Lipidomics, a branch of metabolomics, is the large-scale study of pathways and networks of cellular lipids in biological systems such as cells, tissues, or organisms. The recent advance in mass spectrometry technologies have enabled more comprehensive lipid profiling in the biological samples. In this review, we compared four representative lipid profiling technologies including GC-MS, LC-MS, direct infusion-MS and imaging-MS. We also summarized representative lipid database, and further discussed the applications of lipidomics to the diagnostics of various diseases such as diabetes, obesity, hypertension, and Alzheimer diseases.

**Key Words:** Biomarker, Database, Lipidomics, Mass spectrometry, Profiling

**INTRODUCTION**

Lipids are broadly defined as fat-soluble molecules that include a wide range of molecular structures [1]. Lipids exhibit a wide variety of cellular functions such as cellular structural support, energy storage, protein trafficking, maintenance of electrochemical gradients, and cell signaling. They also play a vital role in Alzheimer’s disease [2,3], cardiovascular diseases [4,5], inflammation [6], and metabolic diseases such as diabetes [7,8], hyperlipidemia [9], hypertension [10,11], and obesity [12,13]. Lipidomics, a branch of metabolomics, is the large-scale study of pathways and networks of cellular lipids in biological systems such as cells, tissues, or organisms [14]. Lipidomics can also be defined as “the full characterization of lipid molecules and their biological functions with respect to expression of proteins involved in lipid metabolism and function, including gene regulation” [15]. The field covers the quantitative and qualitative determination of lipids in time and space, the study of lipid transporters and lipid-metabolizing enzymes, and lipid-lipid and lipid-protein interactions [16,17]. Lipids are classified as fatty acids, steroids, glycerolipids [monoacylglycerol (MG), diacylglycerol (DG), triacylglycerol (TG), cardiolipin (CL), cholesterol ester (CE)], glycolipids [monogalactosylDG (MGDG), digalactosylDG (DGDG), sulfogalactosylDG (SQDG)], phospholipids [phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), lysophospholipids], sphingolipids (ceramides, sulfatides, gangliosides), prenol lipids, and polyketides. Of the 40,000 metabolites recorded in the Human Metabolome Database (HMDB), nearly 70%, up to 28,000, are lipid metabolites, the highest percentage among the various types of metabolites [17].
LIPIDOME PROFILING

In the field of metabolite research, nuclear magnetic resonance (NMR) and mass spectrometry (MS) are frequently used in metabolite profiling. NMR has advantages in analytic reproducibility but has low sensitivity, making it unsuitable for analysis of small sample concentrations, such as those involved in lipid metabolite analysis. Thus, analytic equipment based on MS is most commonly used in lipidomics research. Based on the method of sample introduction, MS is classified as gas chromatography (GC)-MS, liquid chromatography (LC)-MS, or direct infusion-MS, with each method having its own advantages.

1. GC-MS-based lipid profiling

GC-MS is best for the analysis of lipids such as free fatty acids (FFAs) and steroids. Generally, FFAs and steroids are analyzed by transforming the compounds into volatile esters via silylation derivatization (Table 1). The most prominently used derivatization reagent for silylation is N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), while ammonium iodide (NH4I), dithioerythritol (DTE), trimethylchlorosilane (TMCS), trimethylsiloisilimazole (TMSIm) are often added to accelerate the process [18]. FFA and steroid analyses often require methylsiloxane or 5% diphenylpolysiloxane columns. High temperature-compatible columns like a silicoel steel capillary column (MXT-1) are used when increasing the GC oven temperature for the analysis of low volatile compounds like fatty acid esters of steroids and cholesterol esters [19].

2. LC-MS-based lipid profiling

For the analysis of phospholipids, neutral lipids, and sphingolipids, which are greater in molecular weight and less volatile than FFAs and steroids, LC-MS is mainly used. Lipid profiling via LC-MS is more advantageous than GC-MS in that it does not require a derivatization reaction. Generally, reverse phase columns like C8/C18 or hydrophilic interaction liquid chromatography (HILIC) columns are used for lipid analysis (Table 2). To increase the separation of lipids, modifiers can be added to the mobile phase,
## Table 2. Chromatographic conditions for the profiling of phospholipids, neutral lipids, and sphingolipids

| Analyte | Sample | Column | Mobile phase | Analytical platform | Reference |
|---------|--------|--------|--------------|--------------------|-----------|
| PLs, DG, TG, CE, CL Ceramide | Plasma, urine | HILIC column (3 μm, 2.1 × 100 mm) | A: CH₃CN/CH₃OH (9:1, v/v), B: H₂O/CH₃OH/CH₃CN (5:4:1, v/v) Modifier: 5 mM NH₄HCO₃ and 0.05% NH₄OH | UPLC-ESI-MS/MS (+/- mode) | [25] |
| PLs, DG, TG, CE, CL Ceramide | Plasma, urine | C18 column (3 μm, 0.075 × 60 mm) | A: H₂O/CH₃CN (9:1, v/v), B: CH₃OH/CH₃CN/i-PrOH (2:3:5, v/v) Modifier: 1 mM NH₄HCO₃ and 0.05% NH₄OH | Nano LC-ESI-MS/MS (+/- mode) | [25] |
| PC, PE, SM, DG, TG, Ceramide | Tissue C8 column (1.7 μm, 2.1 × 100 mm) | A: CH₃CN/H₂O (3:2, v/v), B: i-PrOH / CH₃CN (9:1, v/v) Modifier: 10 mM NH₄HCO₃ | UPLC-ESI-MS/MS (+ mode) | [27] |
| PLs | Plasma | C18 column (3 μm, 0.05 × 85 mm) | A: H₂O/CH₃CN (9:1, v/v), B: i-PrOH / CH₃CN (9:1, v/v) Modifier: 0.1% FA or 0.05% NH₄OH | Nano LC-LCQ MS (+/- mode) | [119] |
| PLs | Urine | RPLC column (0.075 × 50 mm) | A: H₂O/CH₃CN (9:1, v/v), B: CH₃OH/CH₃CN/i-PrOH (2:3:5, v/v) Modifier: FA/NH₄HCO₃/NH₄OH/NH₄Ac | Nano LC-LTQ MS (+/- mode) | [26] |
| PC, PE, PI, SM | Serum C18 column (1.7 μm, 2.1 × 100 mm) | A: CH₃CN/10 mM NH₄Ac + 0.1% AcOH (3:2, v/v) B: i-PrOH:CH₃CN:10 mM NH₄Ac + 0.1% AcOH (88:10:2, v/v) Modifier: FA/NH₄HCO₃/NH₄Ac | LC-Orbitrap MS (+/- mode) | [24] |
| PIP | Cell C8 column (3.5 μm, 1.0 × 150 mm) | A: MeOH/H₂O/70% Ethylamine (20:80:0.13, v/v) B: i-PrOH/70% Ethylamine:100 mM:0.13, v/v) | LC-LTQ-Orbitrap MS (+/- mode) | [20] |
| LPC, LPE Sphingolipid | Serum C18 column (3 μm, 2.1 × 150 mm) | A: H₂O with 0.1% FA, B: CH₃CN with 0.1% FA | LC-QTOF MS (+ mode) | [120] |
| FA | Tissue C8 column (1.7 μm, 2.1 × 100 mm) | A: 10 mM Ammonium acetate (pH 5), B: CH₃CN | UPLC-ESI-MS/MS (- mode) | [27] |
| FA | Plasma Diphenyl column (1.9 μm, 3.0 × 100 mm) | A: H₂O with 5 mM NH₄Ac + 2.1 mM AcOH B: CH₃CN/ i-PrOH (4:1, v/v) | LC-ESI-MS/MS (- mode) | [121] |
| FA (AMPP derivatization) | Serum C18 column (1.7 μm, 2.1 × 100 mm) | A: H₂O with 0.1% FA, B: CH₃CN with 0.1% FA | UPLC-ESI-MS/MS (+ mode) | [21] |
| Acylcarnitine | Tissue Silica-based bonded column (1.8 μm, 2.1 × 100 mm) | A: H₂O with 0.1% FA, B: CH₃CN | UPLC-ESI-MS/MS (+ mode) | [27] |
including ethylamine [20], formic acid [21,22], ammonium acetate [23,24], and ammonium formate [25-27]. Bang et al. [26] compared the phospholipid analysis sensitivity of different types of mobile phase additives (ammonium hydroxide, ammonium acetate, and ammonium formate) and reported that a modifier containing a mixture of 0.05% ammonium hydroxide and 1 mM ammonium formate (pH 9.3) yielded the greatest improvement in analytical sensitivity. In addition, MS-selected reaction monitoring (SRM) [28-31] is used for the quantitative analysis of lipids, while precursor ion scanning or neutral loss scanning is used for type-specific selective lipid profiling [32-35]. Recently, Bollinger et al. [21] derived N-(4-aminomethylphenyl)pyridinium (AMPP) from a fatty acid and analyzed it via LC-MS in SRM mode, reporting a 60,000-fold increase in analytical sensitivity compared with underivatized fatty acids.

