

Tandem mass spectrometry analysis of prostaglandins and isoprostanes

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Overview

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

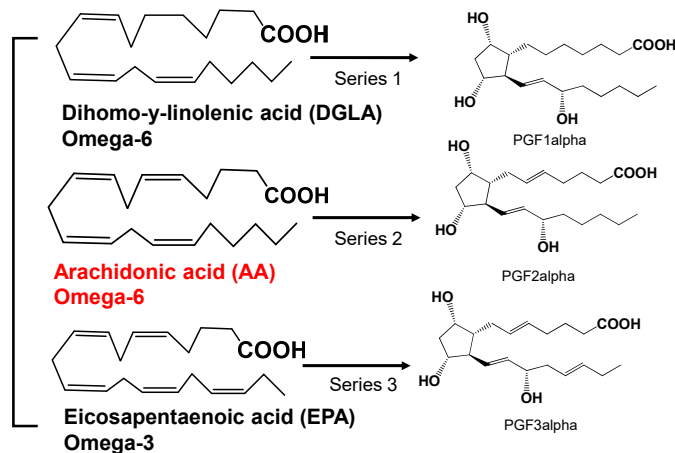
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Prostaglandins

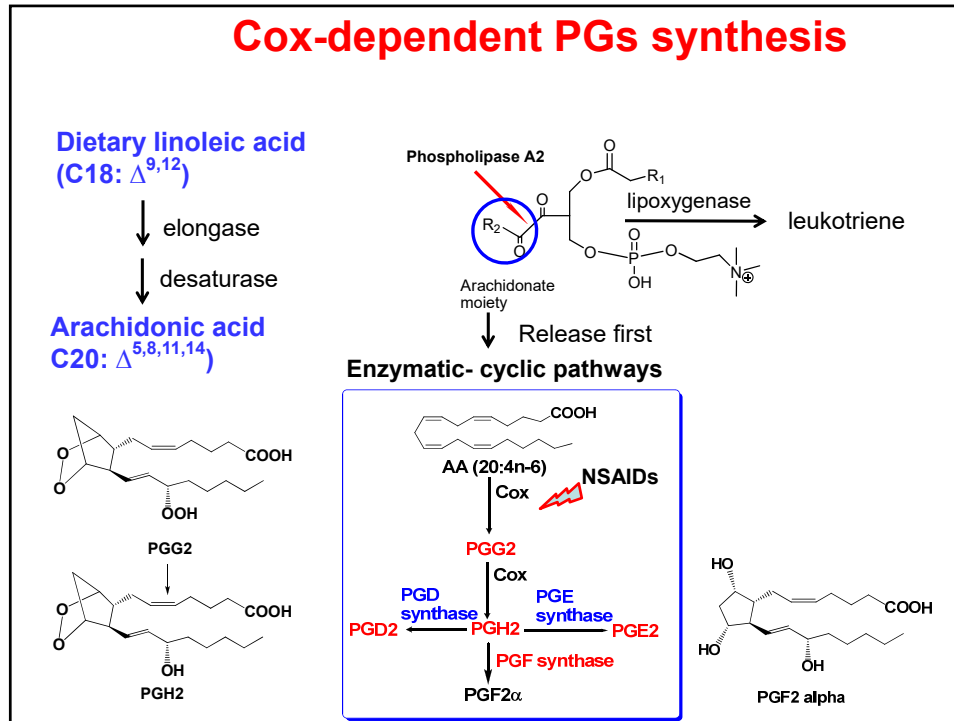
- Derived from 20 carbon PUFA, have short half-lives and act as local hormones
- Bind to specific cell surface G-protein coupled receptors and implicated in a number of physiological processes including reproductive function.
- NSAIDs acts through inhibiting Cox and hence PGs and exert various effects, including infertility. However, the genetics of prostaglandin synthesis and action have largely been unexplored *in vivo*.
- Mammalian systems are not well suited for discovering new genes and molecular mechanisms involved in PG reproductive functions.
- The nematode *C. elegans* provides a platform for discovering roles of genes and mechanisms that would provide an ideal complement to mammalian systems.

