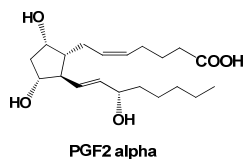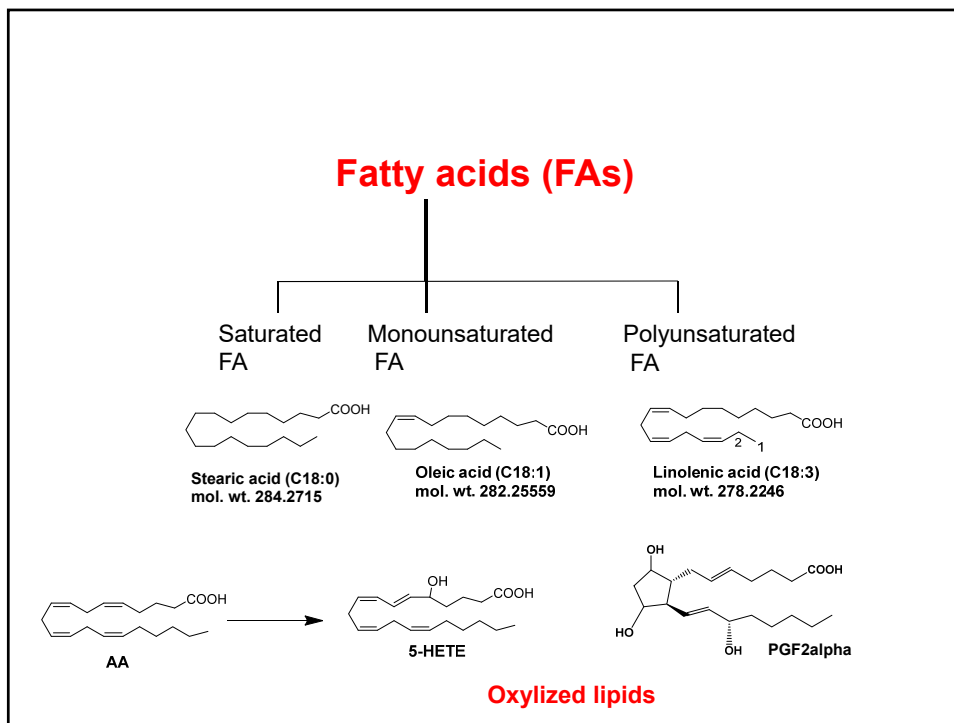# Tandem mass spectrometry analysis of prostaglandins and isoprostanes

PGF2 alpha
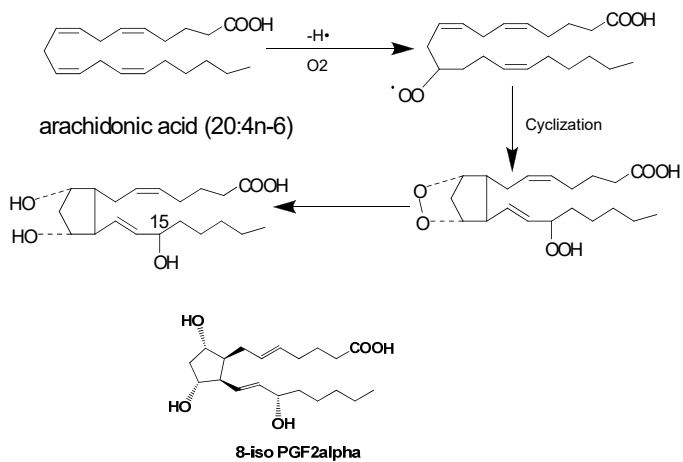
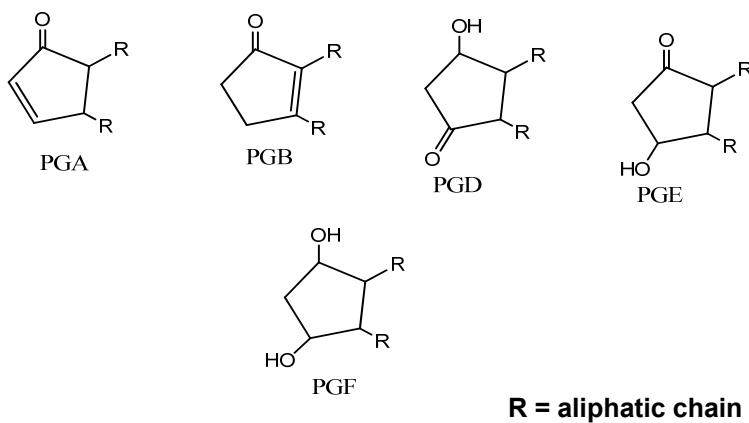Jeevan Prasain
jprasain@uab.edu
6-2612

