Mediator Lipidomics: dissecting the role of PUFA-derived metabolites

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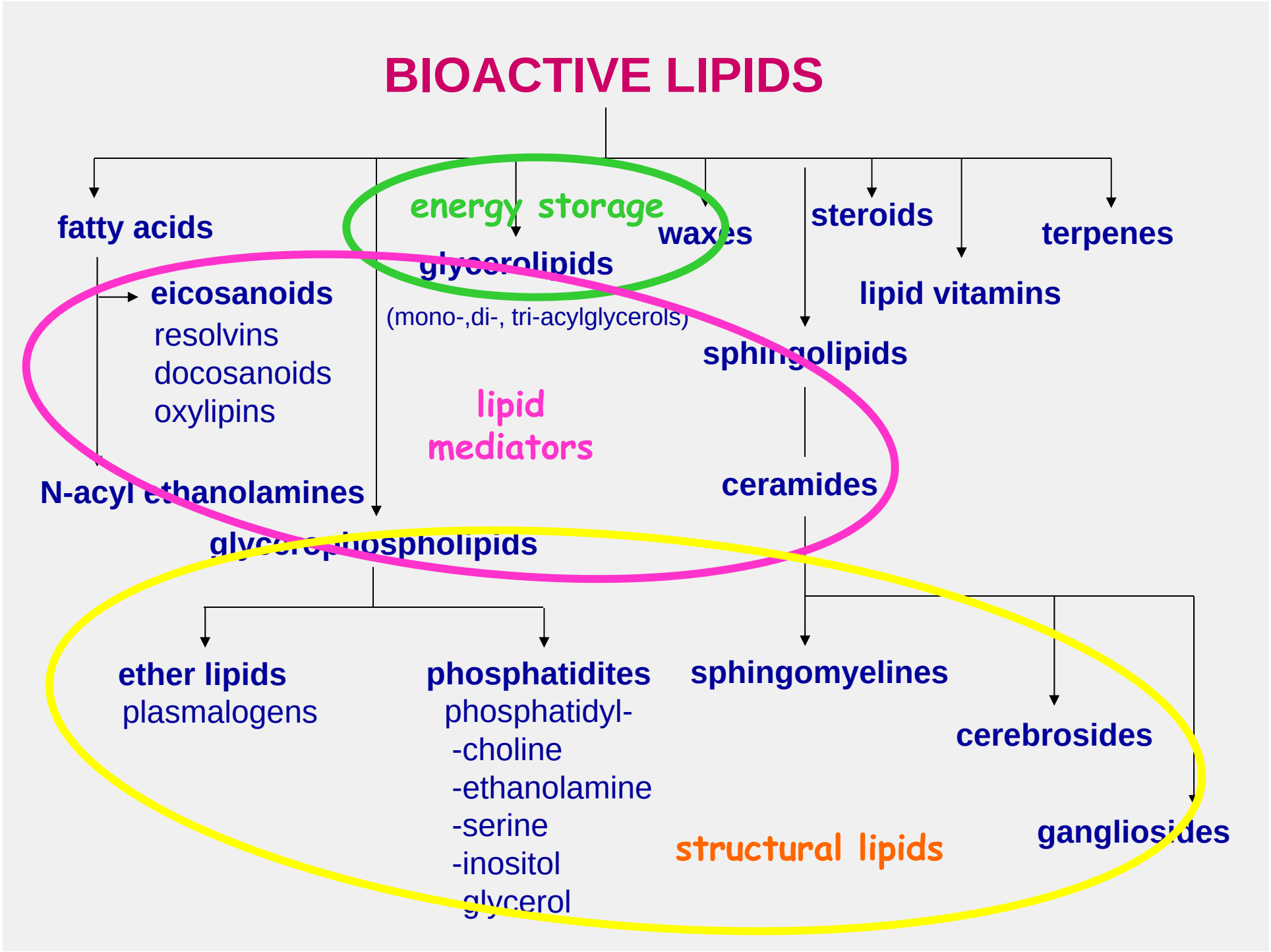
