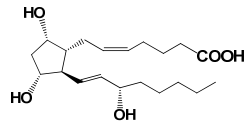
Tandem mass spectrometry analysis of prostaglandins and isoprostanes



PGF2 alpha

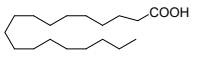
Jeevan Prasain
jprasain@uab.edu
6-2612

Overview

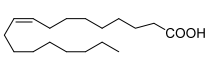
- **Introduction to PGs and their synthesis**
- **Mass spectrometry characterization of PGs and isoprostanes**
- **PGs in Cox-dKO pups and *C. elegans***

Fatty acids (FAs)

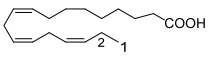
Saturated FA | Monounsaturated FA | Polyunsaturated FA



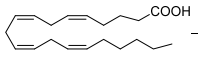
Stearic acid (C18:0)
mol. wt. 284.2715



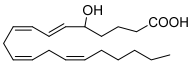
Oleic acid (C18:1)
mol. wt. 282.25559



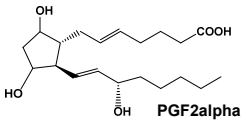
Linolenic acid (C18:3)
mol. wt. 278.2246



AA



5-HETE



PGF2alpha

Oxylized lipids

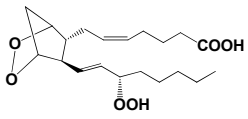
Cox-dependent PGs synthesis

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

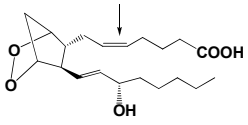
↓ elongase

↓ desaturase

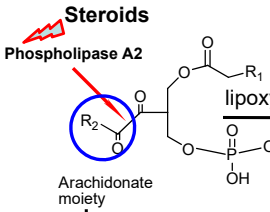
Arachidonic acid
C20: $\Delta^{5,8,11,14}$)



PGG2

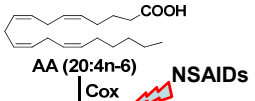


PGH2



↓ Release first

Enzymatic- cyclic pathways



AA (20:4n-6)

