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**Introduction to lipidomics:
biological sample preparation
and LC-MS/MS analysis**





Introduction to lipidomics:

- Part one:** **Introduction to LC-MS in lipidomics:**
- 1. general approach for lipid LC/MS analysis**
 - 2. data acquisition (DDA vs DIA, MS^E)**
 - 3. lipid extraction**
- Part two:** **Sample preparation and advanced analysis**
- 4. challenges in sample preparation. MW**
 - 5. advanced LC-MS in lipidomics with research examples for eicosanoids**

Chromatography or direct infusion?

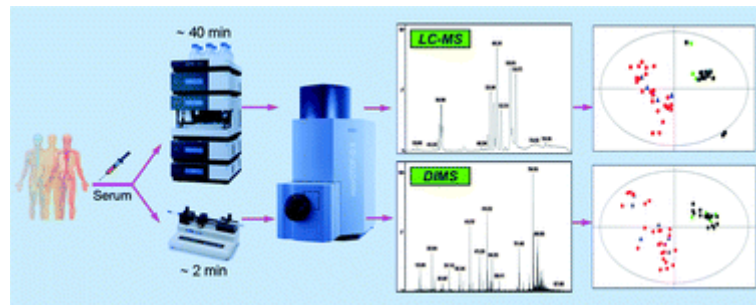
Isobaric ions

Time

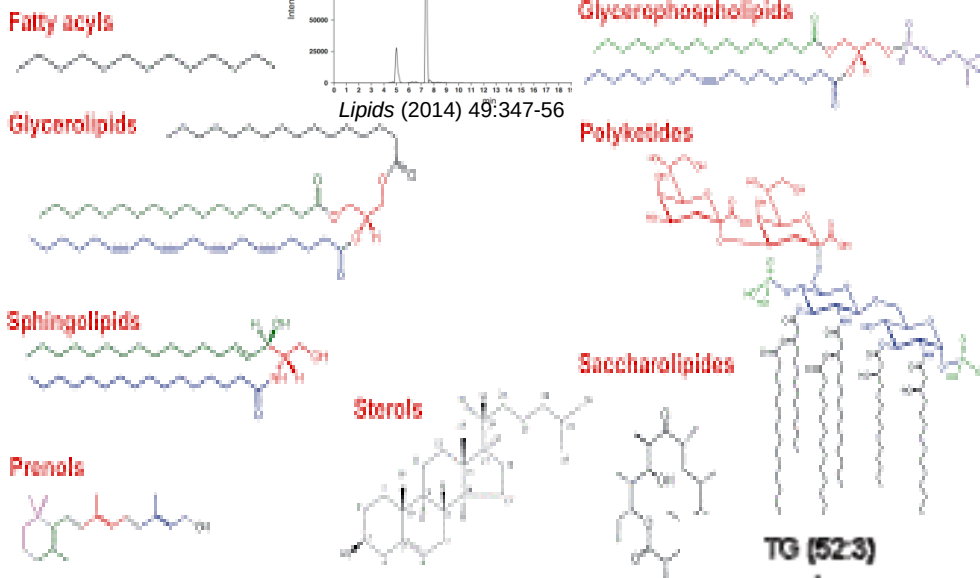
Fast vs comprehensive analysis

Noise

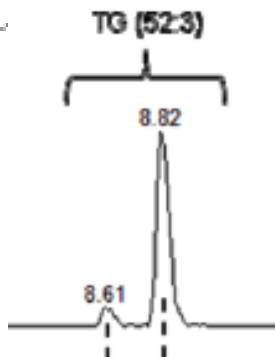
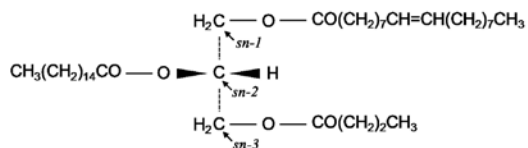
Ion suppression^A

Signal to noise¹⁰⁰

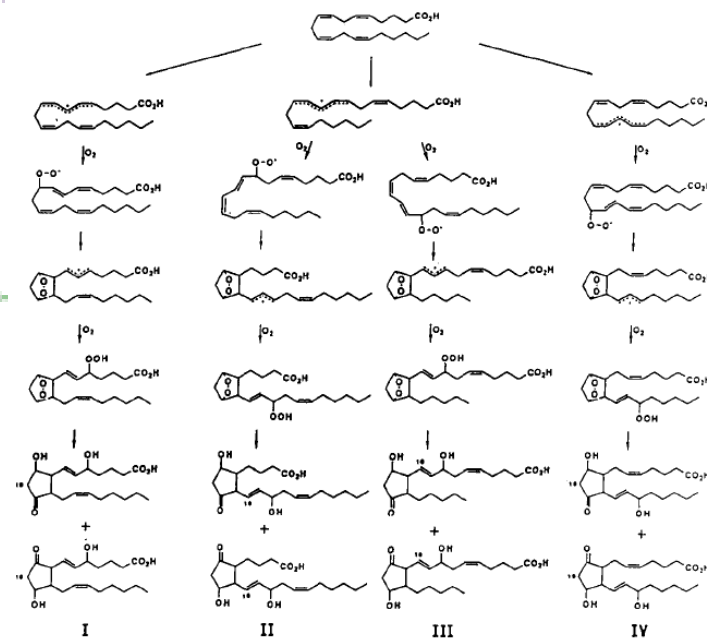
Analyst (2010) 135:2970-8



Anal. Chem. (2018), 90:4249–57

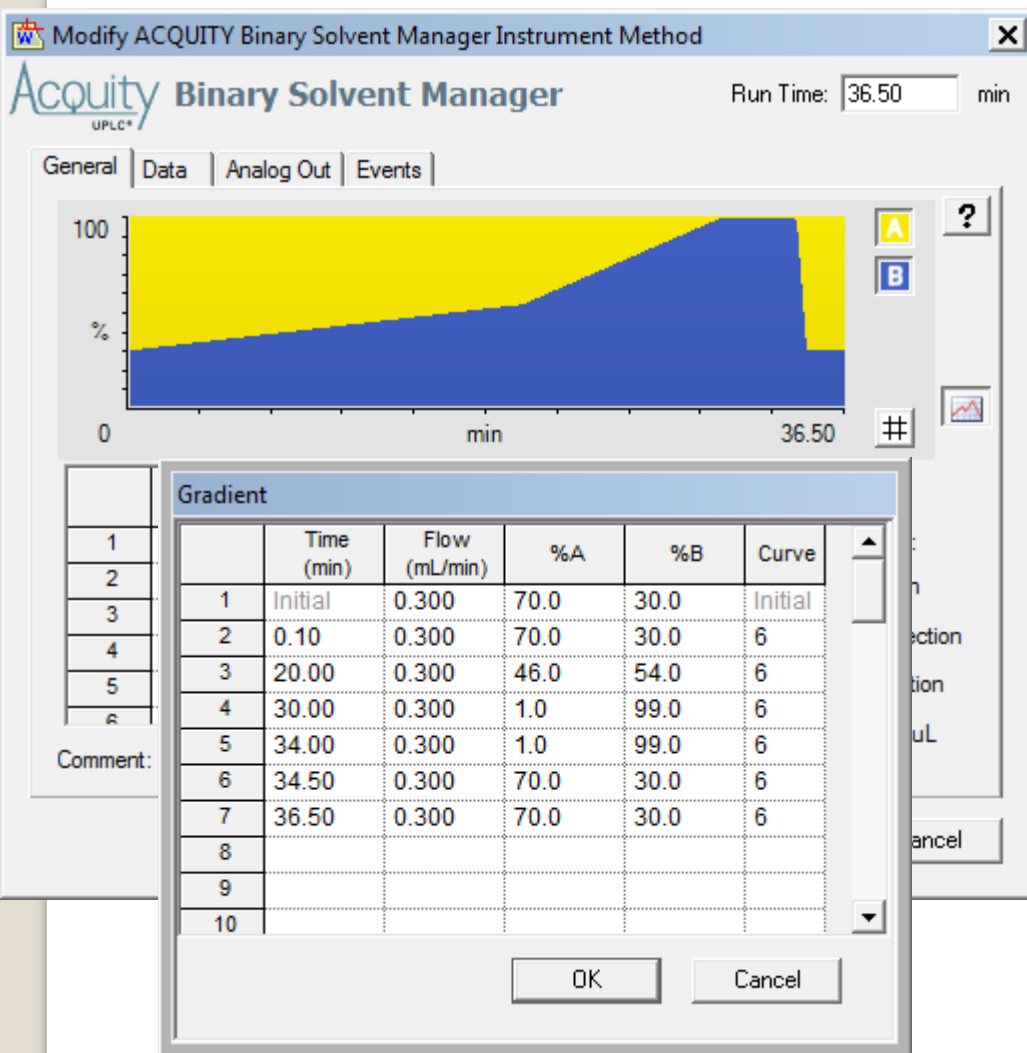


J of Proteome Research (2011) 10:3303-8



J Biol Chem. (1994) 269:4317-26

Plasma lipids, long run for FA, MS^E



A1: H₂O (60%), ACN (40%), AmAc (10 mM), AcAc (0.025%)

B1: IP (90%), ACN (10%), AmAc (10mM), AcAc (0.02%); Heat, Sonicate

Column: ACQUITY UPLC HSS T3 C18 1.8um 2.1x150mm

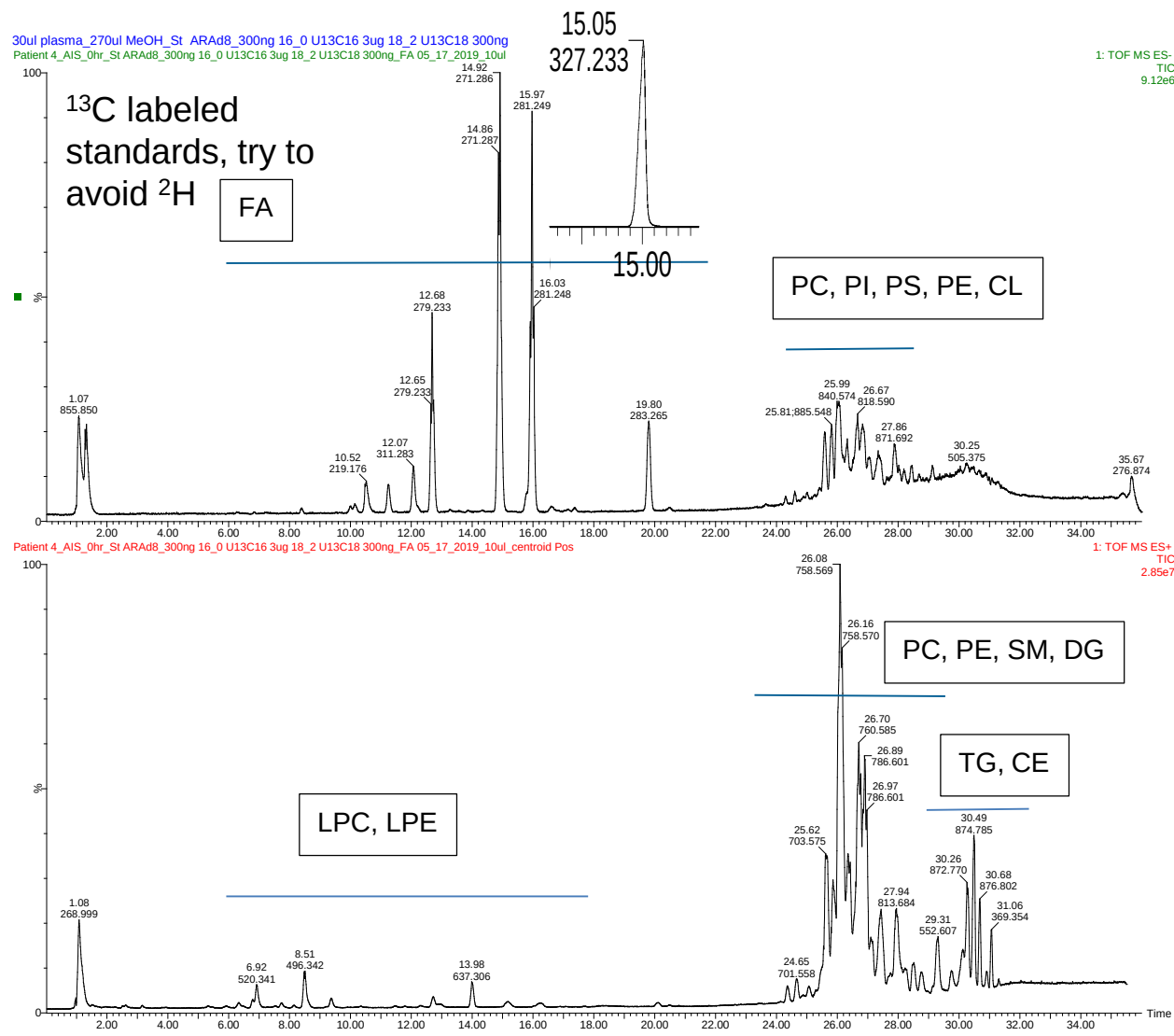
After each 20-25 samples, wash with B1 gradient three times

