The use of mass spectrometry in lipidomics

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Outlines

- **Brief introduction to lipidomics**
- **Analytical methodology: MS/MS structure elucidation of phospholipids**
- **Phospholipid analysis in lean and ob/ob mice by mass spectrometry**

Lipids are very important!!

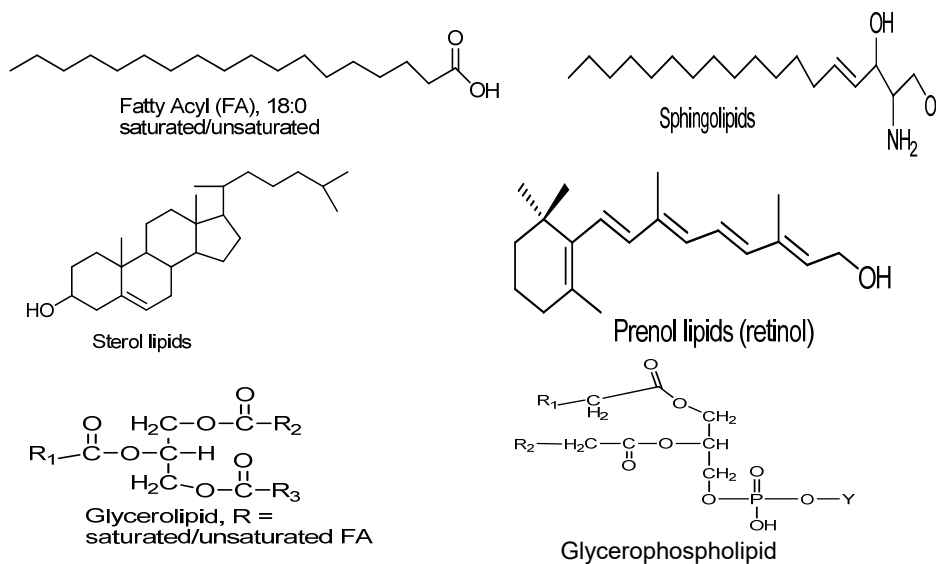**Structural components of cell membrane**
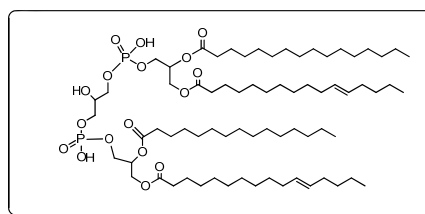**Nutrition**
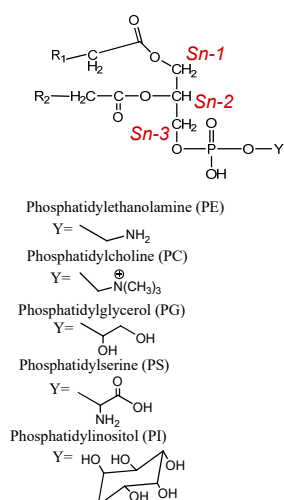-Energy source
-Energy storage
Signaling pathways

Lipidomics- A comprehensive analysis of lipid molecules in response to cellular stress and challenges

Structures of different lipids classes

Fatty Acyl (FA), 18:0
saturated/unsaturated

Sphingolipids

Sterol lipids

Prenol lipids (retinol)

Glycerolipid, R =
saturated/unsaturated FA

Glycerophospholipid

Structures of main phospholipids

Sn-1
Sn-2
Sn-3

Phosphatidylethanolamine (PE)

Y=   NH₂

Phosphatidylcholine (PC)

Y=   N(CH₃)₃

Phosphatidylglycerol (PG)

Y=

Phosphatidylserine (PS)

Y=

Phosphatidylinositol (PI)

Y=

Cardiolipin (diphosphatidylglycerol)

Extraction of lipids by Bligh/Dyer method

- **To a homogenized sample (1 ml containing internal standards) add methanol (2.5 ml) and chloroform (1.25 ml), sonicate by 4-5 bursts and added 1.0 ml water and 1.25 ml chloroform additionally and vigorously shaken.**
- **Centrifuge (1,000 x g) for 2 min and separate the chloroform layer (bottom layer) and repeat the process twice.**
- **Combine the chloroform soluble phase and evaporate to dryness and stored at -20 °C untill analysis.**


Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

| Group	Electrical Propensity	Lipid Classes
Anionic lipids	Carry net negative charge(s) at physiological pH	Cardiolipin, acylCoA, sulfatide, PtdIns (PtdInsP, PtdInsP$_2$, PtdInsP$_3$), PtdGro, PtdSer, PtdH, etc.
Weak anionic lipids	Carry a net negative charge at alkaline pH	PE, lysoPE, ceramide, NEFA, eicosanoids, etc.
Neutral polar lipids	Neutral at alkaline pH	PC, lysoPC, SM, glycolipid, TAG, etc.
Special lipids	Vary	Acylcarnitine, sterols, etc.