NSAIDs

Cox

PGG2

Cox

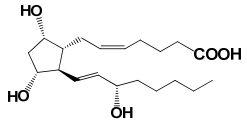
PGD synthase

PGE synthase

PGD2 ← PGH2 → PGE2

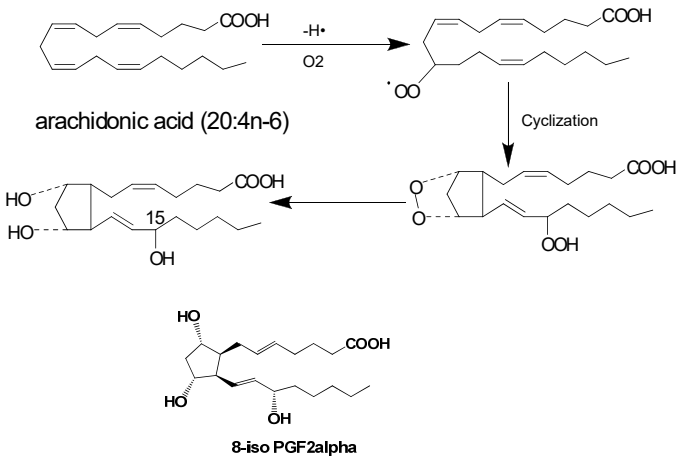
PGF synthase

PGF2α

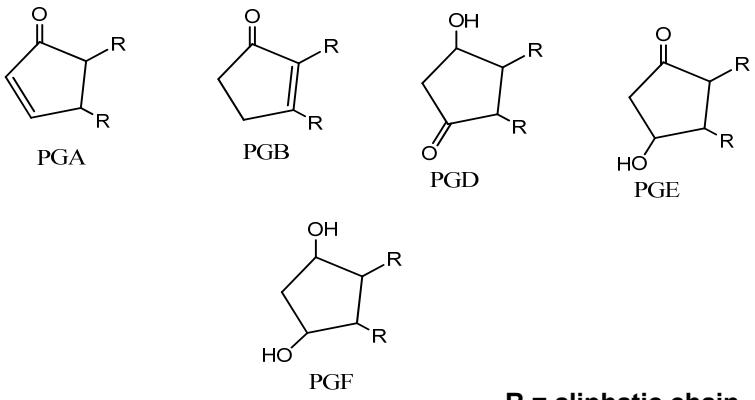


PGF2 alpha

Non-enzymatic isoprostane synthesis

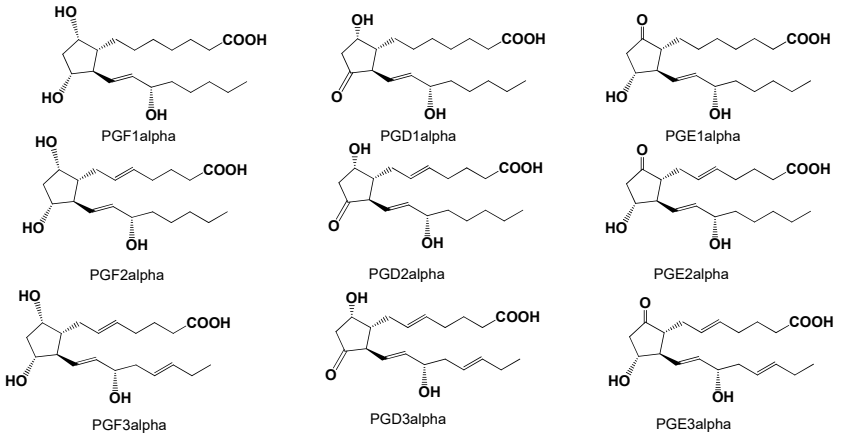


Structural representation PG based on ring features

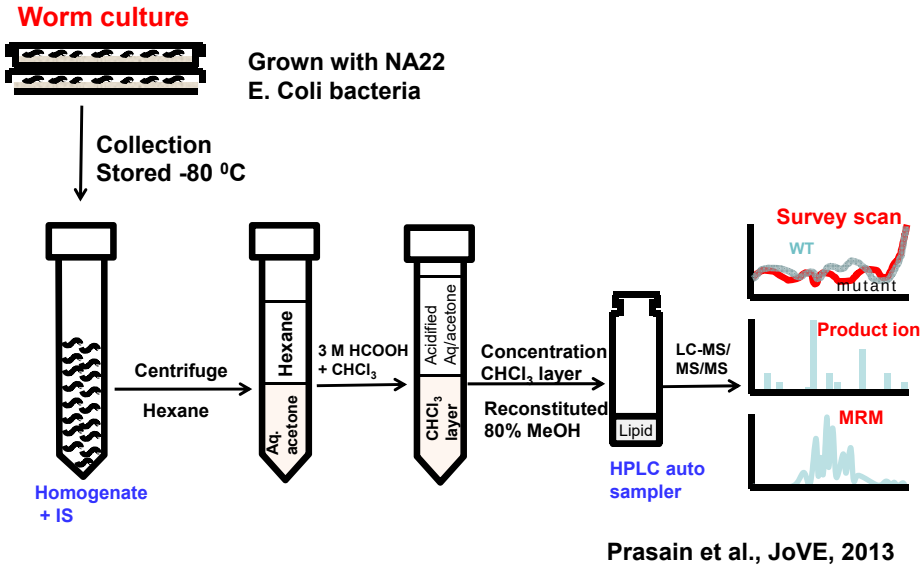


R = aliphatic chain

Structures of PGs D, E and F series



C. elegans culture, lipid extraction and mass spectrometry analysis



Prasain et al., JoVE, 2013

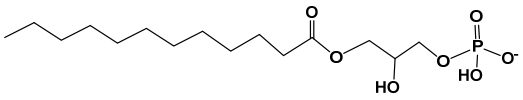
LC-MS/MS data summary for chemically synthesized F-series PG standards.

