Reactivity and Biological Functions of Oxidized Lipids

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Outline

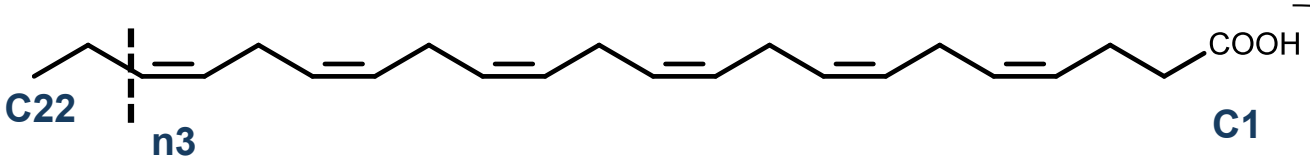
1. Lipid Oxidation Mechanisms and Products
2. Biological Mechanisms
3. *n*-3 vs *n*-6 PUFA Oxidation

USDA ARS United States Department Of Agriculture Agricultural Research Service

1. Fatty acids
2. Phospholipids
3. Cholesterol

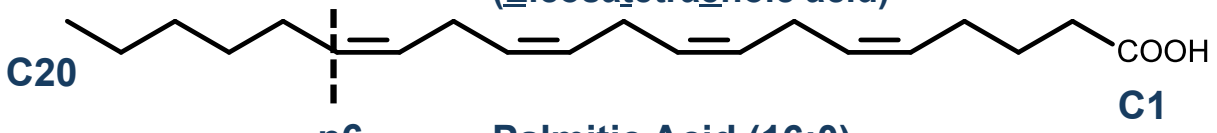
Basic LIPID Biochemistry

Docosahexaenoic Acid (22:6; n-3)

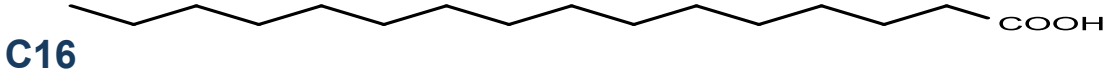


Unsaturated

Arachidonic Acid (20:4; n-6)
(Eicosatetraenoic acid)



Palmitic Acid (16:0)



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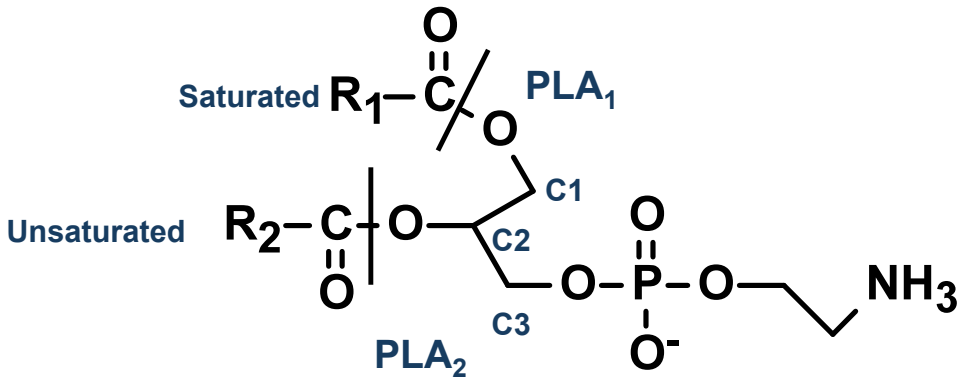
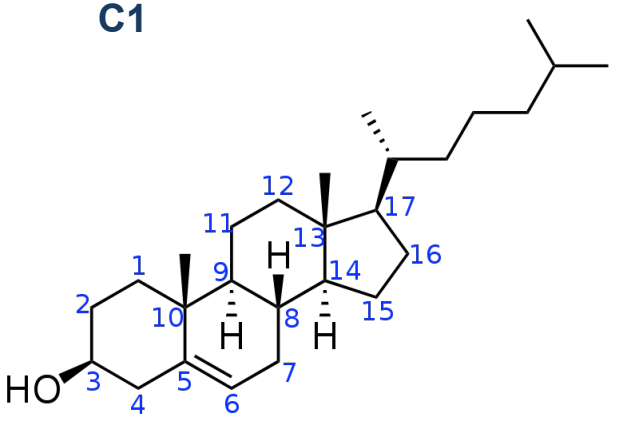
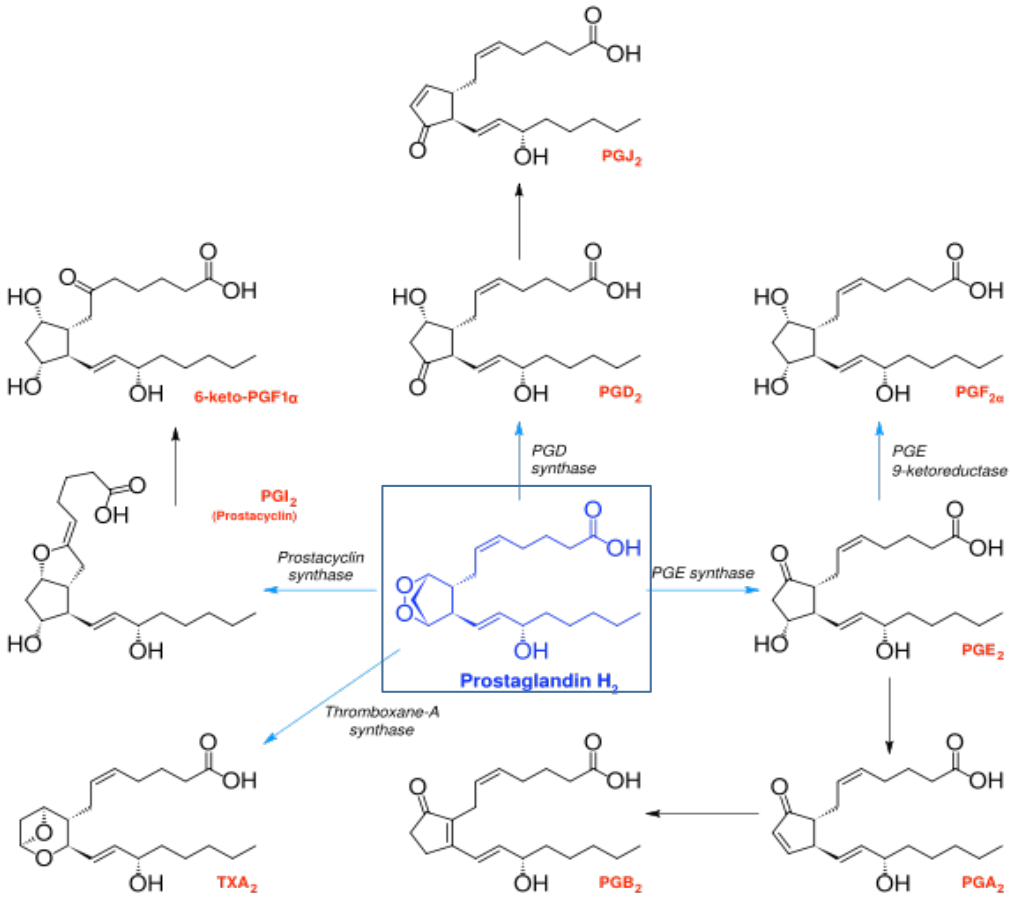
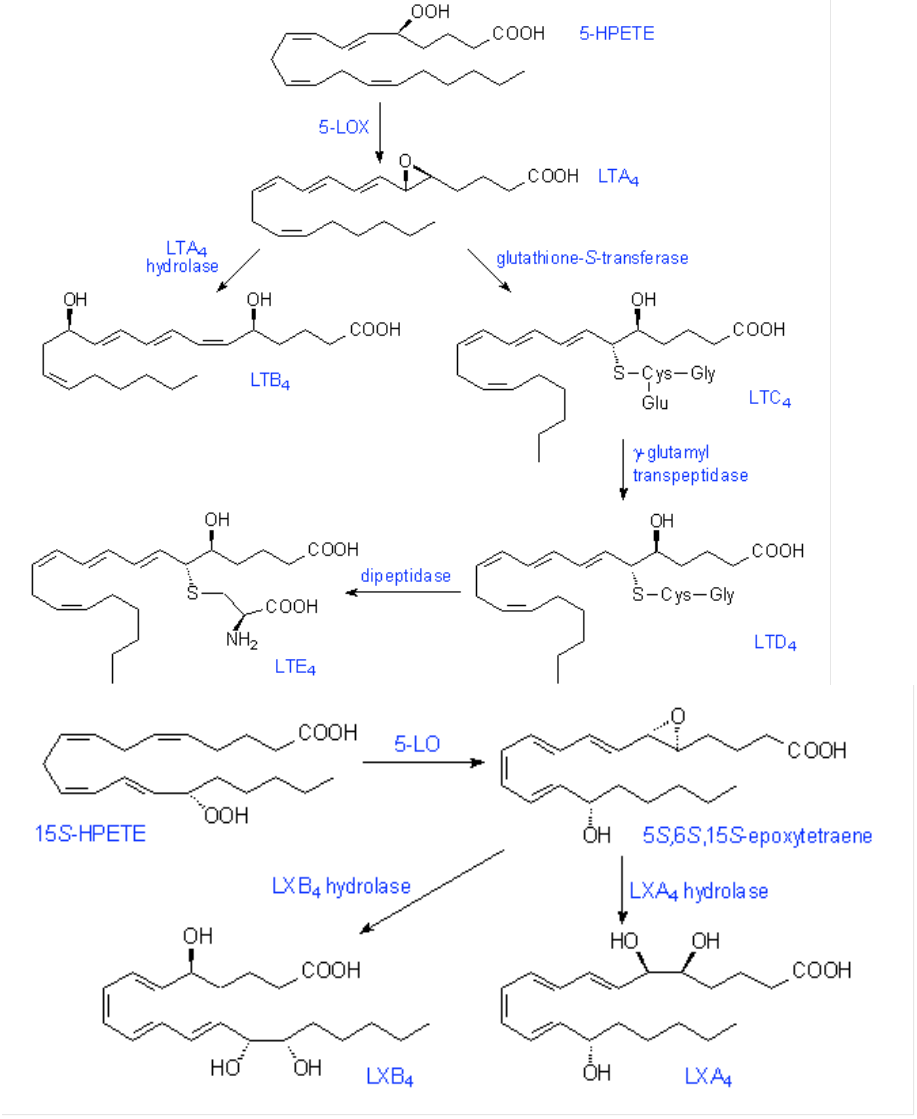




