serving both to define all of the possible lipids and to serve as a basis for structural organization of large lipid databases [8]. Many important concepts were recognized and included in the classification system, although the complexity of the problem of bringing an intuitive solution to classifying such a disparate group of biomolecules as lipids will prolong the process. The value of pursuing such an integrative approach can be seen immediately in the use of computational tools for building and polling databases structured on the intact lipidome. One approach has combined analytical data with classification to create a system of virtual lipid arrays [9]. Lipid compositional data, by various methods, were assembled and displayed in ways consistent with existing array-based technologies, both a technological and a conceptual advance.

### Analytical chemistry and lipidomics

Analytical chemistry and instrumentation has improved dramatically in the past 10 years, providing orders of magnitude increased sensitivity, specificity and efficiency of molecular measurements. Analytical strategies now couple different modalities – separation science and spectroscopy, including high performance liquid chromatography and mass spectrometry – into complementary platforms. These analytical systems further increase sensitivity and efficiency of molecular measurements. When it became clear that the analytical power being applied to measure single molecules could be leveraged into platforms capable of measuring all molecules in a mixture with the same analytical precision [3], the field

of metabolomics was off and running. To date, most lipidomic research studies rely on the output of a single platform designed to analyze all of the lipids in a bio-analytical category rather than a complete lipidome.

The latest analytical platforms are based either on liquid chromatography–mass spectrometry or tandem liquid chromatography–gas chromatography–mass spectrometry methods. The enabling capabilities of high-performance liquid chromatography coupled to electrospray mass spectrometry to simultaneously identify and analyze the full range of phospholipids from a complex mixture is bringing lipidomics to practice [10,11\*,12–14]. Challenges remain to simultaneously identify a broad range of intact lipid metabolites and accurately quantify each molecule in the sample. The inherent advantages of mass spectrometry to identify molecules by virtue of precise molecular mass measurement has the power to identify large numbers of complex lipids. Two important problems remain, however. Mass spectrometer ion sources are still inconsistent, and mass spectrometry is not as quantitatively accurate as metabolite measurements need to be for many applications. Furthermore, many molecular species of glycerolipids have identical molecular weights because of similarities in the fatty acid structures on the glycerol backbone. A recent approach to resolve lipid species with overlapping molecular weights uses high-performance liquid chromatography coupled directly to tandem mass spectrometry [15–17]. This approach offers a means to identify intact lipid molecules, followed by further fragmentation in order to identify individual molecular components. Pacetti *et al.* [16] used normal phase high-performance liquid chromatography coupled to electrospray ion-trap mass spectrometry to simultaneously examine changes in multiple phospholipid classes (phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin) and the individual molecular species within each class without prior derivatization, thin layer chromatography, or solid phase extraction of the bulk lipid extract. Tandem liquid chromatography coupled to mass spectrometry can also be used to separate complex lipid species prior to ionization and detection by mass spectrometry [18]. Combined, tandem methods are steadily increasing the ability to perform sensitive, high throughput, quantitative, and comprehensive analysis of lipid metabolites.

Current quantification by mass spectrometry requires analyte to internal standard peak area ratios. These methods provide relative quantification but still require multiple internal standards. At present, most reports have taken the approach of sacrificing quantification of metabolites for higher throughput identification. This decision is indeed propelling the field of lipid biochemistry toward recognizing which lipids are present in different tissues, cells, subcellular organelles,

among others. Because quantitative analyses are, however, needed to detect the small differences that are the basis of varying states of metabolic health – healthy versus diseased, overweight versus lean, among others – the decision to forgo quantitative precision limits the future utility of the integrated databases of metabolites that are produced by these analyses. It will presumably be necessary then to revisit these studies to rebuild quantitative databases against which individual profiles can be compared to distinguish important differences (i.e., diagnostic applications, unintended side effects of therapeutics) [19]. Rigorous quantitation using mass spectrometry requires, at present, internal standards enriched with one or more stable isotopes. The greater quantitative accuracy offered by this approach was seen in recent studies combining the use of stable isotope tracer molecules with advanced liquid chromatography–mass spectrometry techniques to observe the dynamics of lipid metabolism including lipid conversion, turnover, and signaling [7,20].

In addition to advances in instrumentation *per se*, the varying chemistry of complex lipids can provide opportunities to selectively separate, react, derivatize or complex specific classes of complex lipids for focused analysis at high throughput. The rapid derivatization of phosphatidylethanolamines is a proof of this principle and the combination of rapid throughput derivatization and analytics with lipidomics strategies promises to add an analytical dimension to much of the lipid class analyses [11\*]. To fully understand lipid functions, in addition to measuring lipid composition, the three-dimensional structure of lipid aggregates as found in biological systems must be determined.