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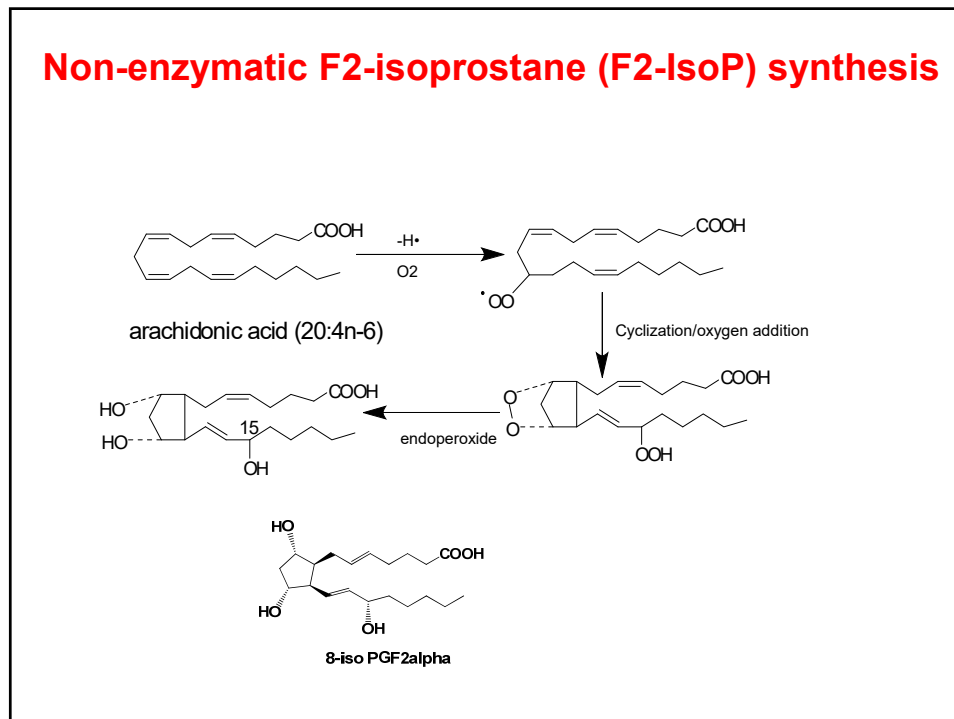
Polyunsaturated fatty acids (PUFAs)- substrates for PGs



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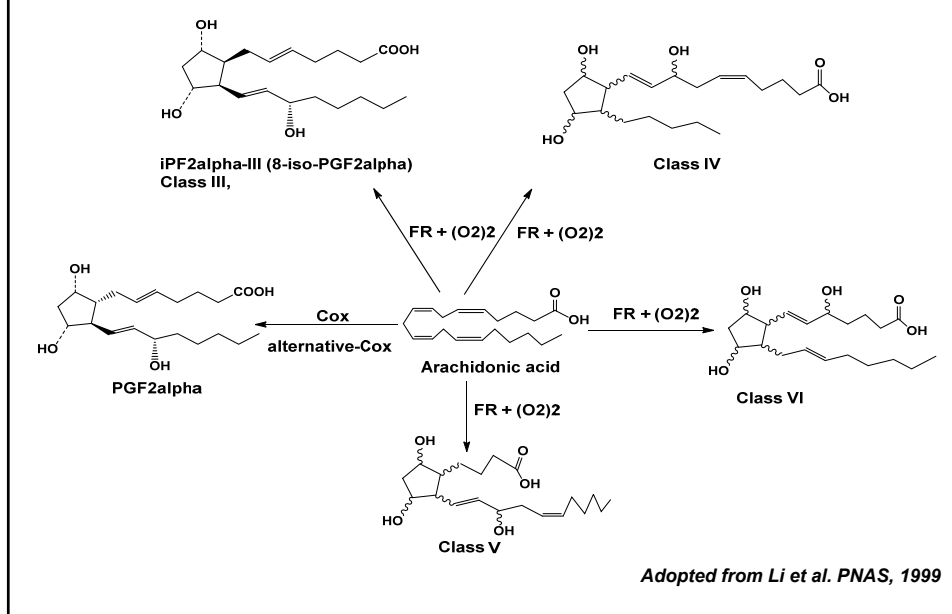


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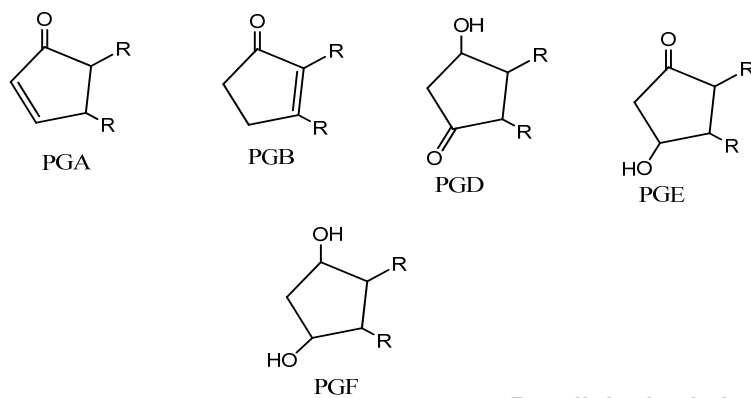
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Four classes of F2-isoprostanes from free-radical initiated reaction of arachidonic acid



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Structural representation PG based on ring features