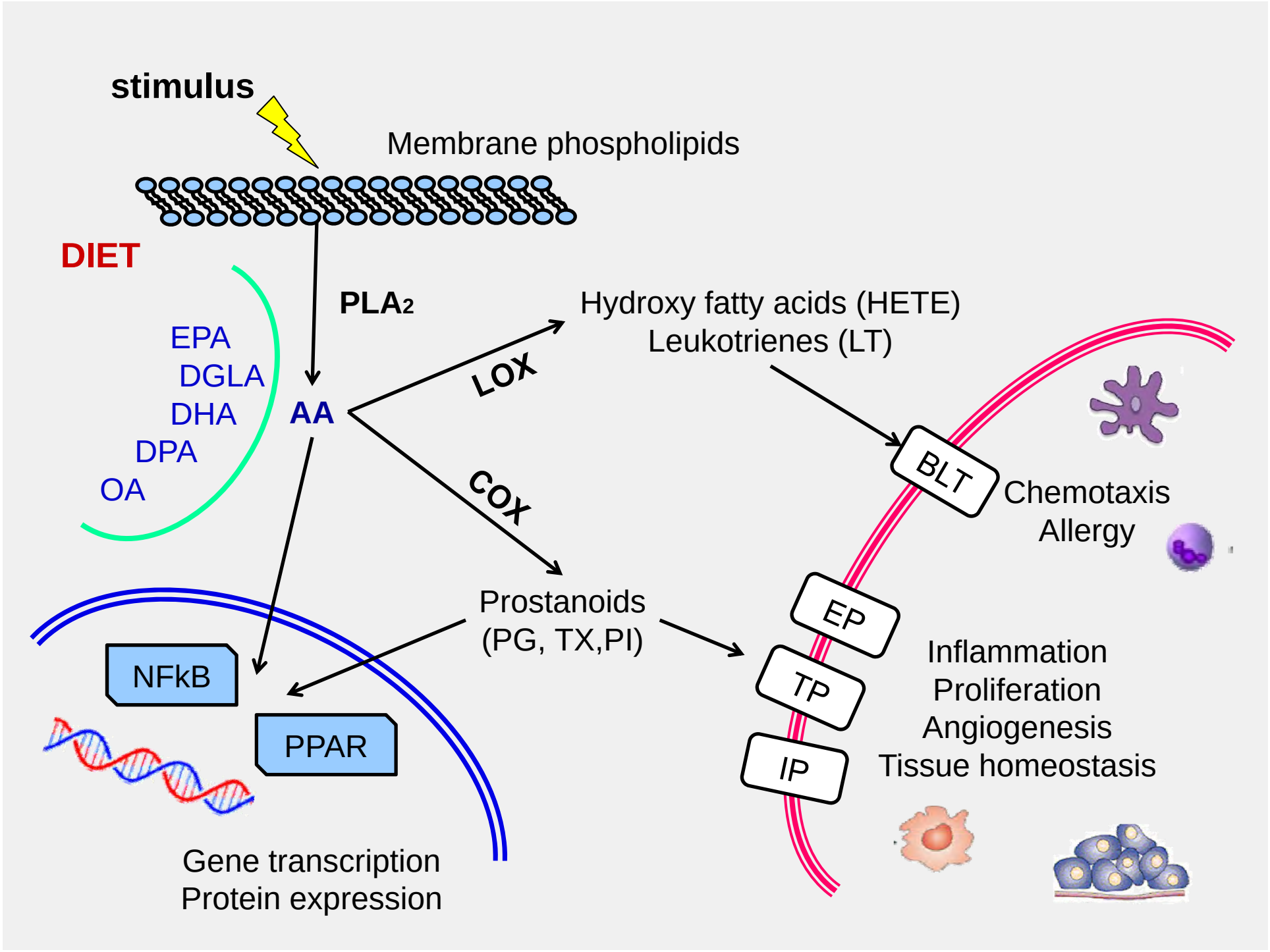
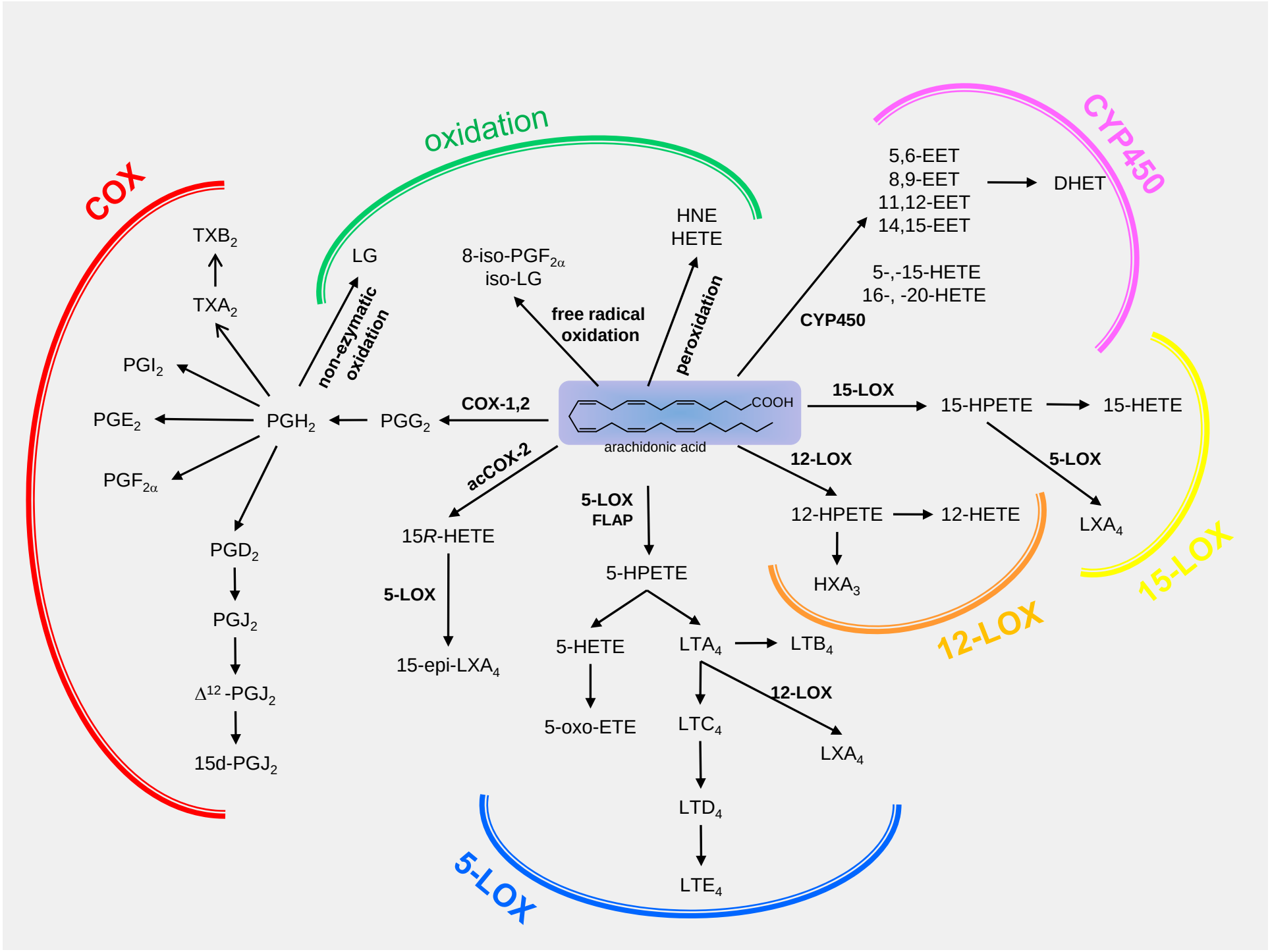
CENTRE FOR SKIN SCIENCES