### **Composition description of specific samples, cell types and subcellular organelles**

Among the first steps towards understanding the structure and function of biomolecules has been to describe their distribution within discrete functional compartments: cells, organelles and subcellular particles. Such strategies have been a hallmark of biochemistry research for decades, and combining this approach with the tools of lipidomics has the potential to add substantially to our knowledge of lipids. Combining lipidomics techniques with specific sample collection, cell isolation and subcellular fractionation methods are beginning to reveal relationships between composition and location, if not yet function. The effects of free cholesterol-loading on cellular phospholipid metabolism in cultured human skin fibroblasts is an example [21]. Lipid biosynthetic pathways were distinguishable from remodeling of existing phospholipid species using stable-isotope labeling. Increasing cholesterol in cells led to increases in phosphatidyl choline concentrations and remodeling of existing phosphatidyl choline species through transacylation

to more unsaturated, longer-chain phosphatidyl choline species, whereas phosphatidyl ethanolamine concentrations decreased accompanied by changes in phosphatidyl ethanolamine to longer, more unsaturated phosphatidyl ethanolamine species via de-novo phosphatidyl ethanolamine synthesis. This study demonstrated how lipid profiling can be used in investigating complex cellular metabolic processes. Hunt [7] provided an insightful approach to identify the nuclear lipidome by isolating nuclear membranes and analyzing in detail the various lipids present within this compartment. These techniques are sufficiently accurate to follow lipid remodeling within the nucleus itself [22].

A primary challenge to understanding the functions of lipids is to isolate functionally distinct subsets of membranes. Otherwise, diagnostic features of lipid composition are lost due to averaging across all membranes. The chemical and physical methods of traditional biochemistry are rarely successful in describing the complex arrangement of lipid molecules found in biological membranes due to their inherently (and perhaps functionally) transient construction. Pike *et al.* [23] have nonetheless achieved some success using carefully selected detergents to isolate and examine the lipidomes of specific membrane clusters or 'rafts' defined by their detergent dispersibility. An alternative strategy to using chemical or physical separation methods is to use biologically guided strategies. An ingenious approach in this direction was reported by Brugger *et al.* [24]. Taking advantage of the recently recognized fact that HIV co-opts the structures of lymphocyte rafts to their own surfaces, Brugger and colleagues isolated HIV particles from different lymphocyte cultures to explicitly provide surrogates of the lymphocyte rafts for lipidomic profiling. The authors took their study one step further, postulating that the lipid composition, particularly the sphingomyelin content of the HIV particles, may reflect a requirement for the virus itself. Indeed, inhibiting sphingolipid biosynthesis in the infected lymphocytes resulted in HIV with four-fold less infectivity. The capabilities illustrated by their study vividly demonstrate that the ability to isolate specific lipid domains, compartments and clusters is the limiting step to this research direction and hopefully will stimulate researchers to find imaginative new ways to capture specific lipid subsets.

Although lipidomic analysis of subcellular compartments will improve the descriptive data of cellular processes, there will still be challenges to move beyond compositional data to functional studies. The experimental logic of separating components in order to characterize their functions is relatively straightforward with proteins as enzymes whose function is the catalytic activity of a particular reaction. Enzymatic activity persists even in an isolated protein. Even though the remarkable diver-

sity of complex lipids distributed heterogeneously in eukaryotic cells implies that they function as discrete aggregates, however, it will be difficult to assign specific functions to specific lipids even as compositionally defined aggregates. Many of the functions already considered for lipids require intact cellular organelles, if not entire cells. That is, the functions of lipids in this respect relate to higher order processes – signal transduction, transport, clustering of multimeric complexes – and these functions require more than an isolated cluster of lipids to be discernible. Nonetheless, detailed compositional analyses will establish the boundaries of composition around which such functions must perform. One of the most attractive approaches and one that has revolutionized lipid biochemistry is the use of selective genetic alterations in simple organisms combined with comprehensive metabolomic strategies. Cowart and Hannun [25] have used this strategy to interrogate sphingolipid metabolism in yeast. Not surprisingly to lipid biologists but not completely reported to date, this study found that sphingolipids were involved in various processes in yeast from plasma membrane signaling to nuclear regulation.

