Analytical Considerations of Stable Isotope Labelling in Lipidomics

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Abstract:

Over the last two decades, lipids have come to be understood as far more than merely components of cellular membranes and forms of energy storage, and are now also being implicated to play important roles in a variety of diseases, with lipid biomarker research one of the most widespread applications of lipidomic techniques both in research and in clinical settings. Stable isotope labelling has become a staple technique in the analysis of small molecule metabolism and dynamics, as it is the only experimental setup by which biosynthesis, remodelling and degradation of biomolecules can be directly measured. Using state-of-the-art analytical technologies such as chromatography-coupled high resolution tandem mass spectrometry, the stable isotope label can be precisely localized and quantified within the biomolecules. The application of stable isotope labelling to lipidomics is however complicated by the diversity of lipids and the complexity of the necessary data analysis. This article discusses key experimental aspects of stable isotope labelling in the field of mass spectrometry-based lipidomics, summarizes current applications and provides an outlook on future developments and potential. High-throughput lipidomics has great potential when investigating the role of lipids in cellular metabolism. A large number of drugs is available to interfere with normal lipid metabolic pathways and contemporary lipidomic techniques can monitor the levels of hundreds of lipids simultaneously. However, true high-throughput lipidomics still involves the subsequent overcoming of several challenges. First, the clean-up of samples so their lipid composition may be measured. Established protocols for extraction of lipids involve liquid-liquid extractions Lipidomics is the study of cellular lipids and their roles in health and disease. The field came to prominence in the early 2000s and has now become indispensable in basic and clinical research, as lipids emerge more and more from being viewed as merely energy-storage molecules, and instead are being implicated in a plethora of pathological conditions, such as metabolic syndrome, cardiovascular and neurodegenerative diseases, as well as cancer. Most lipidomic studies are based on the determination of the levels of lipids and on their comparison either between disease and control groups, at different time points, or before and after treatments. This is by design merely a static measurement of what is really a very dynamic metabolism, and whenever, e.g., differences in the concentrations of circulating lipids in plasma are found, this might either be due to different rates of synthesis or degradation, or due to the release from another storage pool. For example, an increase of plasma triacylglycerols may be due to uptake from food or to release from adipose tissue. While this difference is impossible to assess using pure concentration-based approaches, it may have important different biochemical implications. While the absolute concentration of phosphatidic acid is constantly low, it still has a high rate of metabolic flux, and due to its central position in phospholipid metabolic pathways, is constantly biosynthesized and processed into other phospholipids. Therefore, when it comes to investigating lipid metabolism and dynamics-biosynthesis directly, transport, interconversion and degradation-stable isotope labelling is the technique of choice and provides an additional dimension of information to absolute quantitative values. In all stable isotope labelling studies, a stable isotope label in a substrate, usually ^{13}C or deuterium (^{2}H), is metabolized into all resulting metabolites. Stable isotope tracing therefore enables the determination of the metabolic fate of stable isotope-labelled precursors. This approach has been used since as early as the 1930s, when deuterium labelling was first used to investigate murine fatty acid metabolism and determine that even under a hypocaloric diet, dietary fatty acids were not immediately oxidized, but stored and released from fat tissue. Far before the age of bioanalytical mass spectrometry, the deuterium content was measured using refractometry and densitometry in water after combustion of the labelled biomolecules. Nowadays, modern instrumentation offers a far greater level of detail. Coupling of chromatography and mass spectrometry enables more than just the determination of total labelling; instead, labelling of individual lipid species can be investigated, and even the localization and the enrichment of the label within lipids can be determined using appropriate analysis strategies. Stable isotope labelling has already been applied to numerous studies of lipid metabolism and the techniques used have evolved with time and with the analytical instrumentation available. This article discusses the fundamentals and important experimental aspects of stable isotope labelling for application to mass spectrometry-based lipidomics and offers an outlook on the future and the potentials of this

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technique. Stable isotope labelling is a powerful technique with promising applications. It enables direct analysis of nutrient distribution, metabolism, conversion into metabolites and the fate of the resulting metabolites. In contrast to radioactive labelling, there are no dangers or safety concerns, making this technique particularly well suited for metabolism studies in humans. Future research using stable isotope labelling in lipidomics will likely make use of ultra-high mass resolution, use multiple tracer methods and utilize the information content of tandem mass spectra to localize and quantify label enrichment within lipid building blocks. All this combined will allow the metabolism of lipids to be investigated in unprecedented detail, greatly advancing our knowledge of the roles of lipids in health and disease.

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