Overview

- **Introduction to PGs and their synthesis**

- **Mass spectrometry characterization of PGs and isoprostanes**
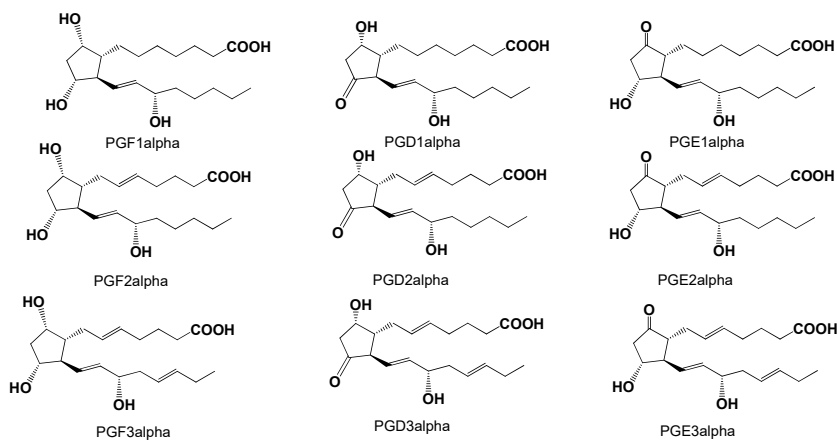
- **PGs in Cox-dKO pups and *C. elegans***

Fatty acids (FAs)

Saturated FA | Monounsaturated FA | Polyunsaturated FA

Stearic acid (C18:0)
mol. wt. 284.2715

Oleic acid (C18:1)
mol. wt. 282.25559

Linolenic acid (C18:3)
mol. wt. 278.2246

AA

5-HETE

PGF2alpha

Oxylized lipids

Cox-dependent PGs synthesis

Steroids

Phospholipase A2

Dietary linoleic acid
(C18: $\Delta^{9,12}$)

↓ elongase

↓ desaturase

Arachidonic acid
C20: $\Delta^{5,8,11,14}$)

lipoxygenase → leukotriene

Arachidonate moiety

↓ Release first

Enzymatic- cyclic pathways

AA (20:4n-6)   **NSAIDs**

| Cox

PGG2

| Cox

PGD
synthase   **PGE**
                 synthase

PGD2 ← **PGH2** → **PGE2**

PGF synthase

PGF2α

PGG2

PGH2

PGF2 alpha

Non-enzymatic isoprostane synthesis

arachidonic acid (20:4n-6)