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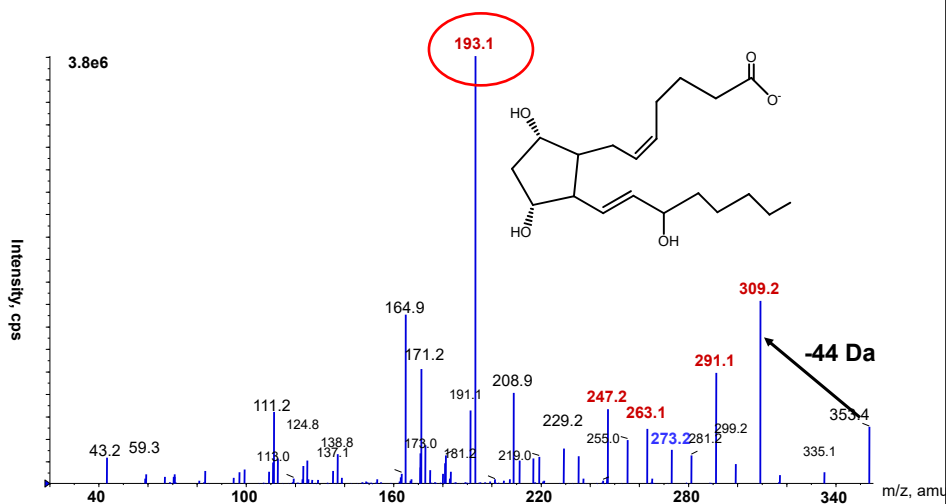
Prostaglandin analysis

Concentration range nM-pM in biological samples

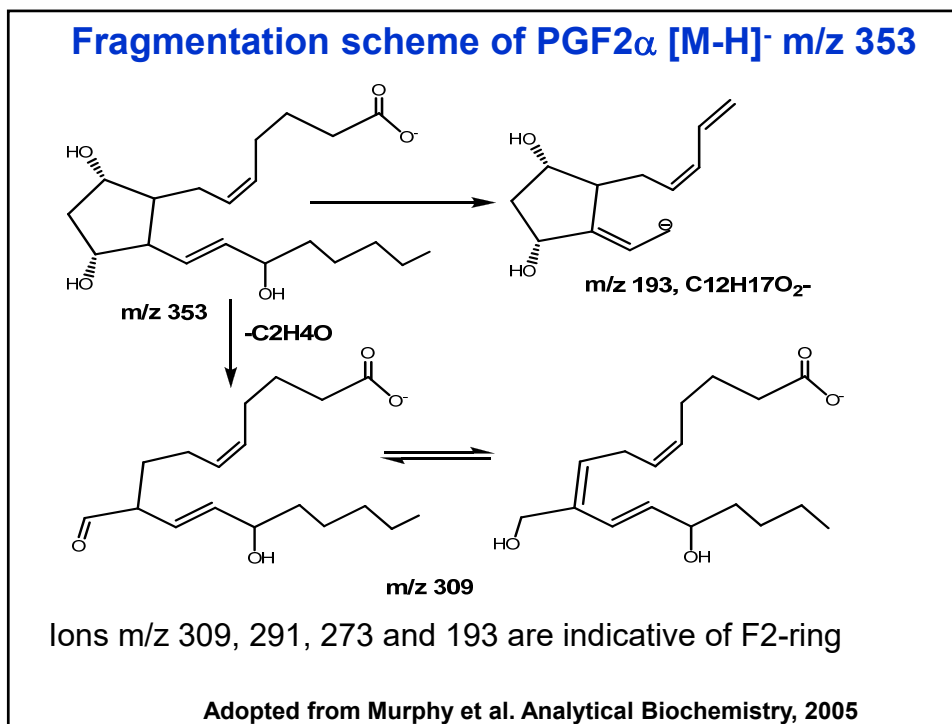
1. Immunoassay (poor specificity for isomeric PGs, and only one or a few compounds/assay)
1. GC-MS (derivatization needed)
1. LC-MS/MS