### Composition description of lipid pathways

Lipidomics combines the tools of metabolomic profiling and specific subsets of lipid biochemistry with the goal of simultaneously achieving greater throughput and greater accuracy. Currently, researchers focus lipidomic technologies on a narrow range of lipids or physiological clusters in which the total number and extent of lipids is constrained. The separation of high-density lipoproteins from plasma of normal individuals and those in acute phase response, combined with a relatively comprehensive analysis of the complex lipids within these particles, provided an early indication of just how powerful the approach would prove to be [26]. Another early example of the principles of lipidomic thinking was an approach to measure many eicosanoids simultaneously [27]. This study was revealing. The basic idea of looking at the entire biochemical pathway system of eicosanoids, although obvious today, was a bold step. The approach directed researchers to interrogate the consequences of altering fluxes within separate pathways. It can be argued that the side-effects of COX-2 inhibitors and similar pharmacologic agents point to the need for such a strategy to study inhibitors of lipid signaling pathways that flux the same precursor to differentially active end products depending on conditions [28]. The ability to combine the high sensitivity of mass spectrometry with separate lipid compartments and the approach of comparative lipidomics is already proving to be very powerful in identifying modified lipids as important to structure function in cells. Kagan *et al.* [29] have made a bold proposal on the basis of this combination of lipidomics and mitochondrial oxidation to propose that

oxidized species of cardiolipin and phosphatidyl serine are the actual signal transduction mechanism of mitochondria initiated apoptosis.

### Composition and function

The technologies to profile lipid metabolites are not new; but there are few reports of quantitative and comprehensive lipid metabolite profiles in the literature. Most reports provide proportional data, but these data consisting solely of percentage compositions are incapable of building a comparable database. Quantitative lipid metabolome data were used to determine the differential effects of dietary  $\alpha$ -linolenic acid and long chain n-3 fatty acids on heart and liver phospholipid metabolism [30]. Although both types of fatty acids led to similar quantitative enrichment of heart tissue with docosahexaenoic acid, the distribution of this fatty acid among the cardiac phospholipid classes depended on the dietary source of n-3 fatty acids. Further, the quantitative distribution within the heart phospholipid classes was altered by diet. Quantitative measurements of the lipid metabolome were mapped to their biochemical pathways. This strategy was also employed to determine the effects of dioxin on liver and plasma lipid metabolism in birds [31] and pregnant primates [32] and the effects of treatment with the insulin-sensitizing drug rosiglitazone on liver, plasma, heart and adipose lipid metabolism in mice [33]. Compositional changes in fatty acids accompany the various stages of differentiation of adipocytes reflecting the development of metabolic pathways in this cell type as a function of its phenotype [34]. The nature of lipidomics makes it possible to re-evaluate data hypothesis-driven studies and in some such cases reveal our prior assumptions of lipid metabolism. Quite unexpected results were observed in a study of myocardium from calorie restricted animals [35]. According to quantitative lipidomic analyses, while in skeletal muscle, as would be expected in response to calorie restriction, triglycerides decreased, in myocardium, triglycerides increased and phospholipids decreased by 25%.

A quantitative approach to measuring specific classes of metabolites can frequently provide detailed information on the molecular basis responsible for changes in metabolism or phenotype. Such examples highlight the value of lipidomics for discovering the underlying causes of genetic polymorphisms, toxicological exposure, dietary intervention as well as investigating drug targets for their complete effects on lipid metabolic pathways. The quantitative comparison of the effects of diet, toxins, drugs or genetic variation on metabolism can be informative to both mechanisms of action and to the subtle differences in effect caused by interactions between compounds. One of the potentially important outcomes of more widespread applications of lipidomics techniques will be to create a growing resource of permanent reference data-

bases of the quantitative results of lipid experimentation. Such databases will extend the influence of lipidomics by allowing direct comparisons of newly generated experimental data with previously archived studies. Annotating these databases will provide a critical knowledge resource for all of lipid biology.

### Lipidomic dynamics, lipid synthesis, remodeling and transport

Mass spectrometry provides the opportunity to use stable isotopic enrichment in specific lipids as the means to follow a label through entire pathways. Because the time course can be defined according to when the label was introduced, measurements of lipid metabolites can provide truly dynamic, kinetic, information.

Cell models of metabolism have been a part of biochemical research for decades. A recent research report illustrated how this basic tool can be moved to personalized assessment of metabolic dysregulation and to define the metabolic responses to candidate therapies. Willard *et al.* [36] used fibroblasts from an individual patient suffering from apparent lipid metabolic disorders to identify a highly unusual deficiency of  $\Delta 6$ -desaturase activity as the causal mechanism. Not only did this approach identify the defective point in metabolism, but it provided a logical strategy and specific candidate molecules for intervention. A similar study protocol [37] was used to identify a lipid metabolic defect responsible for neutrophil aberrations in periodontal disease. The ease of sampling access of this tissue and the capability of lipidomics to identify specific metabolic defects in lipids argues compellingly that as metabolic analyses become more quantitatively accurate, high-throughput lipidomics will become a powerful asset to routine diagnostics.