Name	RT (min)*	$[M-H]^-$ m/z	Key product ions in CID (MS/MS)
PGD_2	12.56	351	315, 271, 233, 203, 189
PGE_2	12.23	351	333, 315, 271, 235, 189, 175, 109
PGH_2	12.23	351	333, 315, 271, 235, 217, 189, 175, 113, 109
$PGF_{1\alpha}$	11.83*	355	337, 319, 311, 301, 293, 275, 265, 249, 237, 211, 195
8-iso $PGF_{1\alpha}$	11.34	355	337, 319, 311, 293, 275, 265, 249, 237, 219, 211, 183
9β-$PGF_{1\alpha}$	11.36	355	337, 319, 311, 301, 293, 275, 265, 237, 211,183, 167
8-iso 9β-$PGF_{1\alpha}$	11.44	355	337, 319, 311, 293, 275, 265, 219, 211, 183
9β, 11β-$PGF_{1\alpha}$	11.99	355	337, 319, 311, 301, 293, 275, 265, 237, 219, 211, 183
$PGF_{2\alpha}$	11.73*	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
ent-$PGF_{2\alpha}$	11.71	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
11β $PGF_{2\alpha}$	11.48	353	335, 317, 309, 291, 273, 247, 209, 193, 173, 165, 111
15(R)-$PGF_{2\alpha}$	11.89	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
8-iso $PGF_{2\alpha}$	11.31	353	335, 307, 309, 291, 273, 247, 209, 193, 181, 171, 165
5-trans $PGF_{2\alpha}$	11.60	353	335, 317, 309, 291, 273, 247, 209, 193, 171, 165, 111
8-iso 15(R)-$PGF_{2\alpha}$	11.39	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
9β-$PGF_{2\alpha}$	11.22	353	335, 317, 309, 291, 273, 255, 247, 193, 173, 171, 165
$PGF_{3\alpha}$	11.26	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 165
8-iso $PGF_{3\alpha}$	10.83	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 171
2,3-Dinor-11β-$PGF_{2\alpha}$	10.67	325	261, 245, 227, 219, 173, 163, 153, 145, 137, 113, 107
19(R)-hydroxy $PGF_{2\alpha}$	9.19	369	351, 333, 325, 315, 307, 263, 235, 209, 193, 171, 165
20-hydroxy $PGF_{2\alpha}$	9.13	369	351, 333, 325, 315, 307, 263, 209, 193, 181, 171, 165

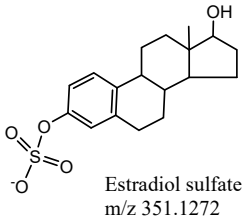
Retention time (RT), parent ion mass ($[M-H]^-$), and key product ion masses are shown for prostaglandin (PG) standards.
*Isomers within each prostaglandin class (i.e. $PGF_{2\alpha}$ isomers) were run together and RTs are directly comparable. PGF_1 and PGF_2 classes were run on different days and a slight RT shift is observed. For example, the RTs for $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are indistinguishable when run together.
doi:10.1371/journal.pgen.1003271.t002