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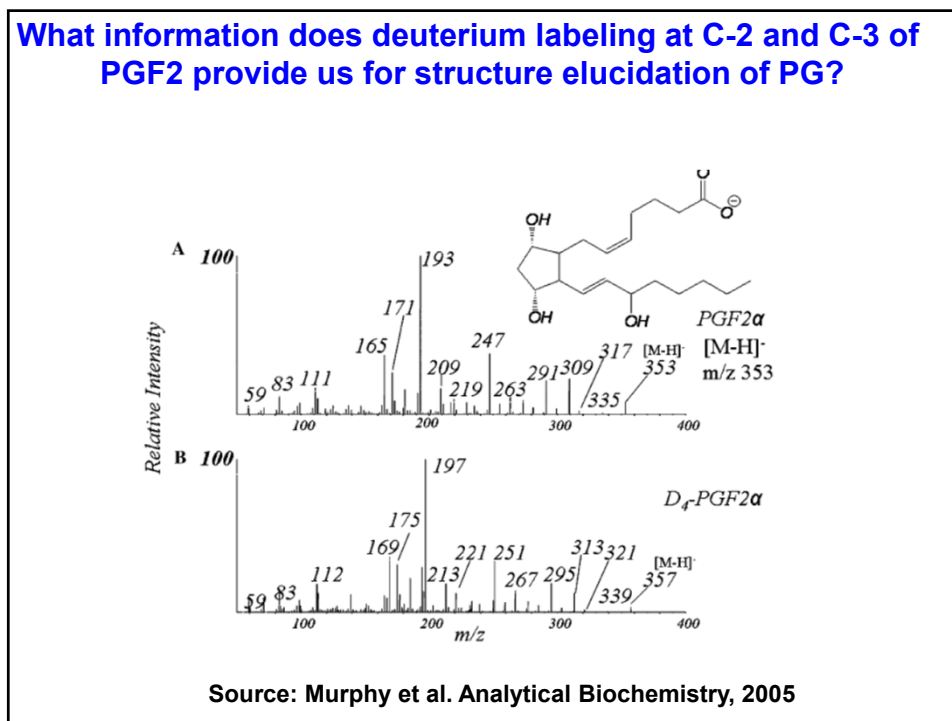
ESI-MS/MS of the [M-H]⁻ from PGF₂α m/z 353 using a quadrupole mass spectrometer



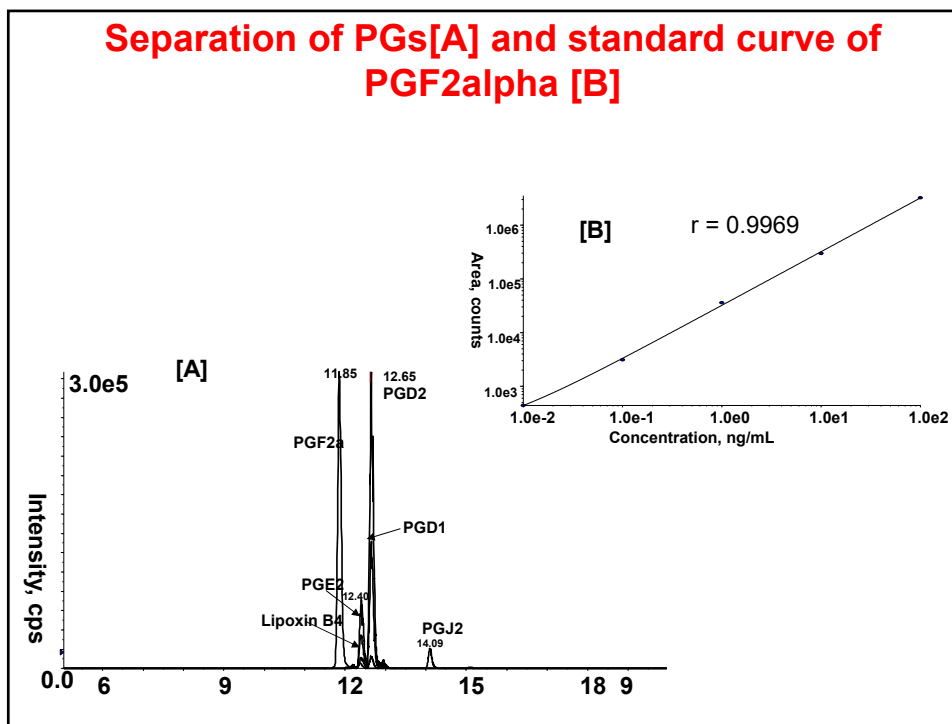
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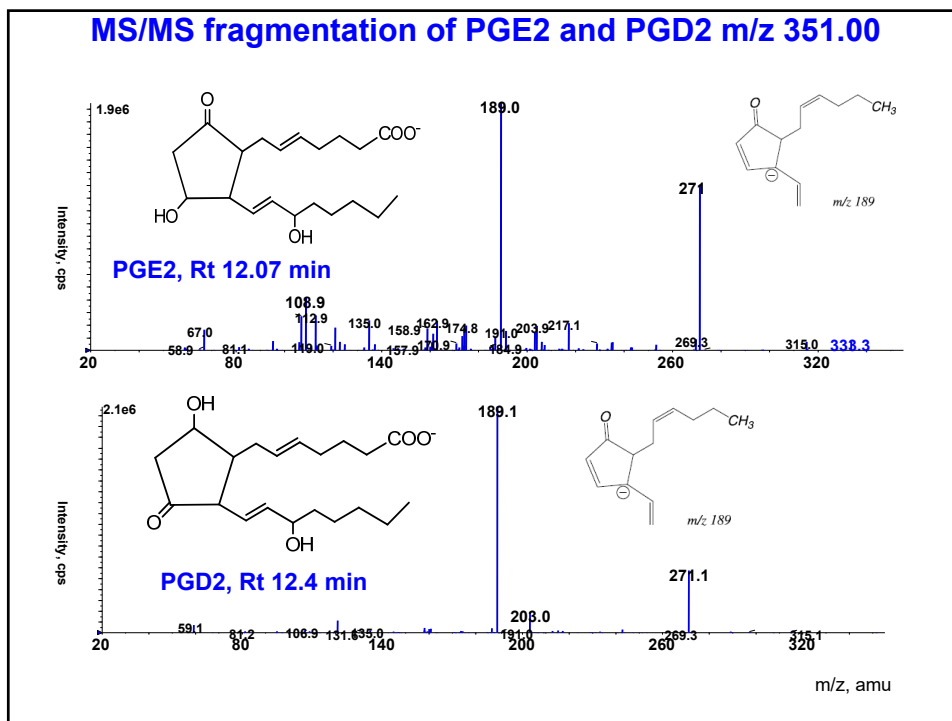
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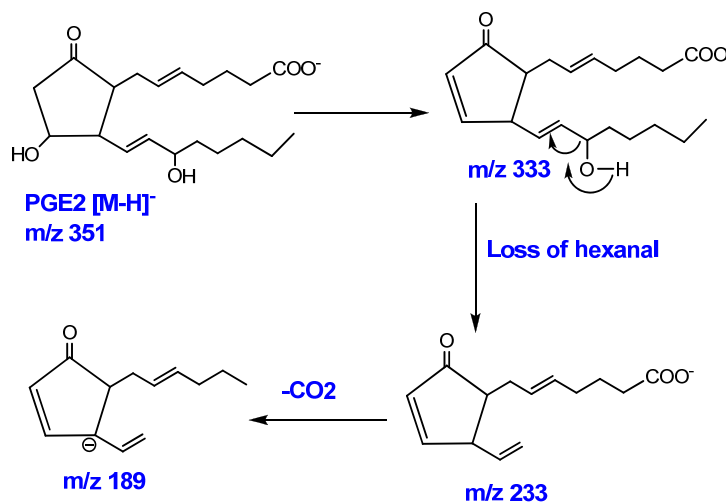