10 mg or µL of sample in 100 µL of injection solvent

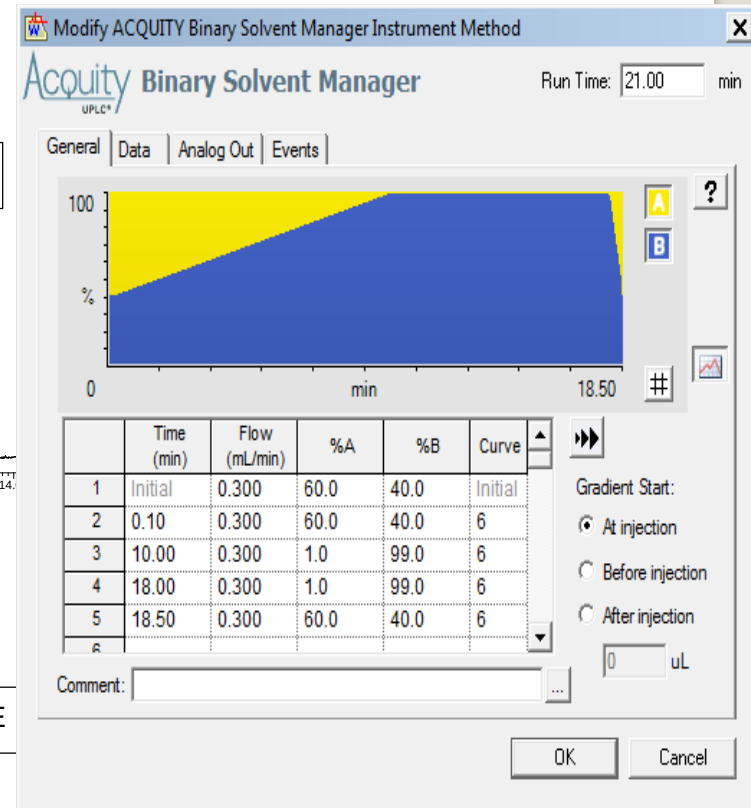
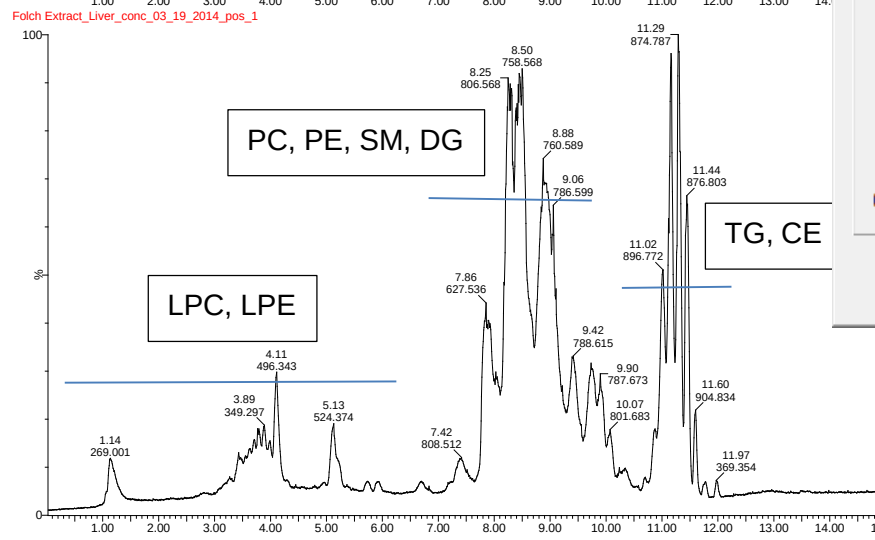
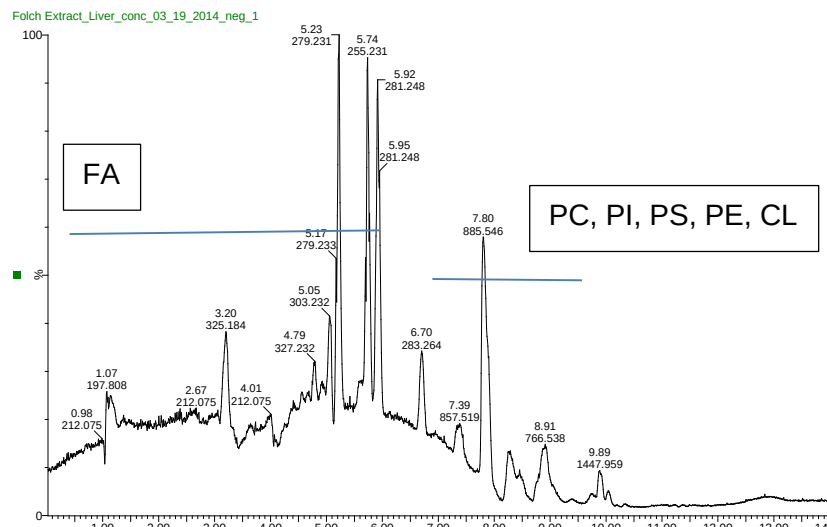
10 µL injected

1x and 100x dilution samples

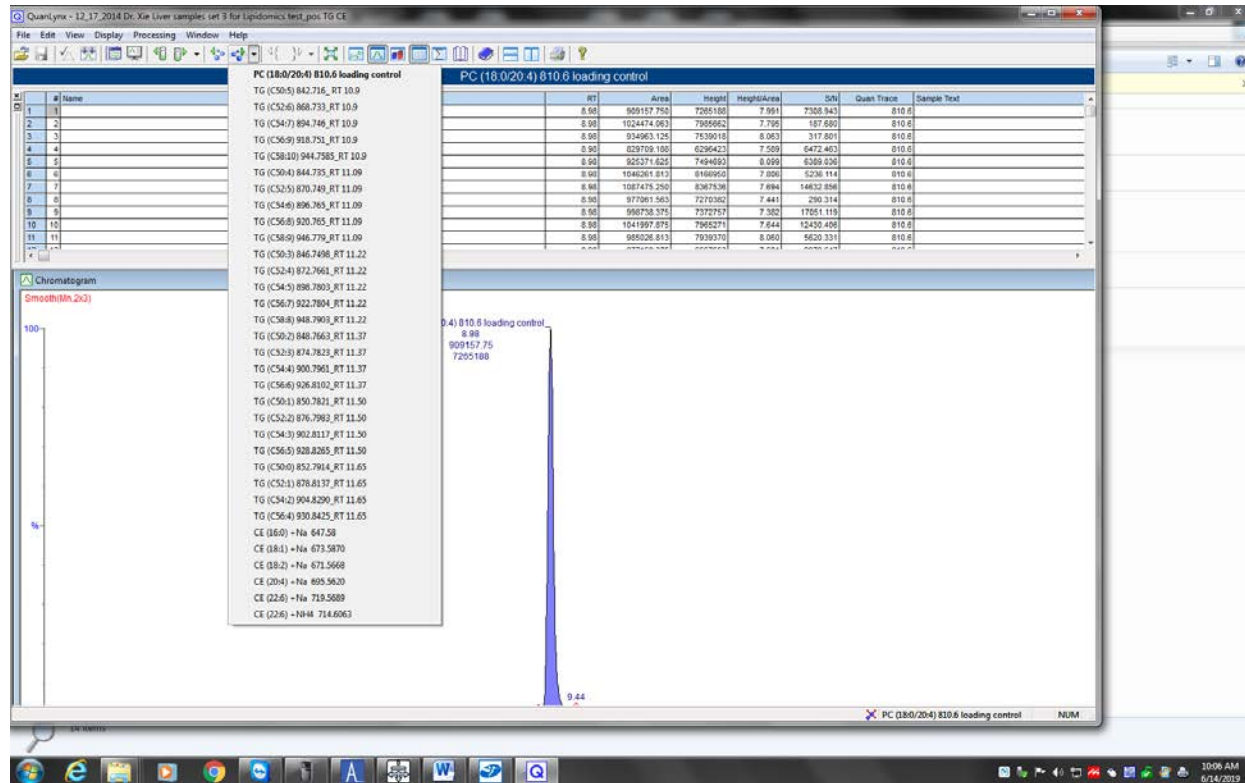
Plasma lipids, long run for FA, MS^E



Liver lipids, short run, MS^E



Plasma TG targeted analysis



Data acquisition: DDA or DIA (MS^E) DDA MS filter (Q) has low resolution

Journal of
research articles **proteome**
•research

Comprehensive LC–MS^E Lipidomic Analysis using a Shotgun Approach and Its Application to Biomarker Detection and Identification in Osteoarthritis Patients

Jose M. Castro-Perez,^{*,†,‡} Jurre Kamphorst,[†] Jeroen DeGroot,[§] Floris Lafeber,^{||} Jeff Goshawk,[⊥]
Kate Yu,[‡] John P. Shockcor,[‡] Rob J. Vreeken,^{†,¶} and Thomas Hankemeier^{†,¶}

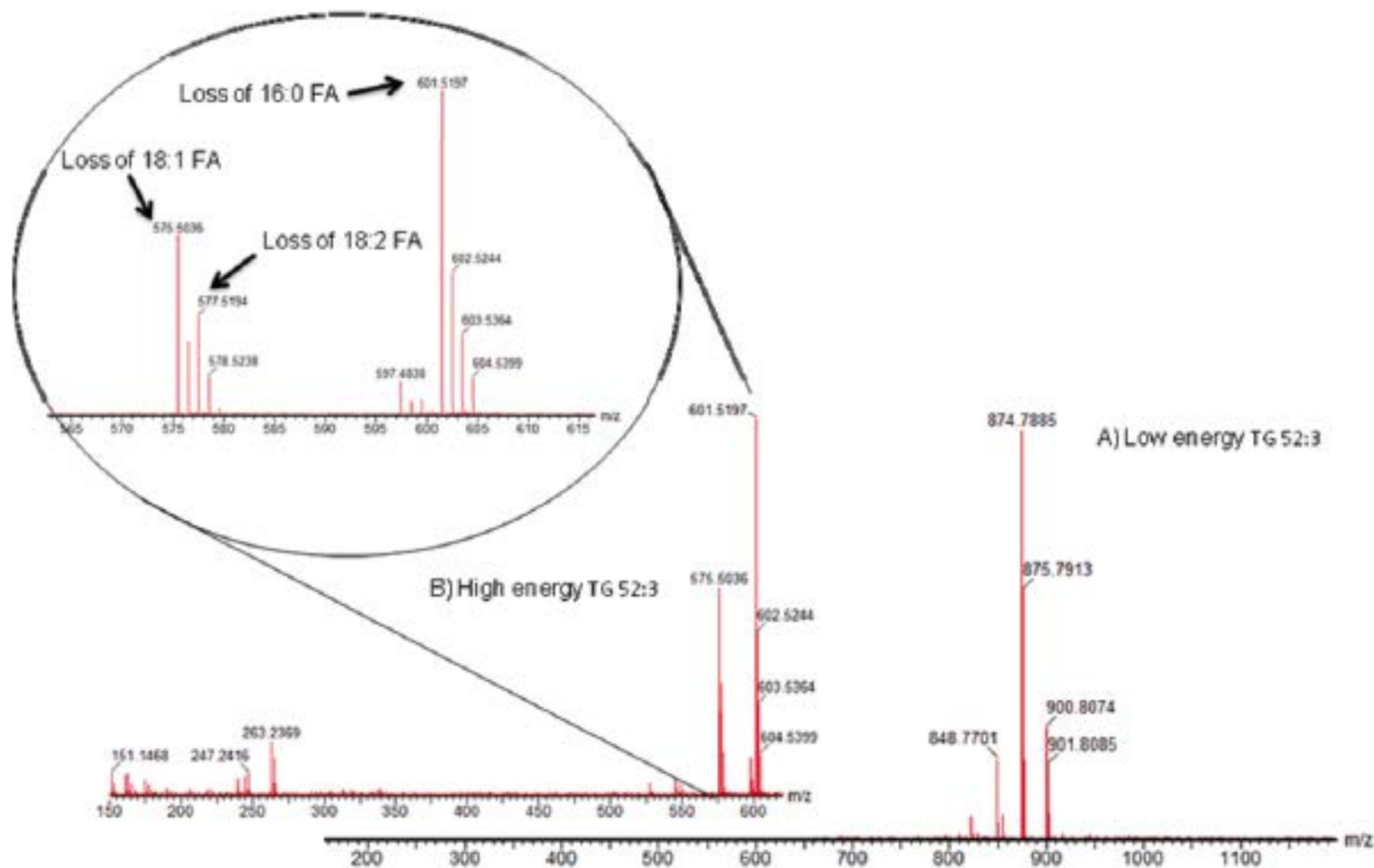
Division of Analytical Biosciences, LACDR, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands, Netherlands Metabolomics Centre, LACDR, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands, Waters Corp, Milford, Massachusetts 01757, Business unit Biosciences, TNO Quality of Life, P.O. Box 2215, 2301 CE Leiden, The Netherlands, Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands, and Waters MS Technology Center, Manchester, United Kingdom