### 3. Direct infusion-MS-based lipid profiling

In 1994, Han et al. [36] directly injected a sample for lipid profiling into a mass spectrometer, avoiding the negative effects of chromatography, increasing the signal to noise ratio, and establishing what is now known as the direct infusion-MS method. Unlike GC-MS and LC-MS, which utilize columns to separate compounds, direct infusion-MS has the advantage of a shortened analysis time but is disadvantageous in that lipid compounds with the same m/z will not separate. To address this issue, MS with high resolution or detection methods specific for certain lipid types (e.g., neutral loss scanning or precursor ion scanning) are used in direct infusion-MS (Table 3) [37-43]. To detect trace amounts of lipids in samples, Wang et al. [44] and Han et al. [34] produced and analyzed derivatives by reacting DG with N,N-dimethylglycine (DMG) [44] and PE with

### Table 3. Diagnostic ions used to identify major lipids in direct infusion-MS

| Analyte           | Adduct ion      | Diagnostic ions | Class information | Acyl chain information | Reference |
|-------------------|-----------------|-----------------|-------------------|------------------------|-----------|
| Glycerophosphate  | [M - H]         | PI* (m/z 153)   |                   |                        | [33]      |
| Cholesterol ester | [M + NH4]+      | PI (m/z 369)    |                   |                        | [122]     |
| DG                | [M + DMG + Li]+ | NL (m/z 103, 87), PI* (m/z 110) | NL (FA) | | [44]      |
| TG                | [M + DMG + Li]+ | NL (FA)         |                   |                        | [32]      |
| Cardiolipin       | [M - 2H]        | PI (m/z 153)    |                   |                        | [45]      |
| MGDG, DGDG        | [M + Na]*       | PI (m/z 243)    |                   |                        | [123]     |
| MGDG              | [M + NH4]*      | NL (m/z 179)    |                   |                        | [124]     |
| DGDG              | [M + NH4]*      | NL (m/z 341)    |                   |                        | [124]     |
| SQDG              | [M - H]         | PI (m/z 225)    |                   |                        | [125]     |
| PC, SM            | [M + H]+        | PI (m/z 184)    |                   |                        | [46]      |
| PC                | [M + Li]+       | NL (m/z 59, 189, 213) | NL (m/z 59+FA, FA) | | [126]     |
| LPC               | [M + Na]*       | NL (m/z 59, 205), PI (m/z 104, 147) | | | [126]     |
| PE, LPE           | [M - H]         | PI (m/z 196)    |                   |                        | [33]      |
| PE, LPE           | [M - H + Fmoc]  | NL (m/z 222)    |                   |                        | [34]      |
| PE                | [M + H]+        | NL (m/z 141)    |                   |                        | [46]      |
| PI, LPI           | [M - H]         | PI (m/z 241)    |                   |                        | [127]     |
| PI                | [M + NH4]*      | NL (m/z 277)    |                   |                        | [46]      |
| PIP               | [M - H]         | PI (m/z 321)    |                   |                        | [128]     |
| PIP2              | [M - H]         | PI (m/z 401)    |                   |                        | [128]     |
| PS, LPS           | [M - H]         | NL (m/z 87)     |                   |                        | [127]     |
| PS                | [M + H]+        | NL (m/z 185)    |                   |                        | [127]     |
| PG, PA, LPG, LPA  | [M - H]         | PI (m/z 153)    |                   |                        | [129]     |
| SM                | [M + Li]+       | NL (m/z 183, 213, 429, 431) | | | [126]     |
| Sphingosine       | [M + H]+        | PI (m/z 264, 282) | | | [130]     |
| Sphingosine       | [M - H]         | NL (m/z 240, 327) | | | [127]     |
| Sphinganine       | [M + H]+        | PI (m/z 266, 284) | | | [130]     |
| Sulfatide         | [M - H]         | PI (m/z 97)     |                   | NL (sphingoids)        | [131]     |

*Precursor ion scan, †Neutral loss scan.
fluorenylmethyloxycarbonyl chloride [34]. Modifiers such as LiOH, LiCl, and ammonium acetate are also often added to improve the formation of adduct ions [44-46].

4. Imaging MS-based lipid profiling

Recently, there has been a significant increase in the identification of biomarkers related to disease through analysis of lipid metabolites present in tissues via imaging MS. The matrix-assisted laser desorption/ionization technique is mainly used in imaging MS to ionize lipid metabolites within samples. Ionization efficiency is increased for specific lipid classes by changing the solvent concentrations or modifier compositions in the matrix added to the samples. The most highly used matrices for lipid profiling by imaging MS include 9-aminoacridine [47], α-cyano-4-hydroxycinnamic acid (CHCA) [47], 2,6-dihydroxyacetophenone (DHA) [48-50], 2,5-dihydroxybenzoic acid (DHB) [48,51], and 2-mercaptobenzothiazole (MBT) (Table 4) [52]. Piperidine

| Analyte          | Matrix                        | Adduct ion                  | Reference |
|------------------|-------------------------------|------------------------------|-----------|
| TG, DG           | 80% MeOH                      | [M + K]+, [M + H]+          | [53]      |
| TG               | 70% MeOH, 50 mg/mL 2,5-Dihydroxybenzoic acid | [M + K]+                   | [51]      |
| Cholesterol      | 80% MeOH, None                | [M + H - H2O]+              | [53]      |
| Cholesterol      | 50% EtOH, 20 mg/mL 2,5-Dihydroxybenzoic acid | [M + H - H2O]+              | [48]      |
| PE, PG, PI, PS, ST, ST, GM1 | 30 mg/mL 2,6-Dihydroxyacetophenone | [M - H]+                   | [49]      |
| PA, PE, PG, PI, PS, ST, ST, GM1 | 7 mg/mL DHA, 7 mg/mL CHCA, 0.1% TFA, 1% Piperidine | [M - H]+                   | [47]      |
| LPC, PC, SM      | 80% MeOH, 10 mM Potassium acetate | [M + K]+, [M + H]+, [M + Na]+ | [53]      |
| LPC, PC          | MeOH, 2-Mercaptobenzothiazole (MBT) | [M + H]+, [M + Na]+, [M + K]+ | [52]      |
| LPC, PC          | 70% MeOH, 50 mg/mL 2,5-Dihydroxybenzoic acid | [M + K]+, [M + H]+, [M + Na]+ | [51]      |
| PC, SM           | Ethyl acetate, 0.5 M 2,5-Dihydroxybenzoic acid, 0.1% TFA | [M + H]+, [M + Na]+, [M + K]+ | [132]     |
| PC               | 50% EtOH, 10 mg/mL 2,6-Dihydroxyacetophenone | [M + H]+, [M + K]+         | [48]      |
| PC               | 50% EtOH, 30 mg/mL 2,6-Dihydroxyacetophenone | [M + H]+, [M + Na]+, [M + K]+ | [50]      |
| PC               | 50% EtOH, 30 mg/mL 2,6-Dihydroxyacetophenone + 100 mM LiCl | [M + Li]+, [M + H]+, [M + K]+ | [50]      |
| PC               | 70% MeOH, 7 mg/mL DHA, 7 mg/mL CHCA, 0.1% TFA, 1% Piperidine | [M + H]+, [M + Na]+, [2M + H]+, [2M + K]+ | [47]      |
| PA, PS           | 70% MeOH, 7 mg/mL DHA, 7 mg/mL CHCA, 0.1% TFA, 1% Piperidine | [M + K]+                   | [47]      |
| PE, SM           | 50% EtOH, 10 mg/mL 2,6-Dihydroxyacetophenone | [M + H]+                   | [48]      |
| SM               | 70% MeOH, 7 mg/mL DHA, 7 mg/mL CHCA, 0.1% TFA, 1% Piperidine | [M + Na]+, [M + H]+, [M + K]+ | [47]      |
| PE               | MeOH, 2-Mercaptobenzothiazole | [M + Cs]+, [M + H]+, [2M + H]+ | [52]      |
| PI, Sulfatide    | 50% EtOH, 20 mg/mL 2,5-Dihydroxybenzoic acid | [M - H]+                   | [48]      |
| PI               | MeOH, 2-Mercaptobenzothiazole | [M + K]+                   | [52]      |
| ST               | 70% MeOH, 7 mg/mL DHA, 7 mg/mL CHCA, 0.1% TFA, 1% Piperidine | [M + K]+                   | [47]      |
| Ceramide         | Ethyl acetate, 0.5 M 2,5-Dihydroxybenzoic acid, 0.1% TFA | [M + Na]+                   | [132]     |
| Ganglioside      | 50% EtOH, 10 mg/mL 2,6-Dihydroxyacetophenone | [M - H]+, [M + Na - 2H]+, [M + K - 2H]+ | [48]      |
and trifluoroacetic acid (TFA) are also used as ion pairing agents to mix such matrices [47]. MBT is low in vapor pressure and acidity, which is suitable for a matrix for lipid profiling, but its background noise is too high to analyze lipids smaller than 500 MW, rendering it inappropriate for lipid profiling [52]. Sugiura et al. [53] conducted research with the addition of alkali metal salts to increase the efficiency of polar lipid analysis.