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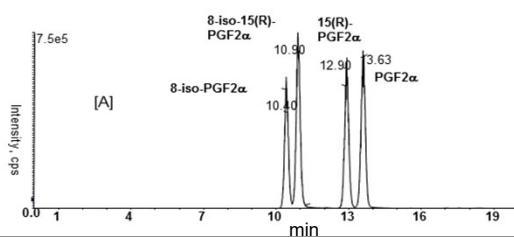
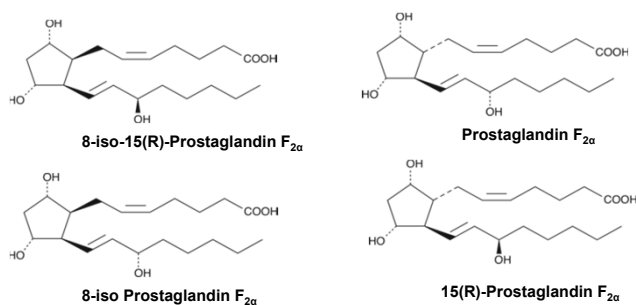
MS/MS fragmentation of PGE₂ [M-H]⁻ m/z 351



The first loss of water, *m/z* 189 and *m/z* 233 are characteristics of PGE₂/PGD₂

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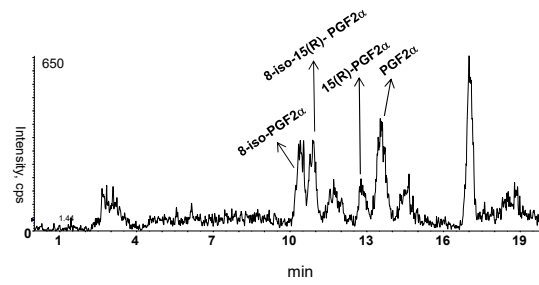
PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS



Prasain et al., J Chrom B. 2013

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SRM chromatogram showing isoprostanes and PG in an AKI patient

