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Review

A matter of fat: An introduction to lipidomic profiling methods☆

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ABSTRACT

In recent years, lipidomics or lipid profiling, an extension of metabolomics where the lipid complement of a cell, tissue or organism is measured, has been the recipient of increasing attention as a research tool in a range of diverse disciplines including physiology, lipid biochemistry, clinical biomarker discovery and pathology. The advancement of the field has been driven by the development of analytical technologies, and in particular advances in liquid chromatography mass spectrometry and chemometric methods. In this review, we give an overview of the current methods with which lipid profiling is being performed. The benefits and shortcomings of mass spectrometry both in the presence and absence of chromatographic separation techniques such as liquid-, gas- and thin layer chromatography are explored. Alone these techniques have their limitations but through a combination many of the disadvantages may be overcome providing a valuable analytical tool for a variety of disease processes.

Contents

1. Introduction 174
2. Mass spectrometry in lipidomics 175
3. Thin layer chromatography in lipidomics 177
4. Gas chromatography–mass spectrometry in lipidomics 177
5. Liquid chromatography–mass spectrometry in lipidomics 179
6. Conclusions 180
Acknowledgements 180
References 180

1. Introduction

Lipidomics has been defined as "the full characterisation of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation" [1]. A distinction is drawn between lipidomics and lipid profiling by some but the differences are subtle and the terms are extensively used interchangeably. Although the lipidome is a sub fraction of the metabolome, the complexity of lipid classes and their distinct chemical properties has necessitated focused approaches to the study of these constituents of the metabolome. The interest in lipidomics has also been driven by the widening role of lipid species in the cell. In the past, the majority of lipids were considered to be either membrane components or an energy store. However, these molecules are now known to have diverse roles in transcriptional and translational control, cel-

Abbreviations: CI, chemical ionisation; CID, chemical induced dissociation; DHB, dihydroxy benzoic acid; EI, electron impact; ESI, electrospray ionisation; FAME, fatty acid methyl ester; GC, gas chromatography; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; MALDI, matrix assisted laser desorption/ionisation; MS, mass spectrometry; MS/MS, tandem mass spectrometry; *m/z*, mass to charge ratio; NCI, negative chemical ionisation; PFB, pentafluorobenzyl; PPAR, peroxisome proliferator activated receptor; SIM, selected ion monitoring; SRM, selected reaction monitoring; TLC, thin layer chromatography; TOF, time-of-flight.

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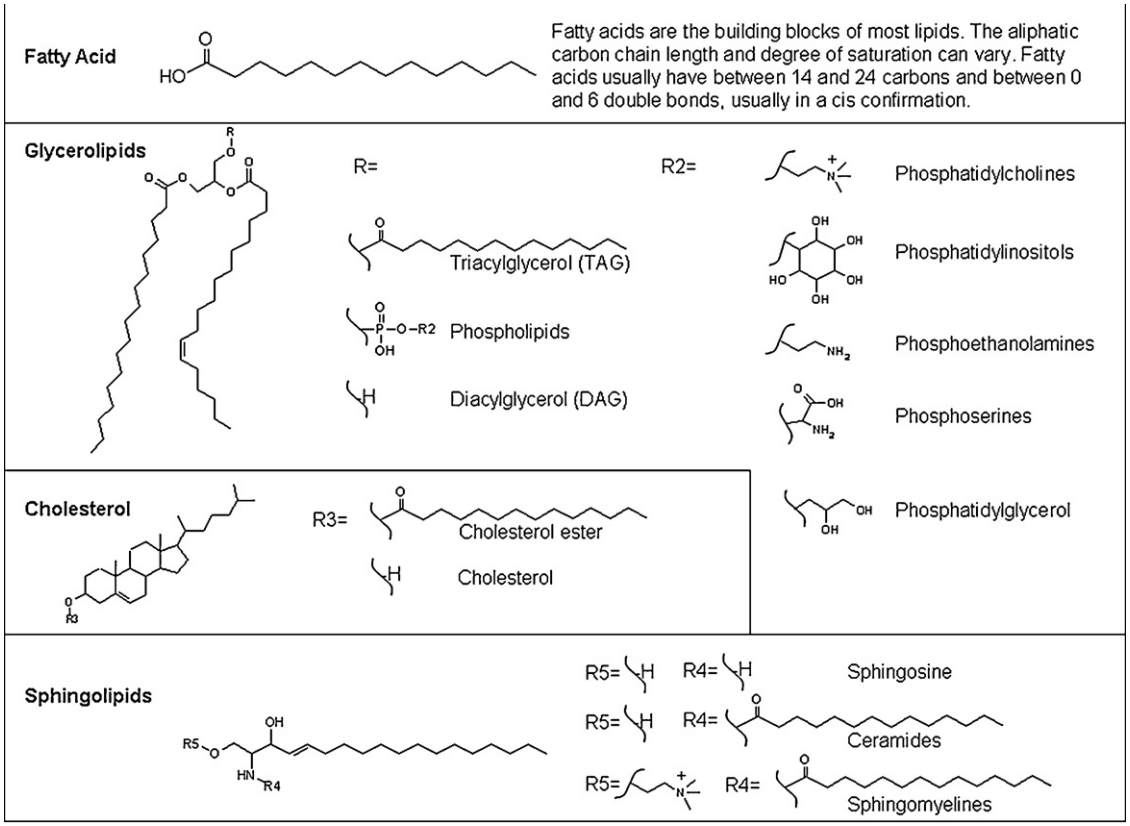