1. Mechanisms of Formation - Enzymatic

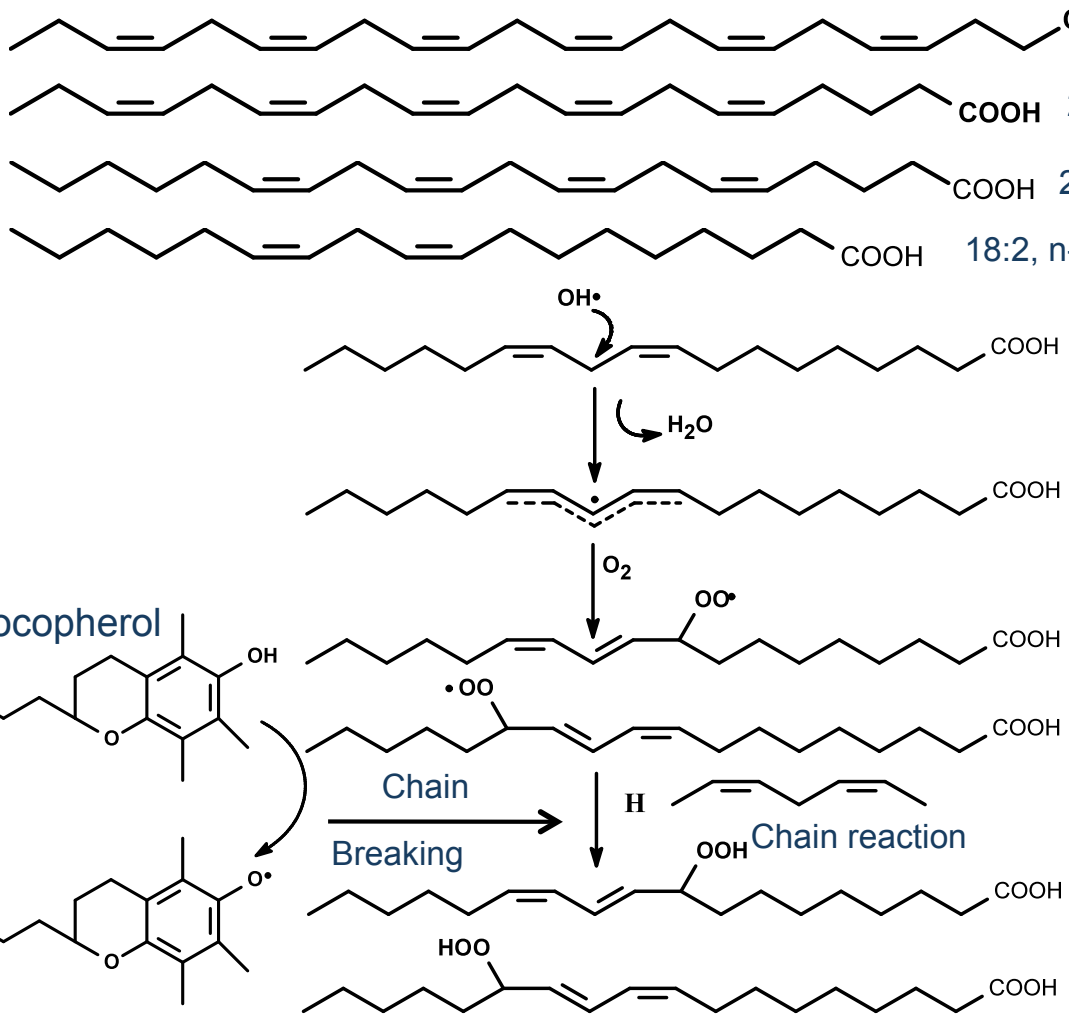
Phospholipid → PLA2 → Cyclooxygenase (1,2)/Lipoxygenase (12/15, 5)





Lipid oxidation is a necessary, physiological event !

