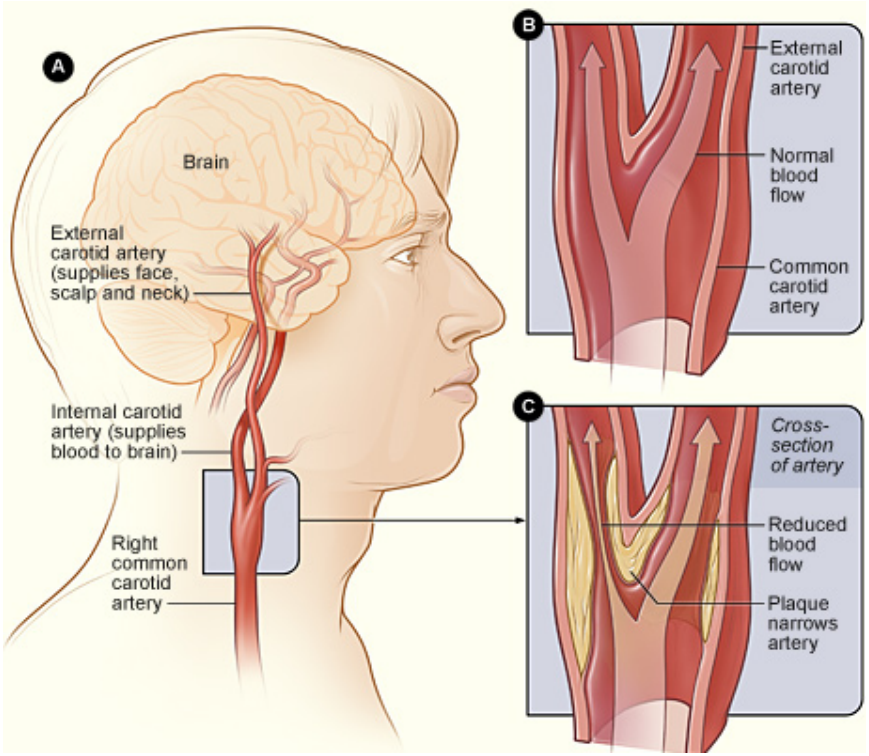
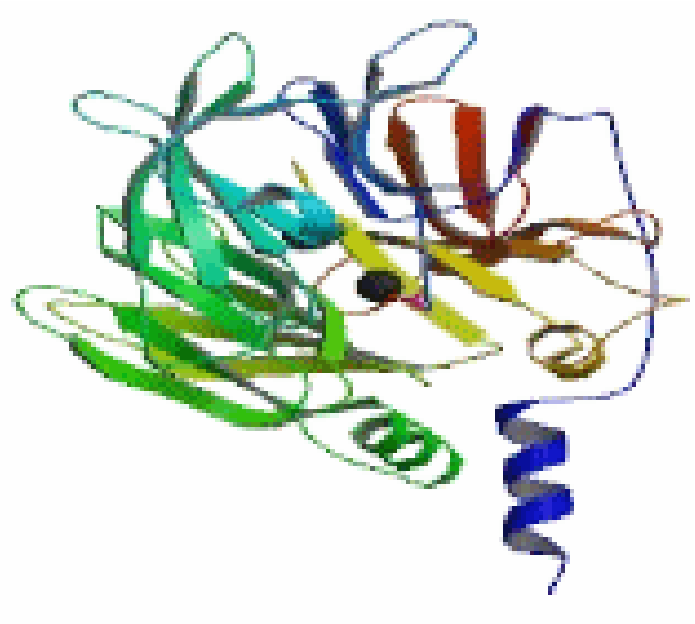
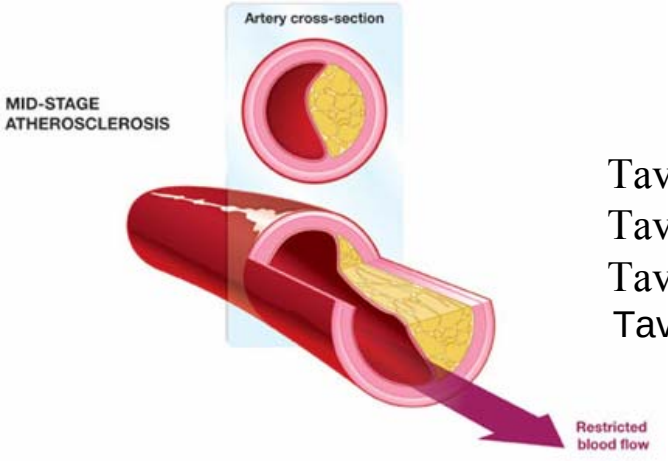
A mutual association between paraoxonase 1 (PON1) and the Human carotid plaque constituents

Atherosclerotic lesion







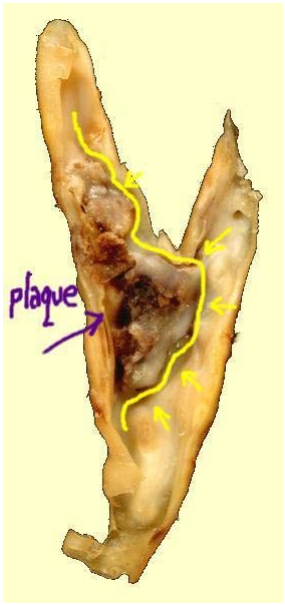
Tavori et al., **(2008).** Bioorg. Med. Chem. 16(15), 7504-9.