The ionization efficiency of an analyte greatly depends on the electrical propensity of an individual analyte in its own microenvironment to lose or gain a charge
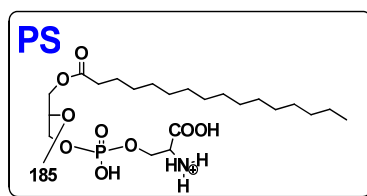
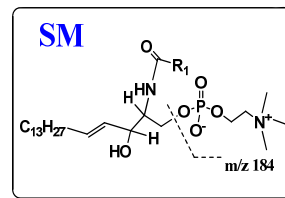Source: Gross and Han, Mass Spec Rev. 2005

Profiling glycerophospholipids
in a complex mixture using MS/MS
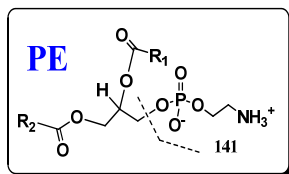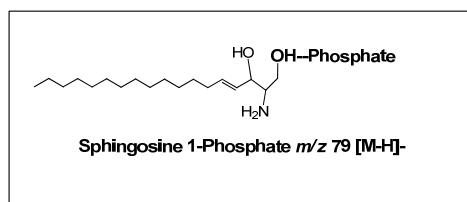

PE
Neutral Loss scan 141
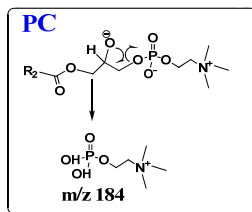
PC & SM
Precursor ion scan 184

PS
Neutral Loss scan 185

How to profile sphingolipids
in a complex mixture using MS/MS?

N-acyl-sphingosine
(Ceramide)
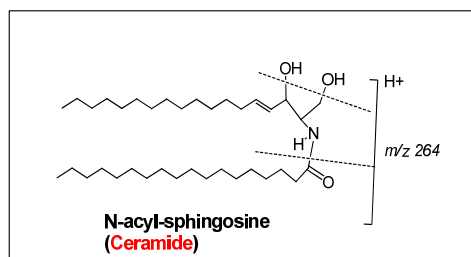
Sphingosine 1-Phosphate *m/z* 79 [M-H]-

m/z 264 is a characteristic ion for all compounds containing a
sphingosine backbone in +ve ion mode