### Conclusion

The field of genomics arose with expectations to build an unprecedented amount of knowledge in biology and to bring this knowledge to practice immediately. While some feel that the promise of genomics has failed to live up to its potential, many of the benefits of genomics are being felt in fields quite far removed from genes and genetic sequence. For lipids, the field of genomics has been enabling a major revolution in scientific research, knowledge creation and practical applications. Now the principles of 'omic technologies' as high-throughput, comprehensive analysis and bioinformatic interpretation have arrived with the field of lipidomics. Although still an emerging field, lipidomics has already established proofs of principle and promising new research possibilities. There are still technological challenges. Most but not all of the lipid metabolites have been identified. The important differences between biological states will be distinguished mainly by quantitative differences in lipids, not qualitative ones. Hence, the technologies of

lipids will need to be quantitatively accurate. As sample sizes get smaller and our ability to measure lipids improves, absolute quantitation becomes more difficult. Beyond technologies, there are also challenges. The field would do well to establish a centralized reservoir for data such that scientific experts in lipid metabolism could participate in the ongoing process of curating and annotating lipid metabolites much as the human genome project is annotating genes. Finally, the nature of lipids is to function as soft matter housing dynamic, ephemeral clusters of aggregated molecules. Lipidomics should stimulate a renewal of the field of separation science to capture this dimension of lipids and build complementary technologies to describe lipids in the multiple forms in which they contribute to biological processes and the discouraging ways that they precipitate biological failures.

## Acknowledgements

The authors gratefully acknowledge the writing support of C.J. Dillard and the support of UC Discovery, NIEHS Superfund Grant P42ES04699 and the CHARGE study (PO1ES11269).

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 89–94).