**LIPIDOME DATABASE**

The bottleneck of lipid metabolite research is in the precision of structural identification of lipid metabolites within samples. For such precise structural identification, it is most important to construct a solid lipidome database (DB) from the various available samples of plants, microbes, and animals. The lipidome mass spectral DB includes two standard databases: a real tandem mass spectrometry (MS/MS) DB acquired from injected reference standards and an in silico MS/MS DB established from lipidome-specific mass fragmentation patterns. Presently, the most utilized lipidome DBs include LipidBank, LIPID MAPS, the Human Metabolome Database, and LipidBlast (Tables 5 and 6). The characteristics of such lipidome DBs are discussed below [54,55].

1. **LipidBank (www.lipidbank.jp)**

Developed from the collaboration of the International Medical Center of Japan and the Japan Science and Technology Corporation, LipidBank DB was released to the public in 1999 (www.lipidbank.jp). LipidBank DB currently has information regarding names, structures, physicochemical properties, and biological functions, including the UV, IR, NMR, and MS data, of 7,009 lipids. The established data of lipid types include neutral lipids, phospholipids, glycolipids, fatty acids, vitamins, steroids, eicosanoids, isoprenoids, and more [56].

| Year | Resource | URL | Lipid category | Number of compounds | Characteristics |
|------|----------|-----|----------------|---------------------|----------------|
| 1999 | LipidBank | www.lipidbank.jp | 17 | 7,009 | The first lipid database |
| 2003 | LIPID MAPS | www.lipidmaps.org | 8 | 37,566 | Physicochemical properties and spectral data |
| 2005 | HMDB | www.hmdb.ca | 7 | 27,440 | "One stop shop" for lipid research |
| 2013 | LipidBlast | http://fiehnlab.ucdavis.edu/projects/LipidBlast | 26 | 119,200 | Lipid classification/nomenclature system |

### Table 6. Lipid classes in the HMDB, LIPID MAPS, LipidBlast, and LipidBank databases

| Lipid classes | HMDB | LIPID MAPS | LipidBlast | LipidBank |
|---------------|------|------------|------------|-----------|
| Fatty acids   | 2,533 | 5,797      | -          | 1,747     |
| Phospholipids | 6,297 | 8,001      | 33,107     | 341       |
| Glycerolipids | 14,001| 7,538      | 29,904     | 574       |
| Glycolipids   | 499   | 1,293      | 16,428     | 696       |
| Sphingolipids | 560   | 4,318      | 1,384      | 145       |
| Steroids      | 867   | 2,678      | -          | 479       |
| Prenol lipids | 3,533 | 1,200      | -          | 112       |
| Polyketides   | -     | 6,741      | -          | -         |
| Phosphatidylinositol mannosides | - | - | 22,752 | - |
| Lipopolysaccharides | - | - | 15,625 | 734 |
| Bile acids    | -     | -          | -          | 674       |
| Vitamins      | -     | -          | -          | 1,219     |
| Total         | 28,290| 37,566     | 119,200    | 6,721     |
2. LIPID MAPS (www.lipidmaps.org)

LIPID MAPS project was started in 2003, is funded by the US National Institutes of Health, and aims to identify and quantify fatty acids, neutral lipids, phospholipids, sphingolipids, steroids, and prenol lipids in samples [57]. A lipid nomenclature and classification system [58], analytical tools for lipid quantification, protocols for lipid separation, and a structural DB of more than 37,566 lipids (LIPID MAPS Structure Database) were established [58] through the project. LIPID MAPS also provides an in silico MS/MS DB for cardiolipins, glycerophospholipids, and mono/di/triacylglycerols that are crucial to lipid metabolite structure identification. With the support of the LIPID MAPS Consortium, biosynthetic pathway maps of nearly 450 types of sphingolipids have been established and are readily accessible [59].

3. Human Metabolome Database (www.hmdb.ca)

The Human Metabolome Database (HMDB) released version 1.0 in 2007 [60], version 2.0 in 2009 [61], and version 3.0 in 2012 [62]. HMDB contains spectroscopic, quantitative, analytic, and physicochemical information on human metabolites, including information on related enzymes and transporters. Currently, data on nearly 40,000 metabolites (20,900 detected metabolites and 19,000 expected metabolites in human biofluids and tissues) have been established, and this includes nearly 28,000 fatty acids, neutral lipids, phospholipids, sphingolipids, steroids, and other lipid metabolites [62]. The specific metabolite information included in HMDB includes physicochemical properties, biofluid/tissue concentrations, human-specific pathway maps, spectral data (NMR, GC-MS, and MS/MS), disease associations, and chemical taxonomy/ontology data.

4. LipidBlast (fiehnlab.ucdavis.edu/projects/LipidBlast)

Research from the University of California at Davis has yielded mass fragmentation patterns of neutral lipids, phospholipids, glycolipids, sphingolipids, and lipopolysaccharides, establishing an in silico MS/MS DB of 119,200 lipid metabolites for use by research personnel [63]. The great advantage of this DB is that the data can be used without compatibility issues due to MS equipment types; in addition, LipidBlast has the largest amount of MS/MS spectrum data, representing up to 212,516 lipid metabolites.

5. Additional lipid MS databases

Other lipidome DBs that are helpful in the identification of lipids found in biological samples include ALEX [64], CyberLipid [65], LipidAT [66], Lipid Data Analyzer [67], LipidHome [68], LipidQA [69], LipidXplorer [70] and MZmine (Table 7) [71].

### Table 7. Representative lipid MS libraries for lipid identification in samples

| Library       | URL                                      |
|---------------|------------------------------------------|
| ALEX          | www.msLipidomics.info                    |
| AMDMS-5L      | shotgunlipidomics.com/programs/programs.htm |
| CyberLipid    | www.cyberlipid.org                       |
| LIMSA         | www.helsinki.fi/science/lipids/software.html |
| LipidAT       | mendel.informatics.indiana.edu/~chuy/LipidAT |
| Lipid Data Analyzer | genome.tugraz.at/Ida              |
| LipidHome     | www.ebi.ac.uk/apweiler-srv/lipidhome     |
| LIPID MAPS MS tools | www.lipidmaps.org/tools/ms/             |
| LipidomeDB    | lipidome.bcf.ku.edu:9000/Lipidomics/     |
| LipidQA       | lipidqa.dom.wustl.edu/                  |
| LipidXplorer  | wiki.mpideg.de/wiki/lipids/index.php/Main_Page |
| LipidView     | www.abschiex.com/products/software/lipidview-software |
| MZmine        | mzmine.sourceforge.net                  |
| TriglyAPCI    | www.uochb.cz/web/structure/626.html      |

APPLICATIONS OF LIPIDOMICS IN DISEASE RESEARCH

1. Applications in metabolic disease

Metabolic disease arises from the failure of individual organs to properly execute metabolism, creating an imbalance in carbohydrates, lipids, proteins, vitamins, minerals, and water. The most well-known of such metabolic diseases are diabetes, obesity, hypertension. Currently, research in early detection methods and treatment response is being actively conducted (Table 8).

Neutral lipids and phospholipids have been reported as lipid metabolite markers related to diabetes. Generally, the neutral lipids TG [72-74] and CE [72] are notably higher in the plasma of diabetic patients than that of normal
Table 8. Plasma lipid biomarker discovery in diabetes, obesity, and hypertension

| Disease       | Sample                  | Biomarker                                | Increase (↑)                  | Decrease (↓)                  | Analytical platform Reference |
|---------------|-------------------------|------------------------------------------|-------------------------------|-------------------------------|--------------------------------|
| Diabetes      | Human plasma            | Total TGs, TG (lower carbon number/double bond) | PC34:2, PC36:2, LPE18:2, PC34:2 | LPC18:0, LPC18:2, LPC20:4, PC34:2 | ESI-MS/MS [72] |
|               | Human plasma            | CE23:2, CE23:3, CE23:4                   | CE23:2, CE23:3, CE23:4        |                                | LC-MS/MS [8] |
|               | Human plasma            | TG (higher carbon number/double bond)    | PC38:6, LPC22:6               |                                | NPLC-TOF/MS [74] |
| Obesity       | Human plasma            | Total TGs, Total DGs                     | PE36:2, PE38:6, PE40:6        |                                | LTQ Orbitrap [78] |
|               | Human plasma            | Total TG                                | LPC18:0                      |                                | UPLC-Q-TOF MS [79] |
|               | Mouse liver             | TG48:0-48:2, TG50:2, TG52:2, TG54:3, DG34:1, DG34:2, DG36:2, DG36:3 | SM (d18:1/24:0), SM (d18:1/24:1) |                                | UPLC-Q-TOF MS [12] |
|               | Human serum             | LPC18:0, LPC18:1                         |                                |                                | UPLC/MS [80] |
|               | Mouse serum             | LPC18:0, LPC18:1                         |                                |                                | UPLC-Q-TOF MS [82] |
|               | Human plasma            | PE38:4                                  |                                |                                | UPLC-IT-TOF MS [13] |
| Hypertension  | Human plasma            | Total TGs, DG (16:0/22:5), DG(16:0/22:6) | PC22:5, PC20:4, PC18:1        |                                | LC-ESI/MS [83] |
|               | Rat plasma              | Total ceramides                         |                                |                                | UPLC-IT-TOF MS [11] |
|               | Human plasma            | Total ceramides                         |                                |                                | LC-ESI/MS [84] |
|               | Rat plasma              | Total ceramides                         |                                |                                | LC-ESI/MS [84] |