Phosphatidylcholine loses a methyl group to form a negatively charged, pseudomolecular ion
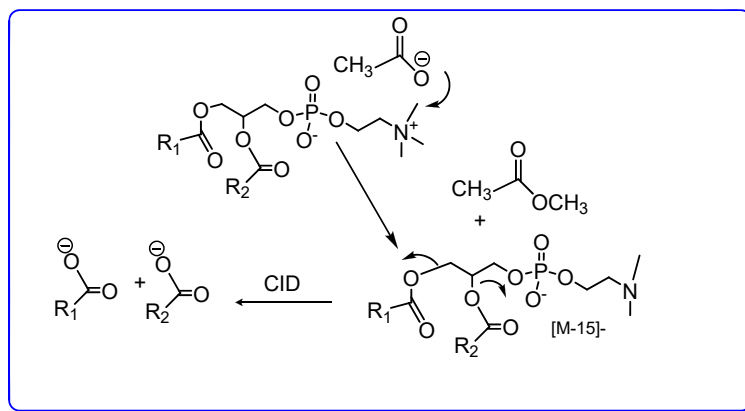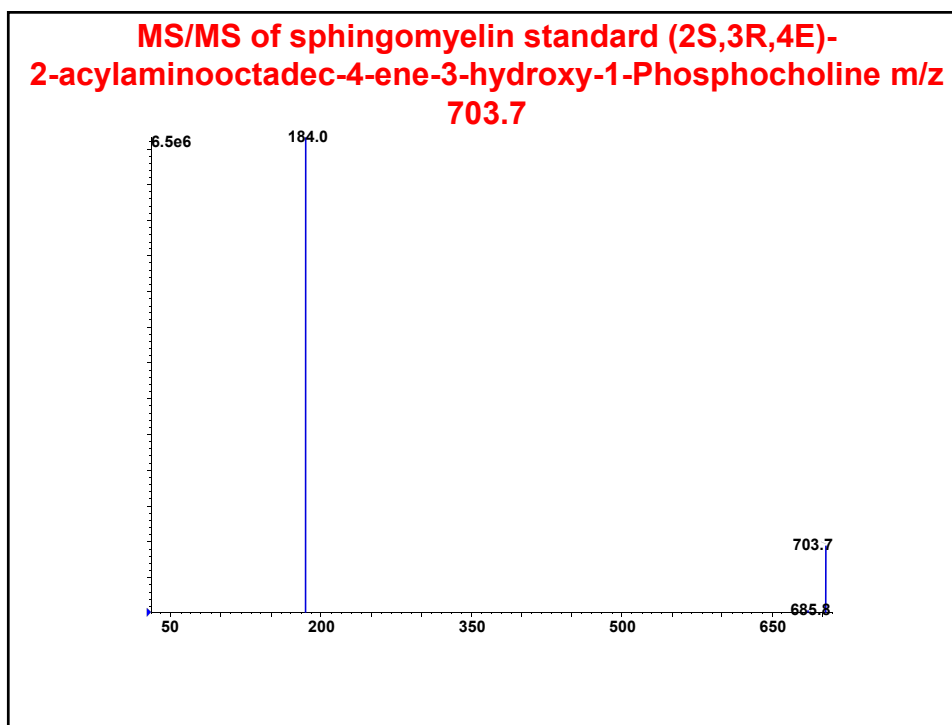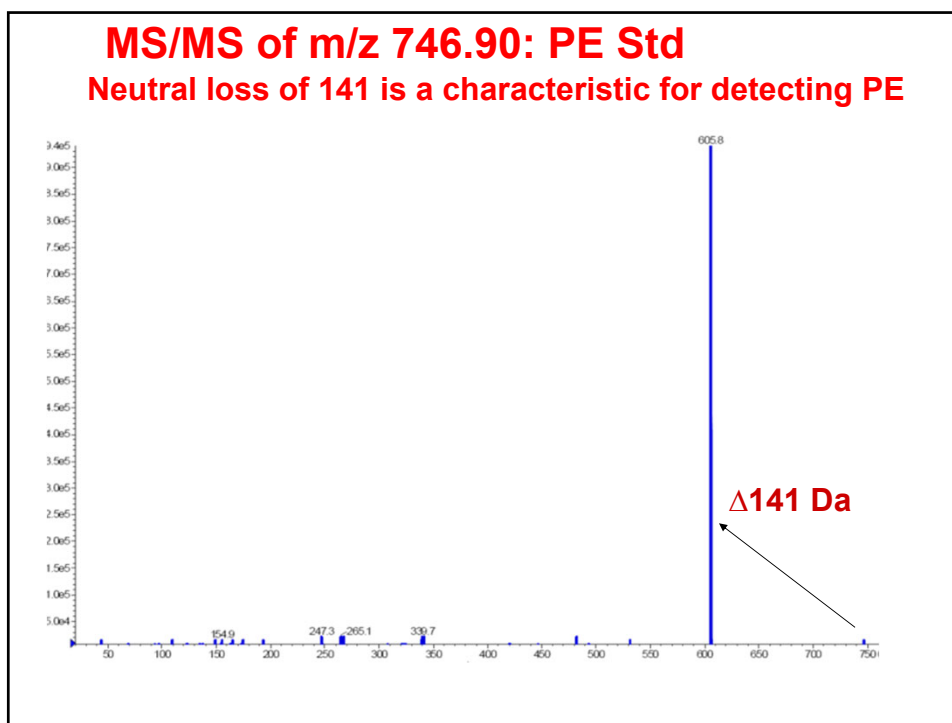

Phospholipids may undergo demethylation and then the loss of the fatty acyl groups from glycerophosphocholine backbone.
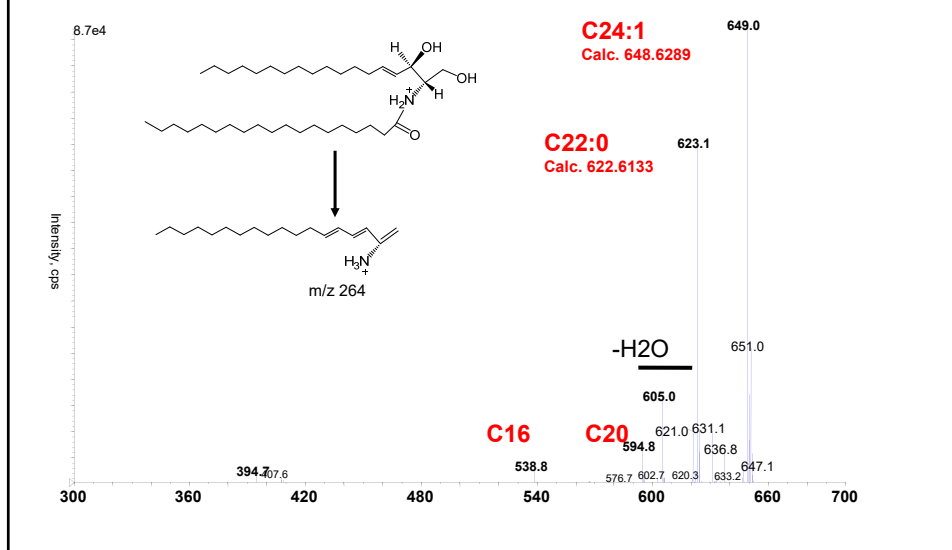
ESI-MS/MS analyses of various lipids