Received November 27, 2009

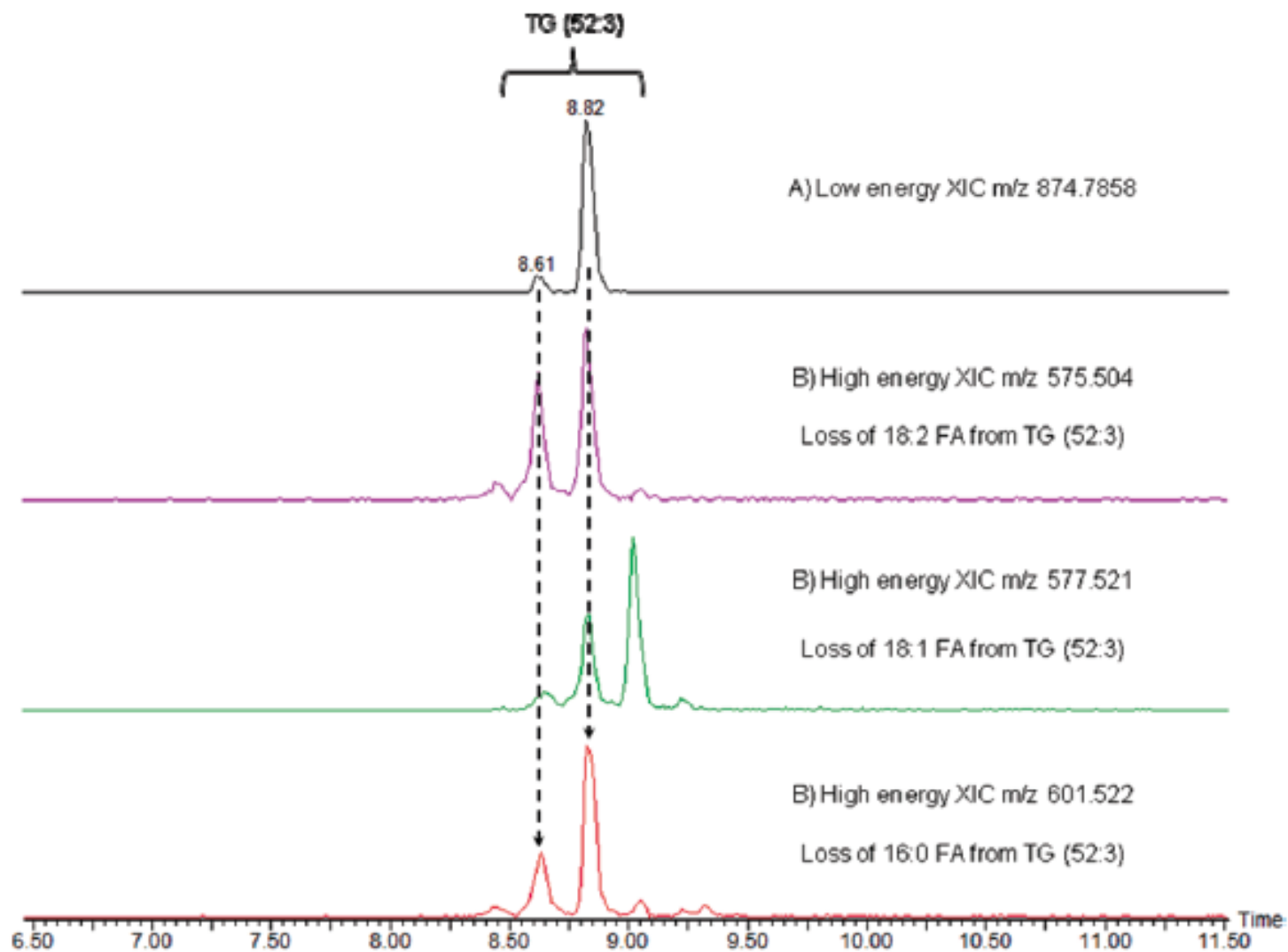
A fast and robust method for lipid profiling utilizing liquid chromatography coupled with mass spectrometry has been demonstrated and validated for the analysis of human plasma. This method allowed quantification and identification of lipids in human plasma using parallel alternating low energy and high energy collision spectral acquisition modes. A total of 284 lipids were identified and quantified (as relative concentrations) in both positive and negative ion electrospray ionization mode. The method was validated with five nonendogenous lipids, and the linearity (r^2 better than 0.994) and the intraday and interday repeatability (relative standard deviation, 4–6% and 5–8%, respectively) were satisfactory. The developed lipid profiling method was successfully applied for the analysis of plasma from osteoarthritis (OA) patients. The multivariate statistical analysis by partial least-squares-discrimination analysis suggested an altered lipid metabolism associated with osteoarthritis and the release of arachidonic acid from phospholipids.

Keywords: lipids • mass spectrometry • metabolite profiling • UPLC • MS^E • osteoarthritis • biomarker

TG analysis after MS^E data acquisition



TG analysis after MS^E data acquisition



Targeted



- RRT vs IS
- RPA (IS)
- response factors
- matrix effect
- ion suppression

Untargeted

- Lipidsearch (Thermo, need to convert raw files from other vendors)
- SimLipid
- LipidView (AB Sciex)
- Progenesis QI (Waters) with LipidMaps or Metlin database



Lipid extraction

Extraction

SPE - purification

Two phase

Single phase

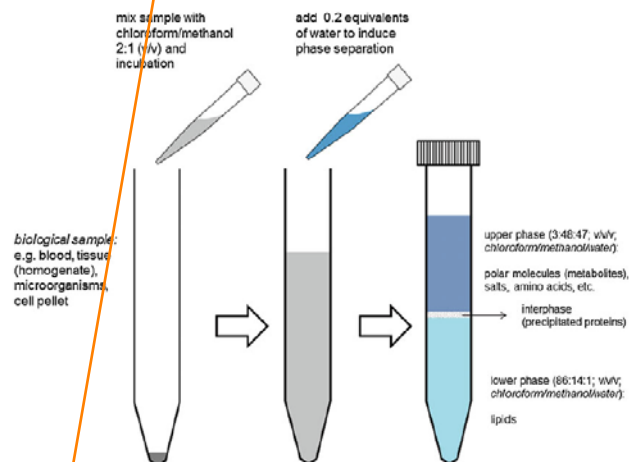
Folch

Bligh and Dyer

HIP

Methanol (labeled standards)

J Lipid Res (2011) 52:850-859



Encyclopedia of lipids (2016)

Acetone/hexane

For omics (MS) should consider a matrix effect (SPE gave 20 fold higher background noise compared to acetone/hexane for PG analysis)

Specific lipid extraction

PIP – low pH

Eicosanoids – acetone

FAMES – ether or hexane

Oxysterols – SPE

Silica columns

Extraction Method	PGE ₂ and PGD ₂	TXB ₂
Methanol followed by solid-phase extraction	3.9 ± 1.0 (0.5 ± 0.1)	17.3 ± 6.2 (2.0 ± 0.7)
Diethyl ether followed by solid-phase extraction	5.6 ± 2.5 (0.7 ± 0.3)	5.5 ± 1.9 (0.7 ± 0.2)
Hexane/2-propanol followed by solid-phase extraction	5.0 ± 1.7 (0.6 ± 0.2)	14.5 ± 3.6 (1.7 ± 0.4)
Hexane/2-propanol followed by acetone	0.5 ± 0.1 (0.1 ± 0.0)	0.7 ± 0.2 (0.1 ± 0.0)
Acetone	0.6 ± 0.1 (0.1 ± 0.0)	0.9 ± 0.2 (0.1 ± 0.0)

J Lipid Res (2008) 49:893-902

Introduction to lipidomics:

Part one: Introduction to LC-MS in lipidomics:

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3. lipid extraction

Part two: Sample preparation and advanced analysis

- 4. challenges in sample preparation. MW**
- 5. advanced LC-MS in lipidomics with research examples for eicosanoids**

Brain metabolome is significantly altered upon 30 sec of brain ischemia-injury

Microwaved:

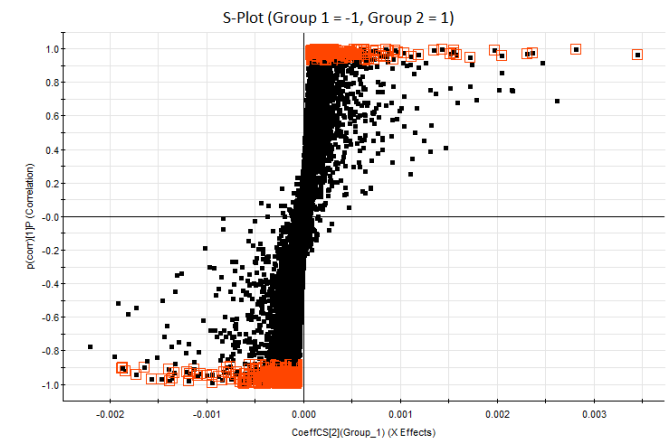
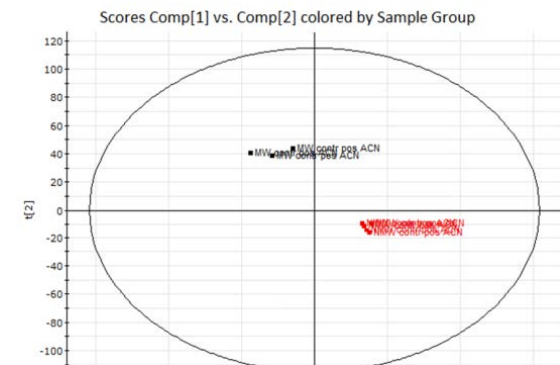
- tissue was fixed with head-focused microwave irradiation;
- protein are heat denatured by 80 - 90°C in 1.2s

6kW
2450 MHz



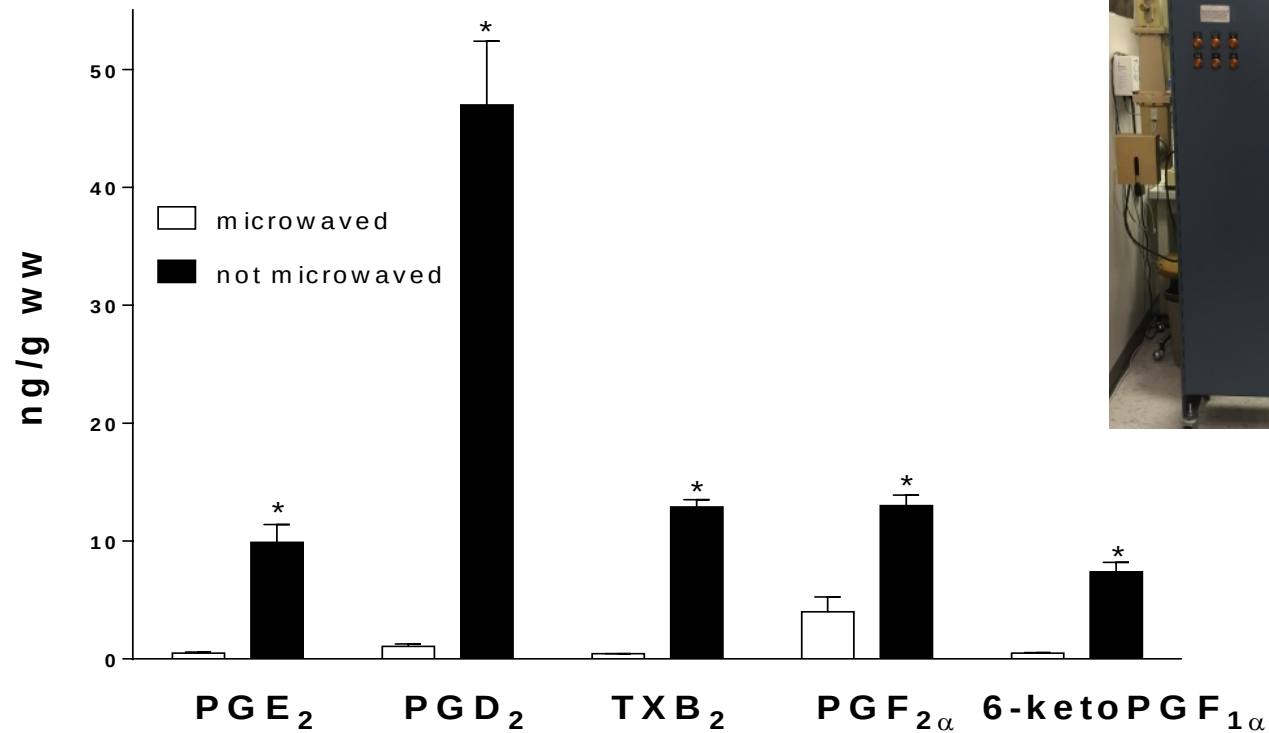
Not Microwaved:

- 20 - 30 s was required to extract and freeze brain in liquid nitrogen



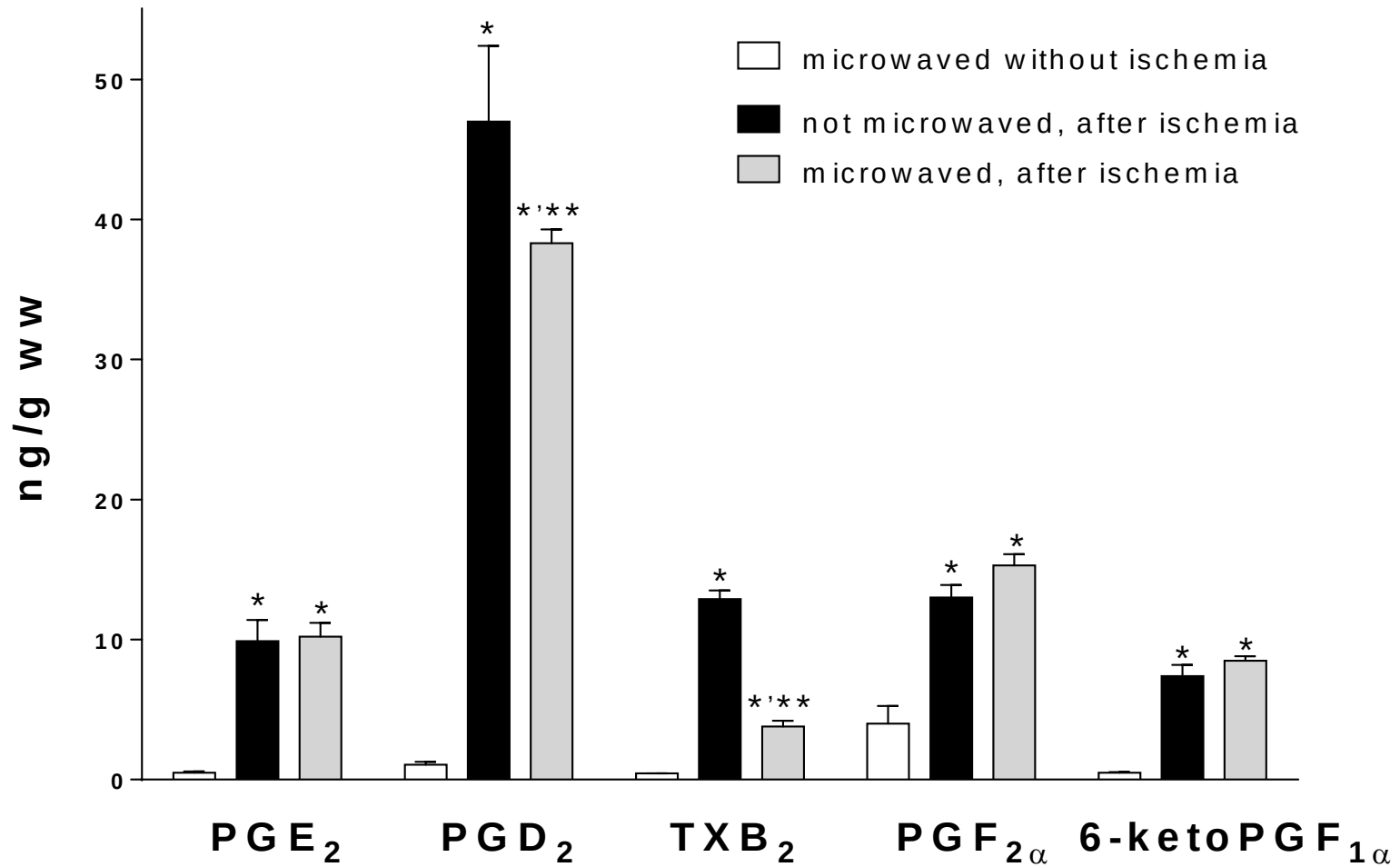
Brain prostanoid levels at basal conditions and 30 sec after brain ischemia

Brain prostanoids are significantly increased upon 30 sec of brain ischemia-injury

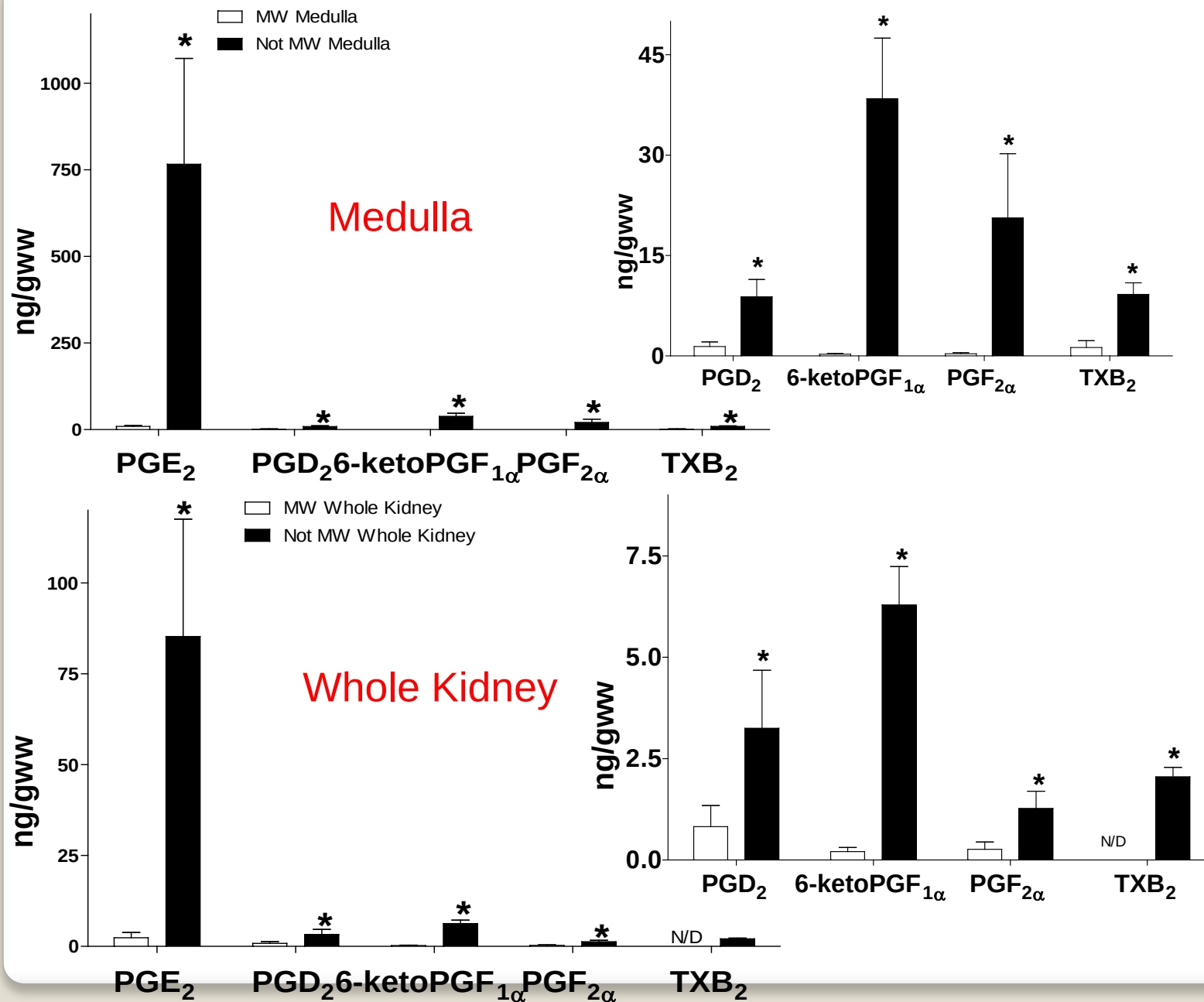


Brain prostanoid levels at basal conditions and 30 sec after brain ischemia

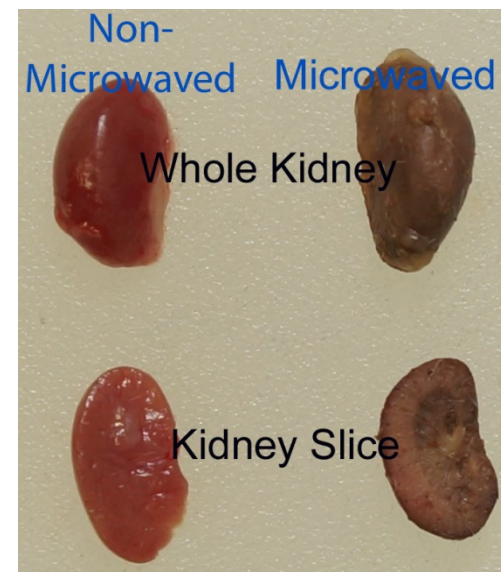
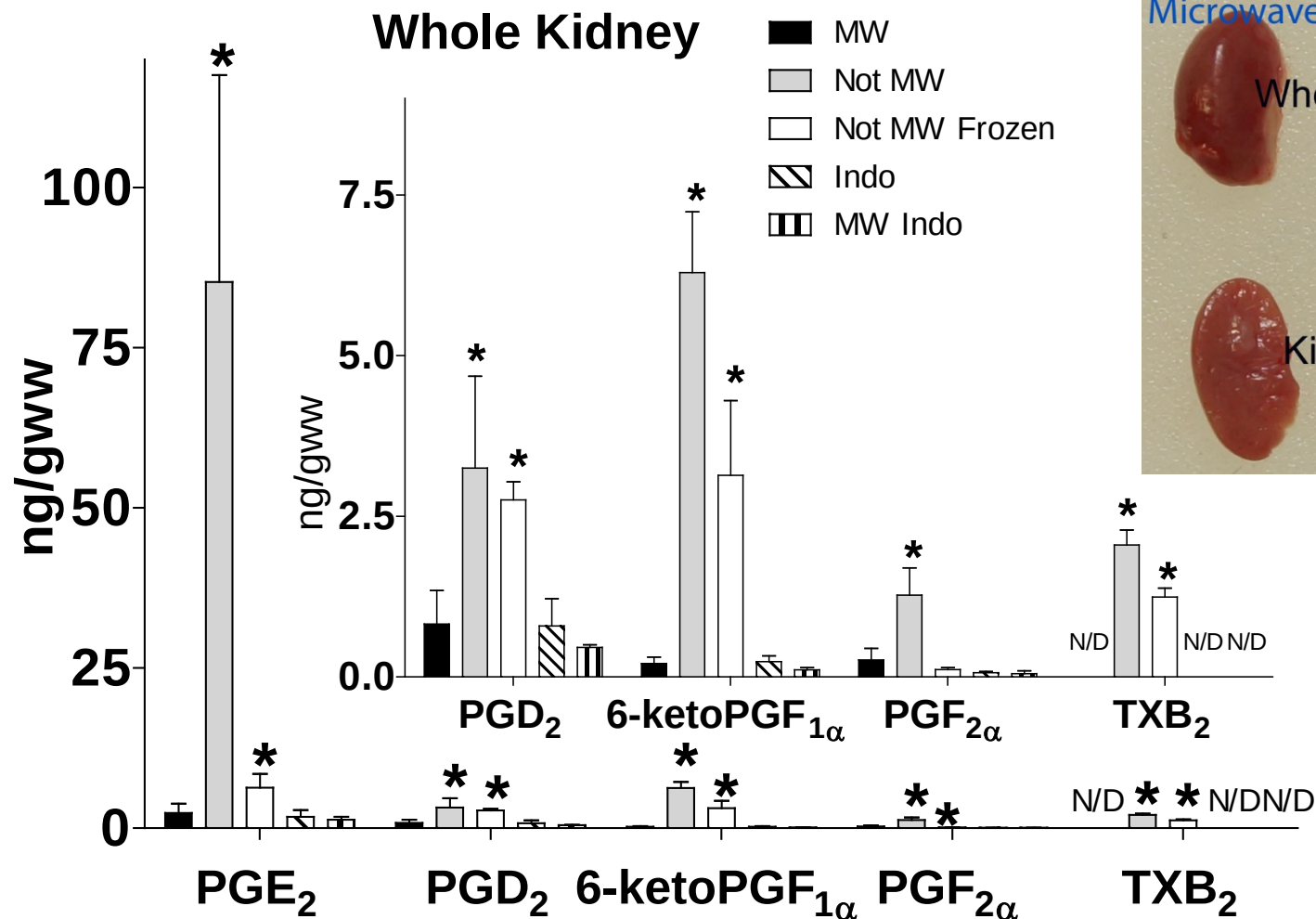
Prostanoids are not destroyed or trapped in denatured proteins upon MW