patients. From the decreased levels of TG and CE in the plasma of diabetic mice treated with oral rosiglitazone [75], increased neutral lipids is a shared phenomenon in both humans and animals suffering from diabetes. According to recently released data from Rhee [8], lipids of lower carbon number and double bond content (44:1, 46:1, 48:0, 48:1, 50:0, 52:1) were associated with an increased risk of diabetes, whereas lipids of higher carbon number and double bond content (56:9, 58:10, 60:12) were associated decreased risk of diabetes. Thus, additional research on the specific relationship between diabetes and acyl chain carbon number and double bond content of lipids is necessary. Lysophosphatidylcholine (LPC) is also increased in diabetic samples compared to the normal sample, with key markers being reported as LPC 18:0, LPC 18:2, and LPC 20:4 [74]. This experiment is concurrent with the report of Huo et al. [76] in that diabetic patients treated with metformin had reduced plasma LPC 16:0, LPC 18:0, and LPC 18:2 levels. However, a decrease in PC (PC 16:0/18:0 and PC 18:0/20:4) with increased LPC was confirmed, while some PC types (PC 16:0/18:2) showed increased levels [74], indicating the need for further confirmatory studies. Rhee et al. reported recently that risk of type II diabetes increased when PC (PC34:2 and PC36:2) with low levels of unsaturation increased, while PC (PC38:6 and LPC22:6) with high levels of unsaturation decreased [8]. For other phospholipids, plasma PE increased [74,77] while PI decreased compared to the levels in healthy patients [74].

As in diabetes, neutral lipids and phospholipids are also used as lipid metabolite markers in obesity. TG [12,78-80], a neutral lipid, was shown to increase in obese patients compared to healthy patients and demonstrated a correlative decrease with decrease in patient weight [81]. DG [12,78], as well as TG, was significantly increased in obese patient plasma. The phospholipids PC [82], PE [13,78], and PI [13] were increased in obese patients, while PC decreased to the normal value with a decrease in patient weight [81]. LPC, on the other hand, differed with acyl chain type, showing an increase with LPC 18:0 [79,80,82] but a decrease with LPC 18:1 or LPC 18:2 [79,82]. These discrepant results in-
dicate the need for further research in order to better understand the roles of these lipids in obesity. SM, a type of sphingolipid, decreased [12] in obese patients, while plasma SM level increased with patient weight loss [81].

Lipidome markers related to hypertension include neutral lipids, sphingolipids, and phospholipids. TG was significantly increased in hypertensive patients compared to that of healthy patients [10,78] but decreased after treatment with anti-hypertensive medication [10]. Some DGs (DG 16:0/22:5 and DG 16:0/22:6) showed significantly higher levels in hypertensive patients plasma compared to that in normal patients [83]. LPC(22:6, 20:4, 18:1), PC(40:6), and SM(16:1, 24:2) were elevated in hypertensive patients compared to the levels in healthy patients, and they decreased after treatment with an herbal medicine (Ping Gan) with anti-hypertensive characteristics [10,11]. Ceramide increased in hypertensive animal models as well as in hypertensive human patients [84].

2. Applications in dermatological disease

In dermatological diseases like psoriasis and atopic dermatitis, lipid metabolites are being actively studied (Table 9). Ceramide (CER), a sphingolipid, is recognized as the most important lipid metabolite in dermatological disease, accounting for nearly 40% of the stratum corneum, the outermost part of the skin layer [85,86]. FFAs, neutral lipids, and cholesterol are also known to be present in the skin. The total ceramide content is decreased in atopic dermatitis patients compared to healthy patients [87-89]. In observing different types of ceramides, the total contents of non-hydroxy acyl 6-hydroxysphingosine ceramide (CER[NH]), non-hydroxy acyl phytosphingosine ceramide (CER[NP]), esterified ω-hydroxy acyl phytosphingosine ceramide (CER[EOS]), and esterified ω-hydroxy acyl 6-hydroxysphingosine ceramide (CER[E0H]), and esterified ω-hydroxy acyl phytosphingosine ceramide (CER[EOP]) decreased in atopic dermatitis patient groups [87,90], while the total content of A-type ceramides, α-hydroxy acyl sphingosine ceramide (CER[AS]), α-hydroxy acyl 6-hydroxysphingosine ceramide (CER[AH]), and α-hydroxy acyl sphingosine ceramide (CER[AS]), increased [87,90]. However, according to a few research groups, these levels differed with carbon content. For instance, non-hydroxy acyl sphingosine ceramide (CER[NS]), non-hydroxy acyl 6-hydroxysphingosine ceramide (CER[NH]), and α-hydroxy acyl sphingosine ceramide (CER[AS]), which have more than 50 carbons in the ceramide portion, were decreased in atopic dermatitis patients compared to those in the healthy group, while ceramides with less than 40 carbons were increased [87]. Thus, additional research should be done on the differences in sphingoid and acyl chain numbers and types of ceramides with respect to dermatologic diseases. As with atopic dermatitis, psoriasis patients indicated a decrease in ceramide content within patient skin [91]. Recent reports have indicated improvements in dermatitis after treatment with ceramide and ceramide-like analogues [92-94], suggesting that research on ceramide function and importance within dermatological diseases should continue.

3. Applications in neurological disease

Lipids are important moderators of brain functions and are strongly related to neurological diseases like Alzheimer’s disease (AD). Currently, the research focus of lipidome markers in neurological disease is indeed for those involved in AD and the related lipid metabolite markers include neutral lipids, sphingolipids, and phospholipids (Table 10). DG

| Table 9. Lipid biomarker discovery in atopic dermatitis and psoriasis |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Disease**     | **Sample**      | **Biomarker**   | **Analytical**  | **Reference**   |
| Atopy           | Human stratum corneum | CER[AS] | Total ceramides, CER[NH], [NP], [EOS], [EOH], [EOP] | LC- ESI/MS | [87] |
|                 | Human stratum corneum | CER[AS], [AH], [AP] | CER[EOS] | HPTLC | [90] |
| Psoriasis       | Skin epidermis | Total ceramides | Total ceramides | TLC | [89] |

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Table 10. Lipid biomarker discovery in Alzheimer’s disease

| Species       | Sample                                | Biomarker                                                                 | Analytical method          | Reference |
|---------------|---------------------------------------|---------------------------------------------------------------------------|----------------------------|-----------|
| Human         | Prefrontal cortex                     | DGs, Ceramides                                                           | PEs, LPCs, TG58:7 (AA-containing) | LC-Qtrap/MS [95] |
| Human         | Entorhinal cortex                     | CEs, SMs, ganglioside GM3, TG36:7 (DHA-containing)                       | PEs, PSs, PIs, LPEs        | LC-Qtrap/MS [95] |
| Mouse         | Forebrain                             | CEs, ganglioside GM3                                                     | PE, pPEs, SMs(acyl chain C16~20) | UPLC-ESI-TOF MS [96] |
| Mouse         | Brain                                 | PC34:2                                                                   | TG60:12 (DHA-containing)   | DHA-conjugated CE |
| Mouse         | Brain                                 | TG54:4 (AA-containing)                                                   |                             | UPLC-ESI-TOF MS [96] |
| Human         | Plasma                                | TGs (DHA-containing)                                                    |                             | UPLC-ESI-TOF MS [96] |
| Human         | Hippocampus                           | GalCer (hydroxy-FA-containing)                                          |                             | UPLC-Q-TOF MS [100] |
| Cell Aβ treated PC12 cells |                                    | PC32:0, PC34:1~2, PC36:2~3                                              |                             | UPLC-MS/MS [28] |
| Human         | Cerebrospinal fluid                   | LPC/PC ratio                                                             |                             | ESI-MS/MS [16] |
| Human         | Brain                                 | pPEs                                                                     |                             | ESI-MS/MS [2] |
| Human         | Plasma                                | PC36:5~6, PC40:6                                                          |                             | LC-MS [102] |

