Mass Spectrometry-based Lipidomics and Its Application to Biomedical Research

Zhexue Wu[†], Jong Cheol Shon[†], Kwang-Hyeon Liu*

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu, Korea

Lipidomics, a branch of metabolomics, is the large-scale study of pathways and networks of all 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 technoligies 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 bio-logical 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), sulfoquinovosylDG (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 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 (NH$_4$I), dithioerythritol (DTE), trimethylchlorosilane (TMCS), trimethyliodosilane, or trimethylsilylimidazole (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 silicosteel-treated stainless 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 1. Derivatization reagents and GC columns for the GC-MS analysis of fatty acids and steroids

Analyte	Sample	Amount	Clean-up	Derivatization	Column	Comment	Reference
Fatty acid	Urine	15 μL	-	MSTFA with 1% TMCS	5% Diphenyl polysiloxane	-	[108]
Fatty acid	Plasma	-	-	MSTFA	35% Phenyl-methylpolysiloxane	-	[109]
Steroid	Urine	2 mL	Oasis HLB SPE	MSTFA/NH4I/DTE (500:4:2)	Methyl siloxane	-	[110]
Steroid	Urine	2 mL	Oasis HLB SPE	Pentafluoropropionic anhydride	MXT-1 stainless steel	-	[111]
Steroid	Serum	0.2 mL	Oasis HLB SPE	MSTFA/NH4I/DTE (500:4:2)	Methyl siloxane	-	[112]
Steroid	Hair	30 mg	Oasis HLB SPE	MSTFA/NH4I/DTE (500:4:2)	Methyl siloxane	-	[113]
Steroid	-	-	-	MSTFA with 1% TMCS	5% Diphenyl polysiloxane	-	[114]
Steroid FA ester	Serum	1 mL	-	MSTFA/NH4I/DTE (500:4:2)	MXT-1 stainless steel	High temperature	[115]
Steroid FA ester	Tissue	10 mg	-	MSTFA/NH4I/DTE (500:4:2)	MXT-1 stainless steel	High temperature	[116]
Steroid, fatty acid	Urine	-	-	MSTFA	5% Phenyl methylpolysiloxane	-	[117]
Steroid, PUFA	Urine	3 mL	Oasis HLB SPE	MSHFB/TMCS/TMSIm (2:2:1)	Methyl siloxane	-	[18]
Oxysterol, Bile acid	Urine	1 mL	C18/Oasis HLB SPE	MSTFA/NH4I/DTE (500:4:2)	Methyl siloxane	-	[118]
Cholesterol ester	Serum	20 μL	SPE	MSTFA/NH4I/DTE (500:4:2)	MXT-1 stainless steel	High temperature	[19]