Tavori et al., **(2009).** *Free Radicals Biology and Medicine*. 46, 607-615

Tavori et al., **(2011).** *Free Radicals Biology and Medicine. 50, 148-56.*

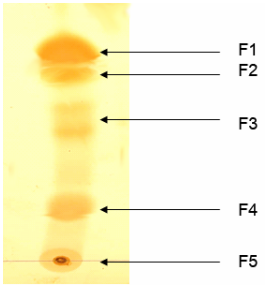
Tavori et al., **(2011).** *Free Radicals Biology and Medicine. In press.*

Human carotid lesion lipid extract (LLE)



Liquid
Nitrogen

EtOAc
extraction

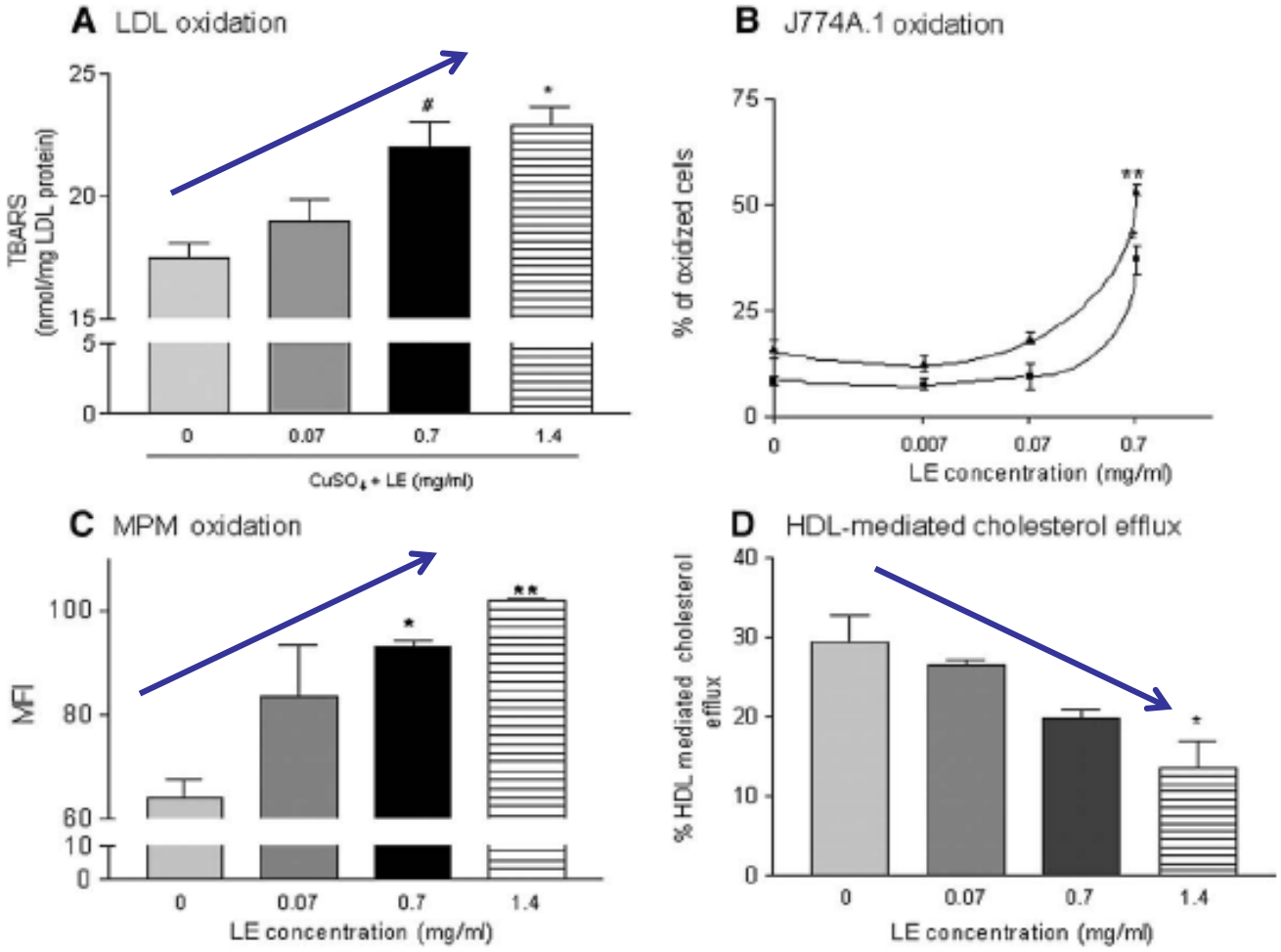


Lesion lipid extraction-LLE
(yellowish paste)

Aim 1:

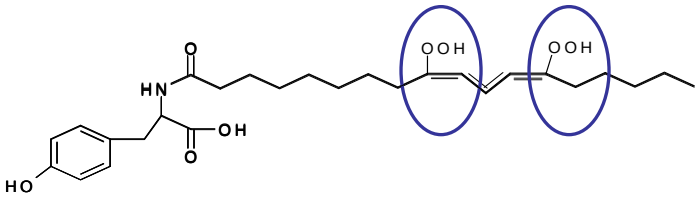
To examine the effect of human carotid lesion lipid extract (LLE) atherogenicity, and the involvement of PON1 in suppressing such LLE atherogenicity

Human LLE oxidize macrophages and LDL, and reduces HDL-mediated cholesterol efflux from macrophages