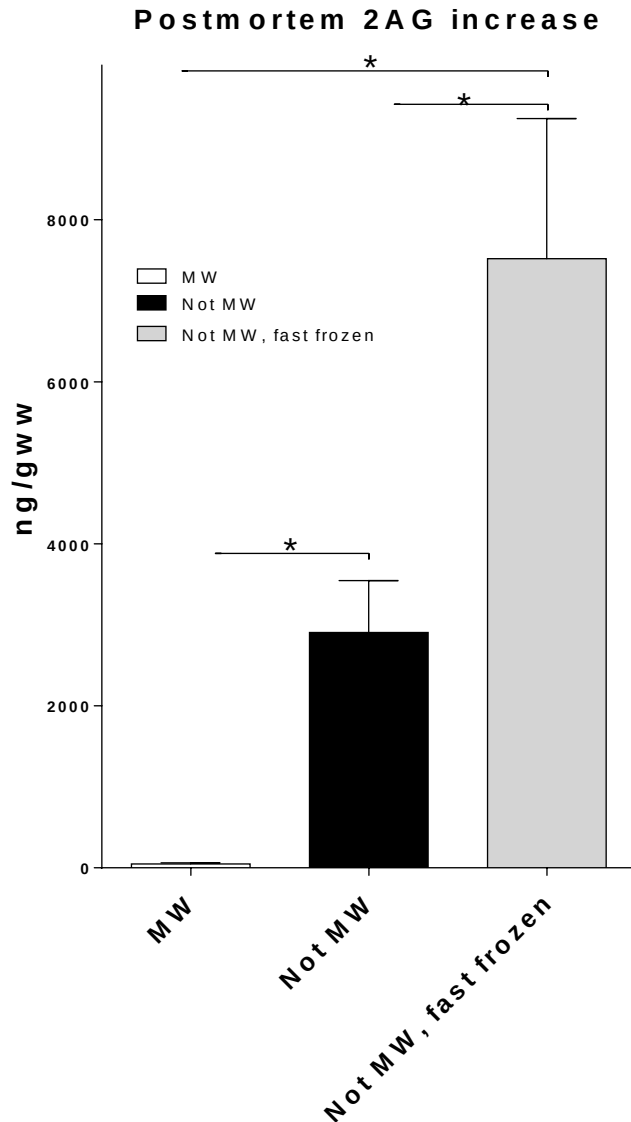
A dramatic increase in PG levels in not-MW kidney



MW is required to measure PG kidneys levels and PG are not destroyed or trapped by MW



A dramatic postmortem 2AG increase is prevented by MW but not by fast freezing of intact head in liquid nitrogen



Not Microwaved:

- 20 - 30 s was required to extract and freeze brain in liquid nitrogen

Microwaved:

- tissue was fixed with head-focused microwave irradiation;
- protein are heat denatured in 1.2s by 80 - 90°C

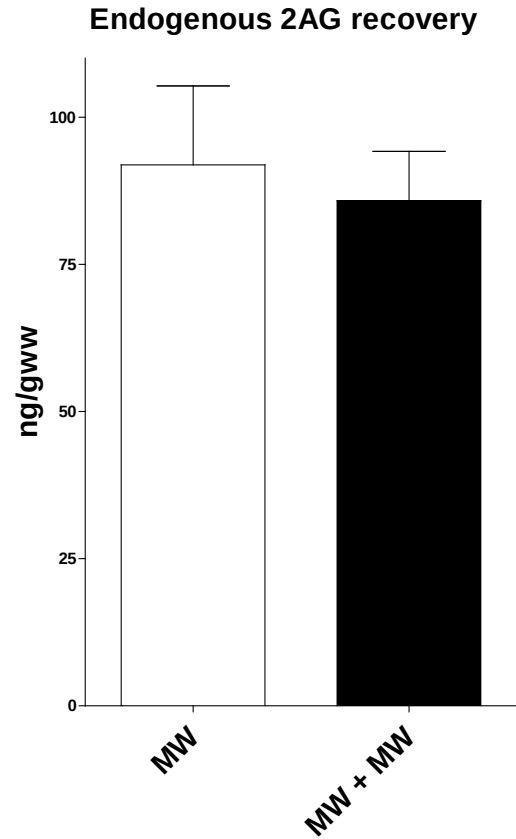
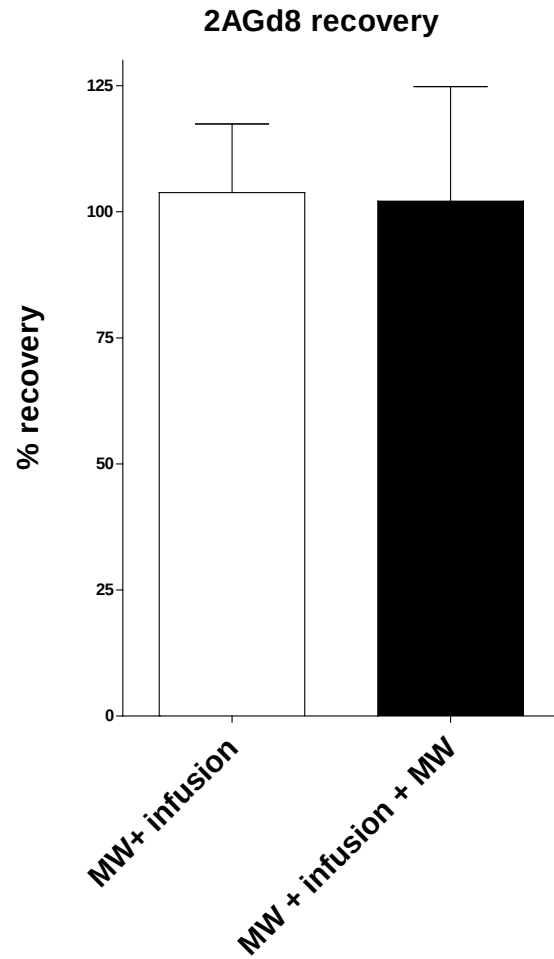
Fast Frozen:

- animals was frozen in liquid nitrogen;
- brain samples kept cold during extraction

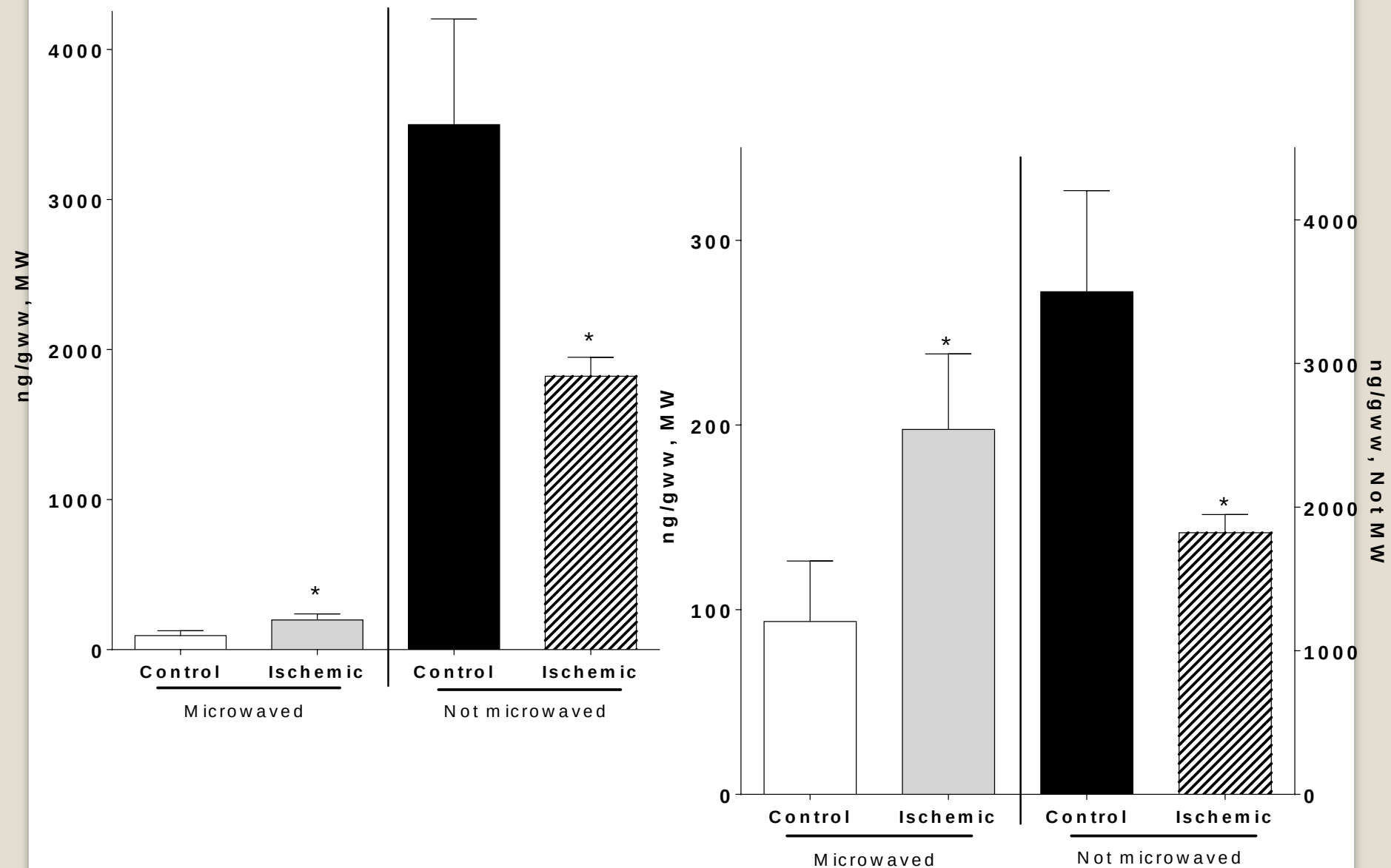


6kW
2450 MHz

MW does not destroy endogenous or exogenous 2AG



MW is required to measure 2AG alterations under ischemia/injury



MW is required to measure many other key metabolites

Prostanoids

- **PGE₂, PGD₂, PGF₂α, 6ketoF₁α, TxB₂**

Lipoxygenase products

- **HETEs**

Endocannabinoids

- **2-AG and other MG, anandamides**

Other lipids

- **PL, PIs, FFA, Acyl-CoA**

Nucleotide phosphates

- **ATP, ADP, AMP**

Nucleosides

- **Adenosine**

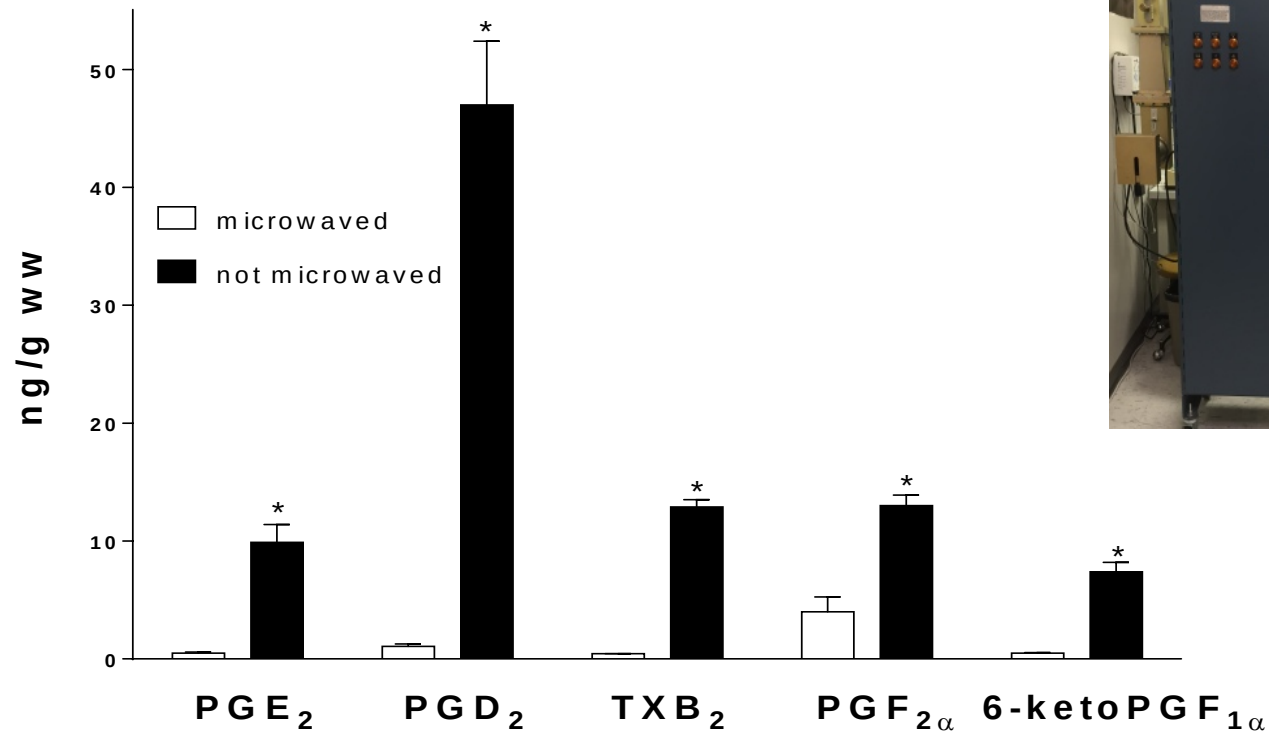
Carbohydrates

- **Glucose, Glycogen**

Carboxylic acids

- **Lactate**

Brain prostanoids are significantly increased upon 30 sec of brain ischemia-injury