Lipid Class(s)	Precursor Ion	MS/MS Mode & Conditions	Fragment
cardiolipin	$[M-2H]^{2-}$	PI, m/z 153.0, 35 eV	glycerol phosphate derivative
PtdGro, PtdH	$[M-H]^-$	PI, m/z 153.0, 35 eV, *	glycerol phosphate derivative
PtdIns	$[M-H]^-$	PI, m/z 241.1, 45 eV	cyclic Inositol phosphate
		PI, m/z 153.0, 35 eV	glycerol phosphate derivative
PtdInsP	$[M-H]^-$	PI, m/z 321.1, 53 eV	phosphoinositol phosphate
PtdInsP$_2$	$[M-H]^-$	PI, m/z 401.1, 62 eV	diphosphoinositol phosphate
PtdSer	$[M-H]^-$	NL, 87.0 amu, 25 eV, *	serine
		PI, m/z 153.0, 35 eV	glycerol phosphate derivative
sulfatide	$[M-H]^-$	PI, m/z 97.0, 65 eV	sulfate
acylCoA	$[M-2H]^{2-}$	PI, m/z 339.0, 30 eV, *	doubly-charged CoA derivative
PE, lysoPE	$[M-H]^-$	PI, m/z 196.0, 50 eV	glycerol phosphoethanolamine derivative
ceramide	$[M-H]^-$	NL, 256.2 amu, 32 eV *	
		NL, 327.3 amu, 32 eV	
		NL, 240.2 amu, 32 eV *	2-*trans*-palmitoyl alcohol
PC, lysoPC, SM	$[M+Li(Na)]^+$	NL, 59.1 amu, -28 eV, *	trimethylamine
	$[M+Li(Na)]^+$	NL, 183.1 amu, -32 eV	phosphocholine
	$[M+Li]^+$	NL, 189.1 amu, -42 eV	lithium cholinephosphate
	$[M+Na]^+$	NL, 205.1 amu, -35 eV	sodium cholinephosphate
	$[M+H]^+$	PI, m/z 184.1, -30 eV, *	phosphocholine
	$[M+Cl]^-$	NL, 50.0 amu, 24 eV, *	methylchloride
cerebroside	$[M+Li]^+$	NL, 162.2, -50 eV, *	
	$[M+Cl]^-$	NL, 36.0 amu, 30 eV	hydrogen chloride
MGDG	$[M+Li(Na)]^+$	PI, m/z 227(243), -45 eV	Li(Na)+galactose derivative
DGDG	$[M+Li(Na)]^+$	PI, m/z 227(243), -66 eV	Li(Na)+galactose derivative
acylcarnitine	$[M+H]^+$	PI, m/z 85.1, -20 eV, *	carnitine
chol. ester	$[M+NH_4]^+$	PI, m/z 369.3, -50 eV, *	cholestane cation
TAG	$[M+Li]^+$	NL, X amu, -35 eV	a fatty acid

Source: Gross and Han, Mass Spec Rev. 2005

MS/MS of m/z 746.90: PE Std
Neutral loss of 141 is a characteristic for detecting PE

Δ141 Da

MS/MS of sphingomyelin standard (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-Phosphocholine m/z 703.7

Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample

Profiling of phospholipids using precursor ion m/z 184 and neutral loss scan 141 for PC, SM and PE
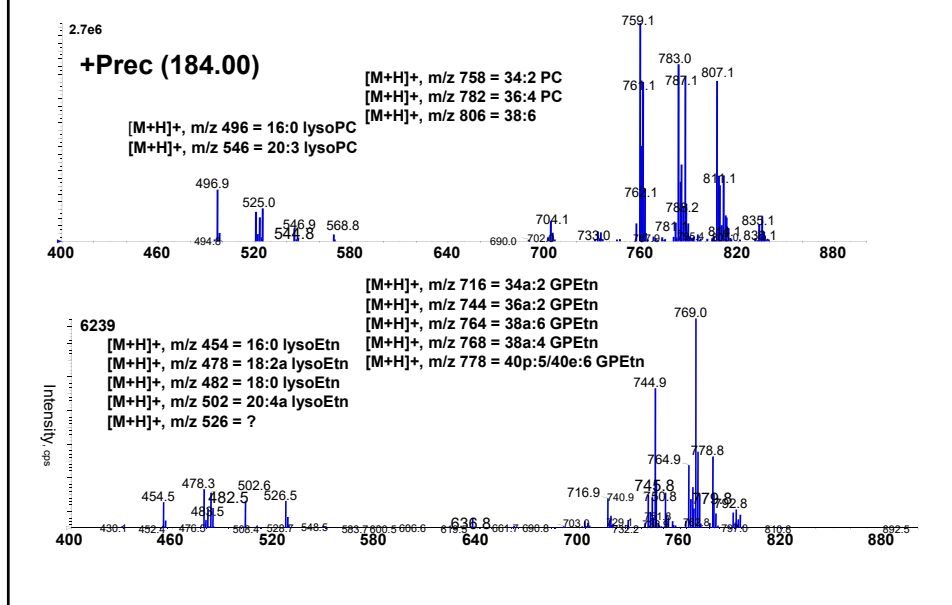
Library search for eicosanoid http://www.lipidmaps.org/