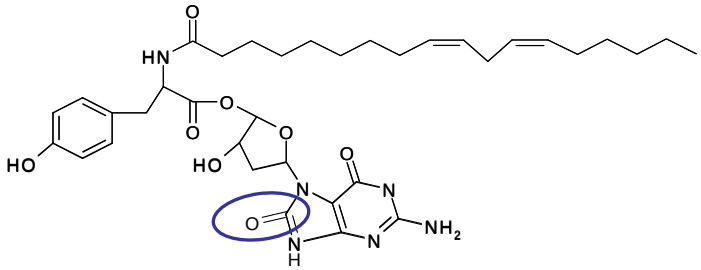


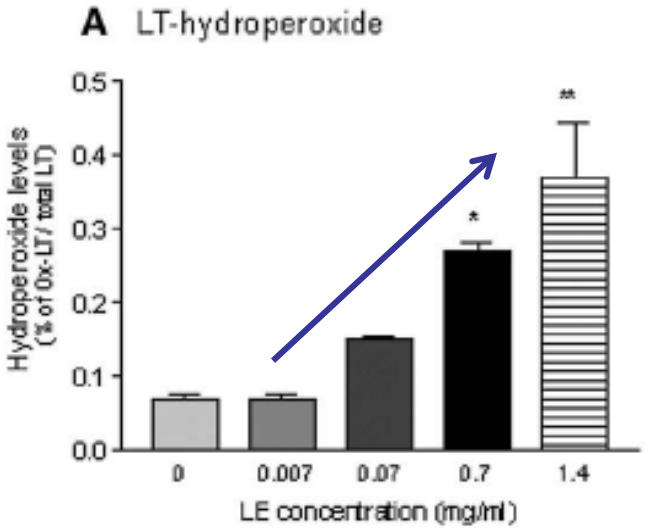
LLE direct oxidation capability

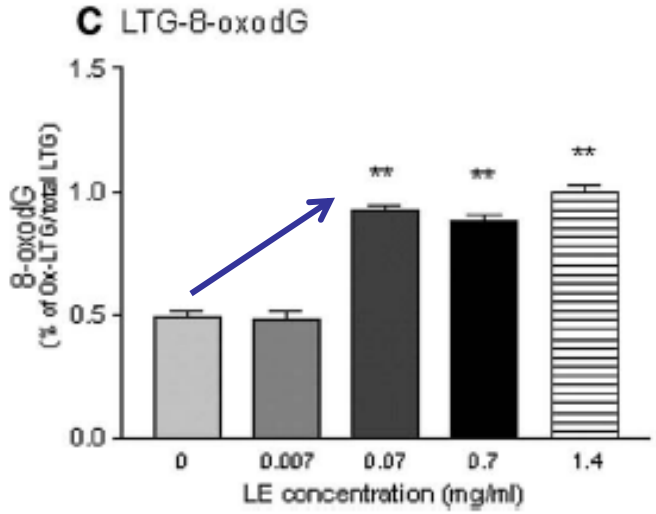
N-linoleoyl tyrosine
(LT)



N-linoleoyl tyrosine 2 –deoxyguanosine (LTG)





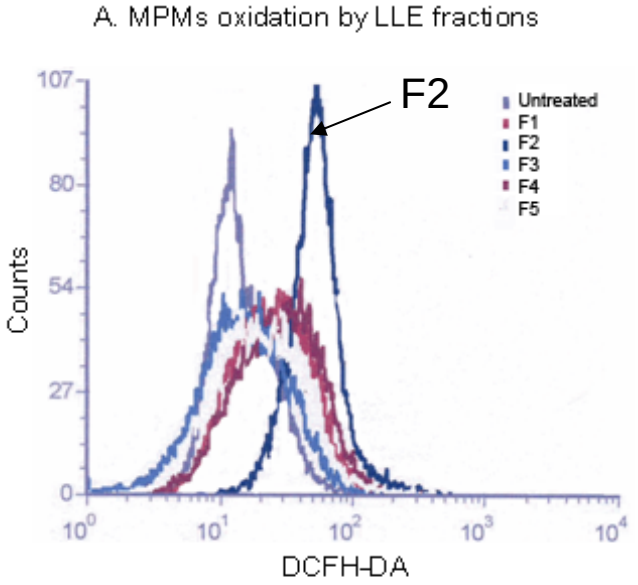


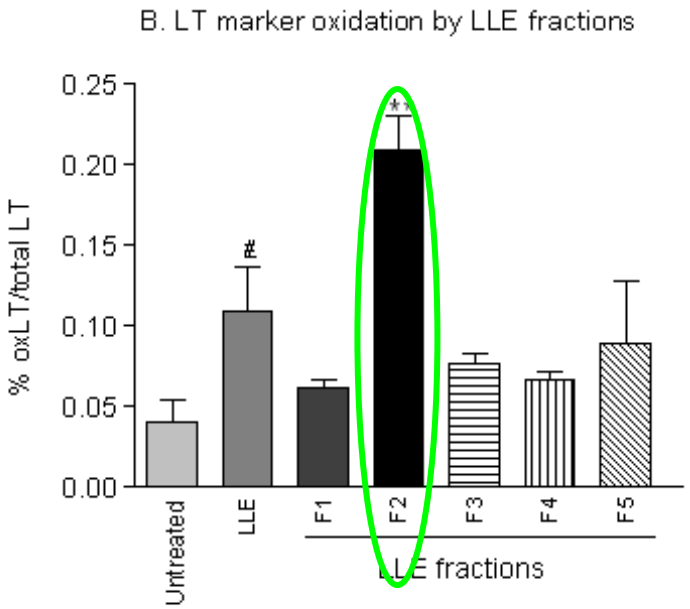
- LLE oxidized lipids and nucleic acids but not amino-acids

Aim:

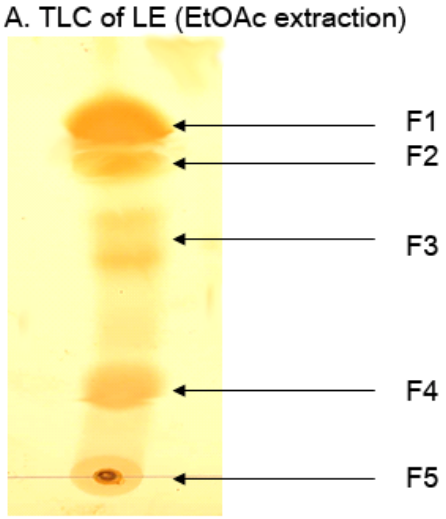
To isolate and identify the chemical structure of the fraction responsible for LLE atherogenic properties