Fig. 1. Summary of the structural diversity of the most commonly analysed lipid species.

lular signaling, cell–cell interactions, and as indicators of changes to the environs of a cell or organism over time. To study the various functions of lipids, a multidisciplinary approach has been employed within lipidomics. These have encompassed: profiling of the lipid species using a range of chromatographic and spectrometric techniques and multivariate statistical data analysis [2], use of traditional biochemical techniques to study lipid–lipid or lipid–protein interactions such as immobilised lipid assays [3] and lipid–protein complex antibody assays [4], and localisation of lipids using imaging methods, for instance, fluorescently tagged lipids or optical probes for their detection [5]. Lipidomics has also proved a powerful means of investigating pathophysiological questions and defining the part lipids play in pathological states, both for diseases where lipids are known to play a prominent role such as diabetes [6] and where their roles are less well characterised such as Alzheimer's disease [7]. The development of the lipidomic field has composed an additional tier to data from proteomics and genomics, furthering our knowledge of the function of lipids within the cell [8] (Fig. 1).

This review will focus on the profiling aspect of lipidomics, giving an overview of a sample of the current chromatographic and spectrometric techniques utilised, with the aim of providing an introduction to readers interested in pursuing research within the field. The advantages and disadvantages of mass spectrometry (MS) and separation techniques such as liquid chromatography (LC), gas chromatography (GC) and thin layer chromatography (TLC) will be discussed alongside a selection of their applications in lipidomic analysis.

2. Mass spectrometry in lipidomics

The field of lipidomics has been largely driven by developments in MS. In any mass spectrometric analysis there are three distinct events: analyte ionisation, mass dependant ion separation and ion detection. The separation can be accomplished by various types of mass spectrometer such as time-of-flight (TOF), quadrupoles, magnetic and/or electric sectors or Fourier transform ion cyclotron resonance [9]. Mass analysers vary in their resolution, mass accuracy, dynamic range and capability to perform tandem-MS experiments. Generally, higher resolution and mass accuracy augments the identification of analytes and improves the number of analytes that can be separated by mass to charge ratio (m/z). Nevertheless, because the cost of high mass accuracy mass spectrometers is relatively high, a range of instruments are in use in lipidomics. Tandem-MS capability can compensate to a degree for the shortcomings of lower resolution and mass accuracy.

The primary methods employed within lipidomics for analyte ionisation are electrospray ionisation (ESI), electron impact (EI) ionisation and to a lesser extent matrix assisted laser desorption/ionisation (MALDI).

EI ionisation requires the analyte to be volatile and so it is employed almost exclusively in combination with GC. By bombarding analyte vapour with high energy electrons, EI ionisation causes analytes to fragment, producing a reproducible pattern of signals [9], which can be exploited to identify substances by searching spectral databases. However, as more complex mixtures are analysed the interpretation decreases in reliability. It can also be a challenge to identify similar species, for example, isomers of unsaturated fatty acids of identical chain length. This disadvantage is circumvented by the extraordinary resolving power of GC which reduces the propensity for analytes to co-elute. The role of GC–MS within lipidomics will be discussed in further detail later.

During the late eighties, the so called soft ionisation techniques ESI [10] and MALDI [11,12] were developed, allowing the mass spectrometric detection of non-volatile and high mass analytes such as