increase and sphingomyelinase activation-mediated increase in ceramide due to the hydrolysis of \( \beta \)-amyloid peptide (A\( \beta \))-stimulated PIP2 were observed in the prefrontal cortex of AD patients, while PE and LPC were decreased [95]. A decrease in PE in an Alzheimer’s model mouse brain has also been reported [96]. CE, amyloidogenesis-related SM, and ganglioside GM3 increased in the entorhinal cortex, indicating tissue specificity in regard to lipid content changes in AD [95]. For sphingolipids, the brain SM content in the AD model mouse [95] and AD patients [95], especially those SM species with medium chain fatty acids (C16-C20), decreased as a result of the increase in SM degradation due to A\( \beta \)42-mediated sphingomyelinase activation [97,98]. Meanwhile, a hydroxylated fatty acid-containing galactosylceramide (GalCer) was increased in the AD brain due to an increase in fatty acid hydroxylase activation [28]. In the forebrain of AD model mice, similar to that of the human entorhinal cortex, CE and ganglioside GM3 contents increased, while the phospholipids PG, PS, PI, and LPE decreased [95]. GM3 recovered to the normal range once the PLD2 gene of AD model mice was removed [95], which indicated that GM3 can be used as an AD-related biomarker. CE also increased significantly in mutant human amyloid precursor protein (APP)-expressing mouse brain, which is indicative of CE use as an AD biomarker [96]. The CE mechanism of AD is related to functions in acyl-coenzyme A-cholesterol acyltransferase (ACAT), which converts cholesterol to CE. Thus, ACAT participates in A\( \beta \) peptide production, and an ACAT inhibitor decreases the production and accumulation of A\( \beta \) [99].

When neurotoxicity was induced by treatment of \( \beta \)-amyloid peptide (A\( \beta \)) to PC12 cells, a model of neuronal differentiation, phospholipase A2 (PLA2) activity was reduced, increasing PC content (PC32:0, PC34:1, PC34:2, PC36:2, and PC36:3) [100]. Mutant human amyloid precursor protein (APP)-expressing mouse plasma and brain also increased significantly in PC34:2 [96]. Lysophospholipid acyltransferase activity, factoring in PC synthesis from LPC, also participated in PC accumulation [101]. Such increases in PC were recovered to normal values by treatment with epigallocatechin gallate, a key compound in green tea polyphenols, indicating that PC can be used as a biomarker for A\( \beta \)-induced neurotoxicity [100]. Among the multiple isoforms for the PLA2 enzyme, some, including cPLA2, were activated by A\( \beta \) peptide, significantly decreasing specific PCs (PC36:5, PC38:6, and PC40:6) in AD patient plasma [102]. The LPC/PC ratio in AD patient cerebrospinal fluid was decreased with statistical significance [16]. Collectively, these results demonstrate that anomalies in the cell membrane metabolism of phospholipids mediated by A\( \beta \) peptide-induced PLA2 affect membrane fluidity, leading to participation in platelet formation, and ultimately to AD [103-106]. When the A\( \beta \) peptide level increased, the reactive oxygen species that oxidize plasmalogen PE (pPE) increased in production, and the pPE level decreased. Thus, pPE38:2 and similar pPEs were greatly decreased in...
APP/tau mice [96] and AD patient brains [2]. A β is known to destabilize alkyldihydroxyacetonephosphate synthase, a plasmalogen-synthesizing enzyme [107].

For the neutral lipids, the characteristics were different with respect to the fatty acid types present in the acyl chains. Levels of TG56:7 and TG 60:12 containing docosahexaenoic acid (DHEA) increased in the AD patient entorhinal cortex as well as in the brain tissue of ten-month-old APP/tau mice [95,96], TG62:14, TG62:13, TG60:11, and TG58:10 containing DHEA significantly increased in the plasma of ten-month-old AD mice [96]. DHEA-conjugated CE increased in APP/tau mice brains, indicating that DHEA accumulates in the AD patient brain in the form of TG and CE. Unlike DHEA, TG54:4 and TG58:7 containing arachidonic acid decreased in the AD patient prefrontal cortex and AD mice brain [95,96].

**PERSPECTIVE AND FUTURE OF LIPIDOMICS**

Lipidomics, a branch of metabolomic research, is a relatively new area of study with increasing numbers of experiments being conducted. Much has been established in the field, including lipid profiling methods, database development for lipid structure identification, and quantitative analytical methods. Furthermore, research regarding the identification of specific lipid functions, functional lipidomics, will be the core of lipidomics research in the years to come. Lipid microarrays for the identification of lipids that interact with protein, RNA, and biomolecules; mass imaging studies of lipid metabolites within tissues; and experiments regarding the flux of lipid metabolites within the body by the utilization of isotopomers are receiving much research attention. Future studies should apply lipidomics to DNA, proteomics, and metabolic research in order to identify the overall lipidome functions and pathophysiology of related diseases.

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**REFERENCES**

1. Hou W, Zhou H, Elisma F, Bennett SA, Figeys D. Technological developments in lipidomics. *Brief Funct Genomic Proteomic* 2008;7:395-409.
2. Han X, Holtzman DM, McKeel DW, Jr. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. *J Neurochem* 2001;77:1168-80.
3. Adibhatla RM, Hatcher JF. Role of Lipids in Brain Injury and Diseases. *Future Lipidol* 2007;2:403-22.
4. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011; 472:57-63.
5. Tan G, Lou Z, Liao W, Dong X, Zhu Z, Li W, Chai, Y. Hydrophilic interaction and reversed-phase ultra-performance liquid chromatography TOF-MS for serum metabolic analysis of myocardial infarction in rats and its applications. *Mol Biochem* 2012;8:548-56.
6. Tam VC, Quehenberger O, Oshansky CM, Suen R, Armando AM, Treuting PM, Thomas PG, Dennis EA, Aderem A. Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* 2013;154:213-27.
7. Meikle PJ, Wong G, Barlow CK, Kingwell BA. Lipidomics: Potential role in risk prediction and therapeutic monitoring for diabetes and cardiovascular disease. *Pharmacol Ther* 2014.
8. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, Carr SA, Vasan RS, Florez JC, Clish CB, Wang TJ, Gerszten RE. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *J Clin Invest* 2011;121:1402-11.
9. Chamulitrat W, Liebisch G, Xu W, Gan-Schreier H, Pathil A, Schmitz G, Stremmel W. Ursodeoxycholyl lysophosphatidylethanolamide inhibits lipoapoptosis by shifting fatty acid pools toward monosaturated and polyunsaturated fatty acids in mouse hepatocytes. *Mol Pharmacol* 2013;84:696-709.
10. Hu C, Kong H, Qu F, Li Y, Yu Z, Gao P, Peng S, Xu G. Application of plasma lipidomics in studying the response of patients with essential hypertension
to antihypertensive drug therapy. *Mol Biochem Pediatr* 2011; 7:3271-9.

11. Jiang H, Nie L, Li Y, Xie J. Application of ultra-performance liquid chromatography coupled with mass spectrometry to metabolomic study on spontaneously hypertensive rats and intervention effects of Ping Gan prescription. *J Sep Sci* 2012;35:483-9.

12. Yetukuri L, Katajamaa M, Medina-Gomez G, Seppanen-Laakso T, Vidal-Puig A, Oresic M. Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis. *BMC Syst Biol* 2007; 1:12.

13. Donovan EL, Pettine SM, Hickey MS, Hamilton KL, Miller BF. Lipidomic analysis of human plasma reveals ether-linked lipids that are elevated in morbidly obese humans compared to lean. *Diabetol Metab Syndr* 2013;5:24.

14. http://en.wikipedia.org/wiki/Lipidomics

15. Spener F, Lagarde M, Geloen A, Record M. Editorial: What is lipidomics? *European Journal of Lipid Science and Technology* 2003;105:481-82.

16. Mulder C, Wahlund LO, Teerlink T, Blomberg M, Veerhuis R, van Kamp GJ, Scheltens P, Scheffer PG. Decreased lysophosphatidylcholine/phosphatidylcholine ratio in cerebrospinal fluid in Alzheimer’s disease. *J Neural Transm* 2003;110:949-55.

17. van Meer G, Leeflang BR, Liebisch G, Schmitz G, Veerhuis R, van Kamp GJ, Scheltens P, Scheffer PG. Decreased lysophosphatidylcholine/phosphatidylcholine ratio in cerebrospinal fluid in Alzheimer's disease. *J Neural Transm* 2003;110:949-55.

18. Kim KM, Jung BH, Lho DS, Chung WY, Paeng KJ, Chung BC. Alteration of urinary profiles of endogenous steroids and polyunsaturated fatty acids in thryroid cancer. *Cancer Lett* 2003;202:173-9.

19. Son HH, Moon JY, Seo HS, Kim HH, Chung BC, Choi MH. High-temperature GC-MS-based serum cholesterol signatures may reveal sex differences in vaso- spastic angina. *J Lipid Res* 2014;55:155-62.

20. Ogiso H, Nakamura K, Yatomi Y, Shimizu T, Taguchi R. Liquid chromatography/mass spectrometry analysis revealing preferential occurrence of non-arachidonate-containing phosphatidylinositol bisphosphate species in nuclei and changes in their levels during cell cycle. *Rapid Comm Mass Spectrom* 2010; 24:436-42.