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: CH_3CN/CH_3OH (9:1, v/v), B: $H_2O/CH_3OH/CH_3CN$ (5:4:1, v/v) Modifier: 5 mM NH_4HCO_2 and 0.05% NH_4OH	UPLC-ESI-MS/MS (+/− mode)	[25]
PLs, DG, TG, CE, CL Ceramide	Plasma, urine	C18 column (3 μm, 0.075 × 60 mm)	A: H_2O/CH_3CN (9:1, v/v), B: $CH_3OH/CH_3CN/$ i-PrOH (2:3:5, v/v) Modifier: 1 mM NH_4HCO_2 and 0.05% NH_4OH	Nano LC-ESI-MS/MS (+/− mode)	[25]
PC, PE, SM, DG, TG, Ceramide	Tissue	C8 column (1.7 μm, 2.1 × 100 mm)	A: CH_3CN /H_2O (3:2, v/v), B: i-PrOH / CH_3CN (9:1, v/v] Modifier: 10 mM NH_4HCO_2	UPLC-ESI-MS/MS (+ mode)	[27]
PLs	Plasma	C18 column (3 μm, 0.05 × 85 mm)	A: H_2O/CH_3CN (9:1, v/v), B: i-PrOH / CH_3CN (9:1, v/v) Modifier: 0.1% FA or 0.05% NH_4OH	Nano LC-LCQ MS (+/− mode)	[119]
PLs	Urine	RPLC column (0.075 × 50 mm)	A: H_2O/CH_3CN (9:1, v/v), B: $CH_3OH/CH_3CN/$ i-PrOH (2:3:5, v/v) Modifier: $FA/NH_4HCO_2/NH_4OH/$ NH_4Ac	Nano LC-LTQ MS (− mode)	[26]
PC, PE, PI, SM	Serum	C18 column (1.7 μm, 2.1 × 100 mm)	A: CH_3CN /10 mM NH_4Ac + 01% AcOH (3:2, v/v) B: i-PrOH: CH_3CN:10 mM NH_4Ac + 01% AcOH (88:10:2, v/v)	LC-Orbitrap MS (+/− mode)	[24]
PIP	Cell	C8 column (3.5 μm, 1.0 × 150 mm)	A: $MeOH/H_2O/70\%$ Ethylamine(20:80:0.13, v/v) B: i-PrOH /70% Ethylamine(100:0.13, v/v)	LC-LTQ-Orbitrap MS	[20]
LPC, LPE Sphingolipid	Serum	C18 column (3 μm, 2.1 × 150 mm) HILIC column (3 μm, 2.1 × 150 mm)	A: H_2O with 0.1% FA, B: CH_3CN with 0.1% FA A: 10 mM NH_4Ac with 0.1% FA, B: CH_3CN 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: CH_3CN	UPLC-ESI-MS/MS (− mode)	[27]
FA	Plasma	Diphenyl column (1.9 μm, 3.0 × 100 mm)	A: H_2O with 5 mM NH_4Ac + 2.1 mM AcOH B: CH_3CN/ 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: H_2O with 0.1% FA, B: CH_3CN with 0.1% FA	UPLC-ESI-MS/MS (+ mode)	[21]
Acylcarnitine	Tissue	Silica-based bonded column (1.8 μm, 2.1 × 100 mm)	A: H_2O with 0.1% FA, B: CH_3CN	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		Reference
		Class information	Acyl chain information	
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]²⁻	PI (*m/z* 153)	PI (FA-H)	[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)	PI (FA-H)	[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)	PI (FA-H)	[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)	PI (FA-H)	[127]
PS	[M + H]⁺	NL (*m/z* 185)		[127]
PG, PA, LPG, LPA	[M − H]⁻	PI (*m/z* 153)	PI (FA-H)	[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-aminoacriaine [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

Table 4. Optimum matrix composition for lipid profiling in imaging MS

Analyte	Matrix		Adduct ion		Reference
	Organic solvent	Modifier/Salt	Major	Minor	
TG, DG	80% MeOH	None	[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	50% EtOH	30 mg/mL 2,6-Dihydroxyacetophenone	[M - H]⁻		[49]
PA, PE, PG, PI, PS, ST, ST, GM1	70% MeOH	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]⁺	[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 + K]⁺	[M + Na]⁺	[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 + K]⁺	[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 paring 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].

Table 5. Online resources and databases with information about lipids

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

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/tri-acylglycerols 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 physiological 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.ucdavs.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

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

Library	URL
ALEX	www.msLipidomics.info
AMDMS-SL	shotgunlipidomics.com/programs/programs.htm
CyberLipid	www.cyberlipid.org
LIMSA	www.helsinki.fi/science/lipids/software.html
LipidAT	mendel.informatics.indiana.edu/~chuyu/LipidAT
Lipid Data Analyzer	genome.tugraz.at/lda
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.mpi-cbg.de/wiki/lipidx/index.php/Main_Page
LipidView	www.absciex.com/products/software/lipidview-software
MZmine	mzmine.sourceforge.net
TriglyAPCI	www.uochb.cz/web/structure/626.html

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].