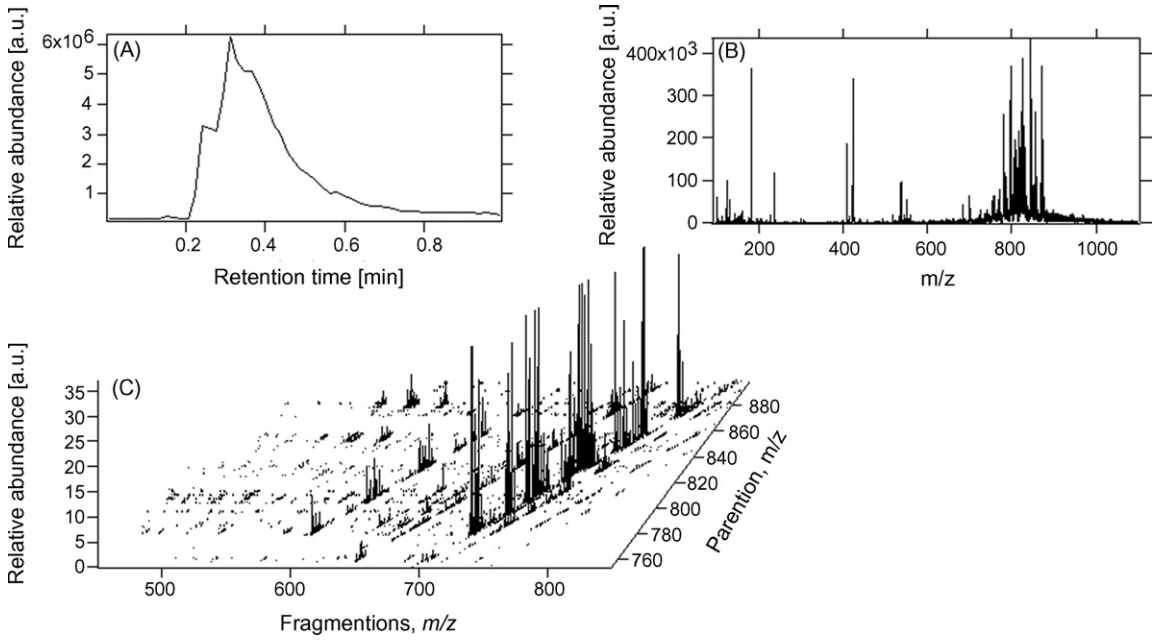

Fig. 2. Fast automated direct infusion of a mouse heart lipid extraction profile experiment in positive mode (McCombie et al. unpublished data). (A) Time course of flushing the lipid sample to the mass spectrometer allowing very short injection cycles. (B) Typical direct infusion mass spectrum of a mouse heart lipid extraction. These profiles can be used for multivariate analysis. (C) Ion map experiment of a pool of all profiled mouse heart extracts. A MS/MS experiment was performed for every *m*/*z* value allowing assignment of the changing signals in the profile.

peptides, proteins or intact lipids. A major advantage of the soft ionisation techniques is that they do not require chemical derivatisations as are necessary for GC–MS analysis. Analyte fragmentation is also minimal, which may assist the analytical interpretation of a mixture.

Concisely, during ESI the analyte is sprayed by elution through a highly charged needle tip. The charged spray droplets are heated causing evaporation of the solvent resulting in the entry of analyte ions into the mass separation unit [13]. ESI is by far the most commonly applied ionisation technique in lipidomics, partially due to the ease of coupling the eluent of an analytical LC experiment to the mass spectrometer.

The most efficient form of analysing mixtures by ESI-MS is to directly infuse the raw analyte mixture into the mass spectrometer (Fig. 2). There are a number of disadvantages to this approach, which will be discussed later; nevertheless, some impressive results have been reported. Indeed, it has been shown that direct infusion lipidomics has enabled the identification and relative quantification of over 450 phospholipids from mammalian cells [14]. Milne et al. used a triple quadrupole mass spectrometer and infused lipid extracts with a syringe from approximately 3×10^6 WEHI-231 cells [14]. Four major lipid classes could be measured in positive and seven in negative ion mode. Characteristic ion fragment patterns were used to identify measured phospholipid species. MS provides significant analyte separation, is highly sensitive [15] and can be utilised to carry out a range of experiments. A mass spectrum of the mixture may be used in metabolic "fingerprinting" experiments. Fingerprinting entails obtaining the required information to decipher metabolic alterations and is so called as it necessitates a rapid biochemical 'fingerprint' of a sample without the time consuming process of metabolite identification [16]. Mass spectrum fingerprinting has been used in the study of raw plant metabolite extractions [17]. Koulman et al. exploited whole spectra as fingerprints before carrying out further experiments to identify the signals with tandem MS (MS/MS) [17]. The advantages of this approach include its speed, relative robustness and capacity for automation. MS/MS is an experimental technique that isolates individual *m*/*z* species for fragmentation. The fragments are then separated by *m*/*z* in a second mass analyser. The *m*/*z* values of the fragments aid the identification of the isolated ion(s). For instance, phospholipid headgroups often give characteristic fragment signals either in the form of a neutral loss or a characteristic signal at a specific *m*/*z* value [18]. Direct infusion ESI has been extensively employed for lipidomic analysis enabling new insights into biological processes [19]. For example, specific lipid changes at very early stages of Alzheimer's disease have been characterised [20]. In this study, brain tissue from 22 subjects with varying degrees of Alzheimer's-induced dementia were analysed post mortem. The study found that sulphatides were far less abundant in subjects with mild dementia at the time of death while ceramides were elevated by three-fold in the very early stages of dementia. As ceramides are interpreted as degradation products of sulphatides, it is pertinent that their concentration peaks at very early stages of dementia. In another case diabetes-induced changes of specific lipid molecular species in rat myocardium [21] have been successfully characterised using direct infusion methods. Several distinct changes in lipid composition were identified after induction of the diabetic state in mice by streptozotocin administration. A remodelling of the triacylglyerols took place with a shift towards less saturated fatty acids. An increase in phosphatidylinositol and plasmenylethanolamine and a decrease in 1-stearoyl-2-arachidonoyl phosphatidylethanolamine was also observed. The authors further found that insulin treatment did not reverse the effects on triglyceride composition, suggesting that peripheral insulin treatment cannot remedy all metabolic changes in diabetic hearts.