Isolation of the oxidizing fraction

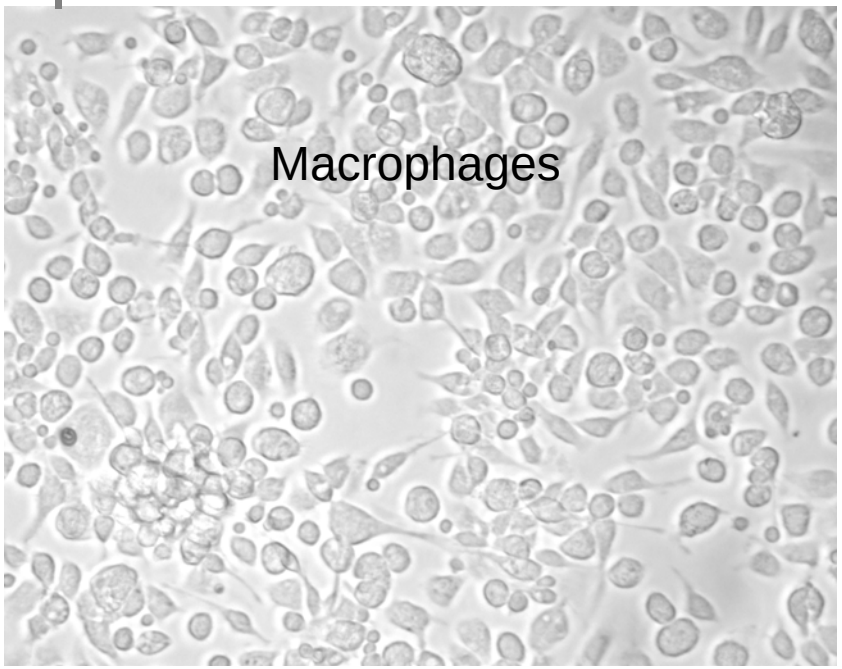


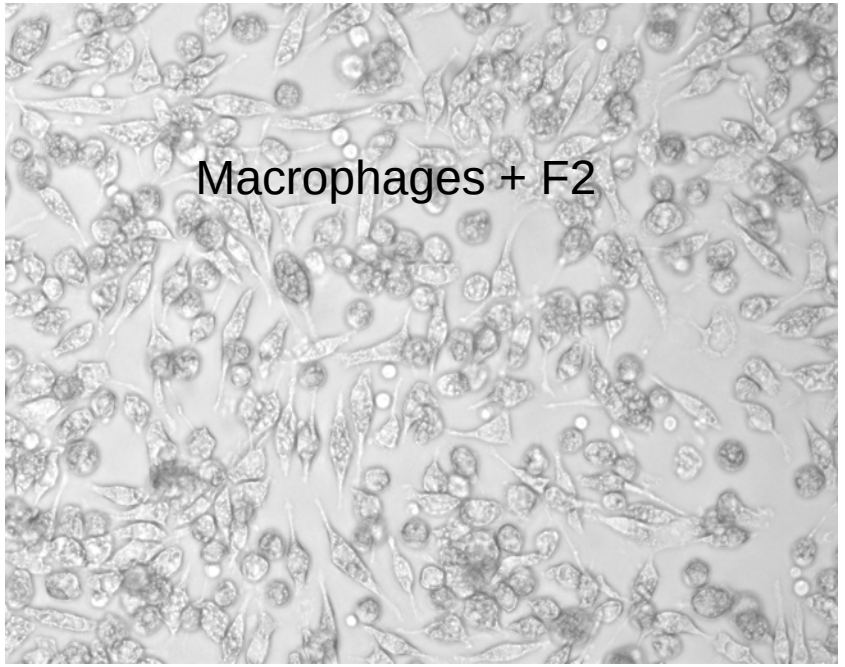


- Fraction number 2 (F2) can oxidize ,macrophages and OS markers in a dose dependent manner