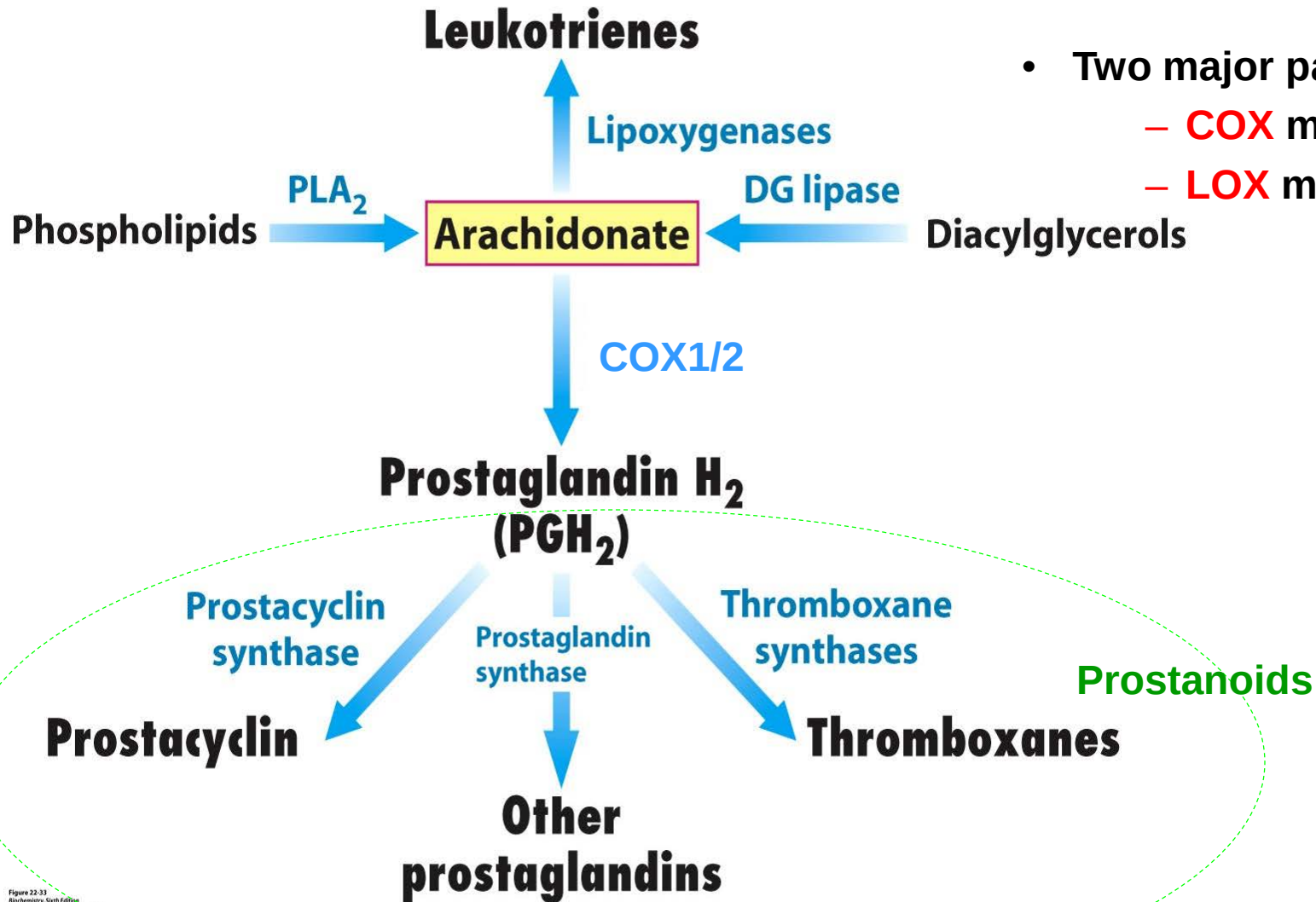


Brain prostanoid levels at basal conditions and 30 sec after brain ischemia

Prostanoids

- Products of polyunsaturated fatty acid enzymatic COX1/2 oxygenation.
- Short lived.
- Local hormones.
- Regulate many functions:
 - neurotransmission,
 - promotion of sleep,
 - pain, inflammation, resolving inflammation, transplant rejection, allergic reaction,
 - PGD₂ synthase is second most abundant protein in CSF,
 - platelet aggregation, vasoconstriction,
 - cell adhesion (cancer).
- Anti-inflammatory and pain relief drugs such as aspirin act by downregulating eicosanoid synthesis.
- There are three families of prostanoids (20"C" prostanoids):
 - prostaglandins,
 - prostacyclins,
 - thromboxanes.

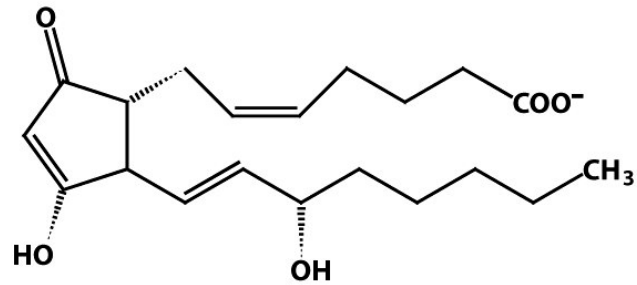
Eicosanoid Biosynthesis



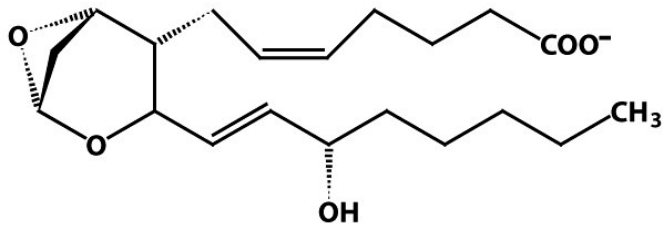
- Two major pathways:

- **COX** mediated
- **LOX** mediated

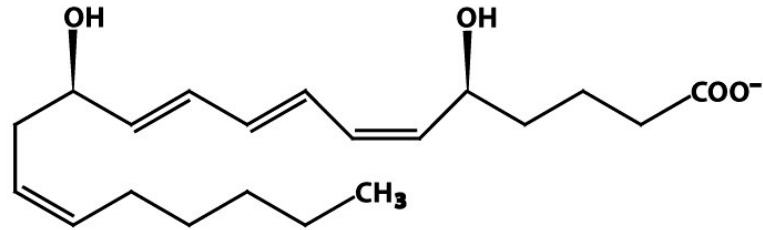
Selected Eicosanoids



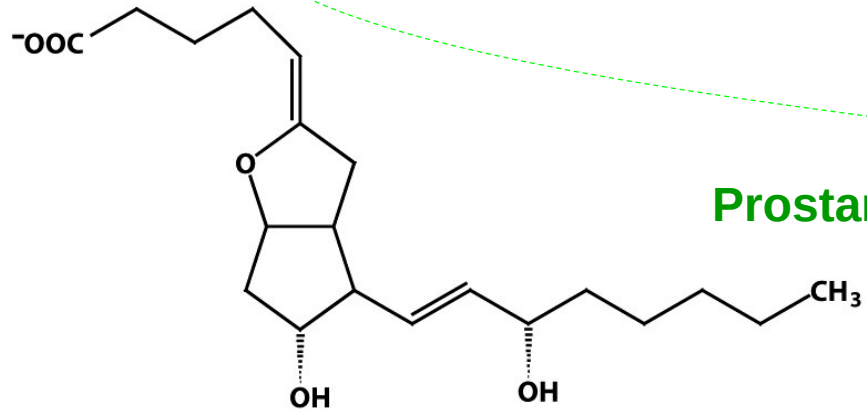
Prostaglandin E₂



Thromboxane A₂ (TXA₂)



Leukotriene B₄

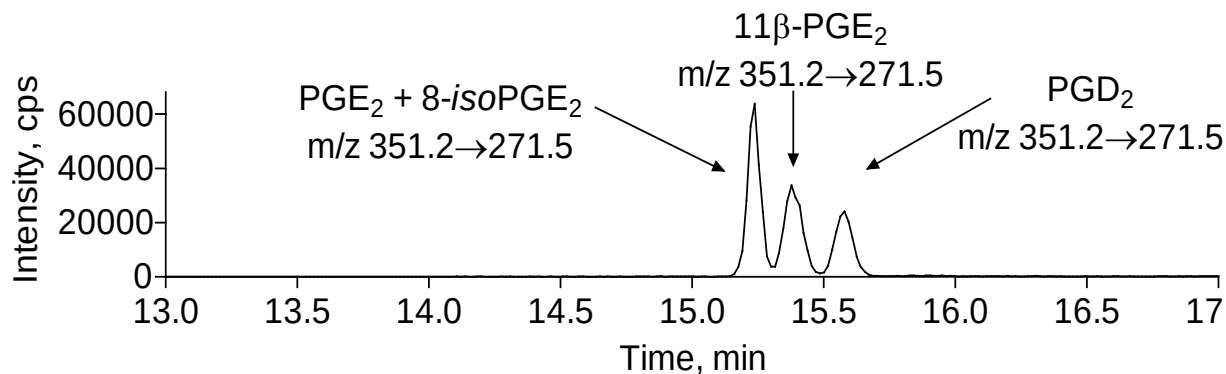
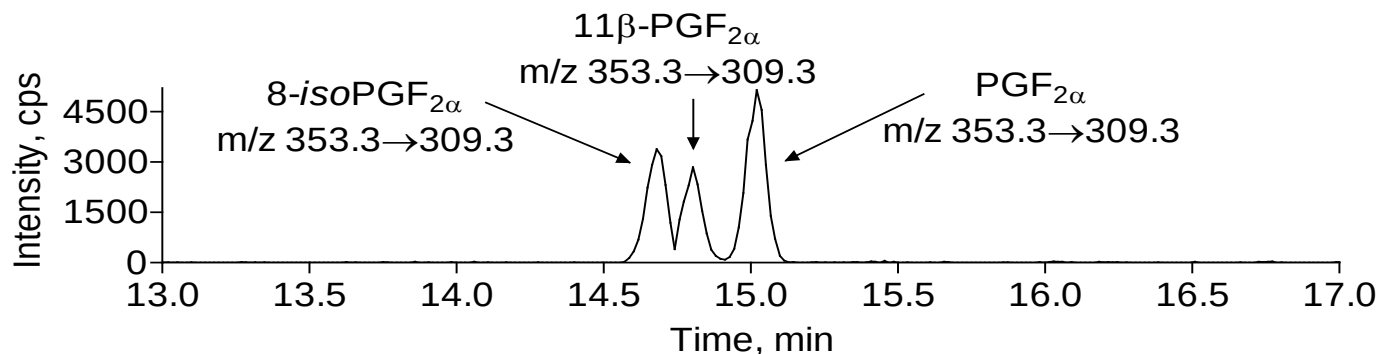


Prostacyclin (PGI₂)