A review on methodologies for direct infusion ESI dubbed the approach "shotgun lipidomics" in reference to the more frequently known shotgun proteomics [22]. The authors draw attention to the possibility of changing the lipid extract's pH in order to maximise the number of lipid species that can be measured and suggest measuring each extract three times. A measurement in negative mode to ionise the anionic lipid species is also recommended. After addition of lithium hydroxide, negative ion mode can detect the

weak anionic lipids and positive mode detects the neutral and polar lipids.

The use of nano-ESI, a means of increasing the sensitivity of the electrospray process by miniaturizing the electrospray tip and reducing the flow rate, has also been successful in the investigation of lipid membrane composition where phospholipid composition could be quantified on sample amounts corresponding to only 1000 cells [23]. This publication represents a significant step forward for nano-ESI-MS measurements on lipids. The authors exploited nano-ESI-MS/MS to detect individual lipid classes from CHO cell lipid extracts using precursor ion and neutral loss scanning. Furthermore, through the use of a 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine internal standard, the authors could quantify phosphatidylcholine, sphingomyelin, phosphatidylinositol, phosphatidylinositol phosphates, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and their plasmalogen analogues.

MALDI requires analyte molecules to be co-crystallised with a so called matrix—often a small organic compound such as dihydroxy benzoic acid (DHB). The crystalline sample is irradiated by a laser pulse, causing the analyte to desorbe and ionise [24]. Although its use is not as wide-spread, MALDI-MS has been successfully applied to lipid analysis [25,26] and its use in lipidomics has been the subject of a recent review [24]. The choice of matrix can have a large impact on the outcome of a MALDI experiment. DHB has been reported to work particularly well with lipid mixtures [27,26] and can be utilised for positive and negative mode measurements. However, the use of *P*-nitroanailine has been reported to enable more sensitive detection of phosphatidylethanolamines in negative ion mode [28]. As MALDI is more resistant to salt in the sample it can be harnessed to measure directly from the surface of biological materials such as brain tissue [29]. McLean et al. were able to separate the lipids from the brain without prior extraction by means of ion mobility and their spatial distribution could be studied and imaged by MALDI-MS [29]. Furthermore, lipid measurements on single zooplankter individuals have also been performed using MALDI [30]. The authors assigned some phosphatidylcholines and were able to obtain relative quantification of structural phospho- versus neutral storage lipids, allowing the study of the role of these two lipid classes during zooplankter development [30]. However, MALDI-MS imaging is a relatively modern research tool and the number of lipids detected is modest compared with other methods described in this article. It remains to be seen where the potential of the technique will lead.

Both the MALDI [31] and ESI [32] ionisation processes can suffer from strong ion suppression effects when analysing lipid mixtures [27]. The ion suppression is generated by analytes competing for charge during the ionisation process, with individual analytes' ionisation efficiencies based on their chemical characteristics [33]. This means that the observed ion count for a particular ion can change, depending on what other analytes or contaminants are being co-ionised. An even greater challenge is that ion suppression can occur even when the interfering compound is not seen in the mass spectrum. It is therefore only possible to quantify analytes in mixtures if the mixture is spiked with a suitable internal standard that has the same ionisation properties as the analyte of interest. Qualitative quantification must assume the mixtures are roughly the same between the groups of samples analysed. The problem may be circumvented, to a degree, by separating the analytes via one or more chromatographic techniques prior to MS analysis [34].

3. Thin layer chromatography in lipidomics

TLC remains infrequently used within lipidomic analysis but demonstrates great potential for the separation of lipids by their class [35]. The separated analyte species can be removed from the spots on the TLC plate and the lipids re-extracted with chloroform and methanol. The lipids can then be measured either by MALDI-, ESI-MS or GC–MS. As, to a great extent, the ionisation efficiency of a lipid is governed by its class membership the reduction in complexity ameliorates some of the ion suppression and resultant peak assignment difficulties. However, the resolution of TLC suffers when compared with other LC methods. Nevertheless, TLC–MALDI-MS has been appropriated in the phospholipid analysis of bronchoalveolar lavage, where the composition differences between human and minipig were identified, demonstrating the potential for direct MALDI measurements after insertion of the TLC plates into a mass spectrometer [36]. Proof of principle has also been demonstrated with the study of phospholipids from egg yoke [37].

4. Gas chromatography–mass spectrometry in lipidomics

Gas chromatography was first proposed in a 1941 paper by Martin and Synge in which it was suggested that the mobile phase in a liquid–liquid chromatography system could be replaced with a vapour [38]. Following advances in technology, it became possible to couple the chromatographic technique to MS. During the 1950's, Gohlke and McLafferty united a gas chromatograph to a TOF mass spectrometer founding the technique of GC–MS [39]. GC–MS has since become a key research technique in a vast array of fields and is a core tool in lipid biochemistry and lipidomics.

GC–MS is a powerful technique for the analysis of metabolites allowing separation, identification and quantification and is routinely utilised within lipidomics to investigate the metabolism of fatty acids. However, due to the complexity of lipid extracts from biological sources it is often considered important to perform a separation process prior to GC–MS analysis. Amongst the methods employed are solid phase extraction [40], high performance liquid chromatography (HPLC) [41] and TLC [42]. For example, Watkins et al. used TLC to separate different lipid species according to polarity prior to analysis of the fatty acids that were present within a particular class of compounds [43].