Hoang et al., 2013

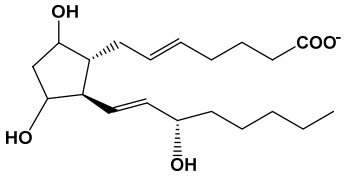
Identifying isomeric and isobaric compounds requires good chromatography separation, high resolution and tandem mass spectrometr



m/z 353.1735



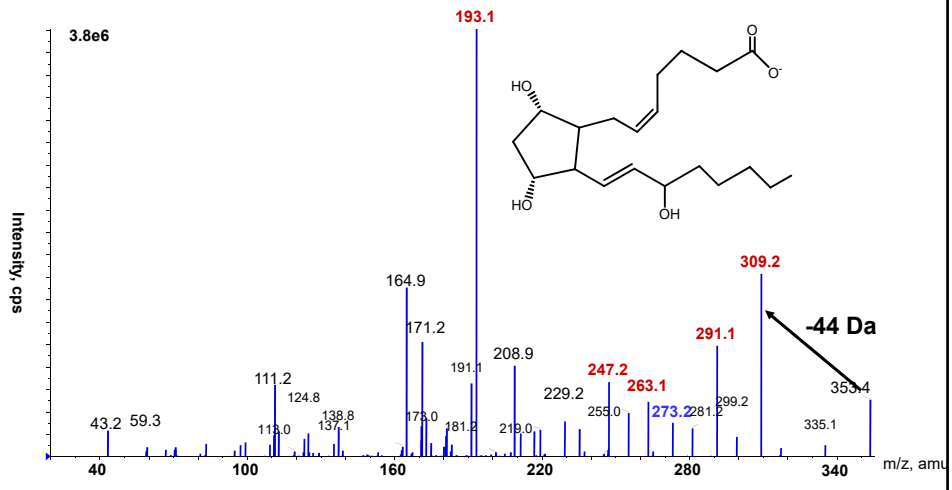
Estradiol sulfate
m/z 351.1272



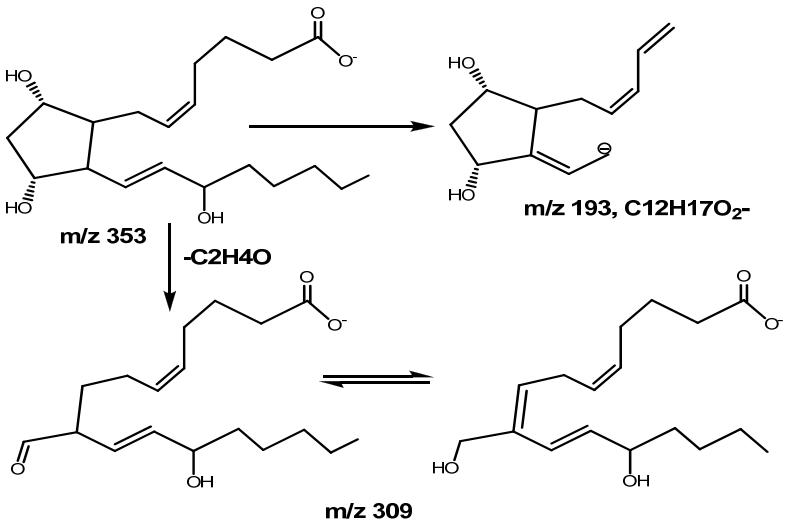
m/z 353.2333

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

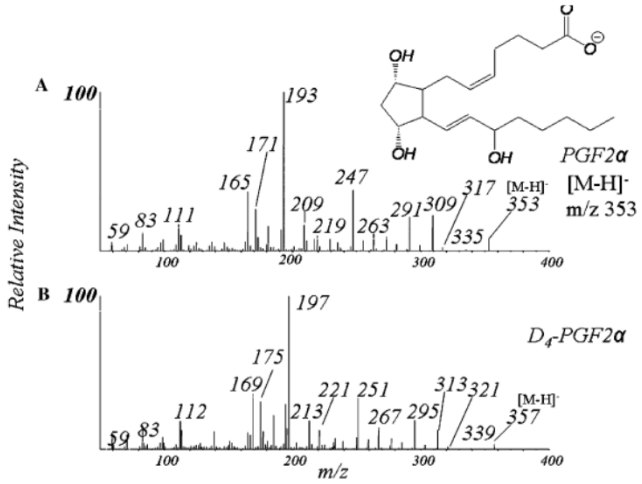


Fragmentation scheme of PGF2α [M-H]⁻ m/z 353



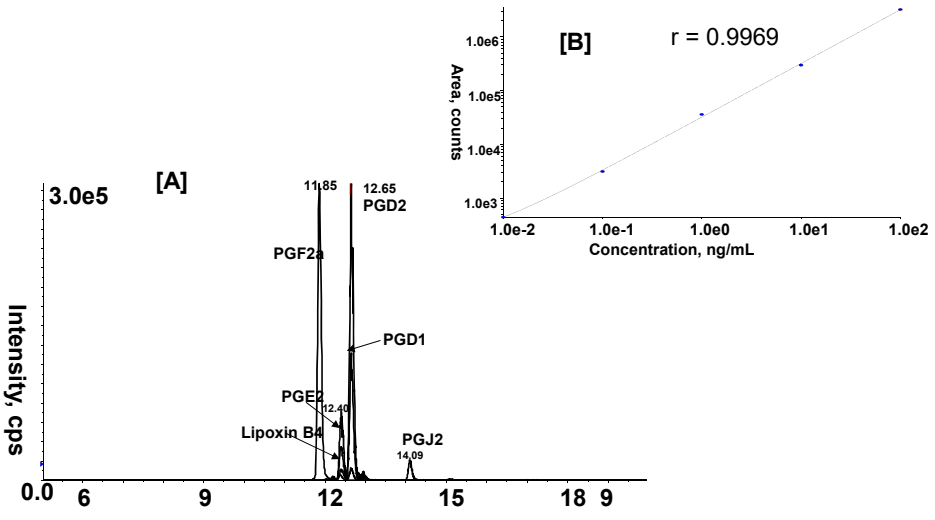
Ions m/z 309, 291, 273 and 193 are indicative of F2-ring

What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?