Isolation of the oxidizing fraction

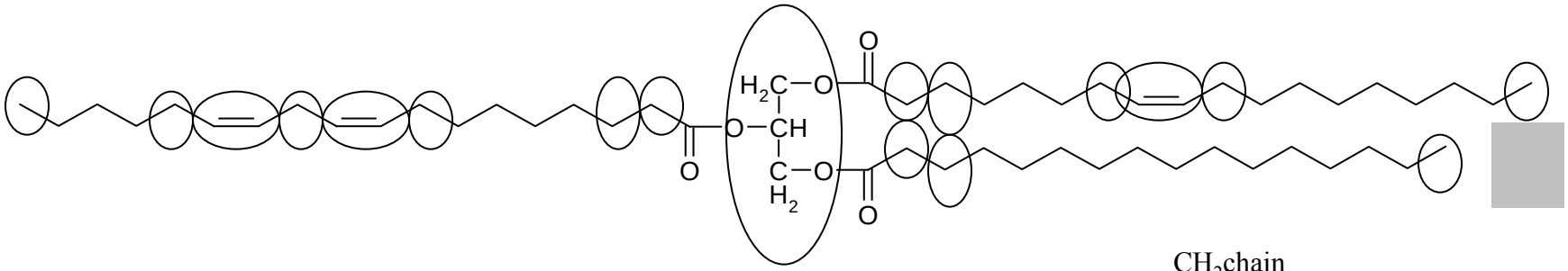


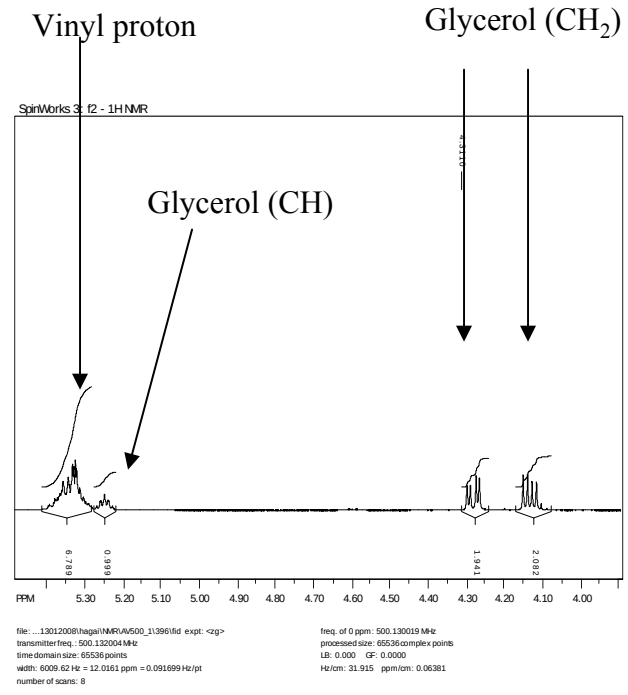


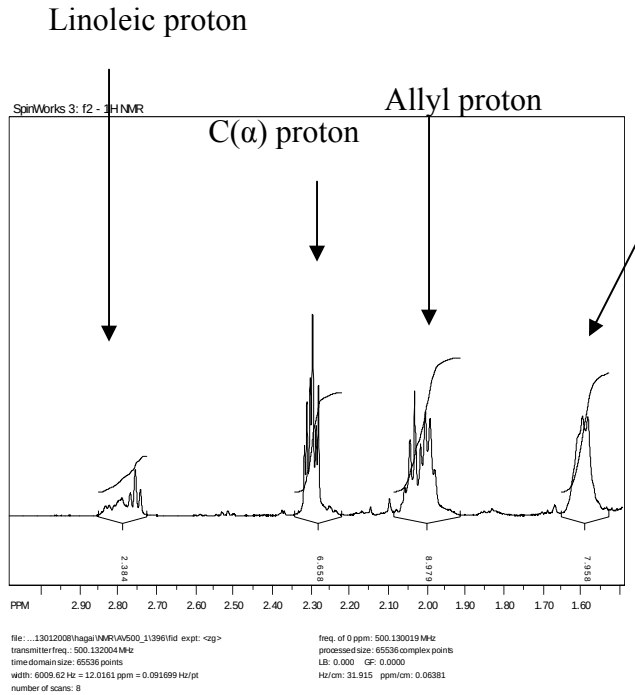
- Macrophages turned into a foam cell appearance upon incubation with F2

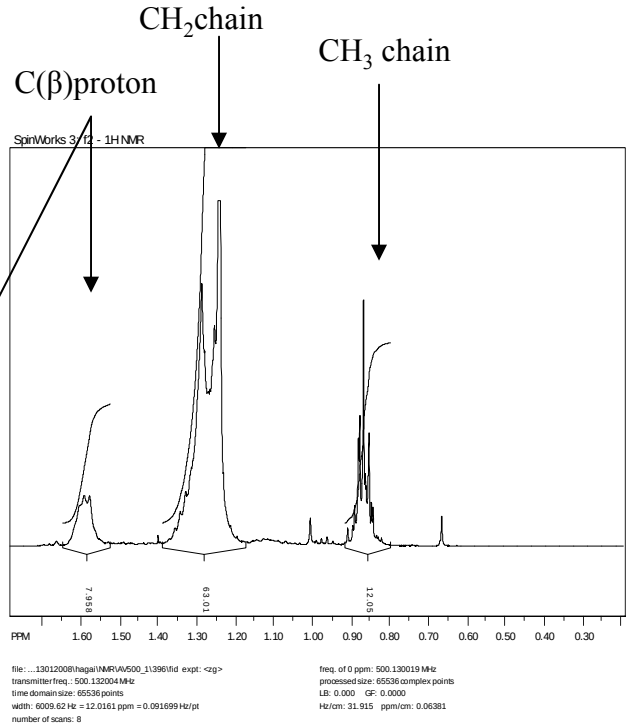
Conclusion: LLE contains element (F2) with atherogenic properties