-H•
O2

Cyclization

15

8-iso PGF2alpha

Structural representation PG based on ring features

PGA

PGB

PGD

PGE

PGF

R = aliphatic chain

Structures of PGs D, E and F series

PGF1alpha

PGD1alpha

PGE1alpha

PGF2alpha

PGD2alpha

PGE2alpha

PGF3alpha

PGD3alpha

PGE3alpha

C. elegans culture, lipid extraction and mass spectrometry analysis

Worm culture

Grown with NA22
E. Coli bacteria

Collection
Stored -80 ^{0}C

Homogenate
+ IS

Centrifuge
Hexane

Hexane

Aq. acetone

3 M HCOOH
+ CHCl$_3$

Acidified
Aq/acetone

CHCl$_3$ layer

Concentration
CHCl$_3$ layer

Reconstituted
80% MeOH

Lipid

HPLC auto
sampler

LC-MS/
MS/MS

Survey scan

WT

mutant

Product ion

MRM

Prasain et al., JoVE, 2013
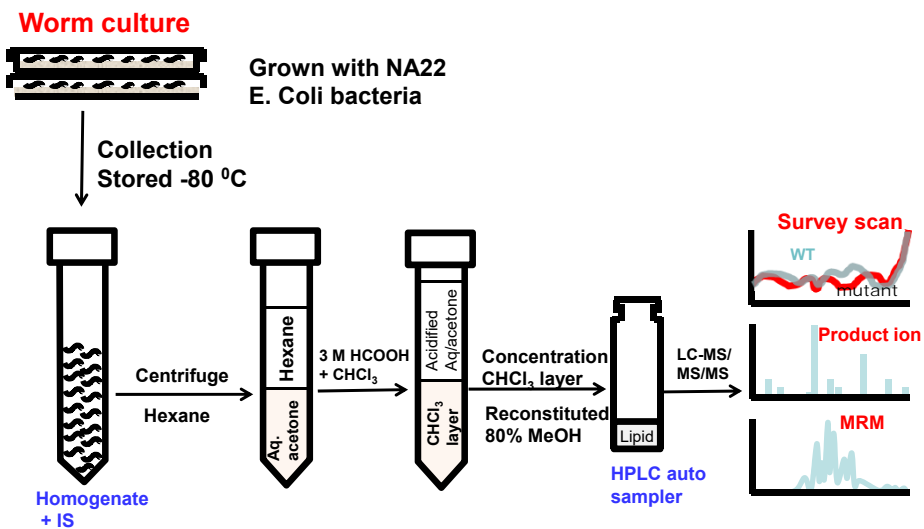
LC-MS/MS data summary for chemically synthesized F-series PG standards.

| Name	RT (min)*	[M-H]⁻ m/z	Key product ions in CID (MS/MS)
PGD₂	12.56	351	315, 271, 233, 203, 189
PGE₂	12.23	351	333, 315, 271, 235, 189, 175, 109
PGH₂	12.23	351	333, 315, 271, 235, 217, 189, 175, 113, 109
PGF₁ₐ	11.83*	355	337, 319, 311, 301, 293, 275, 265, 249, 237, 211, 195
8-iso PGF₁ₐ	11.34	355	337, 319, 311, 293, 275, 265, 249, 237, 219, 211, 183
9β-PGF₁ₐ	11.36	355	337, 319, 311, 301, 293, 275, 237, 211,183, 167
8-iso 9β-PGF₁ₐ	11.44	355	337, 319, 311, 293, 275, 265, 219, 211, 183
9β, 11β-PGF₁ₐ	11.99	355	337, 319, 311, 301, 293, 275, 265, 237, 219, 211, 183
PGF₂ₐ	11.73*	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
ent-PGF₂ₐ	11.71	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
11β PGF₂ₐ	11.48	353	335, 317, 309, 291, 273, 247, 209, 193, 173, 165, 111
15(R)-PGF₂ₐ	11.89	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
8-iso PGF₂ₐ	11.31	353	335, 307, 309, 291, 273, 247, 209, 193, 181, 171, 165
5-trans PGF₂ₐ	11.60	353	335, 317, 309, 291, 273, 247, 209, 193, 171, 165, 111
8-iso 15(R)-PGF₂ₐ	11.39	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
9β-PGF₂ₐ	11.22	353	335, 317, 309, 291, 273, 255, 247, 193, 173, 171, 165
PGF₃ₐ	11.26	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 165
8-iso PGF₃ₐ	10.83	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 171
2,3-Dinor-11β-PGF₂ₐ	10.67	325	261, 245, 227, 219, 173, 163, 153, 145, 137, 113, 107
19(R)-hydroxy PGF₂ₐ	9.19	369	351, 333, 325, 315, 307, 263, 235, 209, 193, 171, 165
20-hydroxy PGF₂ₐ	9.13	369	351, 333, 325, 315, 307, 263, 209, 193, 181, 171, 165

Retention time (RT), parent ion mass ([M-H]⁻), and key product ion masses are shown for prostaglandin (PG) standards.
*Isomers within each prostaglandin class (i.e. PGF₂ₐ isomers) were run together and RTs are directly comparable. PGF₁ and PGF₂ classes were run on different days and a slight RT shift is observed. For example, the RTs for PGF₁ₐ and PGF₂ₐ are indistinguishable when run together.
doi:10.1371/journal.pgen.1003271.t002

Identifying isomeric and isobaric compounds requires good chromatography separation, high resolution and tandem mass spectrometr