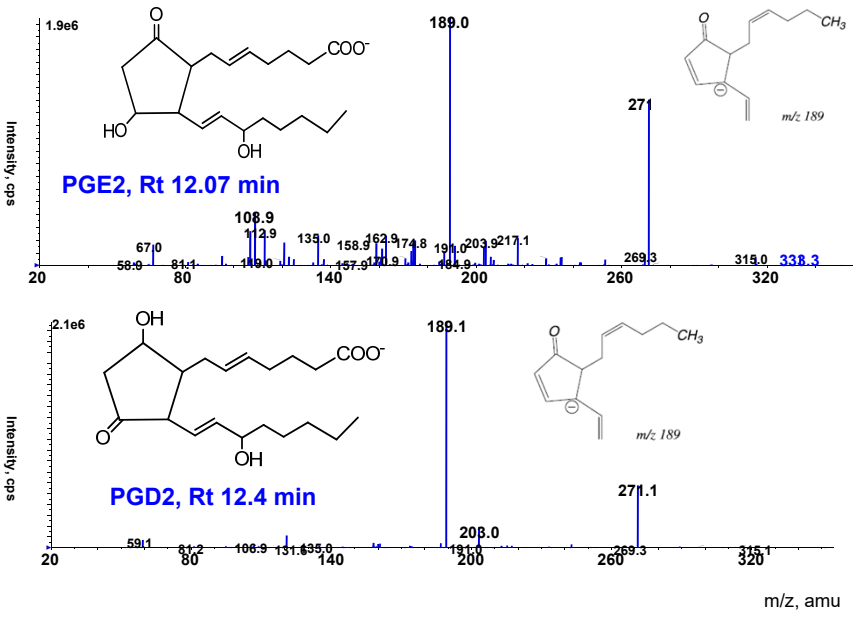


Source: Murphy et al. Analytical Biochemistry, 2005

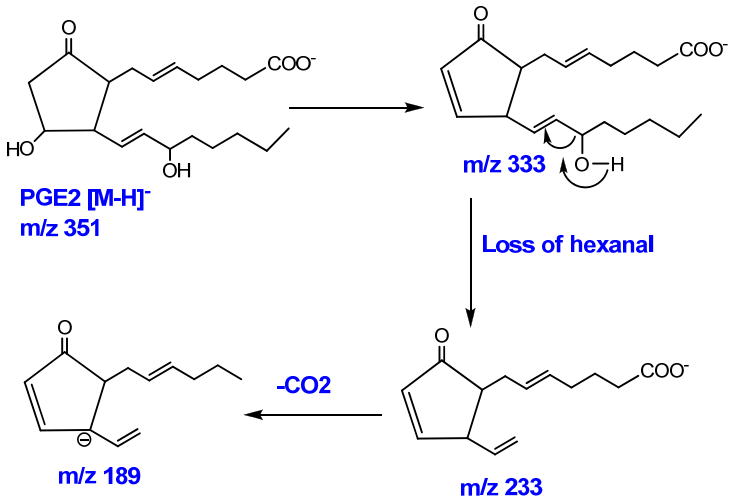
Separation of PGs[A] and standard curve of PGF2alpha [B]



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

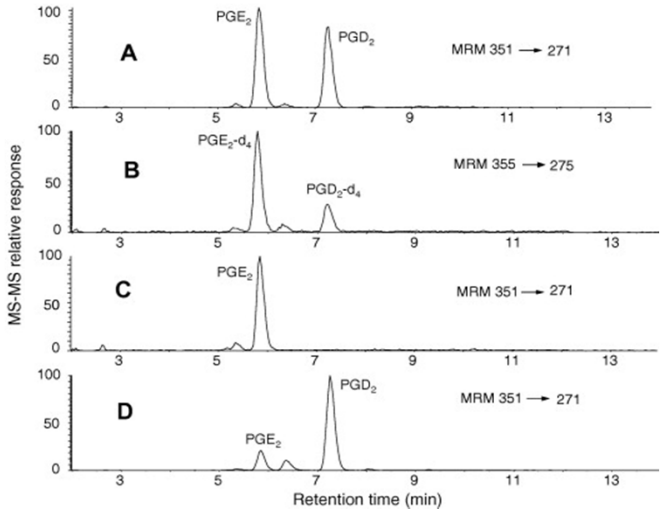


MS/MS fragmentation of PGE_2 $[M-H]^-$ *m/z* 351



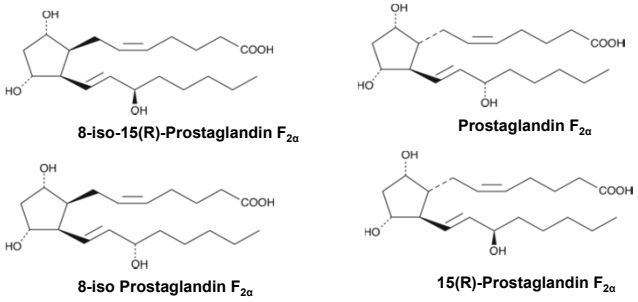
The first loss of water, *m/z* 189 and *m/z* 233 are characteristics of PGE_2/PGD_2

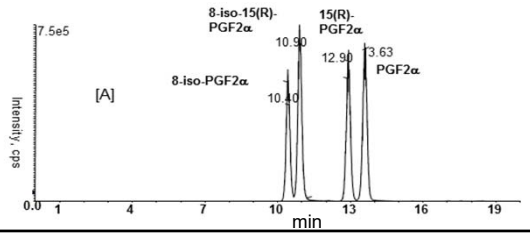
Deuterated PG standards are used for quantitative analysis of PGs in a extract