Following extraction and preliminary chromatographic procedures, applied to biological samples to concentrate the volatile, low molecular mass lipids and fatty acids, a chemical derivatisation step is usually performed. Since GC–MS requires the analytes to be volatile and thermally stable, derivatisation is used to reduce unwanted absorption effects, increase the volatility of polar compounds, remove polar functional groups or generate derivatives as an aid to identification. The most frequent derivatisation of fatty acids is hydrolysis of complex lipids followed by methylation to form fatty acid methyl esters (FAMEs). A range of methods have been described in the literature to perform methylation of fatty acids, including acidic esterification [44], and the use of diazomethane [45]. The chemical derivatisation used is dependent on the focus of analysis, examples include as diverse procedures as basic esterification of *O*-acyl lipids and fatty acids of plant or bacterial origin [46,47], trimethysilyl esterification [48], removal of the polar head group, or base saponification of the two fatty acid groups of glycerophospholipids, formation of pyrrolidides [49] and picolinyl esterification [50].

A key aspect of lipid analysis and lipidomics is the ability to identify the fatty acids once they have been detected. Novel chromatographic and spectrometric techniques have been employed using GC–MS to classify observed fatty acids. The stationary phase of the GC column selected for analysis has a significant impact on the identification process. Non-polar silicone phases separate the fatty acid methyl esters based almost exclusively on molecular weight, where as using high molecular weight hydrocarbons

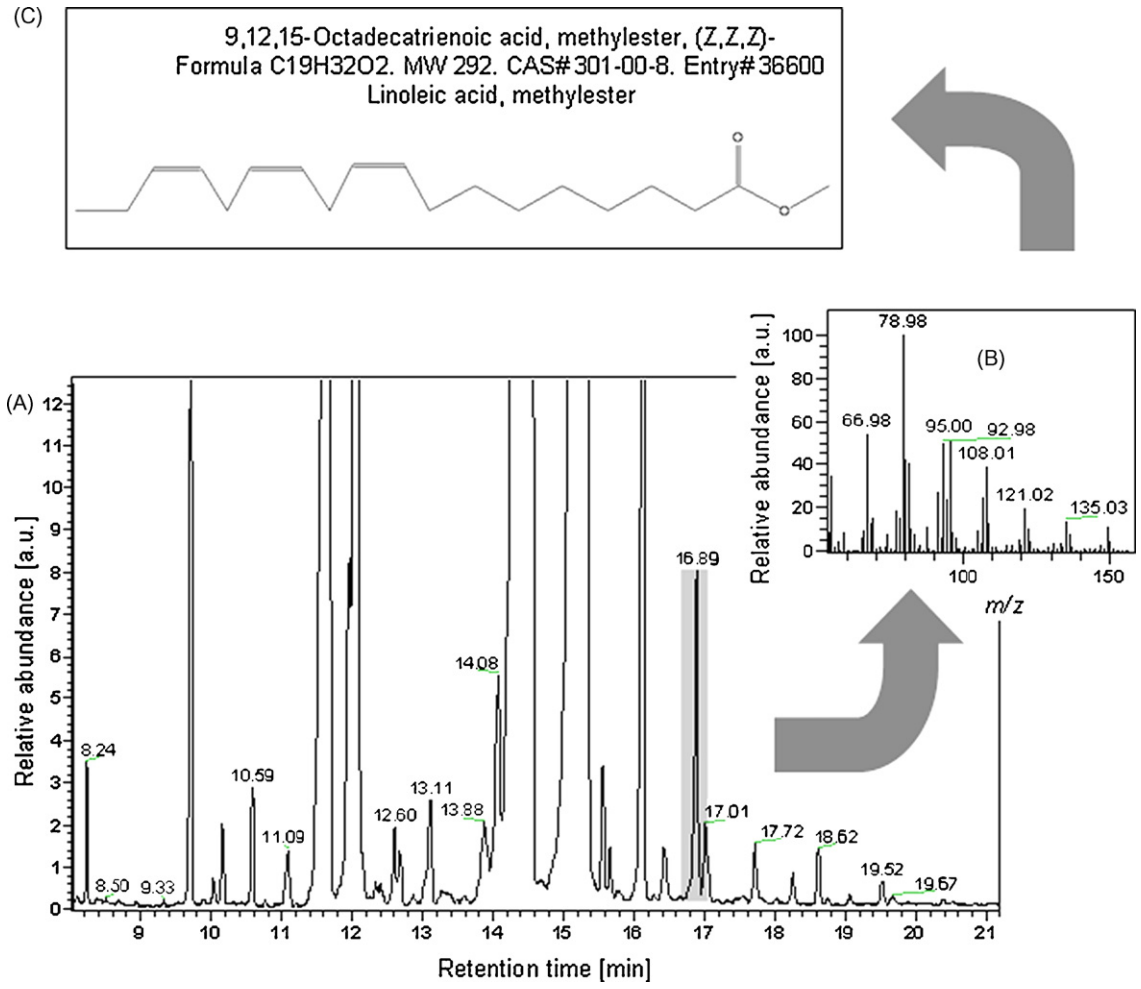

Fig. 3. Example of FAME analysis by GC–MS. The sample comes from an extract of mouse adipose tissue after a 24 h fasting period (Roberts et al. unpublished data). (A) Total ion chromatogram with a peak highlighted at 16.89 min. (B) Mass spectrum of the highlighted peak in A. (C) A National Institute of Standards and technology (NIST) library search result of the mass spectrum. With a probability score of 50 the peak at 16.89 is linolenic acid.