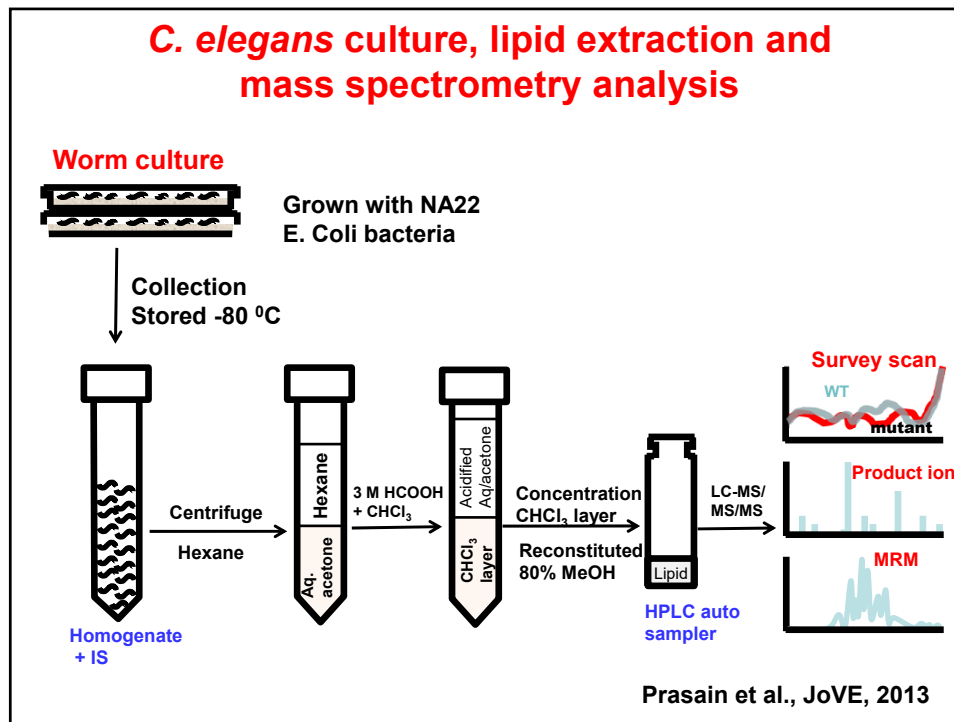


Prasain et al., J Chrom B. 2013

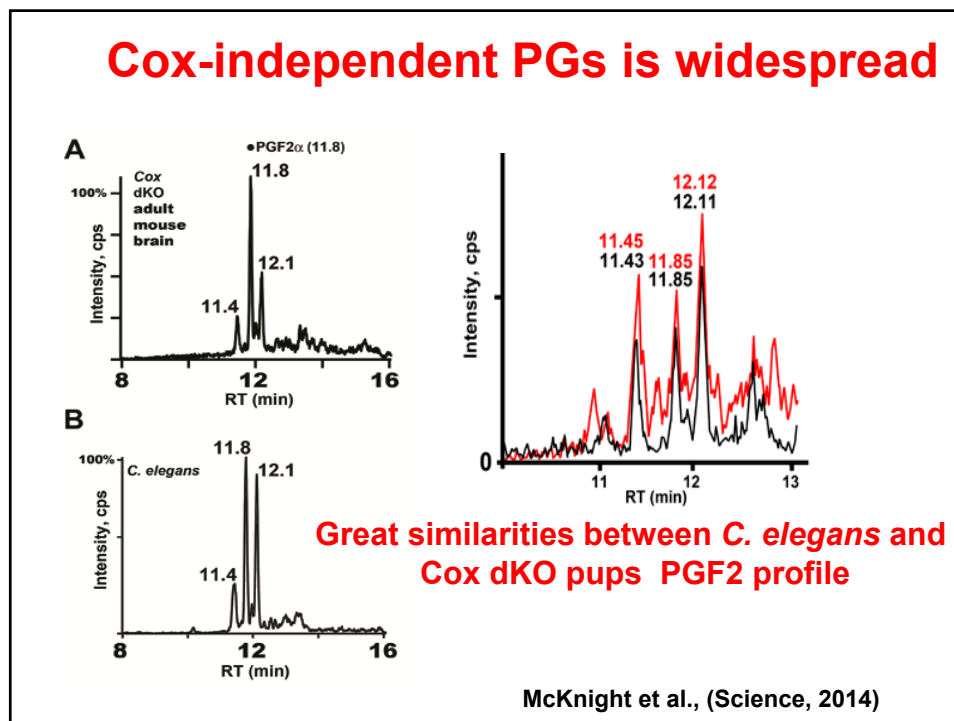
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Cox-independent PGs