Source: Cao et al. Analytical Biochemistry, 2008

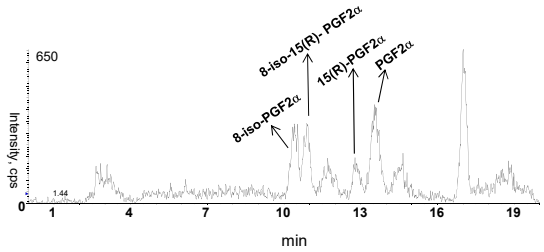
PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS





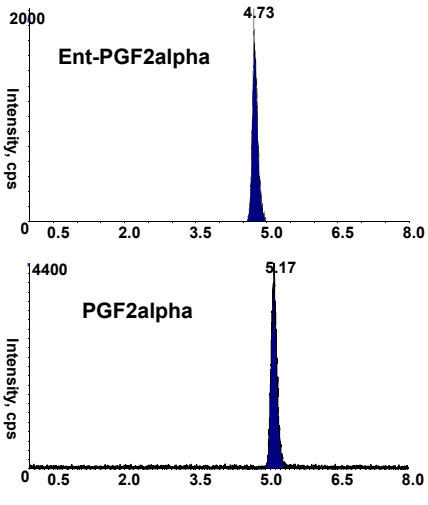
Prasain et al., J Chrom B. 2013

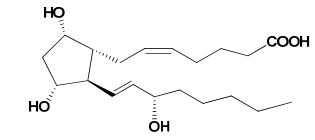
SRM chromatogram showing isoprostanes and PG in an AKI patience