Fraction 2 (F2) -NMR analysis

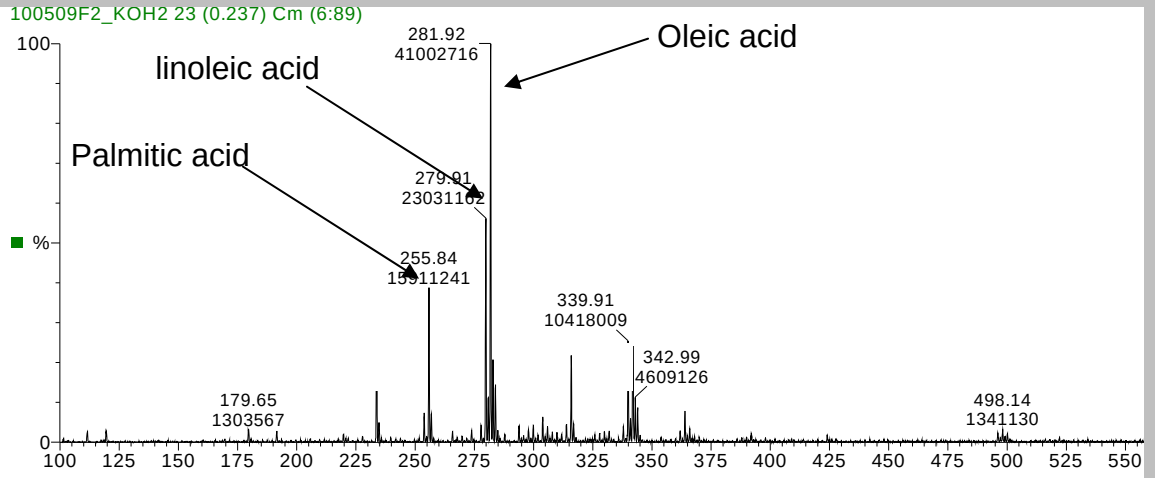






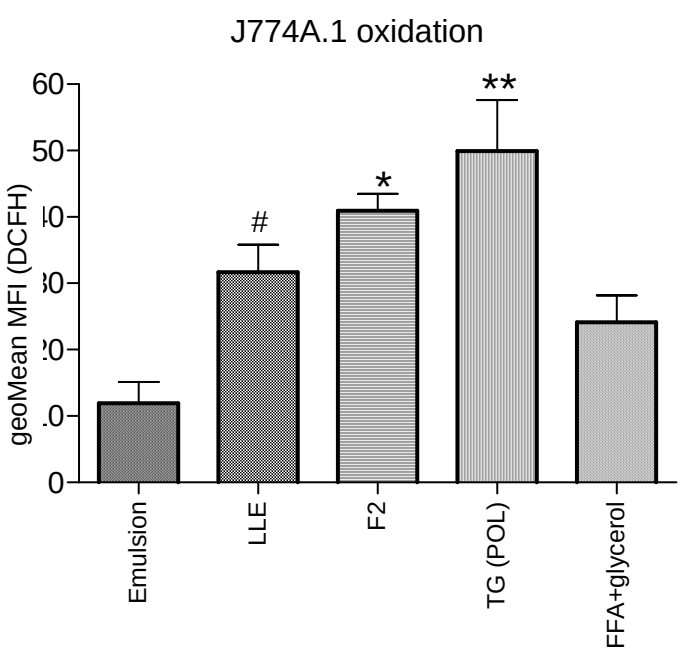


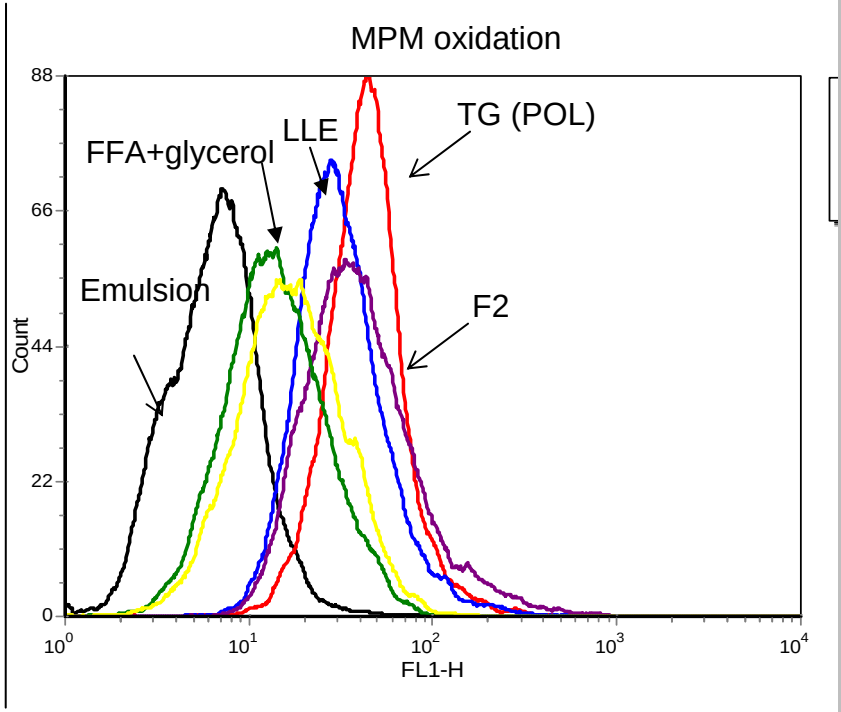
Fraction 2 (F2) –LC/MS analysis



- The fatty acids in F2 are: palmitic, oleic and linoleic acids in 1:1:1 molar ratio.

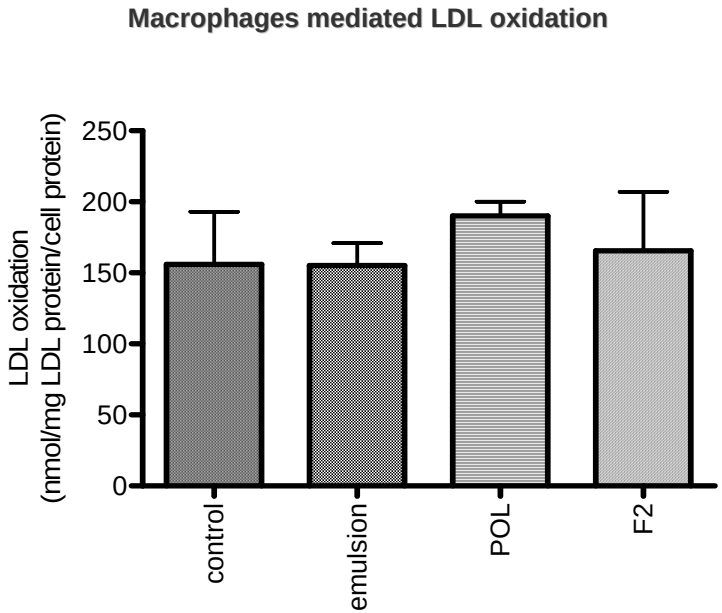
Oxidation with synthetic TG (POL)





- TGs but not FFA + glycerol are responsible for macrophages oxidation

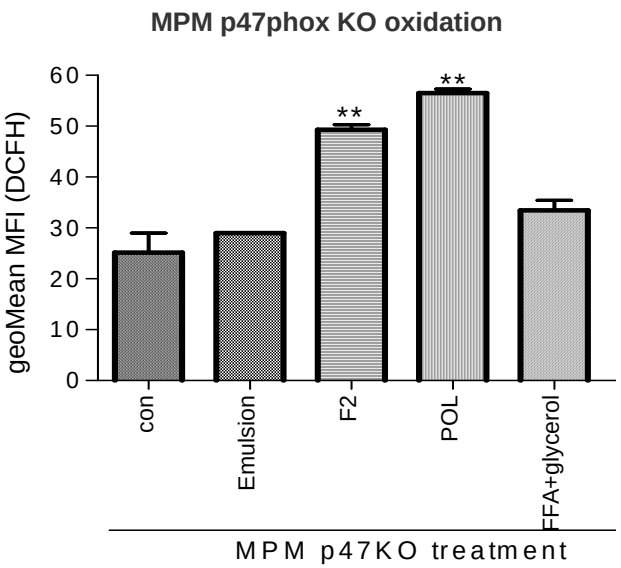
Oxidation with synthetic TG (POL)

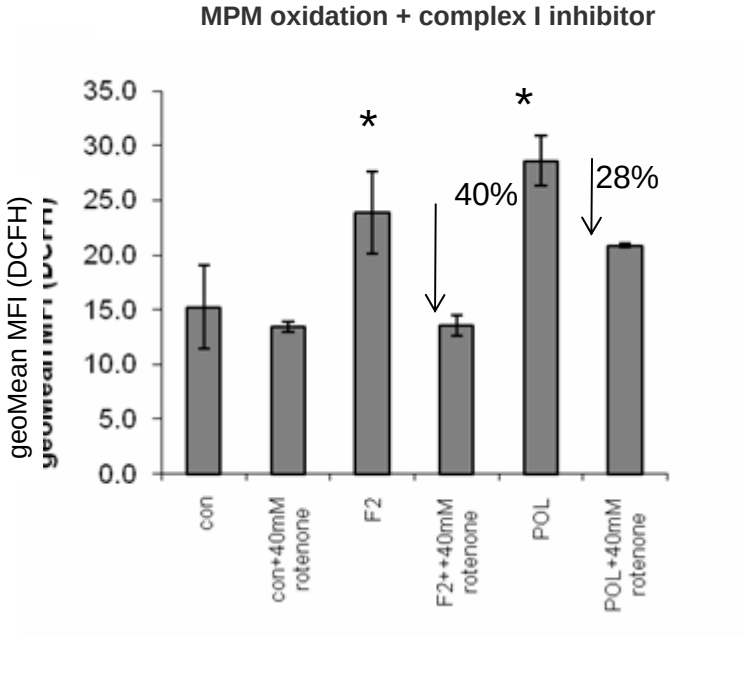


- Although macrophages were oxidized they could not induce LDL oxidation.