LIPID MAPS -- LIPID Metabolites And Pathways Strategy

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LIPID Metabolites And Pathways Strategy

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LMSD: Lipid classification search results

Fatty Acyls [FA] (W) --> Eicosanoids [FA03]

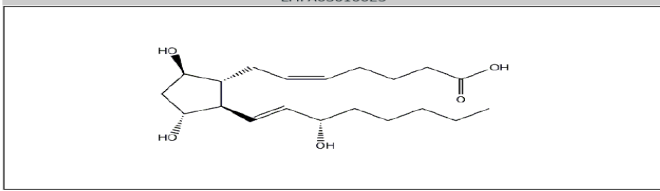| LM_ID	Common Name	Systematic Name	Formula	Mass
LMFA03000001	8(9)-EpETE	(+/-)-8(9)-epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000002	11(12)-EpETE	(+/-)-11(12)-epoxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000003	14(15)-EpETE	(+/-)-14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000004	17(18)-EpETE	(+/-)-17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000005	11(R)-HEDE	11R-hydroxy-12E,14Z-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03000006	17R,18S-EpETE	17R,18S-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000008	15(R)-HEDE	15R-hydroxy-11Z-13E-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03000009	11S-HEDE	11S-hydroxy-12E,14Z-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03010000	Prostanoic acid skeleton	-	-	-
LMFA03010001	6-keto-PGF1α	6-oxo-9S,11R,15S-trihydroxy-13E-prostenoic acid	$C_{20}H_{34}O_6$	370.24
LMFA03010002	PGF2α	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid	$C_{20}H_{34}O_5$	354.24
LMFA03010003	PGE2 (W)	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_5$	352.22
LMFA03010004	PGD2 (W)	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_5$	352.22
LMFA03010005	PGA1	9-oxo-15S-hydroxy-10Z,13E-prostadienoic acid	$C_{20}H_{32}O_4$	336.23
LMFA03010006	PGF2α-d4	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{30}D_4O_5$	358.27
LMFA03010007	PGD2-d4	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{28}D_4O_5$	356.25
LMFA03010008	PGE2-d4	11R,15S-dihydroxy-9-oxo-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{28}D_4O_5$	356.25
LMFA03010009	PGG2	9S,11R-epidioxy-15S-hydroperoxy-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_6$	368.22

LIPID Metabolites And Pathways Strategy

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Structure database (LMSD)

LMFA03010025

LM ID	LMFA03010025
Common Name	PGF2β
Systematic Name	9R,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
Synonyms	-
Exact Mass	354.24
Formula	$C_{20}H_{34}O_5$
Category	Fatty Acyls [FA]
Main Class	Eicosanoids [FA03]
Sub Class	Prostaglandins [FA0301]
LIPIDBANK ID	XPR1764
PubChem Substance ID (SID)	4265968
KEGG ID	-

Total scan of metabolites (Q1 SCAN + ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse

Total metabolomics

Total scan of metabolites (Q1 SCAN -ve ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse
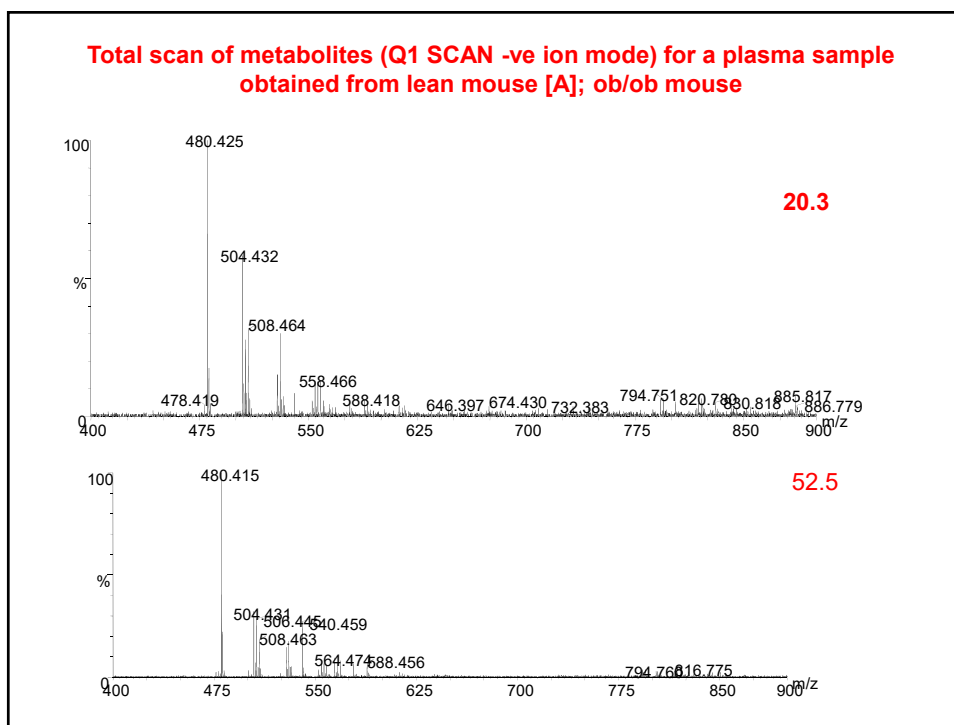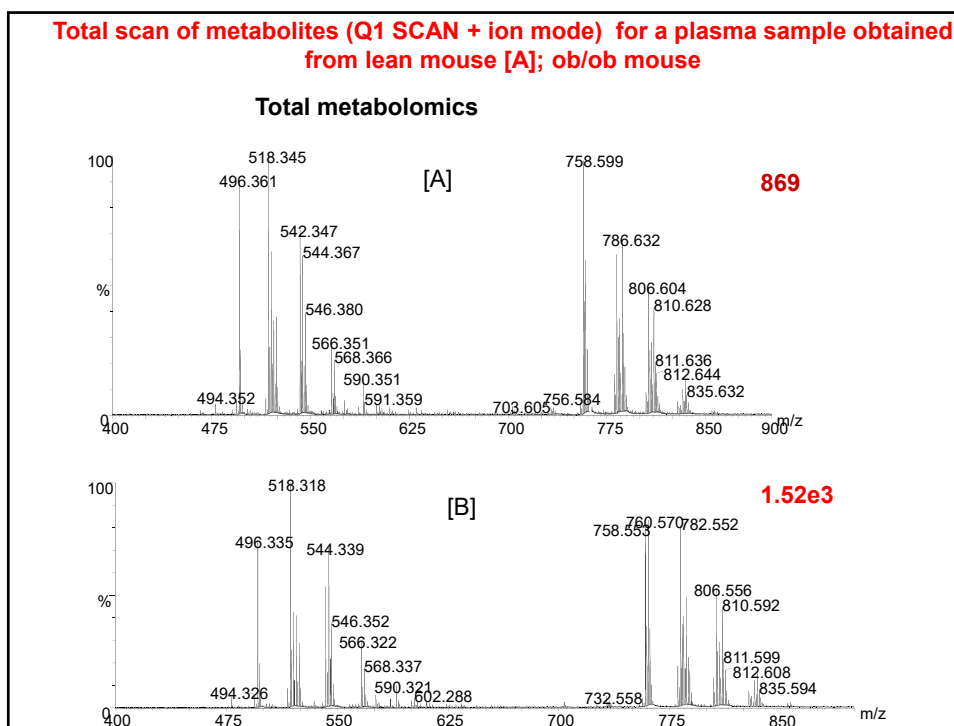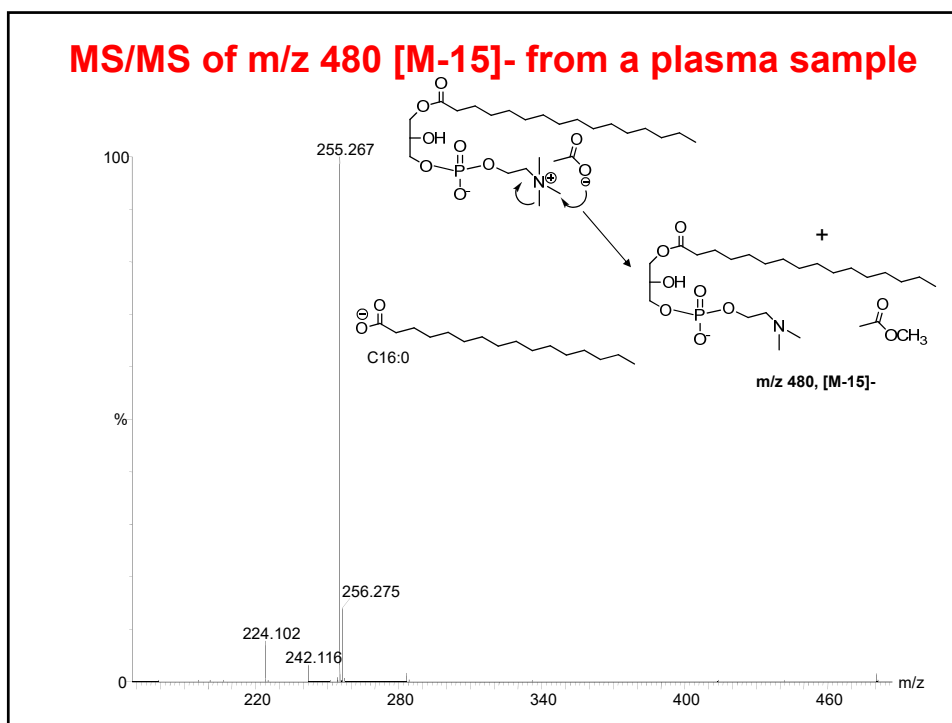