m/z 353.1735

Estradiol sulfate
m/z 351.1272

m/z 353.2333
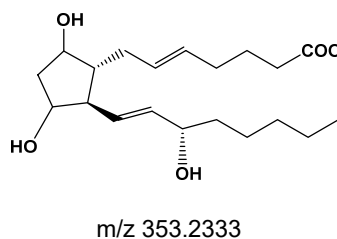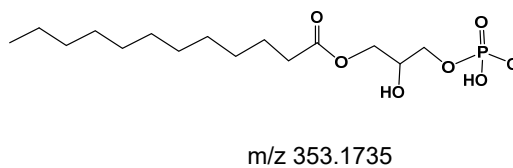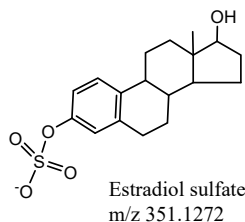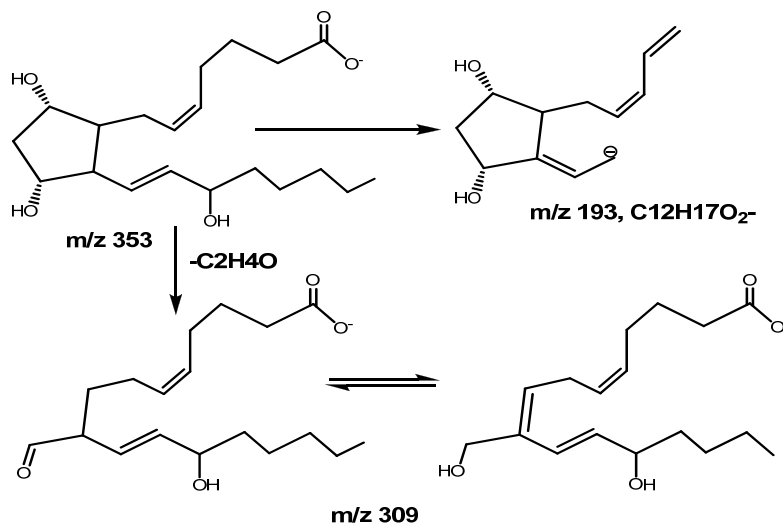
When compounds are not isomeric, it may be possible to distinguish them
Based on their exact masses

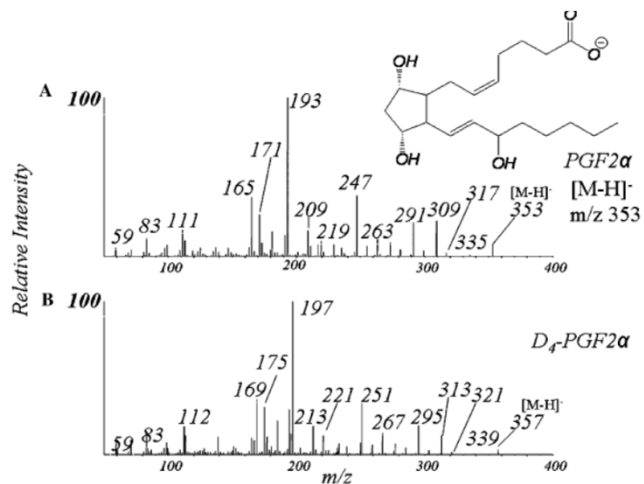## ESI-MS/MS of the [M-H]- from PGF2α m/z 353 using a quadrupole mass spectrometer
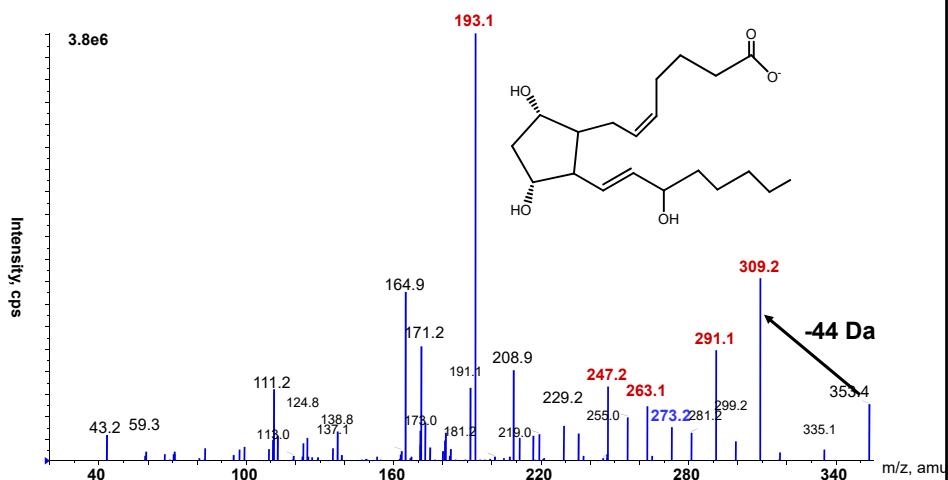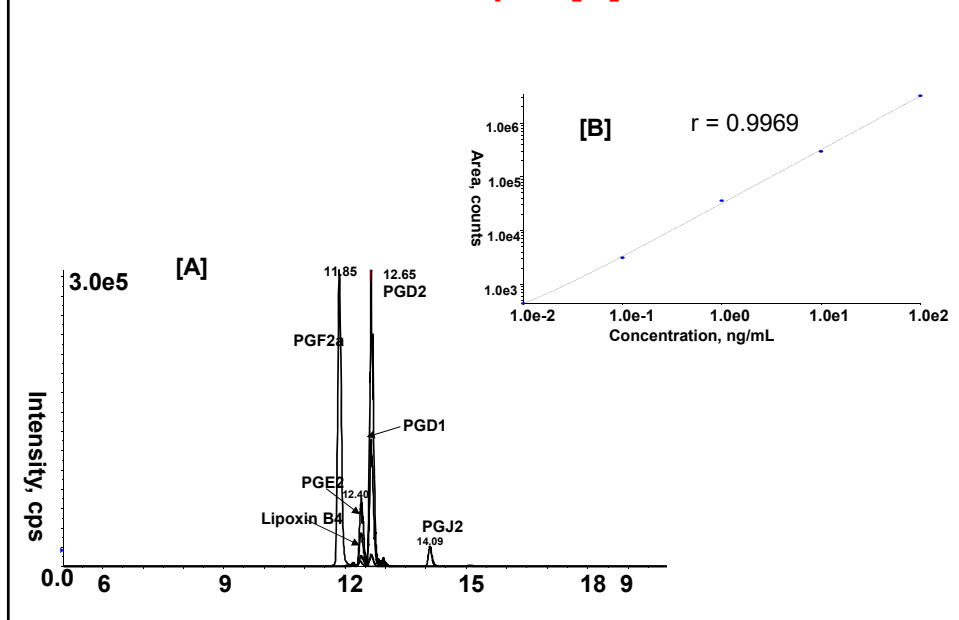