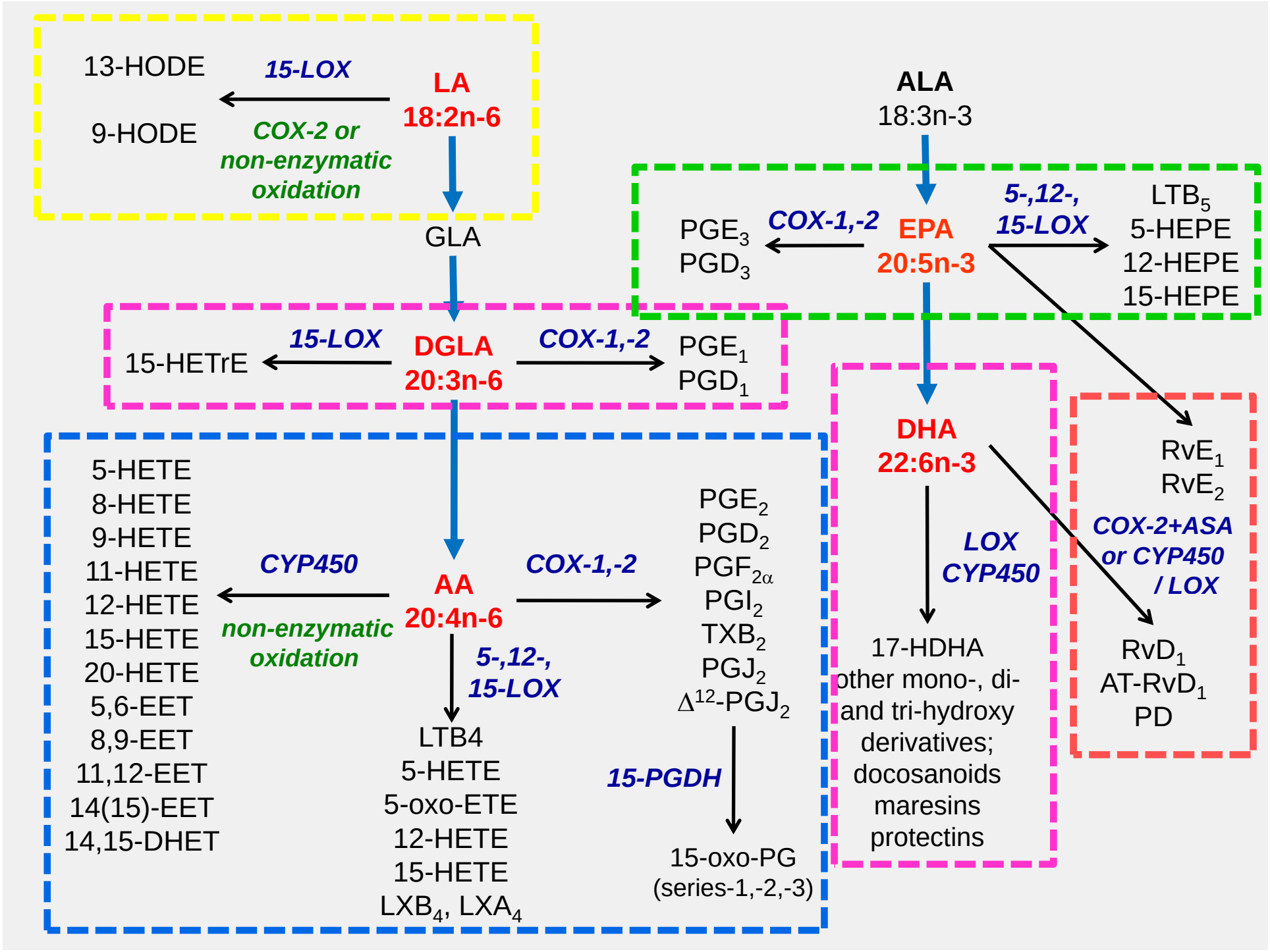
UNIVERSITY OF BRADFORD
MAKING KNOWLEDGE WORK