Conclusion: The mechanism of MQ oxidation by TG (POL) is differ from regular MQ oxidation

Mechanism of macrophages oxidation by F2/POL

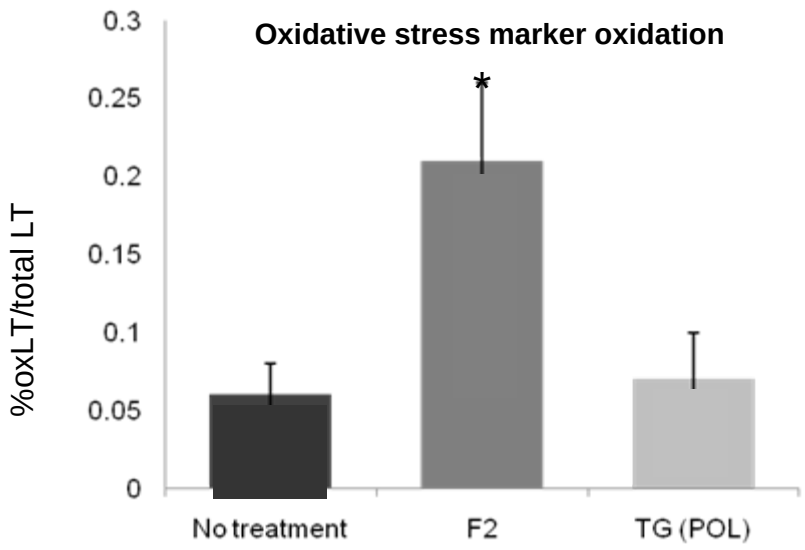


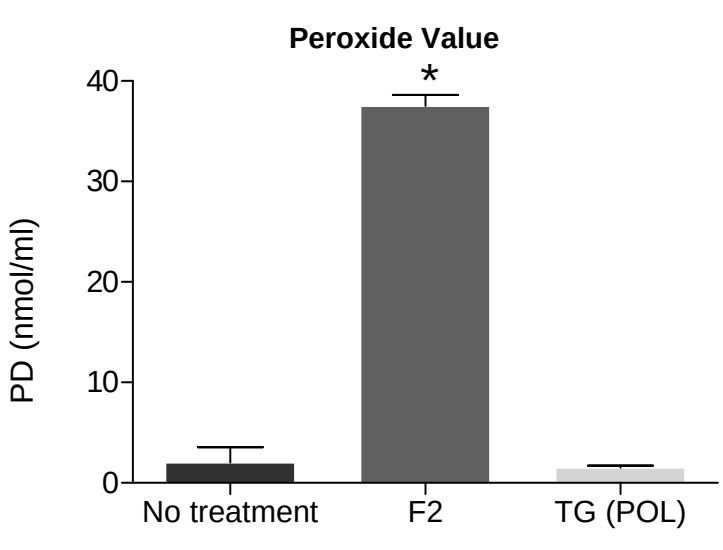


- F2 induces macrophages oxidation via mitochondrial pathway, without the involvements of the NADPH oxidase system.

Tavori et al , FRBM, in press

Oxidation with synthetic TG (POL)





F2 is partially oxidized to hydroperoxide (0.5%) but not the TG (POL). This hydroperoxide impurity seem to be responsible for Marker oxidation .

Summary (1)

- Lesion lipid extract (LLE) fraction # 2 – active in promoting macrophages and OS marker oxidation, but not macrophages mediated LDL oxidation.

- The main component responsible for human lesion lipid extraction oxidizing capacity is a specific TG (POL) which can partially oxidize

- F2 promote oxidation via 2 separate mechanisms:

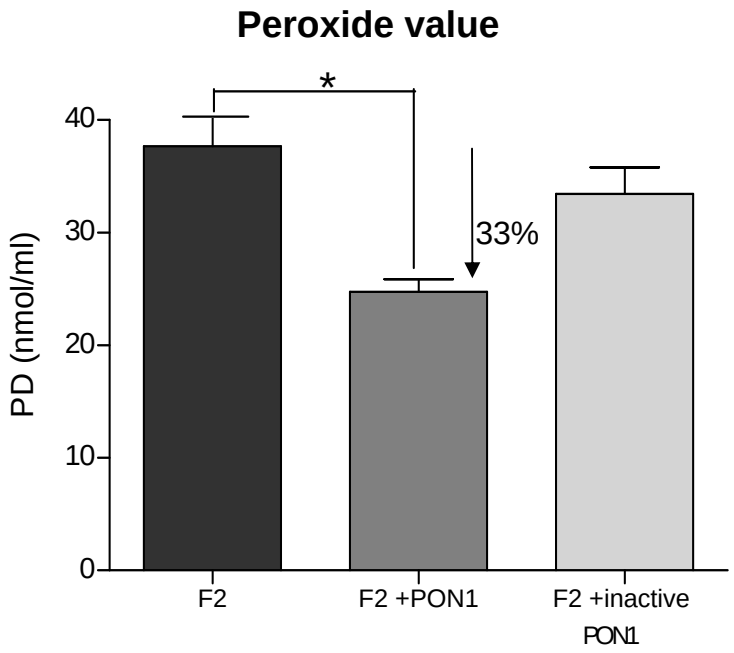
  (A) Direct oxidation via it LA subunit (LT marker)