1. Mechanisms of Formation – Free Radical-Based



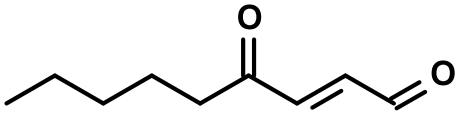
Most basic reaction

More complex products:
Cyclic peroxides, dihydroperoxides

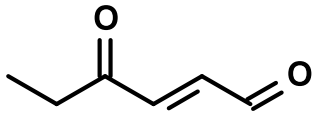
Hydroperoxides can be formed enzymatically - usually *S*-enantiomers
Free-radical catalyzed hydroperoxides are racemic
Usually formed on glycerol backbone (phospholipid/triglyceride)

The multiplicity of potential lipid peroxidation products…

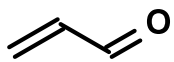
Open Chain – Hydroperoxides/Hydroxy Fatty acids, Aldehydes, Ketones



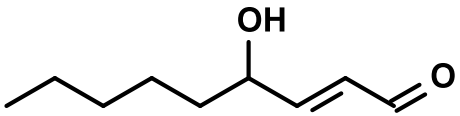
Trans-4-oxo-2-nonenal (4-ONE)



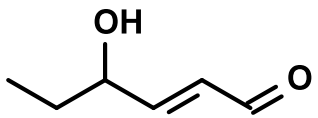
Trans-4-oxo-2-hexenal (4-OHE)



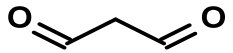
2-propenal (acrolein)



Trans-4-hydroxy-2-nonenal (HNE)



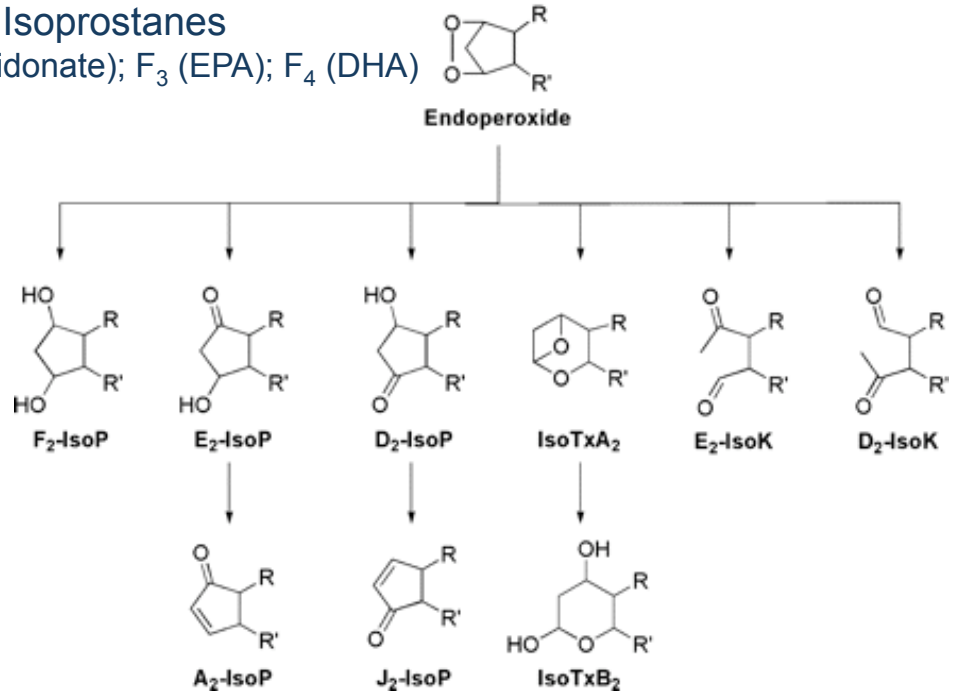
Trans-4-hydroxy-2-hexenal (HHE)



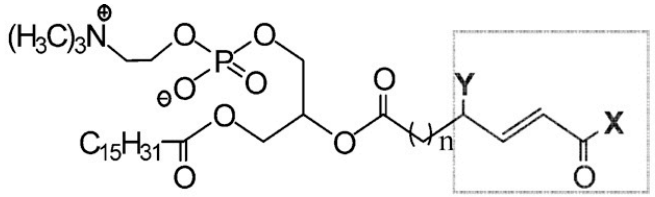
Malondialdehyde

Cyclic – Isoprostanes

F_2 (Arachidonate); F_3 (EPA); F_4 (DHA)