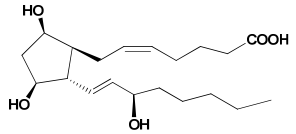
Prasain et al., J Chrom B. 2013

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



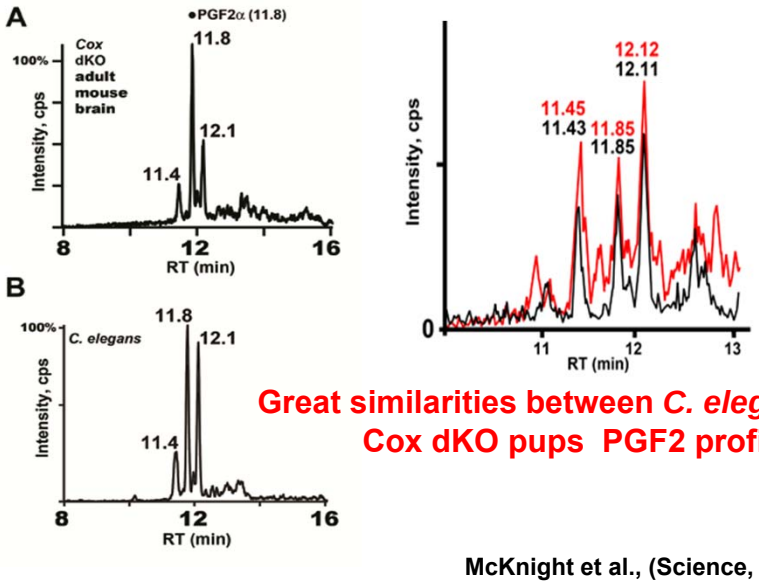


PGF2alpha



ent- PGF2alpha

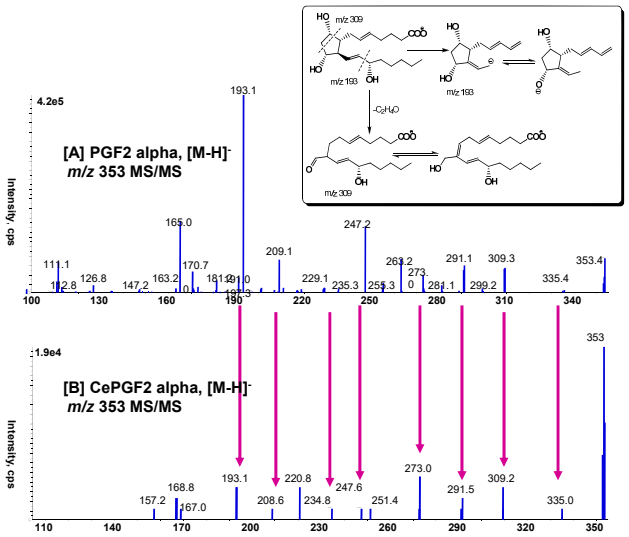
Cox-independent PGs is widespread



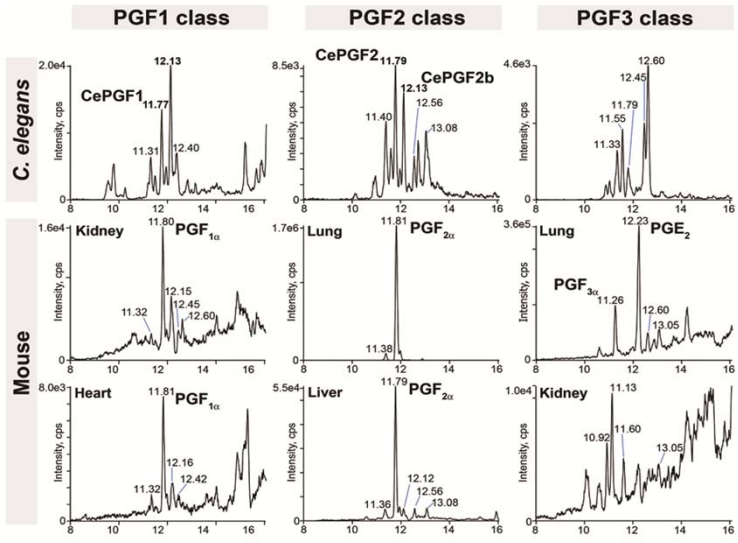
Great similarities between *C. elegans* and Cox dKO pups PGF2 profile

McKnight et al., (Science, 2014)