allow for separation of saturated and unsaturated components of the same chain length. The use of polar polyester columns also permits the separation of esters of the same chain length to the extent that, using highly polar cyanoalkylpolysiloxane phases gives excellent discrimination of *cis*, *trans*-isomers of monounsaturated fatty acids. Given suitable chromatographic separation, provisional identifications can be made using a comparison of the analyte retention times with those of commercially available standard mixtures of known methyl esters of saturated monoenoic and polyenoic fatty acids. However, these identifications must only be viewed as tentative and highlight the benefit of a combined chromatographic-spectrometric technique.

As mentioned previously, EI-MS is most commonly used in combination with GC and offers a very robust system for the identification of fatty acids especially when combined with alternative chemical derivatisation; pyrolidide formation and picolinyl esterification can be used in addition to methyl esterification of fatty acids as an aid to identification. Harvey demonstrated that EI-MS of picolinyl esters resulted in the formation of ions used to determine chain branching and the position of double bonds within fatty acids [51]. In cases where excessive fragmentation of parent ions must be avoided, chemical ionisation (CI) is often utilised. An analyte fatty acid can be identified based on characteristic fragment ions, with alternatively derivatised saturated straight chain fatty acids, unsaturated fatty acids, branch chain fatty acids, carbocyclic fatty acids and oxygenated fatty acids, amongst others, demonstrating unique ions in their mass spectrum (Fig. 3). GC–MS/MS methods capable of analysing complex mixtures of fatty acids that employ electron capture negative chemical ionisation (NCI) of pentafluorobenzyl esters of fatty acids have been established [52]. Furthermore, through the use of a highly selective MS/MS scanning mode known as selected reaction monitoring (SRM) quantitative analysis of very complex mixtures can be achieved. SRM detects a specific chemical species excluding potential interference from chemical noise and coeluting compounds with identical masses. Nevertheless, it is worth noting that MS can rarely provide verification of the arrangement of functional groups in a fatty acid or distinguish the stereochemistry.

GC–MS has been applied within lipid biochemistry and lipidomics in answer to a diversity of biological questions. Analysis of FAMEs extracted from a range of tissues from 1-month-old peroxisome proliferator-activated receptor (PPAR) α knockout mice using GC/EI-MS identified an increase in hepatic saturated fatty acids with a concomitant decrease in unsaturated fatty acids [53]. The authors concluded this may have been an early indicator of steatosis, a common manifestation of dyslipidemia in PPAR α knockout mice. Oursel et al. used a variety of GC–MS techniques to study the lipidome of *Escherichia coli* [54]. GC/EI–MS analysis of the *E. coli* fatty acid picolinyl esters combined with the GC/NCI–MS/MS analysis of pentafluorobenzyl esters was used to identify fatty

acids in bacterial membrane extracts. Relative quantification of the fatty acids was also achieved using GC/CI–MS of the *E. coli* FAMEs with a methyl heptadecenoate internal standard. GC/EI–MS lipidomic analysis has also been exploited to identify changes in lipid metabolism upon hormone-induced differentiation of 3T3-L1 cells into mature adipocytes [55]. Using GC/EI–MS, Su et al. analysed dimethyl disulphide derivatised unsaturated FAMEs obtained from triacylglycerol, phosphoethanolamine and phosphocholine HPLC fractions of the total lipid extract. Using the tendency of dimethyl disulphide adducts of alkenes to undergo a characteristic fragmentation, it was shown that during adipocyte differentiation α oxidation of fatty acids precedes Δ9 desaturation prior to being used in the synthesis of triacylglycerols; likewise, it was shown that in unsaturated fatty acyl moieties of the phosphatidylcholines and phosphoethanolamines the double bond was almost solely located at the Δ9 position, therefore confirming unsaturated fatty acids are not metabolised by peroxisomal α oxidation but are used in lipid anabolic processes.

Lipidomic methods have also been developed to monitor secondary metabolism of fatty acids. Kawai et al. used GC/EI–MS with selected ion monitoring (SIM) to measure lipid-peroxidation-derived aldehydes [56]. Following lipid peroxidation, lipid hydroperoxides are formed which decompose to aldehydes. Liver samples from mice intraperitoneally injected with bromobenzene, which increases lipid peroxidation in the liver, were compared to control animals. Aldehydes were extracted, derivatised to pentafluorobenzyl (PFB)-oximes and analysed with GC/EI/MS-SIM. Fragmentation of PFB-derivatised aldehydes produces an ion of *m*/*z* 181 making the SIM experiments possible. Nine aldehydes were detected in the livers of the mice and significant differences were identified between bromobenzene treated and control livers.

