Progress in Lipid Research 54 (2014) 14–31

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Application of stable isotopes to investigate the metabolism of fatty acids, glycerophospholipid and sphingolipid species

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ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form 30 December 2013
Accepted 7 January 2014
Available online 24 January 2014

Keywords:
Lipid biosynthesis
Lipid metabolism
Metabolic profiling
Stable isotope labeling
Mass spectrometry
Lipidomics

ABSTRACT

Nature provides an enormous diversity of lipid molecules that originate from various pathways. To gain insight into the metabolism and dynamics of lipid species, the application of stable isotope-labeled tracers combined with mass spectrometric analysis represents a perfect tool. This review provides an overview of strategies to track fatty acid, glycerophospholipid, and sphingolipid metabolism. In particular, the selection of stable isotope-labeled precursors and their mass spectrometric analysis is discussed. Furthermore, examples of metabolic studies that were performed in cell culture, animal and clinical experiments are presented.

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Abbreviations: BMP, bis(monoacylglycero)phosphate; Cer, ceramide; CoA, Coenzyme A; Des, desaturation; DG, diacylglycerol; DihCer, dihydroceramide; DiSPH, dihydrosphingosine (sphinganine); DMDS, dimethyl disulfide; ESI-MS/MS, electrospray ionization tandem mass spectrometry; FA, fatty acid; FADS, fatty acid desaturase; FAME, fatty acid methyl ester; FASN, fatty acid synthase; FT-ICR, Fourier transform ion cyclotron resonance; GC–IRMS, gas chromatography isotope ratio mass spectrometry; GlcCer, glucosylceramide; GP, glycerophospholipid; HILIC, hydrophilic interaction chromatography; IRMS, isotope ratio mass spectrometry; IS, internal standard; LacCer, lactosylceramides; LC, liquid chromatography; LOD, limit of detection; LPA, lysophosphatidic acid; LPA, lysophosphatidic acid; MIDA, mass isotopomer distribution analysis; MTAD, 4-methyl-1,2,4-triazoline-3,5-dione; NL, neutral loss; NP, normal phase; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine-N-methyltransferase; PFB, pentafluorobenzyl; PG, phosphatidylglycerol; PGP, phosphatidylglycerolphosphate; PI, phosphatidylinositol; PIS, precursor ion scan; PS, phosphatidylserine; RP, reversed phase; S1P, sphingosine-1-phosphate; SCD, stearoyl-CoA desaturase; SL, sphingolipid; SM, sphingomyelin; SMase, sphingomyelinase; SPC, sphingosylphosphorylcholine; SPH, sphingosine; SPP, S1P phosphatase; SPT, serine palmitoyl transferase.

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http://dx.doi.org/10.1016/j.plipres.2014.01.002
0163-7827/

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1. Introduction

Traditionally, radioactive precursors have been applied to investigate lipid metabolism. During the past decade, numerous studies have demonstrated that stable isotopes can replace radioactive reagents for safety reasons and because mass spectrometric analysis can be used to provide data regarding the lipid species. Today, lipidomic technologies are able to cover almost the full lipidome [1–4]. A combination of these methods with stable isotope labeling is perfectly suitable for analyzing the metabolism of lipid species. This knowledge is of great importance because the reasons why nature provides such an incredible diversity of lipid species are not well understood [5]. In this review, we present an overview of studies that used stable isotopes to analyze the metabolism of fatty acids (FA), glycerophospholipids (GP) and sphingolipids (SL). Labeling strategies and mass spectrometric analysis are discussed.

2. Fatty acids [FA]

2.1. Biosynthesis and metabolism of FA

FAs are key modules for various lipids, including cell membrane lipids such as GPs or triacylglycerides, which are the major components of lipid droplets [4]. Excess lipids or defects in lipid storage are associated with diseases such as metabolic syndrome. Moreover, FAs are the precursors of eicosanoids, which are potent signaling molecules with inflammatory and anti-inflammatory effects [6].