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		Analytical platform	Reference
		Increase ($\uparrow$)	Decrease ($\downarrow$)		
Diabetes	Human plasma	Total TGs CE23:2, CE23:3, CE23:4		ESI-MS/MS	[72]

	Human plasma	TG (lower carbon number/
		  double bond)
		PC34:2, PC36:2, LPE18:2
			TG (higher carbon number/
			  double bond)
			PC38:6, LPC22:6
				LC-MS/MS	[8]

	Human plasma	LPC18:0, LPC18:2, LPC20:4 PC34:2	PI34:0, PI38:4, PI40:6 PC38:4	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	LPC18:1	UPLC-Q-TOF MS	[79]

	Mouse liver	TG48:0~48:2, TG50:2,
		  TG52:2~52:3, TG54:3
		DG34:1,DG 34:2, DG 36:2, DG36:3
		PA34:1, PA34:2, PA36:2
			SM (d18:1/24:0), SM (d18:1/
			  24:1)
				UPLC-Q-TOF MS	[12]

	Human serum	LPC18:0, LPC18:1		UPLC/MS	[80]
	Mice serum	LPC18:0	LPC18:1	UPLC-Q-TOF MS	[82]
	Human plasma	PE38:4		UPLC-Q-TOF MS	[13]
Hypertension	Human plasma	Total TGs LPC22:6, PC40:6, SM16:1, SM24:2		UPLC-IT-TOF MS	[10]
	Human plasma	DG(16:0/22:5), DG(16:0/22:6)		LC- ESI/MS	[83]
	Rat plasma	LPC22:6, LPC20:4, LPC18:1		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 sphingosine ceramide (CER[EOS]),

esterified ω-hydroxy acyl 6-hydroxysphingosine ceramide (CER[EOH]), 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 method	Reference
		Increase (↑)	Decrease (↓)		
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]
	Human stratum corneum		Total ceramides	TLC	[88]
	Human stratum corneum		Total ceramides	HPTLC	[89]
Psoriasis	Skin epidermis		Total ceramides	TLC	[91]

Table 10. Lipid biomarker discovery in Alzheimer's disease

Species	Sample	Biomarker		Analytical method	Reference
		Increase (↑)	Decrease (↓)		
Human	Prefrontal cortex	DGs, Ceramides	PEs, LPCs, TG58:7 (AA-containing)	LC-Qtrap/MS	[95]
Human	Entorhinal cortex	CEs, SMs, ganglioside GM3, TG56:7 (DHA-containing)		LC-Qtrap/MS	[95]
Mouse	Forebrain	CEs, ganglioside GM3	PGs, PSs, PIs, LPEs	LC-Qtrap/MS	[95]
Mouse	Brain	PC34:2 TG60:12 (DHA-containing) DHA-conjugated CE	PEs, pPEs, SMs(acyl chain C16~20) TG54:4 (AA-containing)	UPLC-ESI-TOF MS	[96]
Mouse	Plasma	TGs (DHA-containing)		UPLC-ESI-TOF MS	[96]
Human	Hippocampus	GalCer (hydroxy-FA-containing)		UPLC-MS/MS	[28]
Cell	Aβ treated PC12 cells	PC32:0, PC34:1~2, PC36:2~3		UPLC-Q-TOF MS	[100]
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 β-amyloid peptide (Aβ)-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 Aβ42-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 Aβ peptide pro-

duction, and an ACAT inhibitor decreases the production and accumulation of Aβ [99].

When neurotoxicity was induced by treatment of β-amyloid peptide (Aβ) 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 Aβ-induced neurotoxicity [100]. Among the multiple isozymes for the PLA2 enzyme, some, including cPLA2, were activated by Aβ 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 Aβ peptide-induced PLA2 affect membrane fluidity, leading to participation in platelet formation, and ultimately to AD [103-106]. When the Aβ 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, TG60:13, 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 metabolomic research in order to identify the overall lipidome functions and pathophysiology of related diseases.

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