- 1 Delneri D, Brancia FL, Oliver SG. Towards a truly integrative biology through the functional genomics of yeast. *Curr Opin Biotechnol* 2001; 12:87–91.
- 2 Fiehn O. Metabolomics—the link between genotypes and phenotypes. *Plant Mol Biol* 2002; 48 (1–2):155–171.
- 3 Glassbrook N, Ryals J. A systematic approach to biochemical profiling. *Curr Opin Plant Biol* 2001; 4:186–190.
- 4 Raamsdonk LM, Teusink B, Broadhurst D, *et al.* A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat Biotechnol* 2001; 19:45–50.
- 5 Trethewey RN. Gene discovery via metabolic profiling. *Curr Opin Biotechnol* 2001; 12:135–138.
- 6 Wilson JF. Long-suffering lipids gain respect. *The Scientist* 2003; 17:34.
- 7 Hunt AN. Dynamic lipidomics of the nucleus. *J Cell Biochem* 2006; 97:244–251.
- 8 Fahy E, Subramaniam S, Brown HA, *et al.* A comprehensive classification system for lipids. *J Lipid Res* 2005; 46:839–861.
- 9 Ivanova PT, Milne SB, Forrester JS, Brown HA. LIPID arrays: new tools in the understanding of membrane dynamics and lipid signaling. *Mol Interv* 2004; 4:86–96.
- 10 Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J Lipid Res* 2003; 44:1071–1079.
- 11 Han X, Gross RW. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. *Mass Spectrom Rev* 2005; 24:367–412. This paper was a first proof-of-principle for the lipidomics methods using electrospray ionization mass spectrometry and provides a glimpse of what is becoming possible in measurement breadth and throughput.
- 12 Han X, Yang J, Cheng H, *et al.* Toward fingerprinting cellular lipidomes directly from biological samples by two-dimensional electrospray ionization mass spectrometry. *Anal Biochem* 2004; 330:317–331.
- 13 Pulfer M, Murphy RC. Electrospray mass spectrometry of phospholipids. *Mass Spectrom Rev* 2003; 22:332–364.
- 14 Taguchi R, Houjou T, Nakanishi H, *et al.* Focused lipidomics by tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 823:26–36.
- 15 Masoodi M, Nicolaou A. Lipidomic analysis of twenty-seven prostanoids and isoprostanones by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2006; 20:3023–3029.
- 16 Pacetti D, Boselli E, Hulan HW, Frega NG. High performance liquid chromatography-tandem mass spectrometry of phospholipid molecular species in eggs from hens fed diets enriched in seal blubber oil. *J Chromatogr A* 2005; 1097 (1–2):66–73.
- 17 Takatera A, Takeuchi A, Saiki K, *et al.* Quantification of lysophosphatidylcholines and phosphatidylcholines using liquid chromatography-tandem mass spectrometry in neonatal serum. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006; 838:31–36.
- 18 Sommer U, Herscovitz H, Welty FK, Costello CE. LC-MS-based method for the qualitative and quantitative analysis of complex lipid mixtures. *J Lipid Res* 2006; 47:804–814.
- 19 Watkins SM, Hammock BD, Newman JW, German JB. Individual metabolism should guide agriculture toward foods for improved health and nutrition. *Am J Clin Nutr* 2001; 74:283–286.
- 20 Sun D, Cree MG, Wolfe RR. Quantification of the concentration and 13C tracer enrichment of long-chain fatty acyl-coenzyme A in muscle by liquid chromatography/mass spectrometry. *Anal Biochem* 2006; 349:87–95.
- 21 Binder M, Liebisch G, Langmann T, Schmitz G. Metabolic profiling of glycerophospholipid synthesis in fibroblasts loaded with free cholesterol and modified low density lipoproteins. *J Biol Chem* 2006; 281:21869–21877.
- 22 Hunt AN, Alb JG, Koster G, *et al.* Use of mass spectrometry-based lipidomics to probe PITPalpha (phosphatidylinositol transfer protein alpha) function inside the nuclei of PITPalpha+/+ and PITPalpha-/- cells. *Biochem Soc Trans* 2004; 32 (Pt 6):1063–1065.
- 23 Pike LJ, Han X, Gross RW. Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids: a shotgun lipidomics study. *J Biol Chem* 2005; 280:26796–26804.
- 24 Brugger B, Glass B, Haberkant P, *et al.* The HIV lipidome: a raft with an unusual composition. *Proc Natl Acad Sci U S A* 2006; 103:2641–2646. This study elegantly applied a biologically based isolation strategy (HIV released from lymphocytes) to acquire lipids that are within raft structures by biological criteria (HIV co-opted host membrane rafts) and then determine detailed compositional information to identify and confirm a host lipid metabolic target of viral infectivity.
- 25 Cowart LA, Hannun YA. Using genomic and lipidomic strategies to investigate sphingolipid function in the yeast heat-stress response. *Biochem Soc Trans* 2005; 33 (Pt 5):1166–1169.
- 26 Pruzanski W, Stefanski E, de Beer MC, *et al.* Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. *J Lipid Res* 2000; 41:1035–1047.
- 27 Newman JW, Watanabe T, Hammock BD. The simultaneous quantification of cytochrome P450 dependent linoleate and arachidonate metabolites in urine by HPLC-MS/MS. *J Lipid Res* 2002; 43:1563–1578.
- 28 Egan KM, Lawson JA, Fries S, *et al.* COX-2-derived prostacyclin confers atheroprotection on female mice. *Science* 2004; 306:1954–1957.
- 29 Kagan VE, Borisenko GG, Tyurina YY, *et al.* Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine. *Free Radic Biol Med* 2004; 37:1963–1985.
- 30 Watkins SM, Lin TY, Davis RM, *et al.* Unique phospholipid metabolism in mouse heart in response to dietary docosahexaenoic or alpha-linolenic acids. *Lipids* 2001; 36:247–254.
- 31 Stanton B, Watkins S, German JB, Lasley B. Interaction of estrogen and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with hepatic fatty acid synthesis and metabolism of male chickens (*Gallus domesticus*). *Comp Biochem Physiol C Toxicol Pharmacol* 2001; 129:137–150.
- 32 Moran FM, Hendrickx AG, Shideler S, *et al.* Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on fatty acid availability and neural tube formation in cynomolgus macaque, *Macaca fascicularis*. *Birth Defects Res B Dev Reprod Toxicol* 2004; 71:37–46.
- 33 Watkins SM, Reifsnnyder PR, Pan HJ, *et al.* Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. *J Lipid Res* 2002; 43:1809–1817.
- 34 Su X, Han X, Yang J, *et al.* Sequential ordered fatty acid alpha oxidation and Delta9 desaturation are major determinants of lipid storage and utilization in differentiating adipocytes. *Biochemistry* 2004; 43:5033–5044.
- 35 Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry* 2004; 43:15584–15594.
- 36 Williard DE, Nwankwo JO, Kaduce TL, *et al.* Identification of a fatty acid delta6-desaturase deficiency in human skin fibroblasts. *J Lipid Res* 2001; 42:501–508.
- 37 Gronert K, Kantarci A, Levy BD, *et al.* A molecular defect in intracellular lipid signaling in human neutrophils in localized aggressive periodontal tissue damage. *J Immunol* 2004; 172:1856–1861.