GC–MS using high-resolution capillary columns and MS detection is a versatile tool for use in lipidomic studies and allows for accurate identification of biological sample constituent metabolites. Nevertheless, the technique is limited by its dynamic range, preventing the analysis of larger lipid species. The technique also requires that compounds be thermally stable with high enough vapour pressure to volatilise during injection. Chemical derivatisation for GC–MS has been used to overcome this problem but in itself can introduce variability to the samples and can mask lipid structural information. Chemical derivatisation also increases sample preparation and when combined with a long chromatographic run reduces the throughput of GC–MS as an analytical technique. The multiple procedures required prior to GC–MS analysis also increase the risk of contamination and recovery losses. A number of these concerns can be overcome using a direct thermal desorption interface, the liner of a GC injector can be used as a sample and reaction vessel, which allows the exclusion of the initial lipid extraction stage and thermally assisted methylation to be carried out in automation. Akoto et al. used just such a process to analyse FAME profiles from human plasma and whole blood samples, demonstrating comparable yields of saturated fatty acids and substantially improved yields of polyunsaturated fatty acids to those obtained with the more traditional techniques [57]. However, by using GC–MS in combination with other analytical techniques not only are these restrictions surmounted but the coverage of the lipidome can by significantly increased.

5. Liquid chromatography–mass spectrometry in lipidomics

As previously mentioned, a number of lipidomic studies have directly infused the sample into the ESI source [18,58–60,17,61], but ion suppression by certain prominent phospholipids, especially when using positive ionisation, can affect both low and high concentration analytes [27,8,62]. Ionisation effects can be reduced with an initial, either offline or online, HPLC separation. The use of HPLC for lipid analysis is not a new occurrence. Normal phase chromatography in the separation of lipid classes, particularly phospholipids, is well documented, though HPLC was originally, and often still is, coupled to either evaporative light scattering detection [63,64] or ultra-violet detection [65]. These detectors can suffer from serious constraints in the choice of mobile phase and lack of selectivity [66]. Thus, for lipidomic applications of HPLC the detector of choice is MS, in particular ESI-MS [67] (Fig. 4).

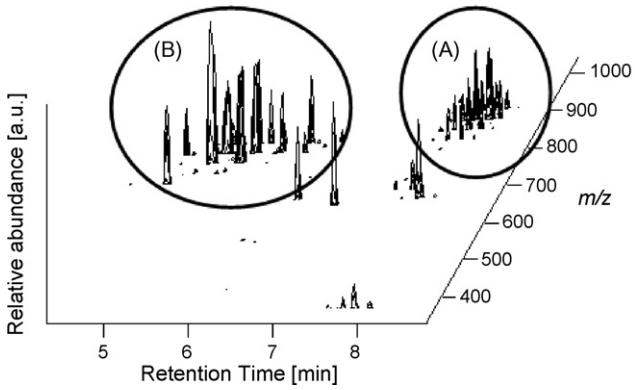

Fig. 4. Two-dimensional representation of a ultra performance liquid chromatography (UPLC)-TOF-MS measurement of a chloroform/methanol lipid extraction of human plasma in positive mode. The area marked A contains predominantly triacylglycerols while region B primarily contains phospholipids (Titman et al. unpublished data).

A major advantage of MS detection is the ability of the technique to deconvolute overlapping peaks. In terms of chromatography, lipidomic procedures performed using normal phase chromatography will separate the lipids into different classes. These methods have been applied to biological fluids, including blood plasma, where HPLC-MS has been used to identify potential biomarkers of type II Diabetes Mellitus, implicating certain phosphoethanolamines and lyso-phosphocholines [68]. Commonly lipidomic analysis is performed with reversed-phase chromatography [69], where separation of lipids within the same class is based on carbon chain length and the number of double bonds present, essentially their lipophilicity [70]. Though, the most detailed map of class and molecular species is obtained with a combination of normal phase fractionation followed by reversed-phase analysis [71].

Of the complex lipids, phospholipids are the most abundant lipid class present in eukaryotic cells [22] and methods of phospholipid analysis have been reviewed elsewhere [34,72]. When analysing phosphatidylcholines the preferred means of detection is positive ionisation; where upon collision induced dissociation (CID) a fragment of *m*/*z* 184 is produced corresponding to the protonated phosphocholine head group [73]. Whereas negative mode demonstrates greater sensitivity towards other phospholipid classes such as the phosphatidyl-ethanolomines, -serines, and -inositols [73–75]. As with other chromatographic methods, the choice of stationary phase and mobile phase, along with the choice of solvents, is paramount in the experimental design. Lipidomic analyses have employed both C_{18} and C_8 reversed phase columns [76,77]. The use of columns with sub-2 μm particle size along with elevated column temperature or temperature gradients is also favoured for higher resolutions and faster separations [77]. Solvents used for elution from the column include methanol, isopropanol, acetonitrile, hexane and chloroform, often with the addition of formic acid and ammonium bases as ion pairs [78]. The choice of solvent is highly dependent on the types of experiment being

carried out and the analytes themselves since the solvent affects factors such as solubility, formation of analyte adducts and ionisation. In combination with ESI, and with the use of a triple quadrupole mass spectrometer, precursor ion scanning, neutral loss scanning and multiple reaction monitoring a means for lipid species detection and quantitation is provided [79,80]. As with direct infusion MS, nano-ESI has been used in combination with HPLC, to reduce the sample requirement for each analysis. Additionally, the use of CI in the analysis of non-polar lipids such as triacylglycerols can improve their detection, by lessening fragmentation, whilst still enabling analysis of polar phospholipid species [81,82].