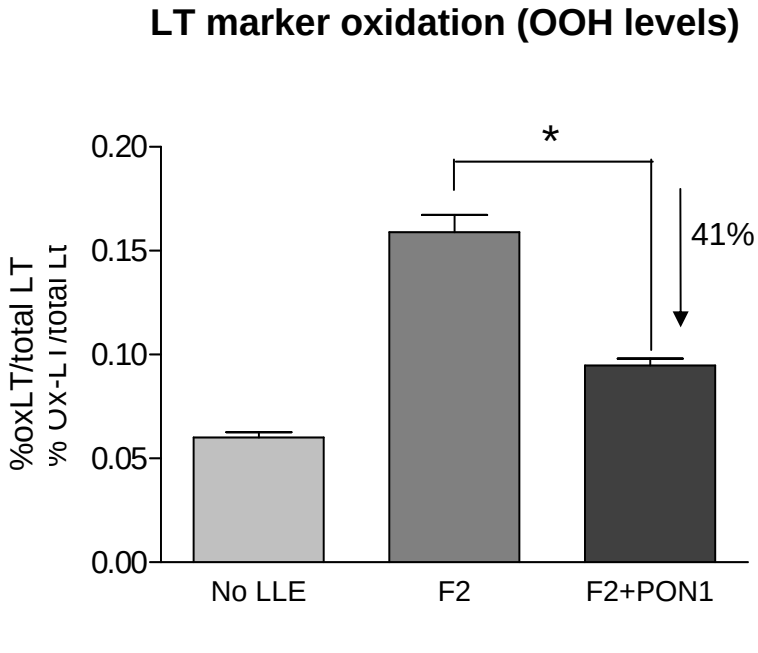
  (B) Macrophages oxidation via mitochondrial pathway

Research Aims

To investigate the effects of PON1 on the atherogenicity of plaque components.

PON1 effect on direct oxidation



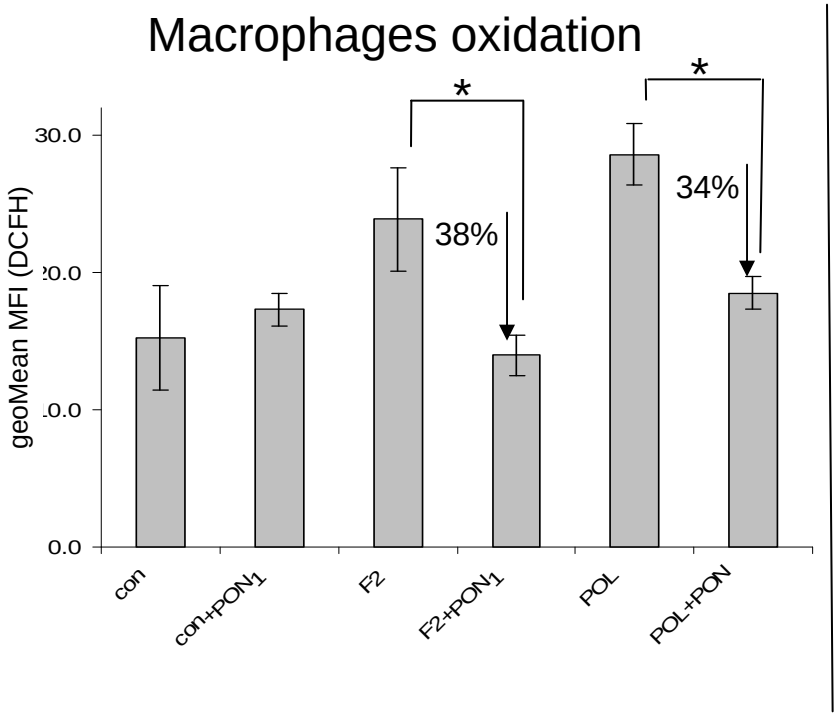


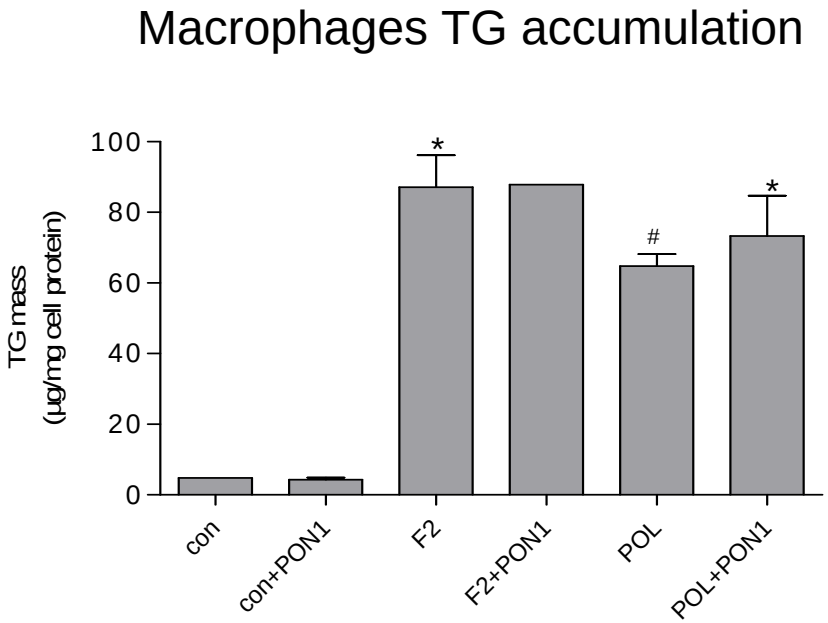
- PON1 reduces F2 hydroperoxide by 33% and F2-mediated marker oxidation by 41%.

- We have recently showed PON1 ability to specifically reduce linoleic acid hydroperoxide (LA-OOH) to hydroxide (LA-OH)

Tavori et al , FRBM, under review

PON1 effect macrophages oxidation





- PON1 reduces both synthetic TG (POL) and F2-mediated macrophages oxidation but, does not affect TG accumulation.

Summary (2)

- PON1 can reduce the F2 atherogenicity via 2 different mechanisms

  (A) Reduce F2 peroxide levels (reduction of LA-OOH to LA-OH), thus acting as peroxidase

  (B) PON1 reduce macrophages oxidation which is induced by the mitochondria.(PON1-mitochndira interaction still need to be tested).

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