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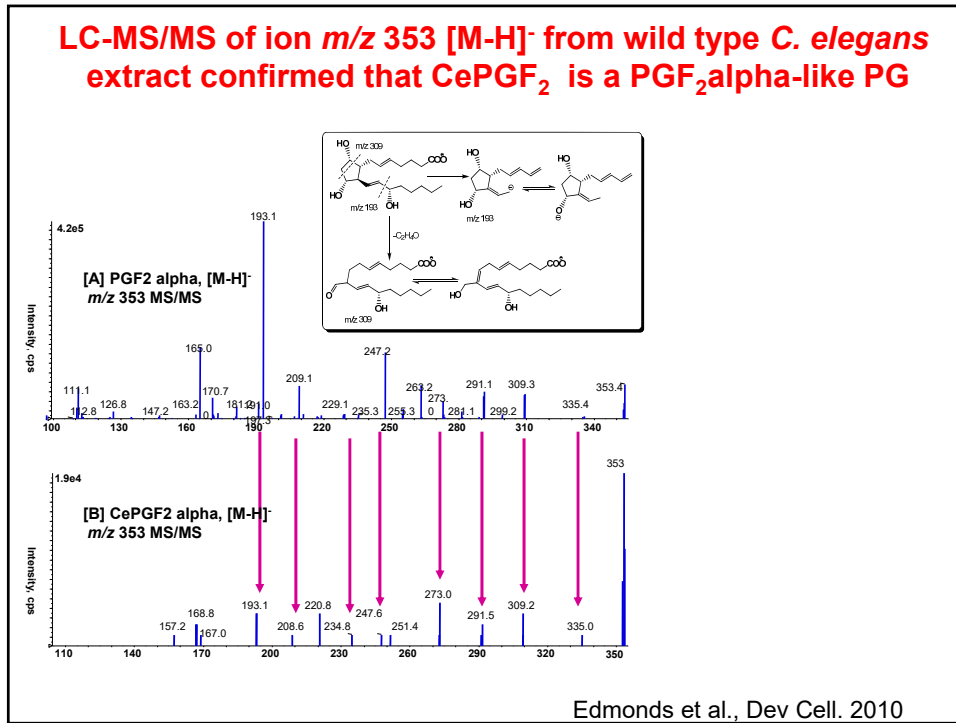


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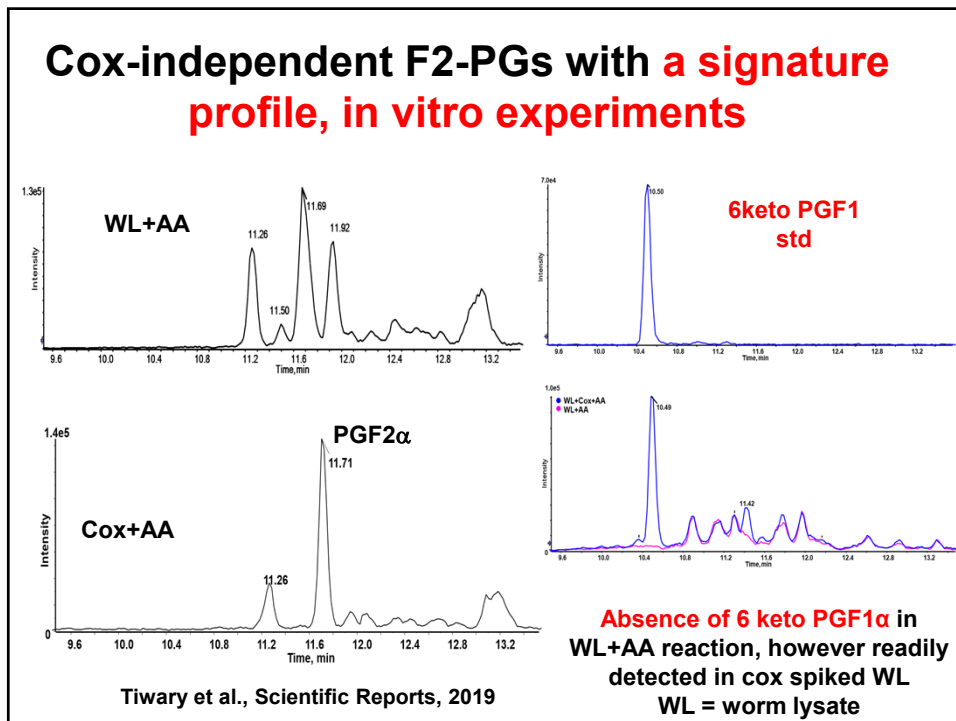
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LC-MS/MS of ion m/z 353 $[M-H]^-$ from wild type *C. elegans* extract confirmed that CePGF₂ is a PGF₂alpha-like PG