Fragmentation scheme of PGF2α [M-H]⁻ m/z 353

Ions m/z 309, 291, 273 and 193 are indicative of F2-ring
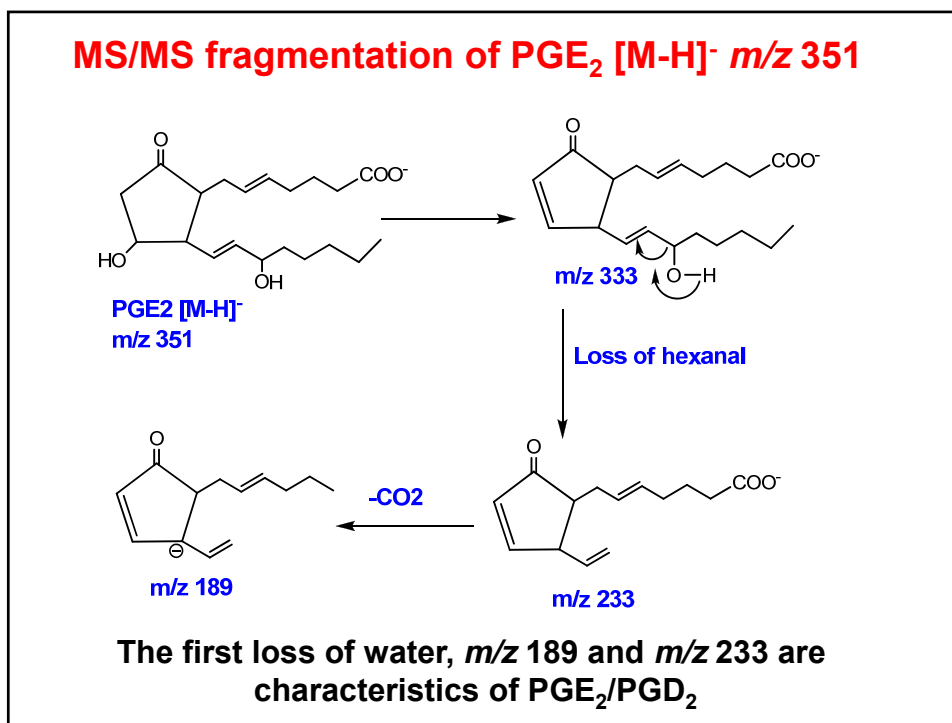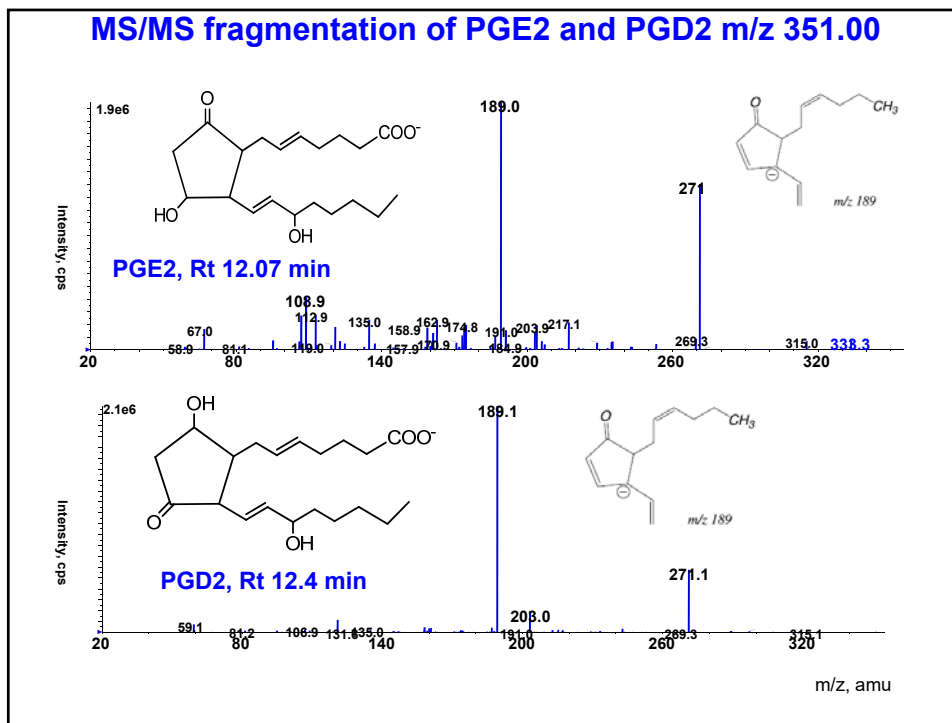
What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?