LC-MS/MS of ion *m/z* 353 $[M-H]^-$ from wild type *C. elegans* extract confirmed that $CePGF_2$ is a PGF_2alpha-like PG

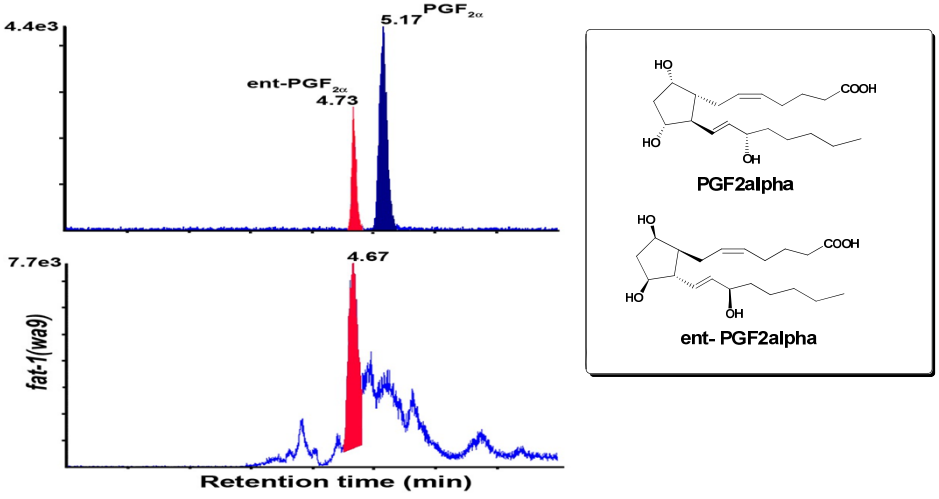


Extracts from healthy mouse tissues and C. elegans Showed a few F-series PGs isomers in common



Hoang et al., PLOS Genetics, 2013

Is CePGF2- PGF_2alpha, co-eluting stereoisomer, ent- PGF_2alpha 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.**