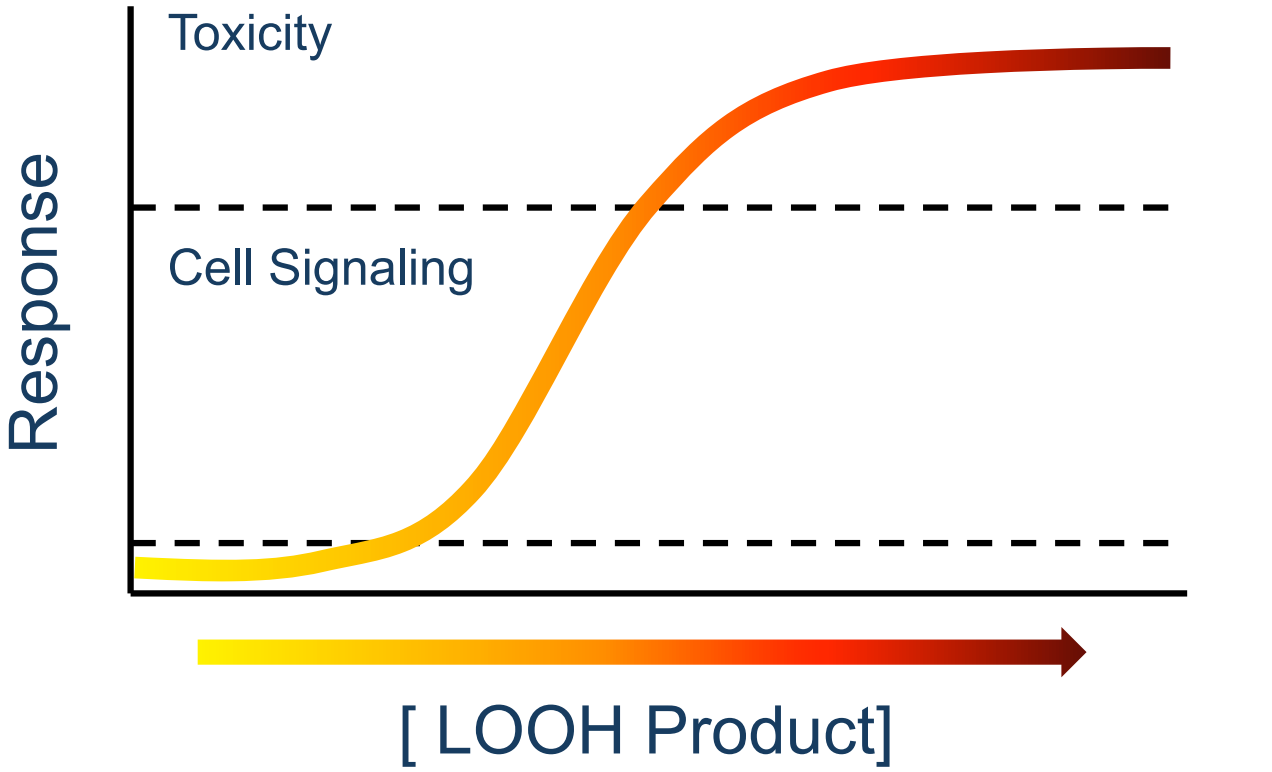


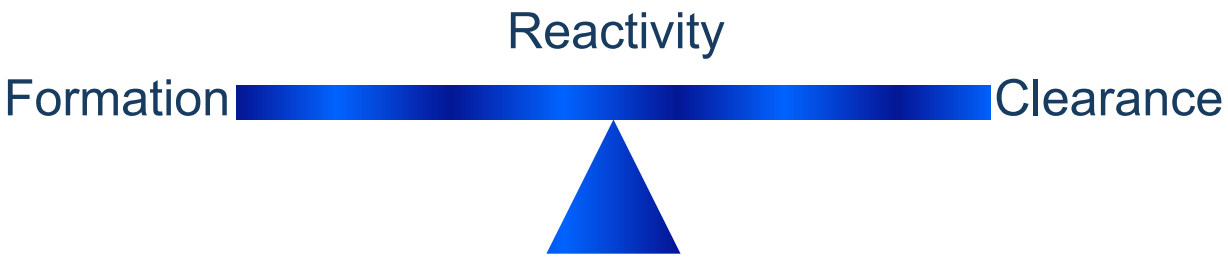
Phospholipid aldehydes



…all have different effects.