Compositional Analysis of Lipids
21 June 2013, Ghent

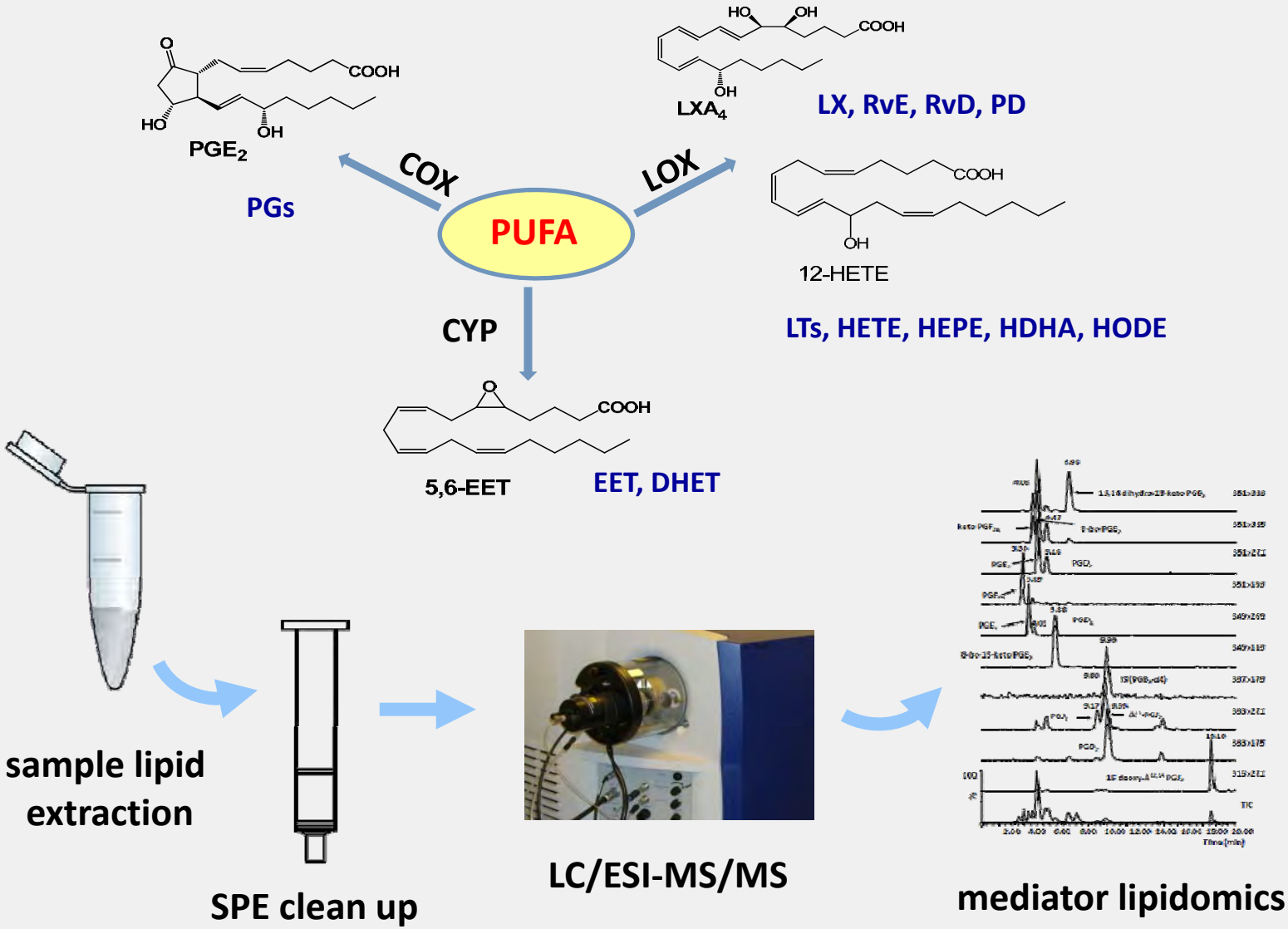

BIOACTIVE LIPIDS
fatty acids
energy storage
waxes
steroids
terpenes
glycerolipids
(mono-,di-, tri-acylglycerols)
eicosanoids
resolvins
docosanoids
oxylipins
lipid vitamins
sphingolipids
lipid mediators
N-acyl ethanolamines
ceramides
glycerophospholipids
ether lipids
plasmalogens
phosphatidites
phosphatidyl-
-choline
-ethanolamine
-serine
-inositol
-glycerol
sphingomyelines
cerebrosides
gangliosides
structural lipids


stimulus
Membrane phospholipids
DIET
EPA
DGLA
DHA
DPA
OA
PLA2
AA
LOX
COX
Hydroxy fatty acids (HETE)
Leukotrienes (LT)
BLT
Chemotaxis
Allergy
Prostanoids
(PG, TX,PI)
EP
TP
IP
Inflammation
Proliferation
Angiogenesis
Tissue homeostasis
NFkB
PPAR
Gene transcription
Protein expression