21. Bollinger JG, Rohan G, Sadilek M, Gelb MH. LC/ESI-MS/MS detection of FAs by charge reversal derivatization with more than four orders of magnitude improvement in sensitivity. *J Lipid Res* 2013;54: 3523-30.

22. Singhkhamanan K, Promdonkoy B, Srikhirit T, Boonserm P. Amino acid residues in the N-terminal region of the BinB subunit of Lysinibacillus sphaericus binary toxin play a critical role during receptor binding and membrane insertion. *J Invertebr Pathol* 2013; 114:65-70.

23. Guo D, Lian J, Liu T, Cox R, Margulies KB, Kowey PR, Yan GX. Contribution of late sodium current (I(Na-L)) to rate adaptation of ventricular repolarization and reverse use-dependence of QT-prolonging agents. *Heart Rhythm* 2011;8:762-9.

24. Gallart-Ayala H, Courant F, Severe S, Antignac JP, Morio F, Abadie J, Le Bizec B. Versatile lipid profiling by liquid chromatography-high resolution mass spectrometry using all ion fragmentation and polarity switching. Preliminary application for serum samples phenotyping related to canine mammary cancer. *Anal Chim Acta* 2013;796:75-83.

25. Bang DY, Byeon SK, Moon MH. Rapid and simple extraction of lipids from blood plasma and urine for liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 2014;1331:19-26.

26. Bang DY, Lim S, Moon MH. Effect of ionization modifiers on the simultaneous analysis of all classes of phospholipids by nanoflow liquid chromatography/tandem mass spectrometry in negative ion mode. *J Chromatogr A* 2012;1240:69-76.

27. Chen S, Hoene M, Li J, Li Y, Zhao X, Haring HU, Schleicher ED, Weigert C, Xu G, Lehmann R. Simultaneous extraction of metabolome and lipidome with methyl tert-butyl ether from a single small tissue sample for ultra-high performance liquid chromatography/mass spectrometry. *J Chromatogr A* 2013;1298: 9-16.

28. Hejazi L, Wong JW, Cheng D, Proschogo N, Ebrahimii D, Garner B, Don AS. Mass and relative elution time profiling: two-dimensional analysis of sphingolipids and steroids in human post mortem brains. *Biochem J* 2011;438: 165-75.

29. Grimm MO, Grosjen S, Riemschneider M, Tanila H, Grimm HS, Hartmann T. From brain to food: analysis of phosphatidylcholines, lyso-phosphatidylcholines and phosphatidylcholin-plasmalogens derivates in Alzheimer's disease human post mortem brains and mice model via mass spectrometry. *J Chromatogr A* 2011;1218:7713-22.

30. Takatera A, Takeuchi A, Saiki K, Morisawa T, Yokoyama N, Matsuo M. Quantification of lysophosphatidylcholines and phosphatidylcholines using liquid chromatography-tandem mass spectrometry in neonatal serum. *Journal of Chromatography. B: Analytical Technologies in the Biom* 2006;838:31-6.

31. Shui G, Guan XL, Gopalkrishnan P, Xue Y, Goh JS, Yang H, Wenk MR. Characterization of substrate preference for Scl1p and Cst26p in Saccharomyces cerevisiae using lipidomic approaches and an LPAAT activity assay. *PLoS One* 2010;5:e11956.
32. Han X, Gross RW. Quantitative analysis and molecular species fingerprinting of triacylglyceride molecular species directly from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem 2001;295:88-100.
33. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD. Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. Proc Natl Acad Sci USA 1997;94:2339-44.
34. 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;47:804-14.
35. Liebisch G, Drobnik W, Reil M, Trumbach B, Arnecke R, Olgemoller B, Roscher A, Schmitz G. Quantitative measurement of different ceramide species from crude cellular extracts by electrospray ionization tandem mass spectrometry (ESI-MS/MS). J Lipid Res 1999;40:1539-46.
36. Han X, Gross RW. Electrospray ionization mass spectrometric analysis of human erythrocyte plasma membrane phospholipids. Proc Natl Acad Sci USA 1994;91:10635-9.
37. Hsu FF, Bohrer A, Turk J. Formation of lithiated ad- ducts of glycerophosphocholine lipids facilitates their identification by electrospray ionization tandem mass spectrometry. J Am Soc Mass Spectrom 1998;9:516-26.
38. Heiskanen LA, Suoniemi M, Ta HX, Tarasov K, Ekroos K. Long-term performance and stability of molecular species directly from lipid extracts of biological samples after one-step in situ derivatization. Analyst Chem 2011;83:1252-9.
39. Milne SB, Ivanova PT, DeCamp D, Hsueh RC, Brown HA. A targeted mass spectrometric analysis of phosphatidylinositol phosphate species. J Lipid Res 2005;46:1796-802.
40. Sewell GW, Hannun YA, Han X, Koster G, Bielawski J, Goss V, Smith PJ, Rahman FZ, Vega R, Bloom SL, Walker AP, Postle AD, Segal AW. Lipidomic profiling in Crohn’s disease: abnormalities in phosphatidylinositol nositols, with preservation of ceramide, phosphatidylycholine and phosphatidylerine composition. International Journal of Biochemistry & Cell Biology 2012;44:1839-46.
41. Hou W, Zhou H, Bou Khalil M, Seebun D, Bennett SA, Figyes D. Lyso-form fragment ions facilitate the determination of stereospecificity of diacyl glycerophospholipids. Rapid Commun Mass Spectrom 2011;25:205-17.
42. Ejsing CS, Duchoslav E, Sampaio J, Simons K, Bonner R, Thiele C, Ekroos K, Shevchenko A. Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. Anal Chem 2006;78:6202-14.
43. Han X, Yang K, Cheng H, Fikes KN, Gross RW. Shotgun lipidomics of phosphoethanolamine-containing lipids in biological samples after one-step in situ derivatization. J Lipid Res 2005;46:1548-60.
44. Wang M, Hayakawa J, Yang K, Han X. Characterization and quantification of diacylglycerol species in biological extracts after one-step derivatization: a shotgun lipidomics approach. Anal Chem 2014;86:2146-55.
45. Han X, Yang K, Yang J, Cheng H, Gross RW. Shotgun lipidomics of cardiolipin molecular species in lipid extracts of biological samples. J Lipid Res 2006;47:864-79.
46. Schwudke D, Oegema J, Burton L, Entchev E, Hannich JT, Ejis ring CS, Kurzchalia T, Shevchenko A. Lipid profiling by multiple precursor and neutral loss scanning driven by the data-dependent acquisition. Analyst Chem 2006;78:585-95.
47. Shanta SR, Zhou LH, Park YS, Kim YH, Kim Y, Kim KP. Binary matrix for MALDI imaging mass spectrometry of phospholipids in both ion modes. Analyst Chem 2011;83:1252-9.
48. Jackson SN, Wang HY, Woods AS. Direct profiling of lipid distribution in brain tissue using MALDI-TOFMS. Analyst Chem 2005;77:4523-7.
49. Jackson SN, Wang HY, Woods AS. In situ structural characterization of glycerophospholipids and sulfatides in brain tissue using MALDI-MS/MS. J Am Soc Mass Spectrom 2007;18:17-26.
50. Jackson SN, Wang HY, Woods AS. In situ structural characterization of phosphatidylcholines in brain tissue using MALDI-MS/MS. J Am Soc Mass Spectrom 2005;16:2052-6.
51. Morita Y, Sakaguchi T, Ikegami K, Goto-Inoue N, Hayasaka T, Hang VT, Tanaka H, Harada T, Shibasaki Y, Suzuki A, Fukumoto K, Inaba K, Murakami M, Setou M, Konno H. Lysophosphatidylcholine acyltransferase 1 altered phospholipid composition and regulated hepatoma progression. J Hepatol 2013;59:292-9.
52. Astigarraga E, Barreda-Gomez G, Lombardero L, Fresneda O, Castano F, Giralt MT, Ochoa B, Rodriguez-Puertas R, Fernandez JA. Profiling and imaging of lipids on brain and liver tissue by matrix-assisted laser desorption/ ionization mass spectrometry using 2-mercaptoethanolziohiole as a matrix. Anal Chem 2008;80:9105-14.
53. Sugiiura Y, Setou M. Selective imaging of positively charged polar and nonpolar lipids by optimizing matrix solution composition. Rapid Commun Mass Spectrom 2009;23:3269-78.
54. Navas-Iglesias N, Carrasco-Pancorbo A, Cuadros-Rodriguez L. From lipids analysis towards lipidomics,
55. Wenk MR. The emerging field of lipidomics. Nat Rev Drug Discov 2005;4:594-610.
56. Watanabe K, Yasugi E, Oshima M. How to search the glycolipid data in "LIPIDBANK for Web" the newly developed lipid database in Japan. Trends Glycosci Glyc 2000;12:175-84.
57. http://www.lipidmaps.org/about/about_consortium.html
58. Fahy E, Sud M, Cotter D. and Subramaniam, S. LIPID MAPS online tools for lipid research. Nucleic Acids Res 2007;35:e606-12.
59. Merril AH, Jr. Sphin GOMAP--a web-based bio-synthetic pathway map of sphingolipids and glycosphingolipids. Glycobiology 2005;15:15G.
60. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S, Fung C, Nikolai L, Lewis M, Coutouly MA, Forsythe I, et al. HMDB: the Human Metabolome Database. Nucleic Acids Res 2007;35:D521-6.
61. Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S, Mandal R, Sinelnikov I, Xia J, Jia L, Cruz JA, et al. HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res 2009;37:D603-10.
62. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djoumbou Y, Mandal R, Aziat F, Dong E, Bouatra S, Sinelnikov I, Arndt D, Xia J, Liu P, et al. HMDB 3.0--The Human Metabolome Database in 2013. Nucleic Acids Res 2013;41:D801-7.
63. Kind T, Liu KH, Lee do Y, DeFelice B, Meissen JK, Fiehn O. LipidBlast in silico tandem mass spectrometry database for lipid identification. Nat Methods 2013;10:755-8.
64. Husten P, Tarasov K, Katafiasz M, Sokol E, Vogt J, Baumgart J, Nitsch R, Ekroos K, Ejsing, CS. Analysis of lipid experiments (ALEX): a software framework for analysis of high-resolution shotgun lipidomics data. PLoS One 2013;8:e79736.
65. Matos M. Cyberlipid Center: What do you want to know about lipids? Biotech Software & Internet Report 2001;2:4.
66. Caffrey M, Hogan J. LIPIDAT: a database of lipid phase transition temperatures and enthalpy changes. DMPC data subset analysis. Chemistry & Physics of Lipids 1992;61:1-109.
67. Hartler J, Trotzmuller M, Chitraju C, Spener F, Kofeler HC, Thallinger GG. Lipid Data Analyzer: unattended identification and quantitation of lipids in LC-MS data. Bioinformatics 2011;27:572-7.
68. Foster JM, Moreno P, Fabregat A, Hermjakob H, Steinbeck C, Apweiler R, Wakelam MJ, Vizcaíno JA. LipidHome: a database of theoretical lipids optimized for high throughput mass spectrometry lipidomics. PLoS One 2013;8:e61951.
69. Song H, Hsu FF, Ladenson J, Turk J. Algorithm for processing raw mass spectrometric data to identify and quantitate complex lipid molecular species in mixtures by data-dependent scanning and fragment ion database searching. J Am Soc Mass Spectrom 2007;18:1848-58.
70. Fernandez C, Schuhmann K, Herzog R, Fielding B, Prayn K, Shevchenko A, James P, Holm C, Strom K. Altered desaturation and elongation of fatty acids in hormone-sensitive lipase null mice. PLoS One 2011;6:e21603.
71. Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 2010;11:395.
72. Zhao C, Mao J, Ai J, Shenwu M, Shi T, Zhang D, Wang X, Wang Y, Deng Y. Integrated lipidomics and transcriptomic analysis of peripheral blood reveals significantly enriched pathways in type 2 diabetes mellitus. BMC Med Genomics 2013;6 Suppl 1:S12.
73. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, Defronzo RA, Kirwan JP. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes 2009;58:337-43.
74. Zhu C, Liang QL, Hu P, Wang YM, Luo GA. Phospholipidic identification of potential plasma biomarkers associated with type 2 diabetes mellitus and diabetic nephropathy. Talanta 2011;85:1711-20.
75. Watkins SM, Reifsnyder PR, Pan HJ, German JB, Leiter EH. Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. J Lipid Res 2002;43:1809-17.
76. Hsu FF, Ladenson J, Turk J. Enhanced identification and quantitation of lipids in high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis. Anal Chem 2005;77:4108-16.
77. Graessler J, Schwudke D, Schwarz PE, Herzog R, Shevchenko A, Bornstein SR. Top-down lipidomics reveals ether lipid deficiency in blood plasma of hypertensive patients. PLoS One 2009;4:e6261.
78. Kim JY, Park JY, Kim OY, Ham BM, Kim HJ, Kwon DY, Jang Y, Lee JH. Metabolic profiling of plasma
in overweight/obese and lean men using ultra-performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). *J Proteome Res* 2010; 9:4368-75.