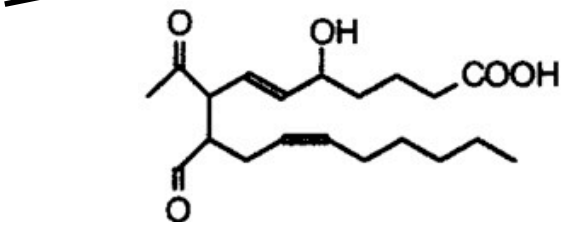
Biological Effects of LOOH Products

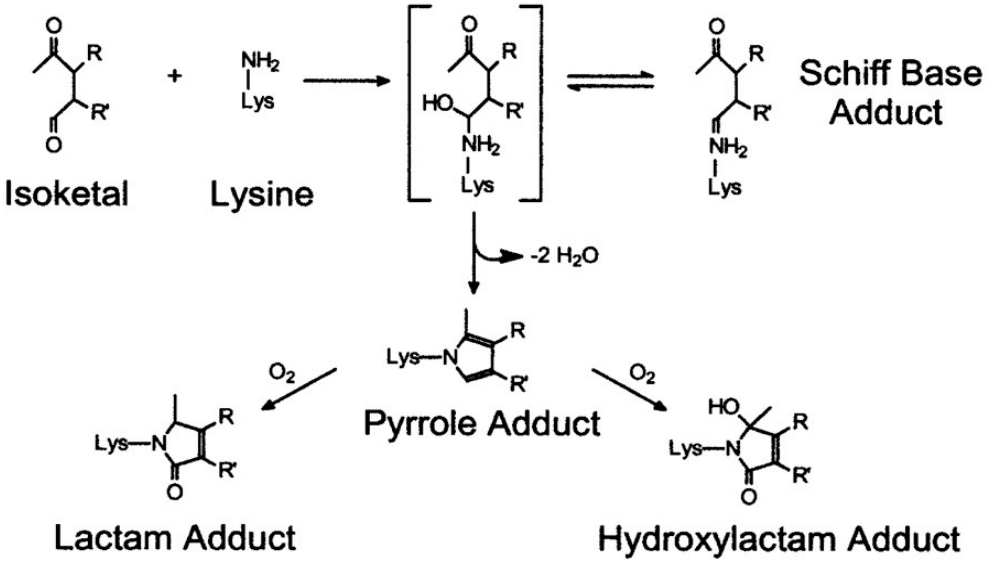


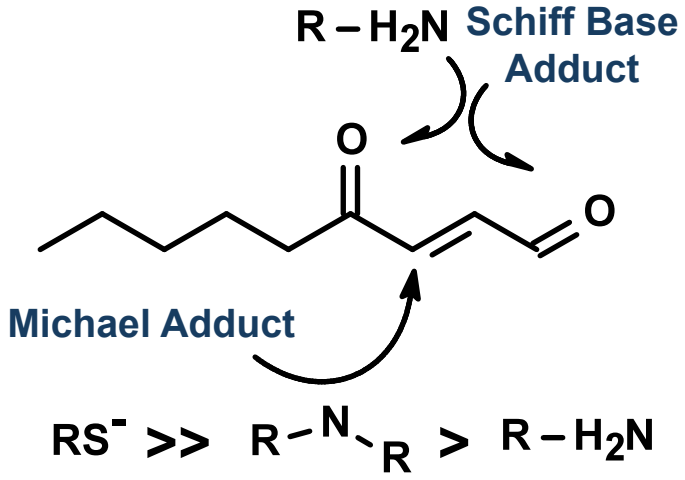


Mechanisms of Biological Response

Nucleophile Alkylation — **Signaling**







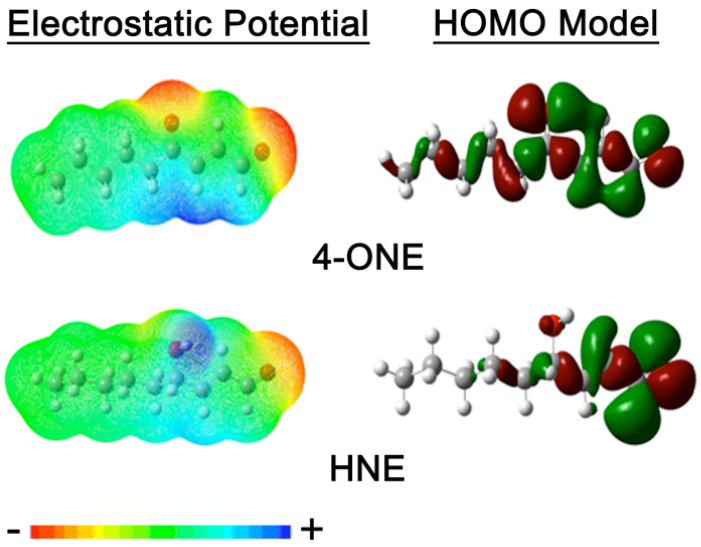
RS$^-$ can be proteins or GSH

Subsequent intra-molecular and intermolecular reactions (e.g. crosslinks) can occur