COX
oxidation
CYP450
5,6-EET
8,9-EET
11,12-EET
14,15-EET
DHET
HNE
HETE
LG
8-iso-PGF2α
iso-LG
5-,-15-HETE
16-, -20-HETE
TXB2
TXA2
non-ezymatic oxidation
free radical oxidation
peroxidation
CYP450
PGI2
PGE2
PGH2
PGG2
COX-1,2
COOH
arachidonic acid
15-LOX
15-HPETE
15-HETE
PGF2α
acCOX-2
12-LOX
5-LOX
PGD2
5-LOX FLAP
12-HPETE
12-HETE
LXA4
15R-HETE
5-HPETE
HXA3
PGJ2
5-LOX
5-HETE
LTA4
LTB4
15-epi-LXA4
Δ12-PGJ2
5-oxo-ETE
LTC4
12-LOX
LXA4
15d-PGJ2
LTD4
LTE4
5-LOX


13-HODE
9-HODE
15-LOX
COX-2 or non-enzymatic oxidation
LA 18:2n-6
GLA
ALA 18:3n-3
PGE3 PGD3
COX-1,-2
EPA 20:5n-3
5-,12-, 15-LOX
LTB5 5-HEPE 12-HEPE 15-HEPE
15-HETrE
15-LOX
DGLA 20:3n-6
COX-1,-2
PGE1 PGD1
DHA 22:6n-3
RvE1 RvE2
COX-2+ASA or CYP450 / LOX
LOX CYP450
17-HDHA other mono-, di- and tri-hydroxy derivatives; docosanoids maresins protectins
RvD1 AT-RvD1 PD
5-HETE 8-HETE 9-HETE 11-HETE 12-HETE 15-HETE 20-HETE 5,6-EET 8,9-EET 11,12-EET 14(15)-EET 14,15-DHET
CYP450
non-enzymatic oxidation
AA 20:4n-6
COX-1,-2
PGE2 PGD2 PGF2α PGI2 TXB2 PGJ2 Δ12-PGJ2
5-,12-, 15-LOX
LTB4 5-HETE 5-oxo-ETE 12-HETE 15-HETE LXB4, LXA4
15-PGDH
15-oxo-PG (series-1,-2,-3)

Mediator Lipidomics: array of >80 lipids



Massey & Nicolaou FRBM 2012

Mediator lipidomics workflow

Sample preparation

Solid: Homogenise in 15 % (v/v) methanol in water
Liquid: Adjust to 15 % (v/v) methanol
Add internal standard(s)

Solid phase extraction

Remove precipitated proteins
Acidify sample (pH 3.0)
Load on C18 SPE cartridge
Elute lipids with methyl formate