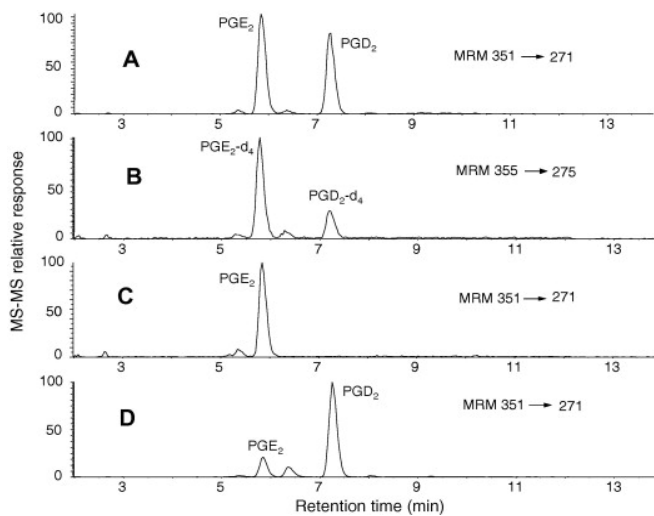**Source: Murphy et al. Analytical Biochemistry, 2005**
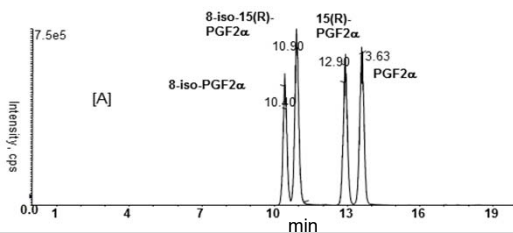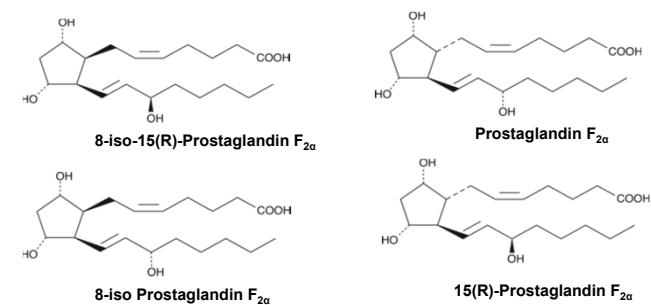
Separation of PGs[A] and standard curve of PGF2alpha [B]

7

MS/MS fragmentation of PGE2 and PGD2 m/z 351.00

MS/MS fragmentation of PGE$_2$ [M-H]$^-$ *m/z* 351

The first loss of water, *m/z* 189 and *m/z* 233 are characteristics of PGE$_2$/PGD$_2$