Brame C J et al. J. Biol. Chem. 2004;279:13447-13451

Chemical Reactivity and Biological Effect

4-ONE vs HNE



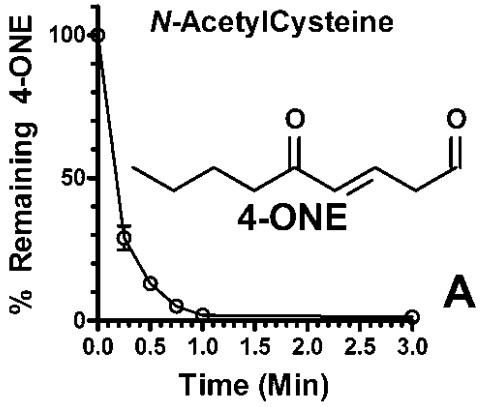
4-ONE is highly electrophilic

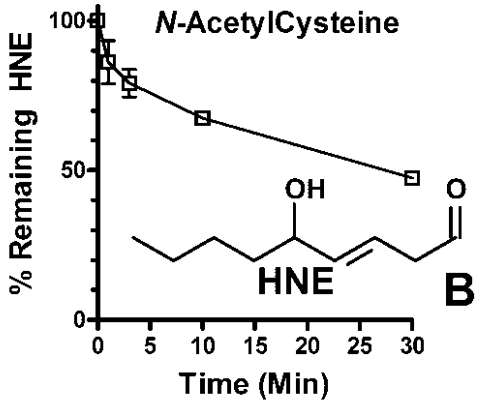
> + ESP than HNE

>150-fold reactive with N-AcCys

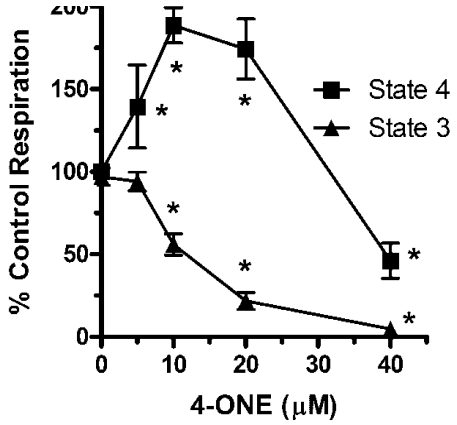
>20-fold at altering respiration

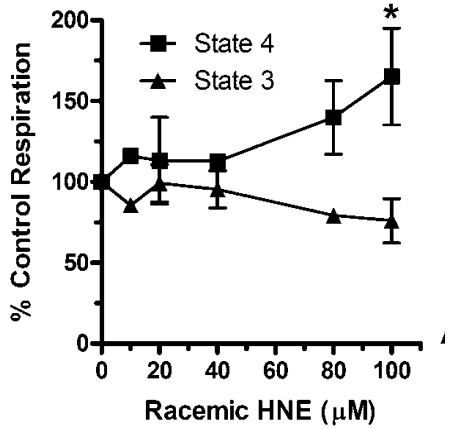
Simple Reaction





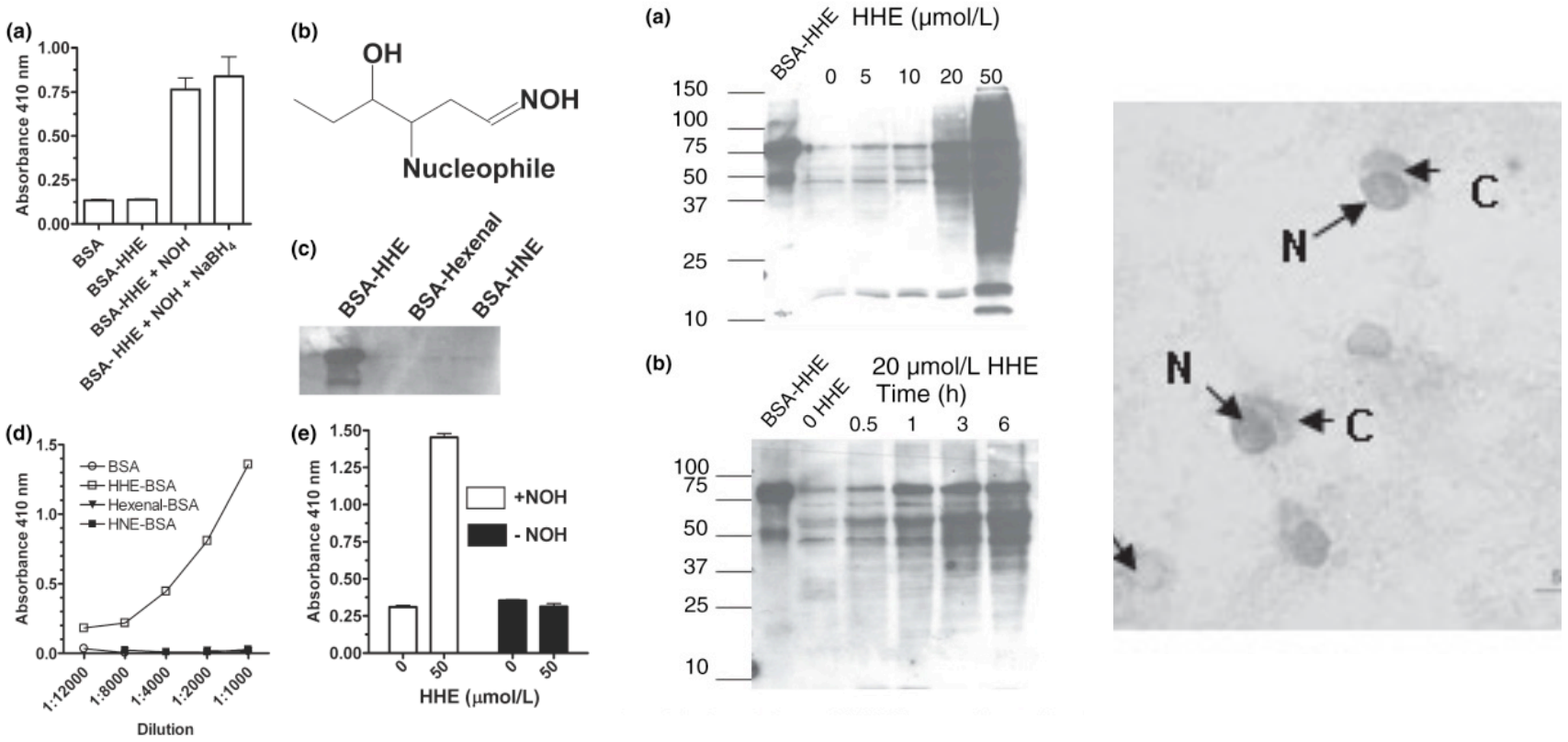
Mitochondrial Respiration





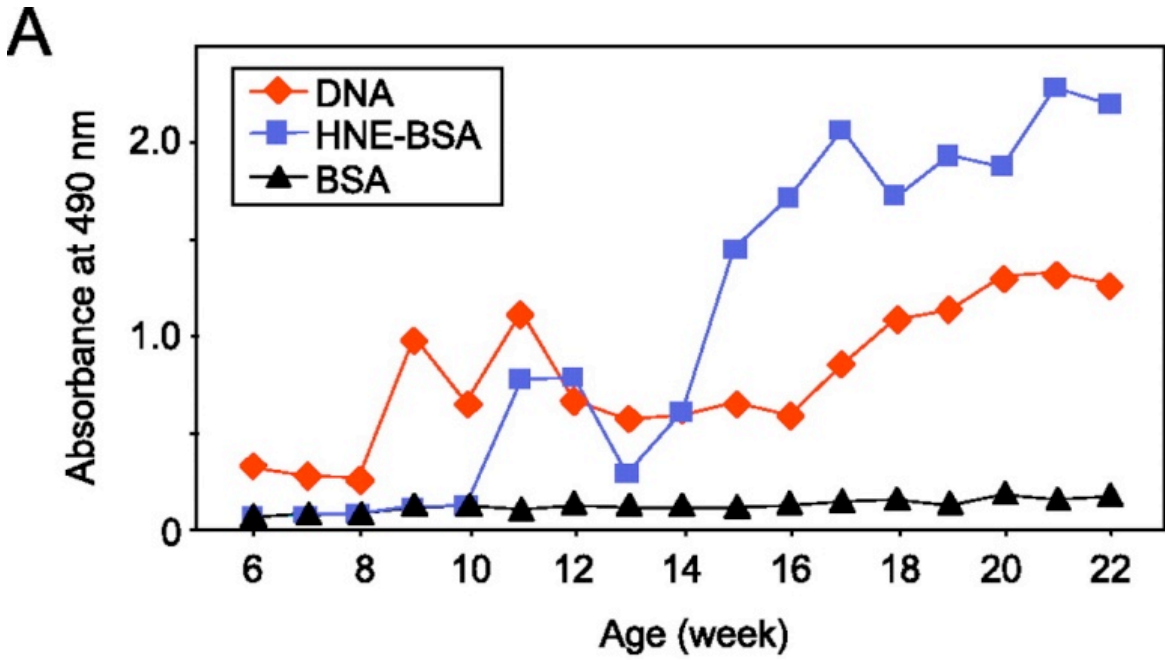
Picklo et al, *FRBM* in press

Protein adducts are immunogenic



Long et al J. Neurochem 2008; 105: 714-724.

HNE/4-ONE protein adducts induce an auto-immune response with resulting antibodies recognizing DNA



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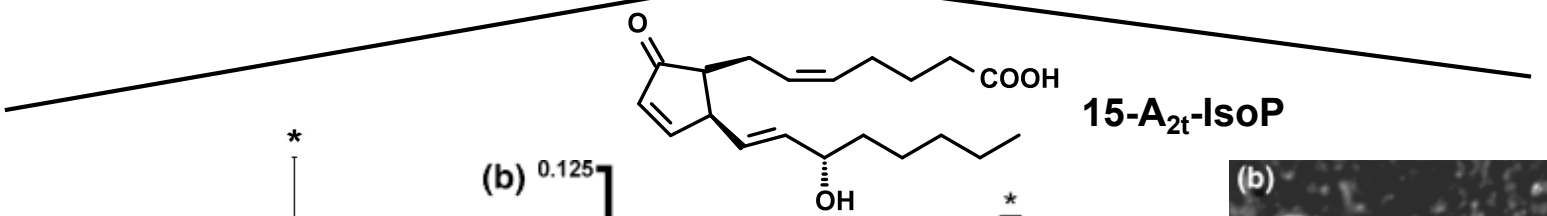
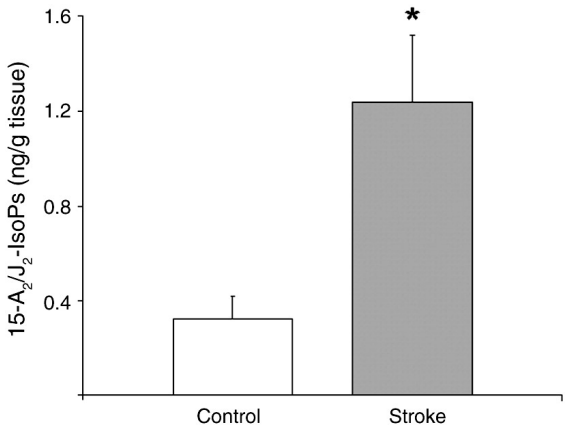
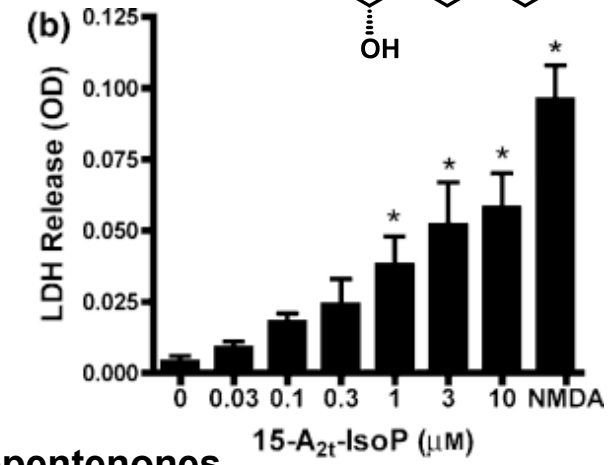
Alkylation to Signaling