80. Pietilainen KH, Sysi-Aho M, Rissanen A, Seppanen-Laakso T, Yki-Jarvinen H, Kaprio J, Oresic M. Acquired obesity is associated with changes in the serum lipid profile independent of genetic effects—a monozygotic twin study. *PLoS One* 2007; 2:e218.

81. Schwab U, Seppanen-Laakso T, Yetukuri L, Agren J, Kolehmainen M, Laaksonen DE, Ruskeepaa AL, Gylling H, Uusitupa M, Oresic M, Group GS. Triacylglycerol fatty acid composition in diet-induced weight loss in subjects with abnormal glucose metabolism—the GENOBIN study. *PLoS One* 2008;3:e2630.

82. Kim HJ, Kim JH, Noh S, Hur HJ, Sung MJ, Hwang JT, Park JH, Yang HJ, Kim MS, Kwon DY, Yoon SH. Metabolomic analysis of livers and serum from high-fat diet induced obese mice. *J Proteome Res* 2011;10:722-31.

83. Egan BM. Plasma lipid profile signature of hypertension in mexican american families. *Hypertension* 2013;62:453-4.

84. Spijkers LJ, van den Akker RF, Janssen BJ, Debets JJ, De Mey JG, Stroes ES, van den Born BJ, Wijesinghe DS, Chalfant CE, MacAlessie L, Eijkel GB, Heeren RM, Alewiinse AE, Peters SL. Hypertension is associated with marked alterations in sphingolipid biology: a potential role for ceramide. *PLoS One* 2011;6:e21817.

85. Norlen L, Nicander I, Lundh Rozell B, Ollmar S, Forslind B. Inter- and intra-individual differences in human stratum corneum lipid content related to physical parameters of skin barrier function in vivo. *J Invest Dermatol* 1999;112:72-7.

86. Homanaka S, Hara M, Nishio H, Otsuka F, Suzuki A, Uchida Y. Human epidermal glucosylceramides are major precursors of stratum corneum ceramides. *J Invest Dermatol* 2002;119:416-23.

87. Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, Kitahara T, Takema Y, Koyano S, Yamazaki S, Hatamochi A. Changes in the ceramide profile of atopic dermatitis patients. *J Invest Dermatol* 2010;130:2511-4.

88. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? *J Invest Dermatol* 1991;96:523-6.

89. Yamamoto A, Serizawa S, Ito M, Sato Y. Stratum corneum lipid abnormalities in atopic dermatitis. *Arch Dermatol Res* 1991;283:219-23.

90. Jungersted JM, Scheer H, Mempel M, Baurecht H, Cifuentes L, Hogh JK, Hellgren LI, Jemec GB, Agner T, Weidinger S. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. *Allergy* 2010;65:911-8.

91. Lew BL, Cho Y, Kim J, Sim WY, Kim NL. Ceramides and cell signaling molecules in psoriatic epidermis: reduced levels of ceramides, PKC-alpha, and JNK. *J Korean Med Sci* 2006;21:95-9.

92. Kircik LH, Del Rosso JQ, Aversa D. Evaluating Clinical Use of a Ceramide-dominant, Physiologic Lipid-based Topical Emulsion for Atopic Dermatitis. *Journal of Clinical & Aesthetic Dermatology* 2011;4: 34-40.

93. Kircik LH, Del Rosso JQ. Nonsteroidal treatment of atopic dermatitis in pediatric patients with a ceramide-dominant topical emulsion formulated with an optimized ratio of physiological lipids. *Journal of Clinical & Aesthetic Dermatology* 2011;4:25-31.

94. Vavrova K, Hrabalek A, Mac-Mary S, Humbert P, Muret P. Ceramide analogue 14S24 selectively reverses perturbed human skin barrier. *Br J Dermatol* 2007;157:704-12.

95. Chan RB, Oliveira TG, Cortes EP, Honig LS, Duff KE, Small SA, Wenk MR, Shui G, Di Paolo G. Comparative lipidomic analysis of mouse and human brain with Alzheimer disease. *J Biol Chem* 2012;287: 2678-88.

96. Tajima Y, Ishikawa M, Maekawa K, Murayama M, Seno Y, Nishimaki-Mogami T, Nakahashi H, Ikeda K, Arita M, Taguchi R, Okuno A, Mikawa R, Niida S, Takikawa O, Saito Y. Lipidomic analysis of brain tissues and plasma in a mouse model expressing mutated human amyloid precursor protein/tau for Alzheimer's disease. *Lipids Health Dis* 2013;12:68.