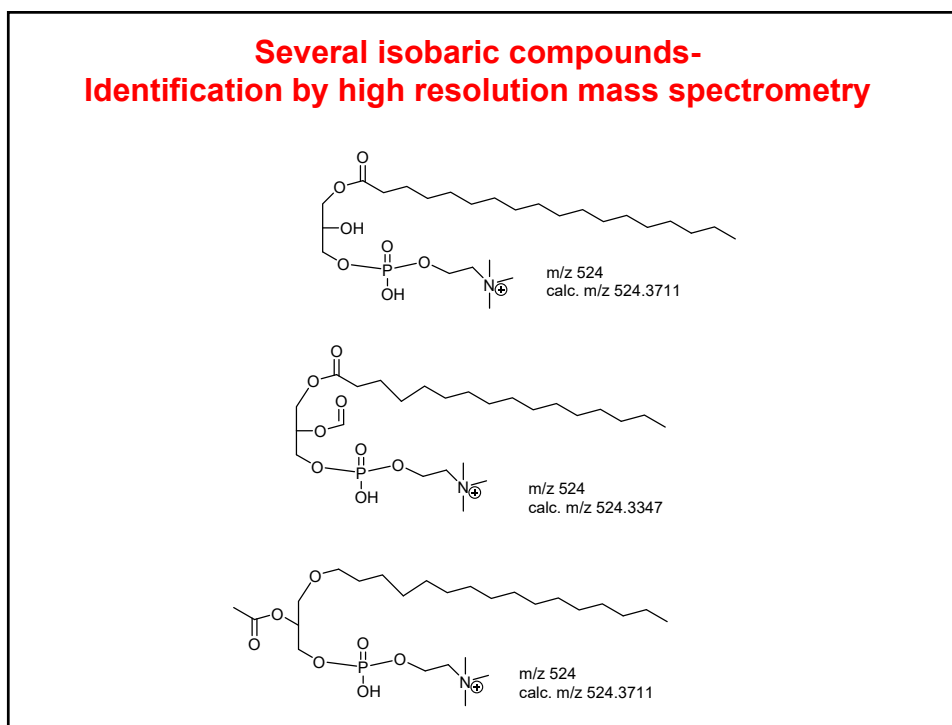## MSMS fragmentation of *m/z* 496 [M+H]+ obtained from a plasma sample in positive ion mode
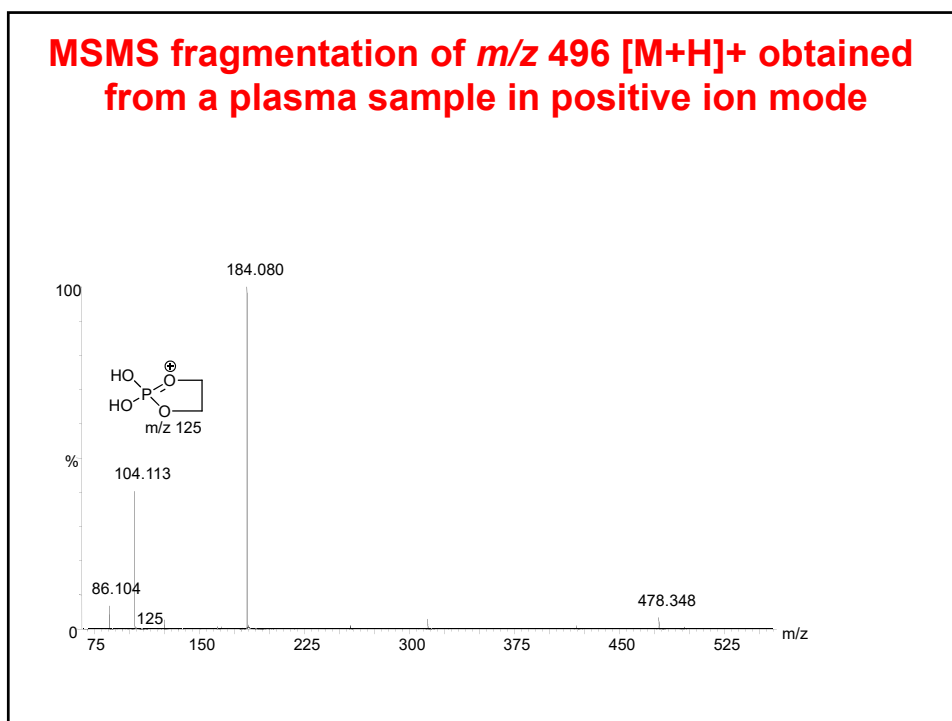
MSMS of m/z 518 [M+Na]+ indicates that it may be a sodiated ion of a phospholipid with mol. Wt. 495 Da
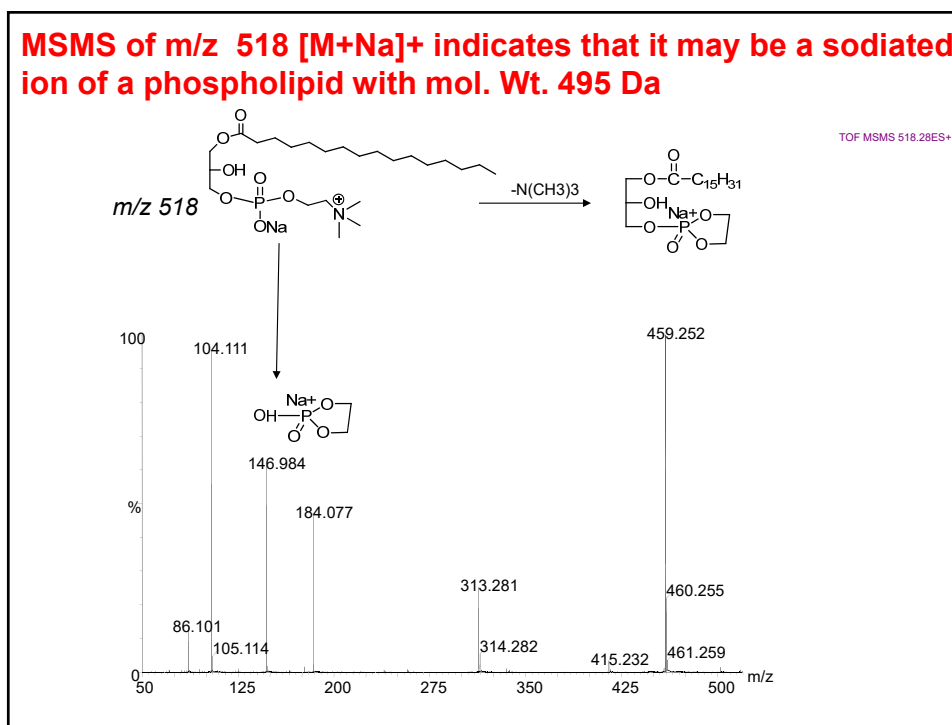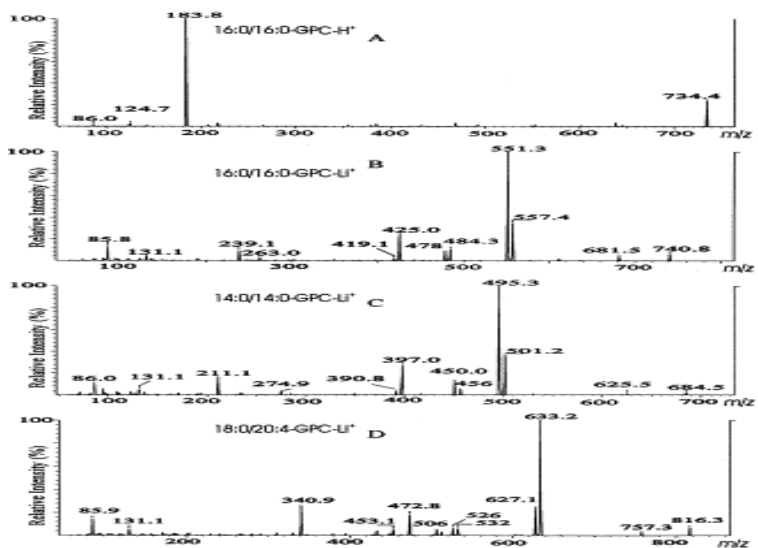
MS/MS of m/z 480 [M-15]- from a plasma sample