# Deuterated PG standards are used for quantitative analysis of PGs in a extract

Source: Cao et al. Analytical Biochemistry, 2008

# PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS

8-iso-15(R)-Prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$

8-iso Prostaglandin $F_{2\alpha}$

15(R)-Prostaglandin $F_{2\alpha}$

Prasain et al., J Chrom B. 2013

SRM chromatogram showing isoprostanes and PG in an AKI patience
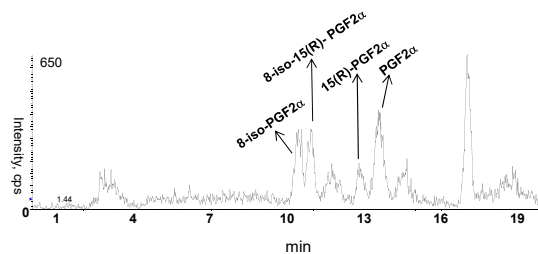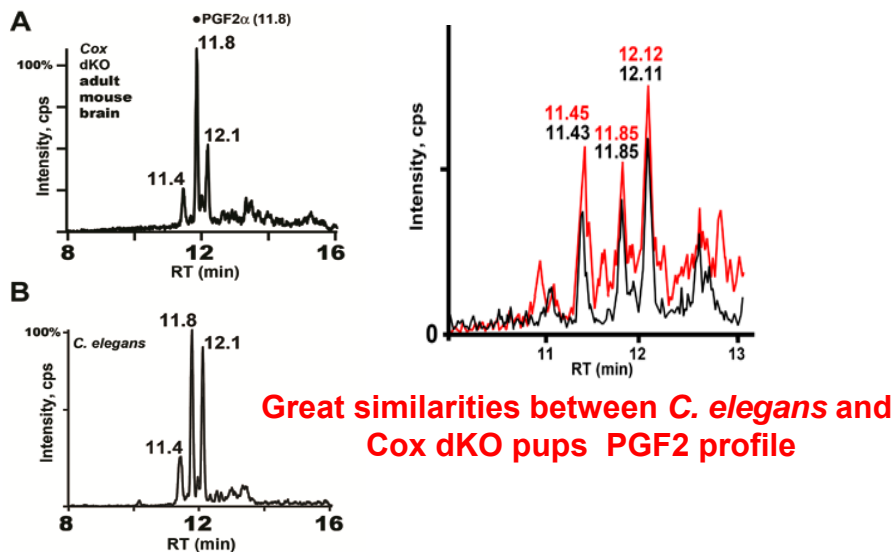