97. Svennerholm L, Gottfries CG. Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). *J Neurochem* 1994;62:1039-47.

98. Grimm MO, Grimm HS, Patzold AJ, Zinser EG, Halonen R, Duering M, Tschape JA, De Strooper B, Muller U, Shen J, Hartmann T. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol* 2005;7:1118-23.

99. Bhattacharyya R, Kovacs DM. ACAT inhibition and amyloid beta reduction. *Biochim Biophys Acta* 2010; 1801:960-5.

100. Zhang H, Wang JR, You LF, Ho HM, Chan CL, Hu P, Liu L, Jiang ZH. A cellular lipidomic study on the Abeta-induced neurotoxicity and neuroprotective effects of EGCG by using UPLC/MS-based glycerolipids profiling and multivariate analysis. *Mol Biosyst* 2012;8:3208-15.
101. Ross BM, Moszczynska A, Erlich J, Kish SJ. Phospholipid-metabolizing enzymes in Alzheimer’s disease: increased lysophosphatidylcholine acyltransferase activity and decreased phospholipase A2 activity. *J Neurochem* 1998;70:786-93.

102. Whiteley L, Sen A, Heaton J, Proitsi P, Garcia-Gomez D, Leung R, Smith N, Thambisetty M, Kloszkowska I, Meccoci P, Soininen H, Toslaki M, Vellas B, Lovestone S, Legido-Quigley C, AddNeuroMed C. Evidence of altered phosphatidylcholine metabolism in Alzheimer’s disease. *Neurobiol Aging* 2014;35:271-8.

103. Gattaz WF, Maras A, Cairns NJ, Levy R, Forstl H. Decreased phospholipase A2 activity in Alzheimer brains. *Biol Psychiatry* 1995;37:13-7.

104. Gattaz WF, Cairns NJ, Levy R, Forstl H, Braus DF, Maras A. Decreased phospholipase A2 activity in the brain and in platelets of patients with Alzheimer’s disease. *European Archives of Psychiatry & Clinical Neuroscience* 1996;246:129-31.

105. Sanchez-Mejia RO, Mucke L. Phospholipase A2 and arachidonic acid in Alzheimer’s disease. *Biochim Biophys Acta* 2010;1801:784-90.

106. Lee JC, Simonyi A, Sun AY, Sun GY. Phospholipases A2 and neural network dynamics: implications for Alzheimer’s disease. *J Neurochem* 2011;116:813-9.

107. Grimm MO, Kuchenbecker J, Rothhaar TL, Grosgen S, Hundsdorfer B, Burg VK, Friess P, Muller U, Grimm HS, Riemensneider M, Hartmann T. Plasmalogen synthesis is regulated via alkyl-dihydroxyacetonephosphosphate-synthase by amyloid precursor protein processing and is affected in Alzheimer’s disease. *J Neurochem* 2011;116:916-25.

108. Song IS, Lee do Y, Shin MH, Kim H, Ahn YG, Park I, Kim KH, Kind T, Shin JG, Fiehn O, Liu KH. Pharmacogenetics meets metabolomics: discovery of tryptophan as a new endogenous OCT2 substrate related to metformin disposition. *PLoS One* 2012;7:e36637.

109. Fiehn O, Kind T. Metabolite profiling in blood plasma. *Methods Mol Biol* 2007;358:3-17.

110. Moon JY, Jung HJ, Moon MH, Chung BC, Choi MH. Heat-map visualization of gas chromatography-mass spectrometry based quantitative signatures on steroid metabolism. *J Am Soc Mass Spectrom* 2009;20:1626-37.

111. Moon JY, Kim KJ, Moon MH, Chung BC, Choi MH. A novel GC-MS method in urinary estrogen analysis from postmenopausal women with osteoporosis. *J Lipid Res* 2011;52:1595-603.

112. Moon JY, Shin HJ, Son HH, Lee J, Jung U, Jo SK, Kim HS, Kwon KH, Park KH, Chung BC, Choi MH. Metabolic changes in serum steroids induced by total-body irradiation of female C57B/6 mice. *Journal of Steroid Biochemistry & Molecular Biology* 2014;141C:52-59.

113. Jung, HJ, Kim SJ, Lee WY, Chung BC, Choi MH. Gas chromatography/mass spectrometry based hair steroid profiling may reveal pathogenesis in hair follicles of the scalp. *Rapid Commun Mass Spectrom* 2011;25:1184-92.

114. Kind T, Wohlgemuth G, Lee do Y, Lu Y, Palazoglu M, Shahbaz S, Fiehn O, FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal Chem* 2009;81:10038-48.

115. Jung HJ, Lee WY, Chung BC, Choi MH. Mass spectrometric profiling of saturated fatty acid esters of steroids separated by high-temperature gas chromatography. *J Chromatogr A* 2009;1216:1463-8.

116. Jung HJ, Lee WY, Yoo YS, Chung BC, Choi MH. Database-dependent metabolite profiling focused on steroid and fatty acid derivatives using high-temperature gas chromatography-mass spectrometry. *Clin Chim Acta* 2010;411:818-24.

117. Kind T, Tolstikov V, Fiehn O, Weiss RH. A comprehensive urinary metabolic approach for identifying kidney cancer. *Anal Biochem* 2007;363:185-95.

118. Kumar BS, Chung BC, Lee YJ, Yi HJ, Lee BH, Jung BH. Gas chromatography-mass spectrometry-based simultaneous quantitative analytical method for urinary oxysterols and bile acids in rats. *Anal Biochem* 2011;408:242-52.

119. Byeon SK, Lee JY, Moon MH. Optimized extraction of phospholipids and lysophospholipids for nanoflow liquid chromatography-electrospray ionization-tandem mass spectrometry. *Analyt 2012;127:451-8.

120. Lin L, Huang Z, Gao Y, Yan X, Xing J, Hang W. LC-MS based serum metabolomic analysis for renal cell carcinoma diagnosis, staging, and biomarker discovery. *J Proteome Res* 2011;10:1396-405.

121. Hellmuth C, Weber M, Koletzko B, Peissner W. Nonesterified fatty acid determination for functional lipidomics: comprehensive ultrahigh performance liquid chromatography-tandem mass spectrometry quantitation, qualification, and parameter prediction. *Anal Chem* 2012;84:1483-90.

122. Duffin, K, Obukowicz M, Raz A, Shieh JJ. Electrospray-tandem mass spectrometry for quantitative analysis of lipid remodeling in essential fatty acid deficient mice. *Anal Biochem* 2000;279:179-88.

123. Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou HE, Rajashekar CB, Williams TD, Wang X. Profiling membrane lipids in plant stress responses. Role of phospholipase D alpha in freezing-induced lipid changes in
Arabidopsis. *J Biol Chem* 2002;277:31994-2002.

124. Yang WY, Zheng Y, Bahn SC, Pan XQ, Li MY, Vu HS, Roth MR, Scheu B, Welti R, Hong YY, Wang XM. The patatin-containing phospholipase A pPLAIIalpha modulates oxylipin formation and water loss in *Arabidopsis* thaliana. *Mol Plant* 2012;5:452-60.

125. Zhang X, Fhaner CJ, Ferguson-Miller SM, Reid GE. Evaluation of ion activation strategies and mechanisms for the gas-phase fragmentation of sulfoquinovosyldiacylglycerol lipids from Rhodobacter sphaeroides. *Int J Mass Spectrom* 2012;316-318:100-07.

126. Yang K, Zhao Z, Gross RW, Han X. Systematic analysis of choline-containing phospholipids using multi-dimensional mass spectrometry-based shotgun lipidomics. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877:2924-36.

127. Han X, Yang J, Cheng H, Ye H, Gross RW. T Toward fingerprinting cellular lipidomes directly from biological samples by two-dimensional electrospray ionization mass spectrometry. *Anal Biochem* 2004;330:317-31.

128. Hsu FF, Turk J. Characterization of phosphatidylinositol, phosphatidylinositol-4-phosphate, and phosphatidylinositol-4,5-bisphosphate by electrospray ionization tandem mass spectrometry: a mechanistic study. *J Am Soc Mass Spectrom* 2000;11:986-99.

129. Yang K, Cheng H, Gross RW, Han X. Automated lipid identification and quantification by multidimensional mass spectrometry-based shotgun lipidomics. *Anal Chem* 2009;81:4356-68.

130. Gu M, Kerwin JL, Watts JD, Aebersold R. Ceramide profiling of complex lipid mixtures by electrospray ionization mass spectrometry. *Anal Biochem* 1997;244:347-56.

131. Hsu FF, Bohrer A, Turk J. Electrospray ionization tandem mass spectrometric analysis of sulfatide. Determination of fragmentation patterns and characterization of molecular species expressed in brain and in pancreatic islets. *Biochim Biophys Acta* 1998;1392:202-16.

132. Ma X, Liu G, Wang S, Chen Z, Lai M, Liu Z, Yang J. Evaluation of sphingolipids changes in brain tissues of rats with pentylenetetrazol-induced kindled seizures using MALDI-TOF-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007;859:170-7.