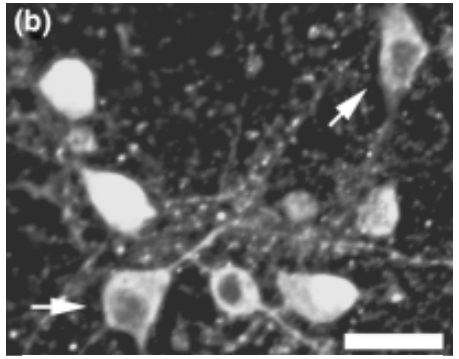
Alkylation → Signaling

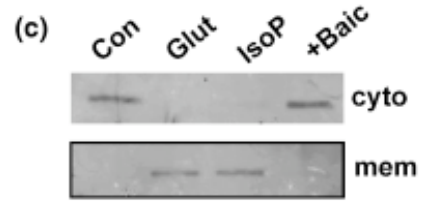


15-A_{2t}-IsoP



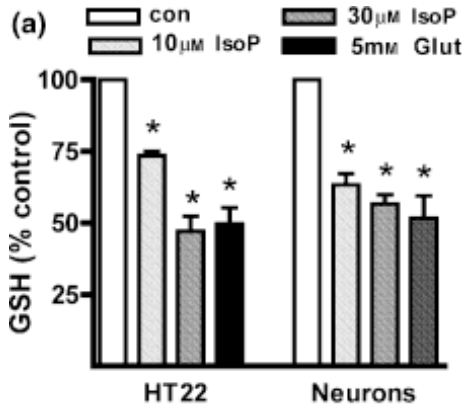


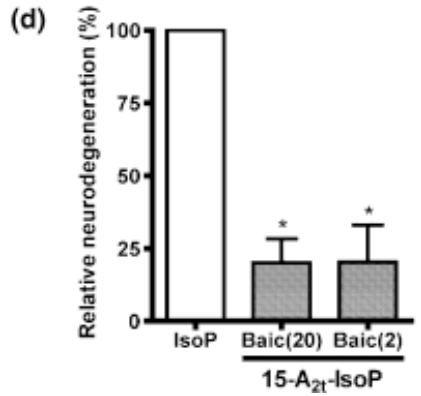




Arachidonate-derived iso-cyclopentenones

- Increase with stroke
- Neurotoxic
- Deplete GSH
- Induce Apoptotic Pathway
  - Activate 12-LOX
- COX-derived PGA_2/J_2
  - Mt Dysfunction/ROS





Zeiger et al. FRBM 2009 (47) 1422-1431
Musiek et al. J Neurochem 2006 (97) 1301-1313
Kondo et al J Biol Chem 2001 (276) 12076-12083

baicalein = 12-LOX Inhibitor

Lipid Peroxidation of n-3 vs n-6 PUFA
Evolutionary Aspects and Disease

Fatty acid intake in human diet has changed, our species developed in a richer, n-3 environment

Increase in n-6 consumption, Decrease in n-3 consumption

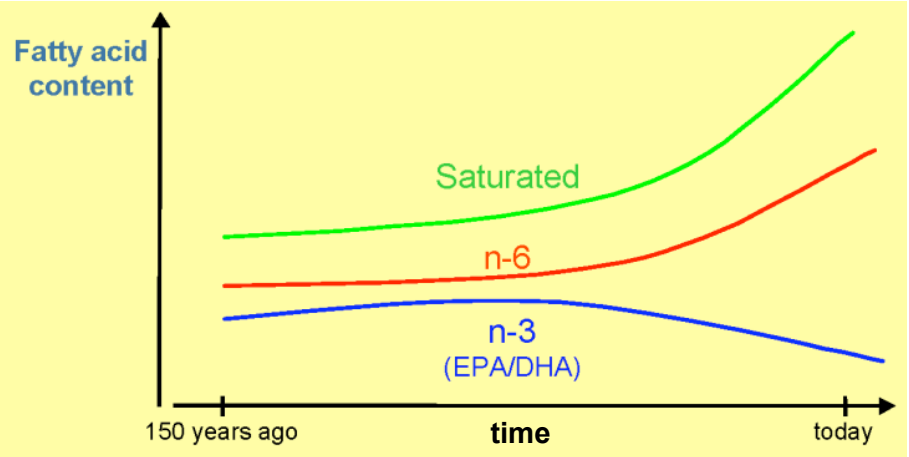
150 yrs ago, n-6:n-3 ratio = 2 : 1

Today, n-6:n-3 ratio = 20 : 1

n-3 derived lipid oxidation products (in some cases):

Anti-inflammatory, vasodilatory

- PGI_3 vasodilatory
- TxB3 no activity
- DHA/LOX-derived resolvins are anti-inflammatory
- n-3 cyclopentenones are anti-inflammatory
- EPA - competitive inhibitor of COX for arachidonate

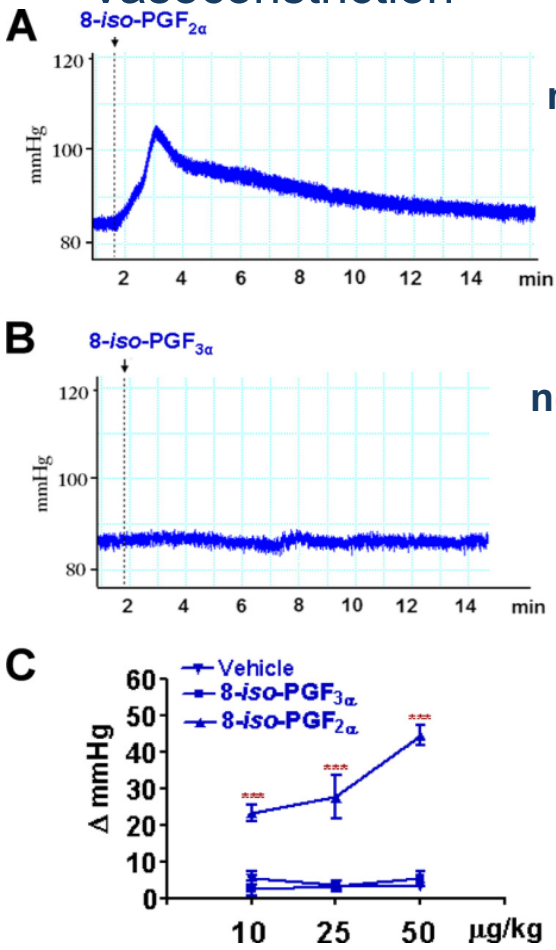


Thus, perhaps the *pathological*, n-6 lipid peroxidation pathways we are studying developed in an environment in which n-3 lipid peroxidation was *physiologically essential*, not pathological.