Libraries to assist in sample identification for LC–MS lipidomic data are limited. Unlike EI mass spectra, ESI-MS is more affected by the instrument type, ion source, ion source potentials, mobile phases and other factors affecting fragmentation patterns [83]. However, a number of resources are available to a newcomer to lipidomics. These provide pertinent reference material and structural information of MS/MS fragmentation. Examples of these include LIPID MAPS (www.lipidmaps.org [84]), The Lipid Library (http://www.lipidlibrary.co.uk/), Cyber Lipid Center (http://www.cyberlipid.org/), LIPIDAT (http://www.lipidat.ul.ie/ [85]) and LipidBank (http://lipidbank.jp/).

In an earlier section, we described how GC–MS has become the method of choice for fatty acid analysis. However, the sensitivity and hence detection capabilities are matched and can be enhanced by the use of LC–MS and LC–MS/MS methods. Samples still require derivatisation but dependent on the derivatisation agent used the method can be sensitive to the fmol range and enable high-quality CID for quantitation, as is the case for trimethylaminoethyl ester derivatives [86]. Here, atherosclerotic plaques were used for lipid extraction and the fatty acid profiles from the carotid artery were analysed.

The potential of lipidomic analysis in drug research has not gone unnoticed due to the importance of lipids in cardiovascular, diabetes and related inflammatory disorders [87]. Determination of lipid changes associated with the conversion of normal high density lipoprotein (HDL) to pro-inflammatory HDL (acute-phase HDL) have been analysed by normal phase LC–MS. Phosphatdiylcholine and sphingomyelin ratios were increased and diacyl and alkenylacyl glycerophosphatidylethanolamine and phosphatidylinositol were decreased, which may be indicative of proinflammatory and proatherogenic roles [88]. Lipidomic analysis of the liver from the *ob*/*ob* mouse, a classic model of obese insulin resistance and hepatic steatosis, using reversed-phase LC–MS revealed an increase of tri- and di-acylglycerols, diacylphosphoglycerols and certain ceramides and a decrease in the concentration of sphingomyelins [89]. Furthermore, the analysis in this study was supported by complementary gene expression data which concurred with the lipidomic results. Lipidomic analysis using LC–MS has also been used in combination with transcriptomic, immuno-histological and other clinical markers in an investigation using the POKO mouse, a model of PPAR γ 2 ablation on an *ob*/*ob* background [76]. The POKO mice were found to have decreased concentrations of triacylglycerides but increased concentrations of diacylglycerides, with an increase of two ceramide species and three lysophosphatidylcholine species compared to the *ob*/*ob* or wild-type mice, and as with the *ob*/*ob* mice decreased sphingomyelin concentrations were detected. Pietilainen et al. used LC–MS lipidomics to study the global serum lipid profiles from monozygotic twins determined that acquired obesity, independent of genetic influences, was linked with an increase in the concentration of lysophosphatidylcholines [70]. Again, these results were correlated and considered alongside clinical measurements revealing the propriety of lipidomic analyses in human studies.

6. Conclusions

The recent and rapid developments in the areas of MS and chromatography have lead to significant advances within the field of lipidomics. An extensive range of profiling technologies is now available to the lipid analyst and their applications have proven to be diverse. This review has outlined a number of considerations that must be taken into account when beginning lipidomic research. The choice of mass spectrometer with regards to sensitivity, resolution, cost, MS/MS capabilities and the ionisation source and the use of chromatographic separation must be suitable for application to the particular lipid species of interest. Direct infusion ESI-MS has been used extensively to examine whole lipid extracts and has shown promise in the identification and relative quantification of phospholipid and fatty acid species in a rapid and robust manner. However, it is important to remember that both ESI- and MALDI-MS are susceptible to the phenomena of ion suppression, a disadvantage that can be overcome to some extent by chromatographic techniques, especially when analysing polar phospholipids. LC–MS can also be used to separate lipids from complex biological fluids into individual lipid classes or separate lipids within the same class based on the chromatographic phase used, though choice of solvent also plays a critical role. GC–MS is a powerful tool for the analysis of fatty acids and their derivatives but is limited by its dynamic range and the requirement that analytes be volatile, hindering analysis of larger lipids. The coverage of the lipidome that any one of the methods described in this review can achieve is still far from complete. However, through a combined analytical approach utilising a range of technologies a more inclusive picture of the lipidome can be acquired by overcoming the limitations of individual techniques. To achieve a fully comprehensive lipidomic map will require a collective approach allying traditional lipid biochemistry, lipidomic profiling and the swiftly evolving area of lipid bioinformatics.

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