Prasain et al., J Chrom B. 2013

Separation of PGF_2alpha and its enantiomer only possible in chiral normal phase column (ChiralPak AD-H column) APCI –ve ion mode
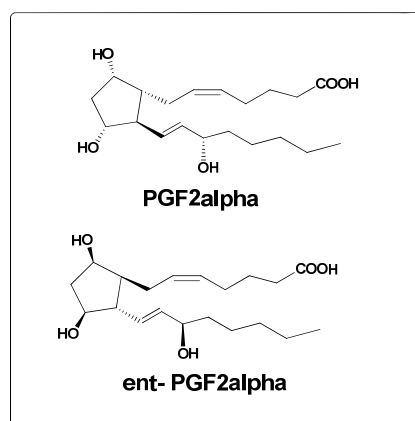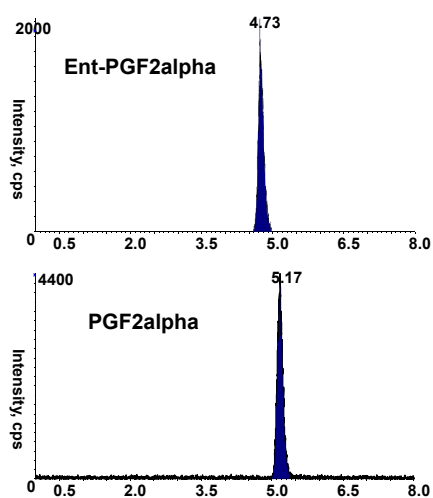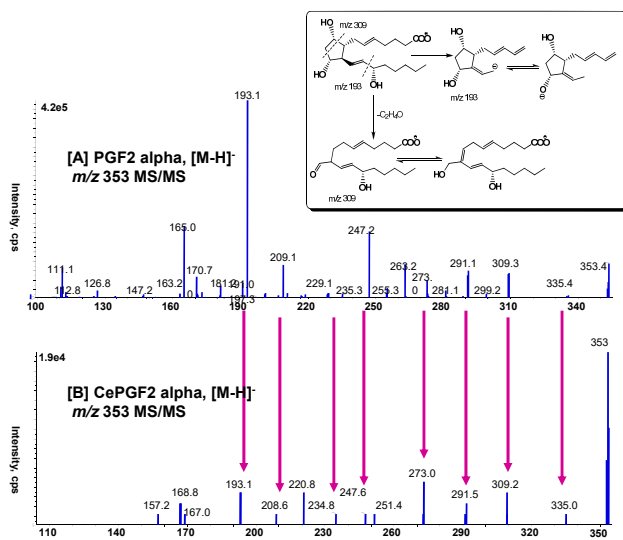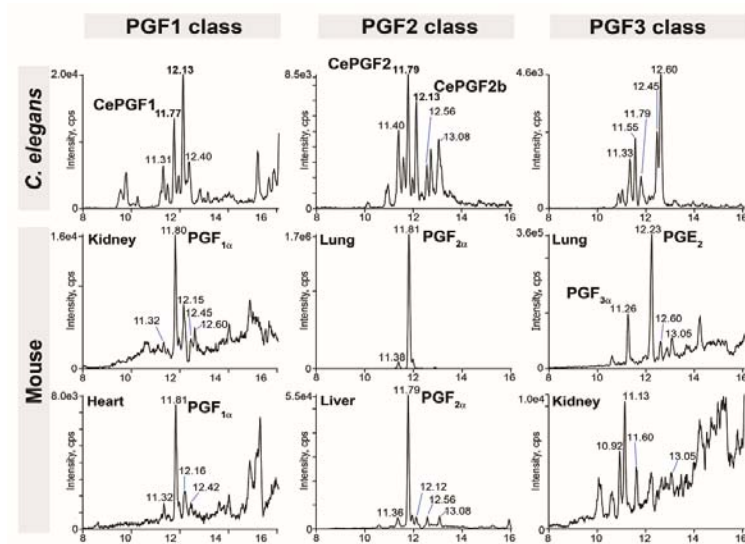
Cox-independent PGs is widespread

Great similarities between *C. elegans* and Cox dKO pups PGF2 profile

McKnight et al., (Science, 2014)

LC-MS/MS of ion *m/z* 353 [M-H]⁻ from wild type *C. elegans* extract confirmed that CePGF₂ is a PGF₂alpha-like PG
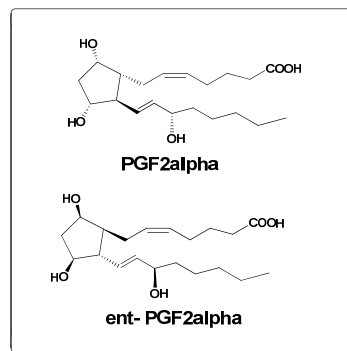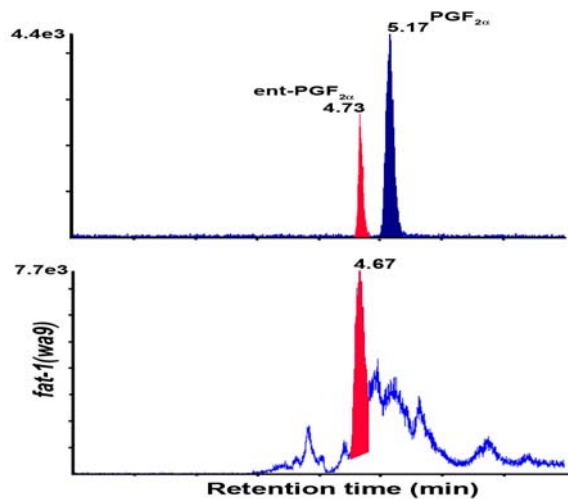
**Extracts from healthy mouse tissues and C. elegans
Showed a few F-series PGs isomers in common**

Hoang et al., PLOS Genetics, 2013

**Is CePGF2- PGF$_2$alpha, co-eluting stereoisomer,
ent- PGF$_2$alpha or a racemic mixture ?**

**CePGF2 Close similarity with ent-PGF$_{2a}$
in chiral normal phase LC-MRM**

Hoang et al., PLOS Genetics. 2013

Conclusions

- **Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), genetic analyses, and bioactivity assays, *C. elegans* synthesizes Cox-independent sperm guiding F-series PGs from PUFA precursors.**

- **F-series PGs are synthesized in Cox-deficient mice, indicating the possible existence of similar mechanisms in other animals.**