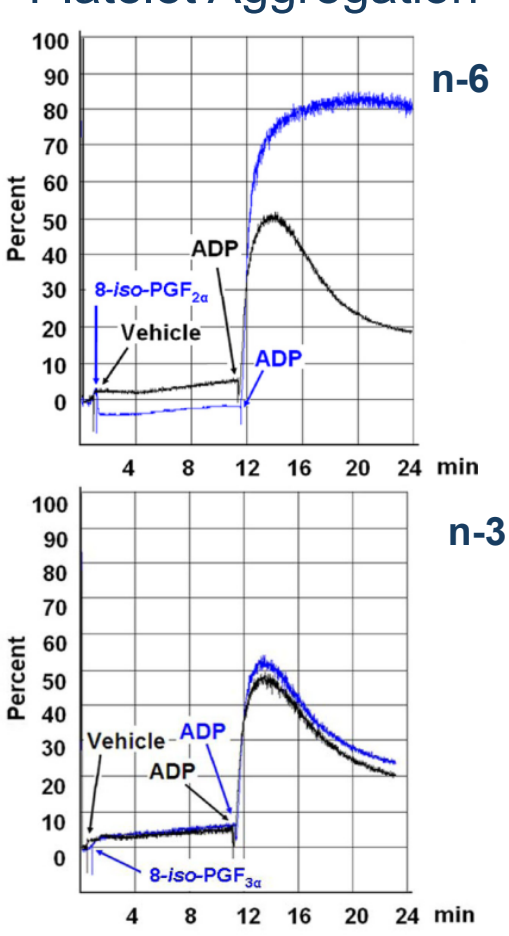
n-3 vs n-6

8-iso-$PGF_{2\alpha}$ vs 8-iso-$PGF_{3\alpha}$

Vasoconstriction

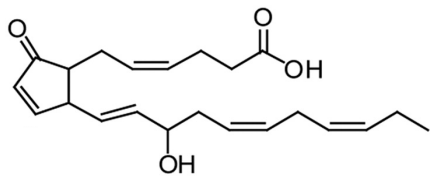


Platelet Aggregation

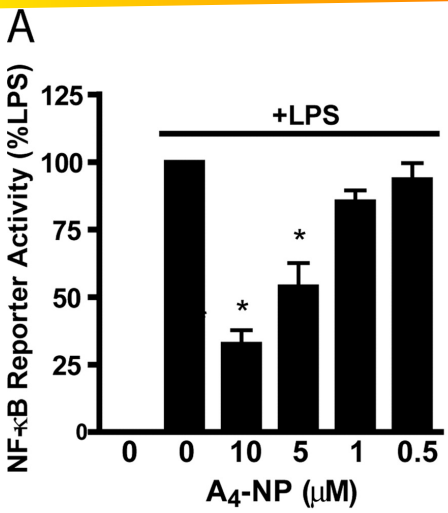


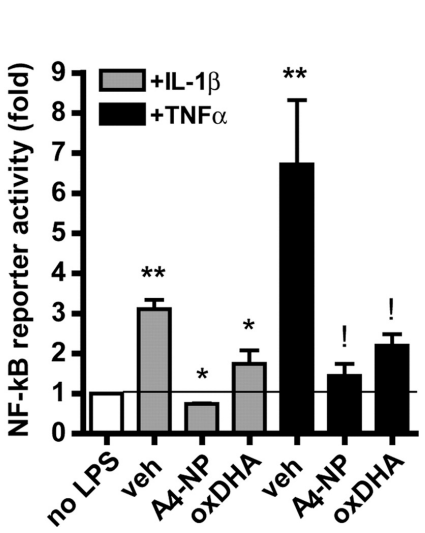
Wen-Liang Song et al JBC
2009 (284) pg 23636

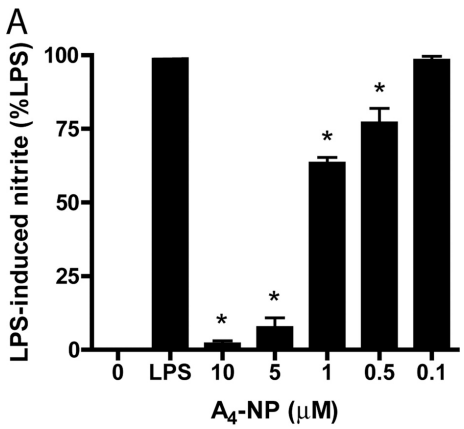
F_4-cyclopentenones

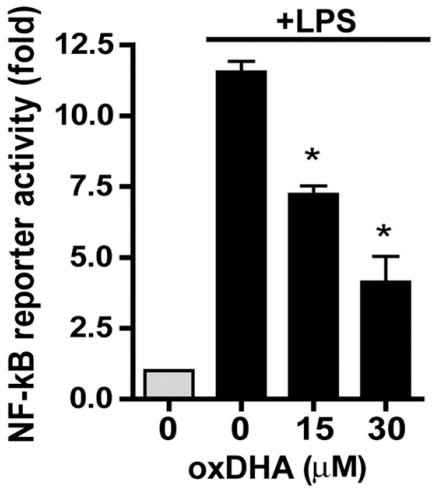


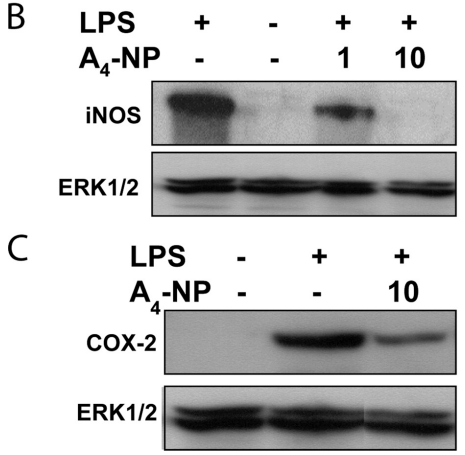
A_4-Neuroprostane











Blocks inflammatory response via NF-κB Signaling Pathway
Inhibit IKK and NF-κB translocation

Musiek et al. J Biol Chem 2008 (283) 19927-35

Summary

1. Lipid oxidation is an enzymatic and non-enzymatic event
    - COX, LOX, P450, Free-radical
2. A number of biologically active products are formed
    - Protein alkylation and signaling mechanisms
3. Data indicate opposing effects of n-3 vs n-6 LOOH products
    - F_3-IsoPs vs F_2-IsoPs

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