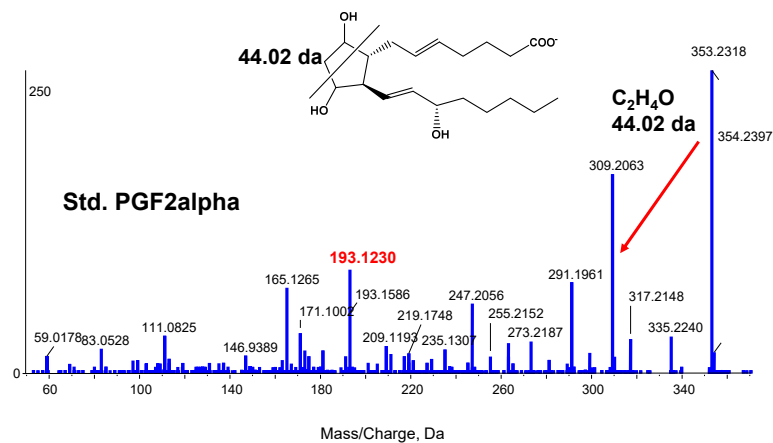
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Cox-independent F2-PGs with a signature profile, in vitro experiments



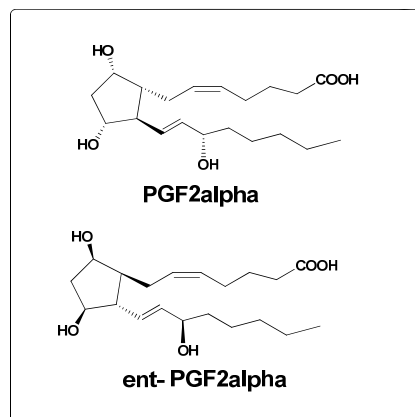
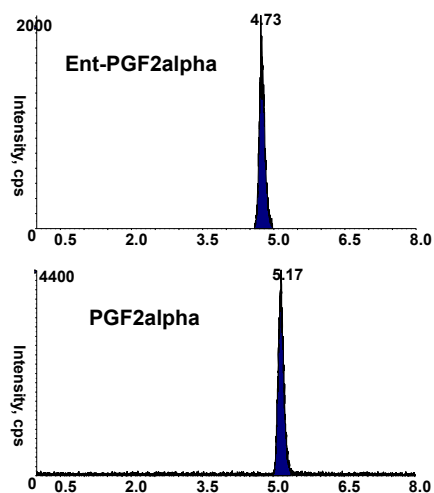
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High-resolution mass spectrometry analysis of PGF₂alpha



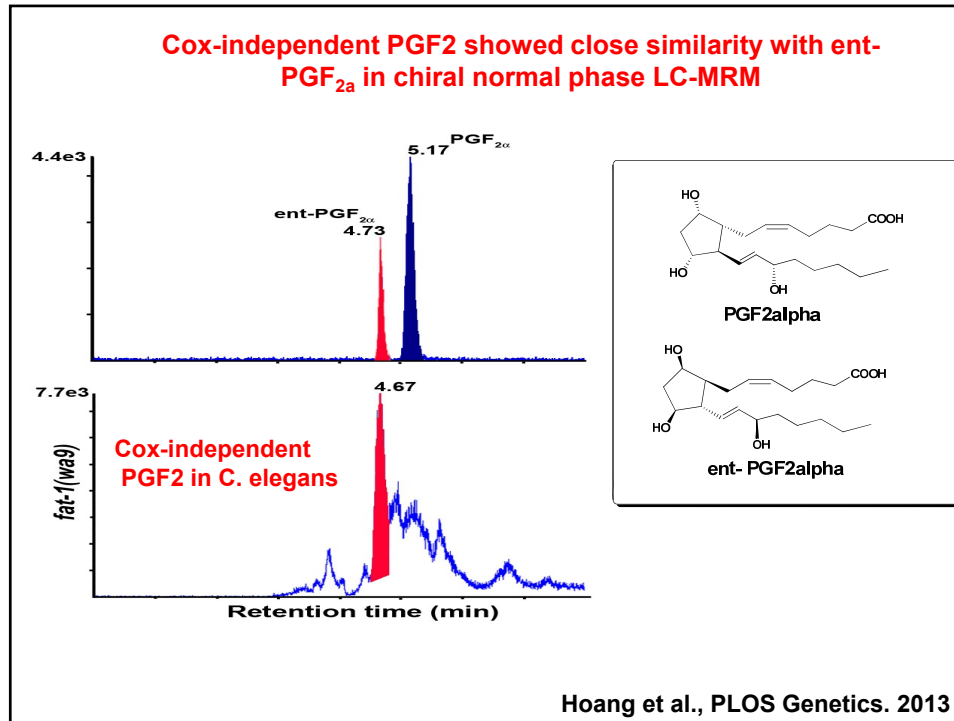
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Separation of PGF₂alpha and its enantiomer only possible in chiral normal phase column (ChiralPak AD-H column) APCI -ve ion mode



Hoang et al., PLOS Genetics. 2013

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Conclusions

- Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), genetic analyses, and bioactivity assays, *C. elegans* synthesizes Cox-independent 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.

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