For numerous cellular processes, such as cell growth or differentiation, as well as for key cellular functions, FAs must be synthesized de novo, primarily as building blocks for cell membrane generation [7]. As indicated in Fig. 1, in mammals, FAs are synthesized de novo from acetyl-CoA by FA synthase (FASN) to yield palmitate (FA 16:0) [8]. Palmitate can either be desaturated to palmitoleate (FA 16:1n-9) by stearoyl-CoA desaturase (SCD1) or elongated by an elongase (ELOVL6) to stearate (FA 18:0). Stearate can be further desaturated to oleate (FA 18:1n-9) by SCD1 [9,10]; palmitoleate can be elongated by ELOVL6 to yield vaccinate (FA 18:1n-7). The precursor for polyunsaturated FAs (PUFA) are linoleic acid (FA 18:2n-6) and alpha-linolenic acid (FA 18:3n-3) [6]. Both are essential FAs obtained from the diet in mammals. Unlike plants, mammals do not contain delta-12 and delta-15 desaturases, which are necessary to desaturate FA 18:1 and FA 18:2 [11]. Linoleic acid (n-6) metabolites include arachidonic acid (FA 20:4n-6) (Fig. 1), whereas alpha-linolenic metabolites (n-3) include eicosapentaenoic acid (EPA, FA 20:5n-3) and docosahexaenoic acid (DHA, FA 22:6n-3). Importantly, the metabolism of n-3 and n-6 PUFAs shares the same series of enzymes. In addition to endogenous metabolism, arachidonic acid, as well as EPA and DHA, can also be obtained directly from the diet. Fish is a typical source of n-3 FAs, such as EPA and DHA, whereas westernized diets primarily contain n-6 FAs, including linoleic acid and arachidonic acid [12]. Whereas arachidonic acid is the key precursor for inflammatory eicosanoids,

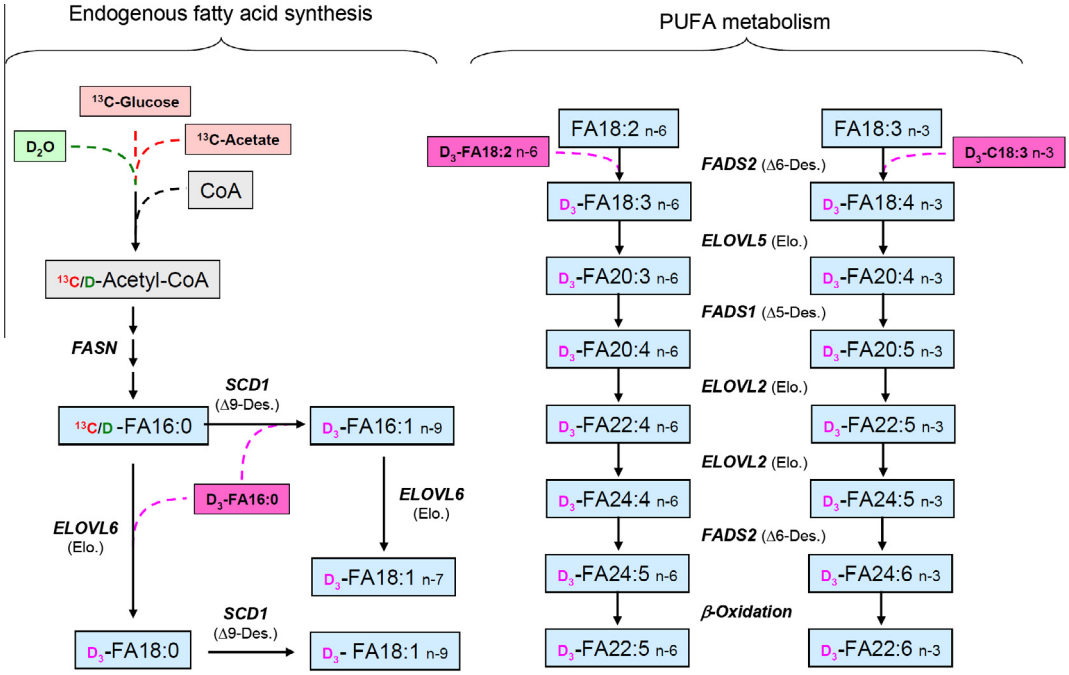

Fig. 1. Labeling of fatty acid synthesis and metabolism. The pathways of human endogenous fatty acid synthesis and PUFA metabolism are displayed. The stable isotope-labeled precursors (compare Table 1) are indicated in green, red and pink squares. The fatty acids are indicated in blue squares. CoA, Coenzyme A; Des., desaturation; Elo., elongation; ELOVL, Fatty acid elongase; FA, fatty acid; FADS, fatty acid desaturase; FASN, fatty acid synthase; SCD, Stearoyl-CoA desaturase.