Several isobaric compounds-
Identification by high resolution mass spectrometry

m/z 524
calc. m/z 524.3711

m/z 524
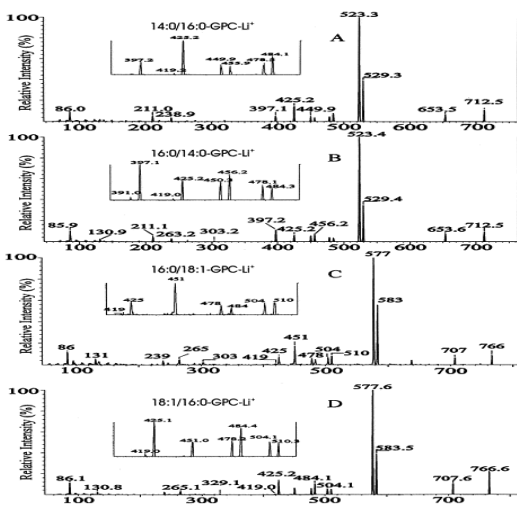calc. m/z 524.3347

m/z 524
calc. m/z 524.3711

Lithiated adducts of phosphocholine provide more structural information in their MS/MS spectra

Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

Relative abundances of product ion can be used to distinguish positional isomers of lithiated phospholipids

Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

Conclusions

- **Shotgun lipidomics approaches are high throughput and applicable to perform profiling as well as quantitative analysis of various lipids in biological samples.**
- **Tandem mass spectrometry analysis of phospholipids in +ve ion mode characterizes phospholipid polar head groups, whereas –ve ion mode provide fatty acid chain structural information**
- **Identification of phospholipids at a molecular level present a great challenge due to their structural diversity and dynamic metabolism.**