Mass spectrometry of lipid synthesis using stable isotopes

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Traditional approaches for metabolic flux analysis using substrates labelled with radioactive or stable isotopes underpin all the basic concepts of metabolic pathways. Early studies based on the incorporations of deuterium or tritium from respectively deuteriated or tritiated water provided the means to determine absolute rates of fatty acid and sterol synthesis, while incorporations of [¹⁴C]-acetate and related precursors were central to the determination of mechanisms of lipid biosynthesis. While such approaches have proved invaluable for the analysis of the synthesis of phospholipid classes, they have inherent limitations for characterising the synthesis of individual lipid molecular species. Advances in tandem MS/MS and high mass accuracy MS have facilitated novel approaches to characterise and quantify the synthesis and turnover of intact lipid molecular species from the incorporation of stable isotope-labelled substrates. This presentation will review both targeted methodologies that monitor incorporations into products of specific pathways and more global approaches that rely on bioinformatics to de-convolute incorporation patterns due to metabolic fluxes through multiple pathways. Analysis of incorporations into phospholipid headgroups from stable isotope-labelled substrates (e.g. methyl-D₉ choline, D₆-myoinositol) relies on the generation of diagnostic fragment ions that retain the isotopic label. Relevant precursor or neutral loss scans then determine the distribution of labelled and unlabelled individual molecular species (e.g. phosphatidylcholine, PC, or phosphatidylinositol, PI). We have applied this methodology in vitro to demonstrate PC synthesis de novo within the nuclear matrix of cultured cells [4] and to monitor the dynamics of PC synthesis and turnover in vivo in human disease [2;3] and in experimental animal models [1;5;6]. The clinical studies have focussed on probing the disrupted lung surfactant metabolism of patients with severe respiratory failure, and the transgenic mouse studies have targeted mechanisms regulating surfactant PC synthesis. A more global approach monitored the incorporation of ¹-[¹³C]-glucose into the lipid fraction of Drosophila larvae. The incorporation pattern here is more complex, as label is incorporated into both fatty acid and glycerol portions of phospholipids and neutral lipids, but multiple precursor and neutral loss scans of labelled fatty acid fragment ions permit the calculation of enrichments of combinations of fatty acids in glycerolipids and their absolute rates of synthesis. In addition, accurate mass determination by FT-ICR MS can resolve labelled and unlabelled triacylglycerol of the same nominal mass. Accurate mass analysis has also been used to quantify incorporation of U-¹³C-palmitate into intact lipid species by monitoring pairs of ions differing in this exact mass. Finally, incorporation of label from D₂O to monitor de novo lipogenesis, coupled with MALDI mass spectrometry imaging, has recently been applied to analyse the location of brain lipid synthesis in vivo.