Prostanoids

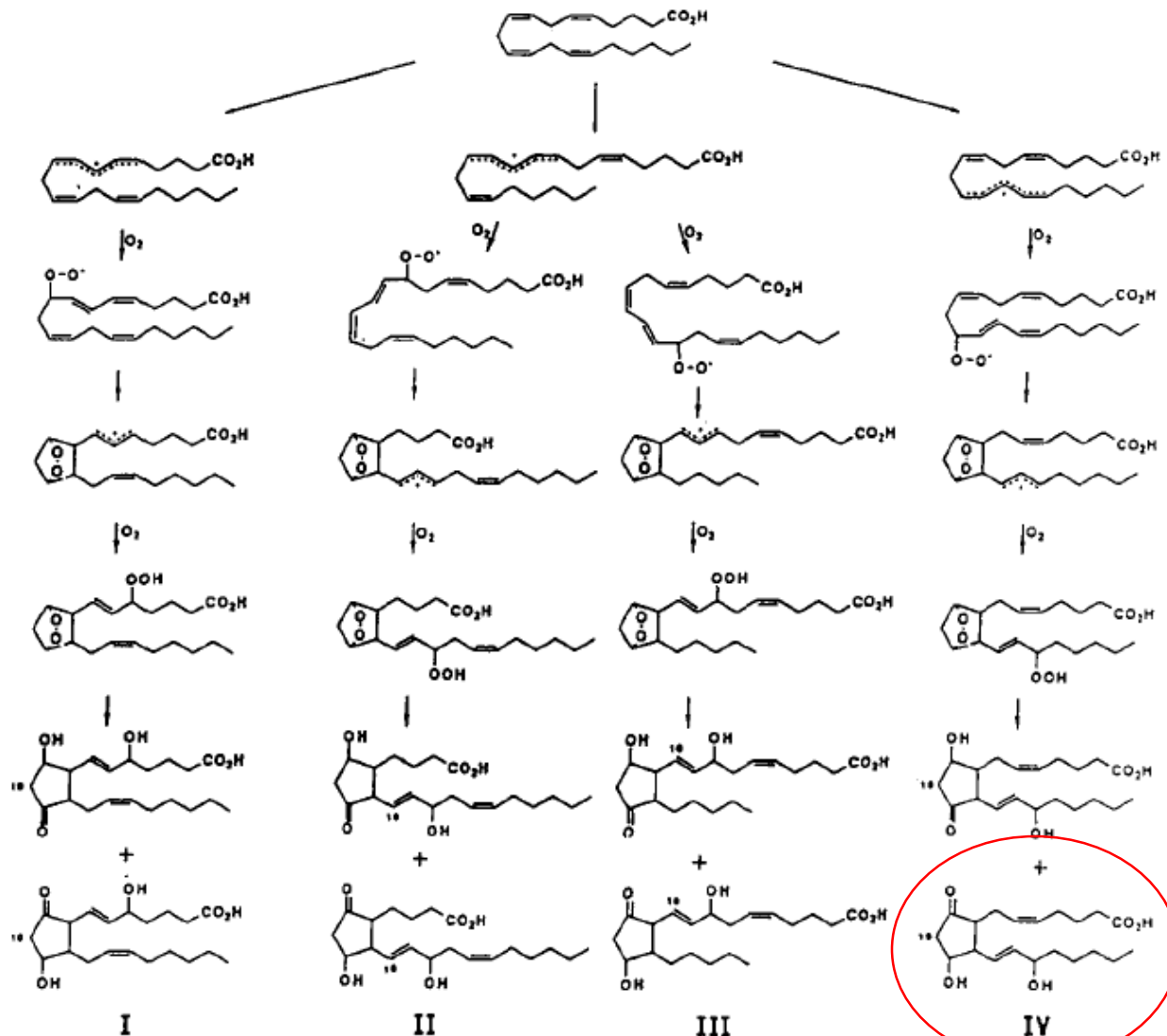
Are prostanoids stored in phospholipids are isoprostanes?

RF HPLC method used for the analysis does not separate PG from all isoprostanes



Are prostanoids stored in phospholipids are isoprostanes?

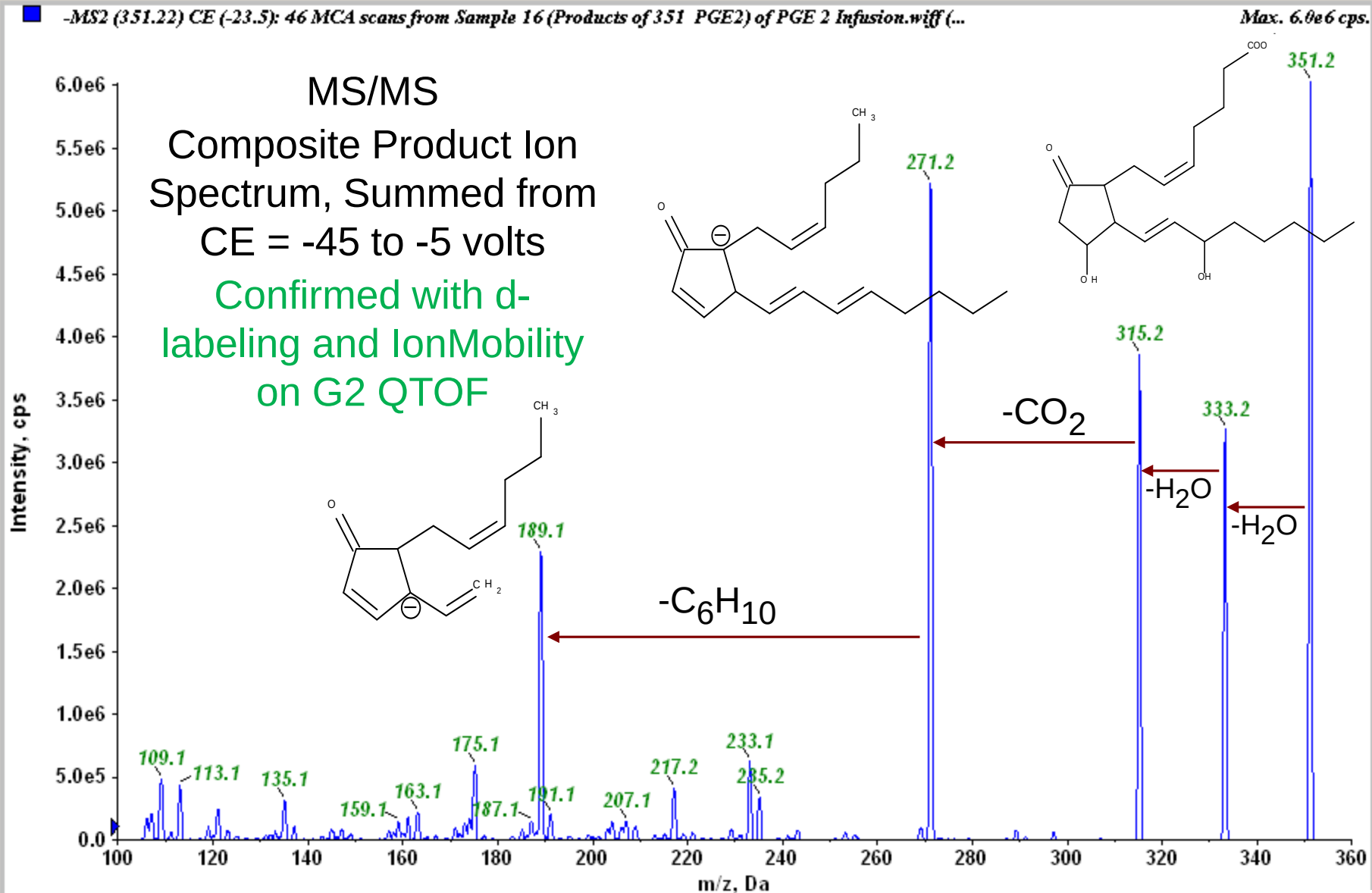
isoPGE₂ formation



J Biol Chem. (1994) 269:4317-26

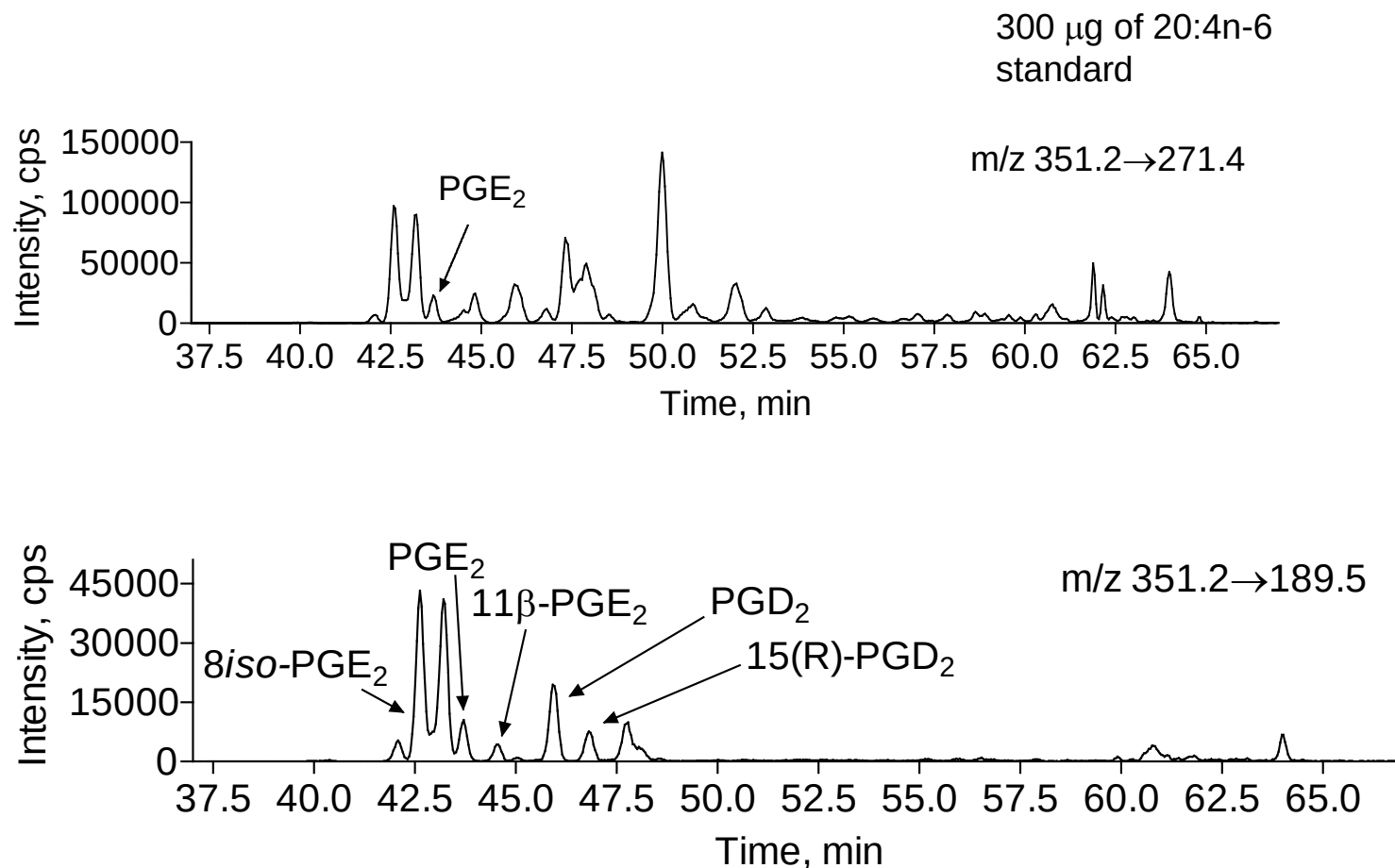
Are prostanooids stored in phospholipids are isoprostanes?

Specificity of MS analysis for isoPGE₂ formation



Are prostanoids stored in phospholipids are isoprostanes?

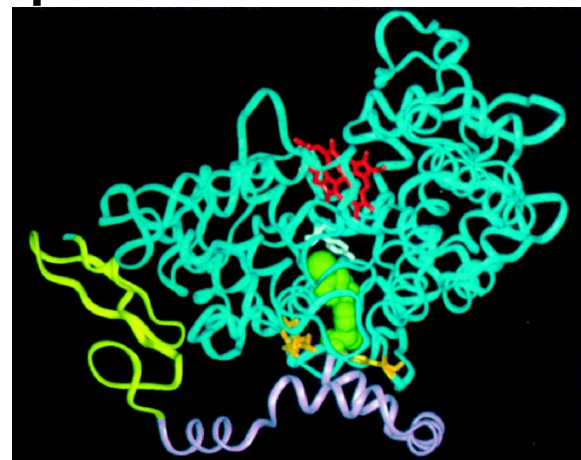
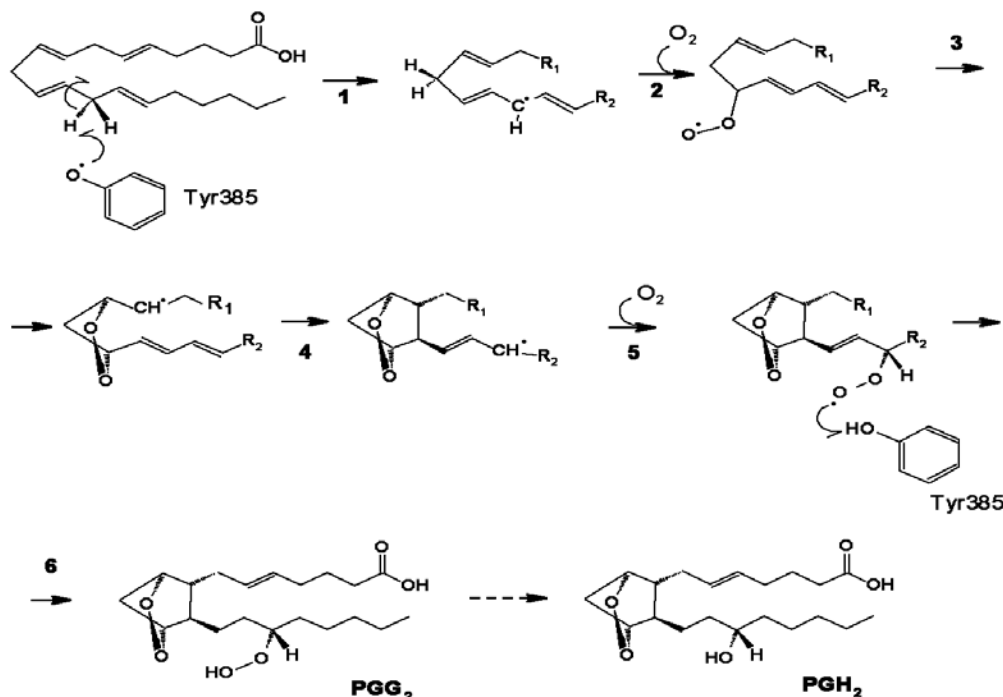
Specificity of a new MS/MS method



Are prostanoids stored in phospholipids are isoprostanes?

Stereochemistry of PGE₂ formation

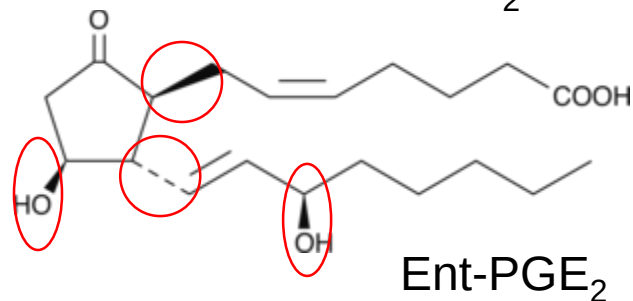
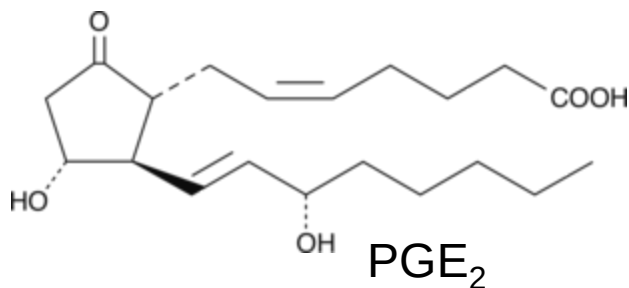
arachidonic acid



JBC (1999) 274: 22903-6

Enzymatic oxidation:
100% PGE₂ + 0%
ent-PGE₂

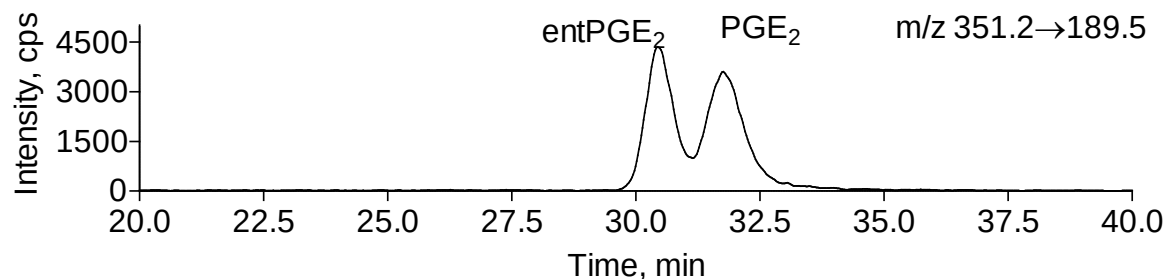
Non-enzymatic
oxidation: 50%
PGE₂ + 50% ent-
PGE₂



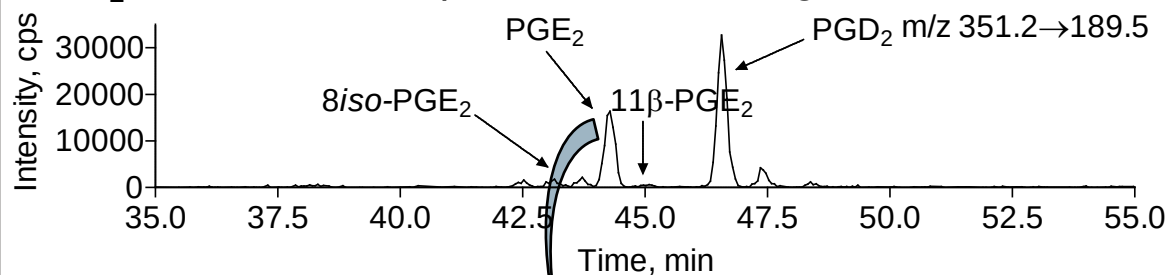
Are prostanoids stored in phospholipids are isoprostanones?

Chiral separation for PGE₂ and ent-PGE₂

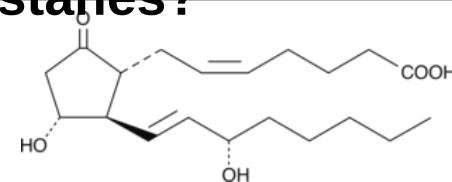
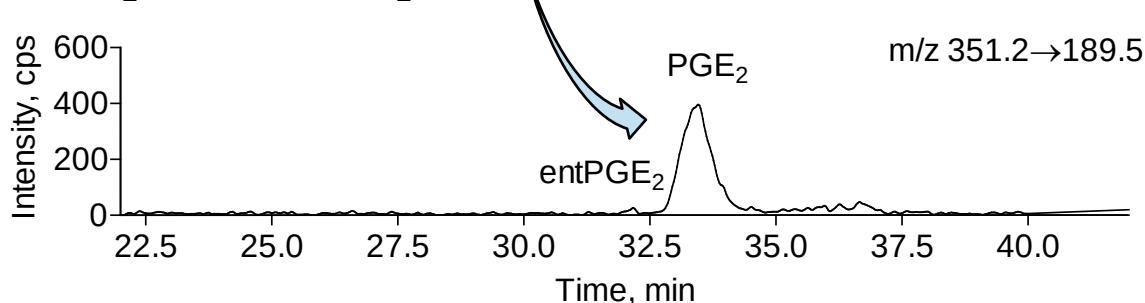
PGE₂ and ent-PGE₂ standards



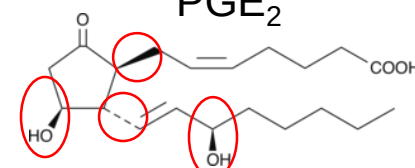
PGE₂ like molecules upon 5 min of brain global ischemia



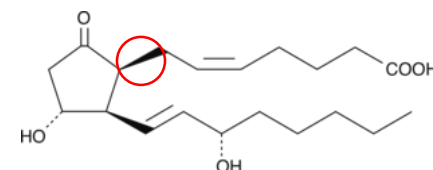
PGE₂ and ent-PGE₂ upon 5 min of brain global ischemia



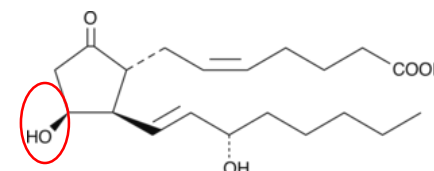
PGE₂



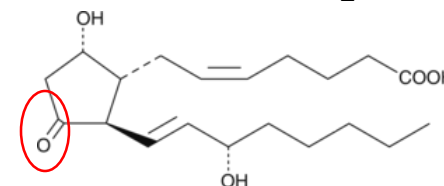
ent-PGE₂



8iso-PGE₂



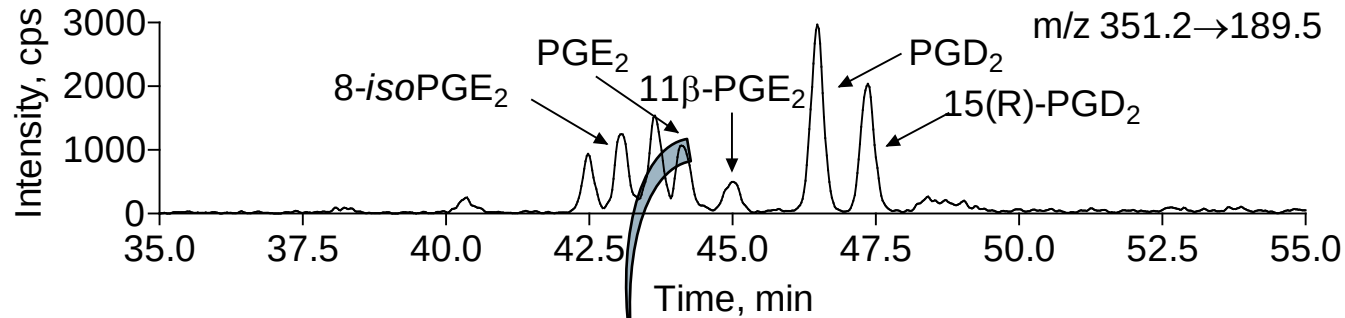
11β-PGE₂



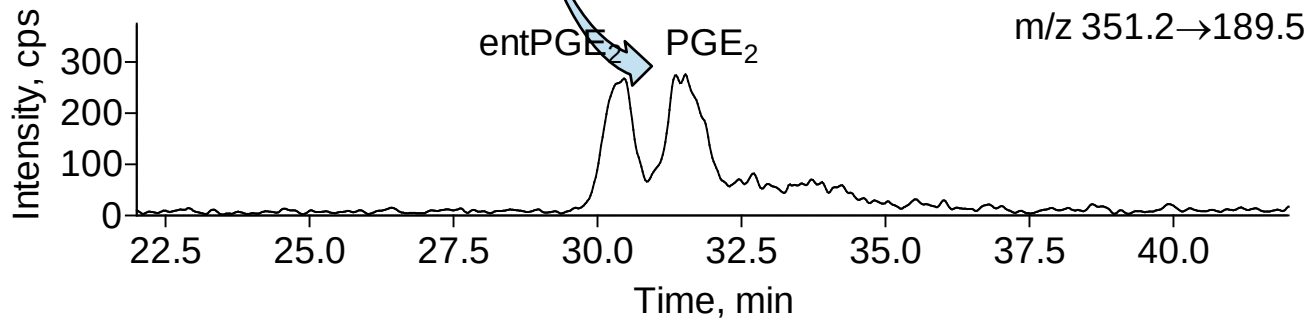
PGD₂

Prostanoids stored in phospholipids are **NOT COX derived PG**

PGE₂ like molecules under basal conditions



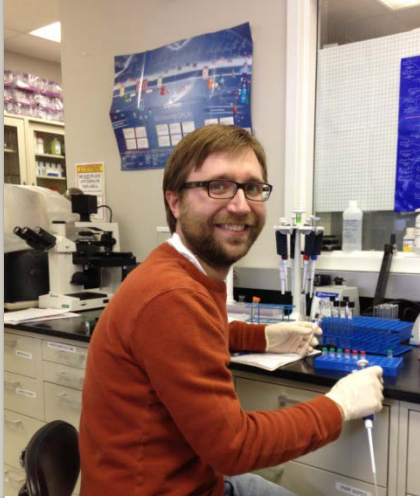
PGE₂ and ent-PGE₂ released from PL



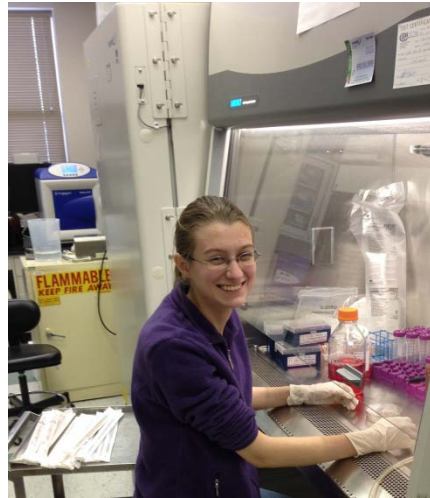
Summary

- LC-MS is invaluable tool for comprehensive lipidomics analysis
- Tissue fixation is required for metabolome analysis under basal conditions
- Tissue fixation is required for measuring metabolome alterations under different conditions
- MW irradiation is an efficient method to tissue fixation without destroying endogenous bioactive lipid metabolites
- 351/189 MS/MS transition combined with chiral chromatography allows for better specificity for PGE₂ series quantification

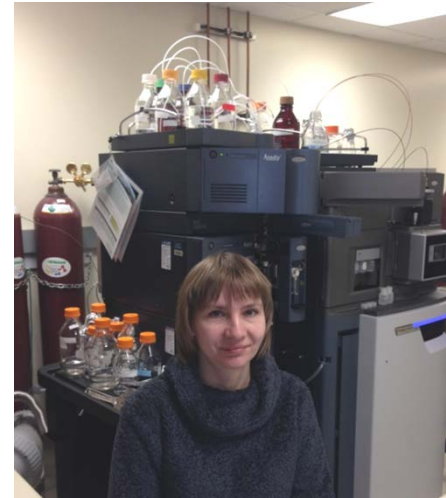
Thanks...



Steve Brose



Amanda Marquardt



Svetlana Golovko



Brock Thuen

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5P30 GM103329 (NIH/GM)

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R21NS109856 (NIH/NINDS)