Lipid extract preparation & storage

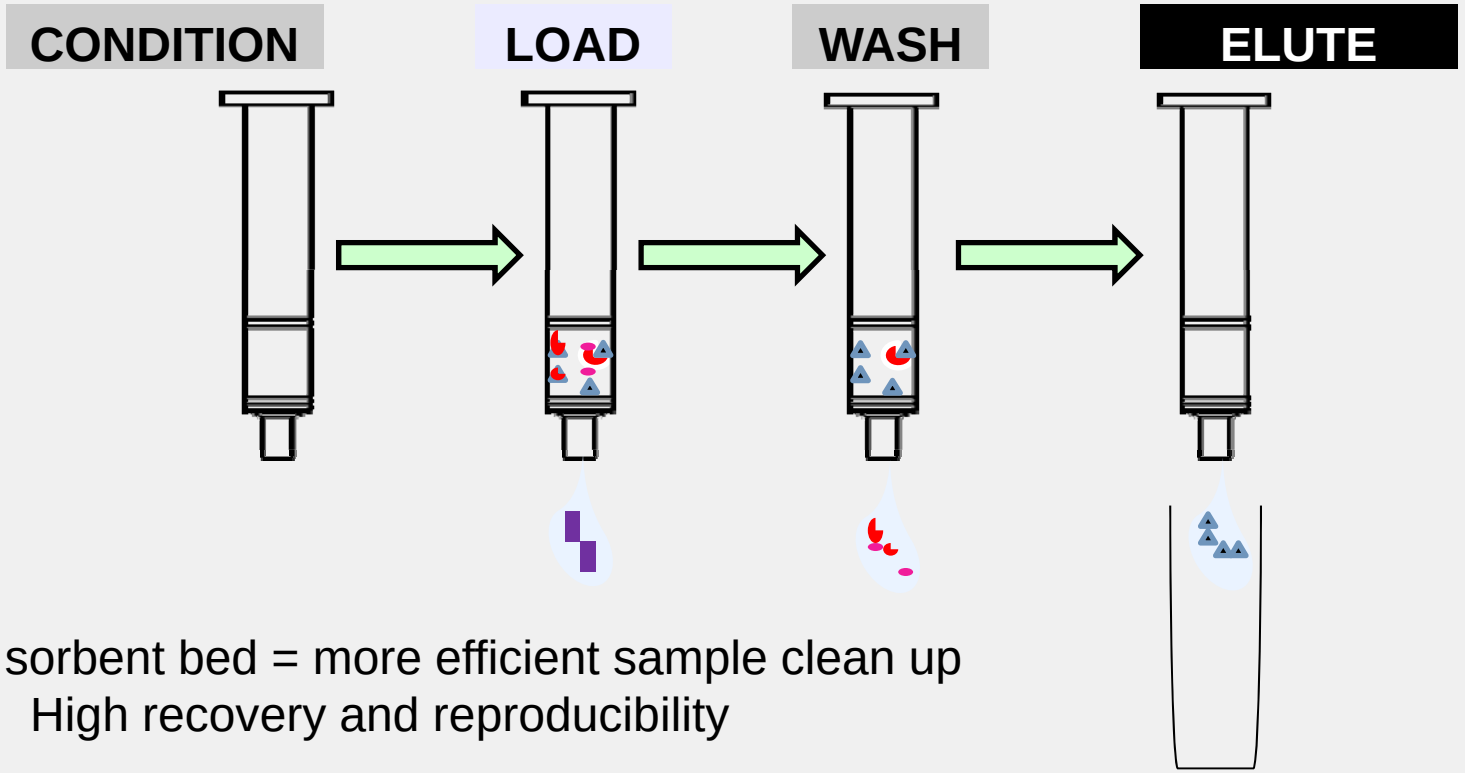
Remove solvents under N_2
Reconstitute extract in small solvent volume
If needed, store at -20 ^{o}C and for up to 1 week

LC/ESI-MS/MS

Prepare fresh calibration standards
Check baseline and instrument sensitivity
Run **COX** assay or
Run **LOX/CYP** assay or
Run **chiral** assay

Data processing and calculations

Solid phase extraction (SPE) clean up



Selective sorbent bed = more efficient sample clean up
High recovery and reproducibility

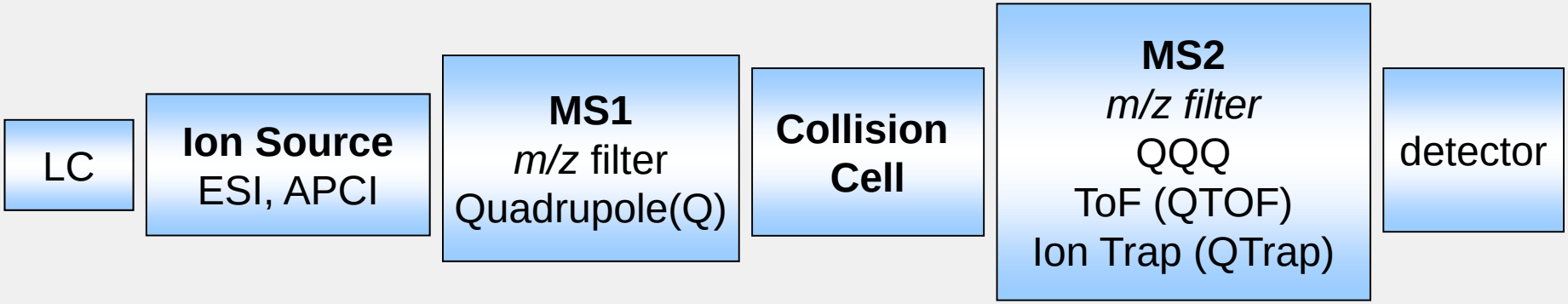
1. **Condition SPE:** Prepare C18 sorbent bed

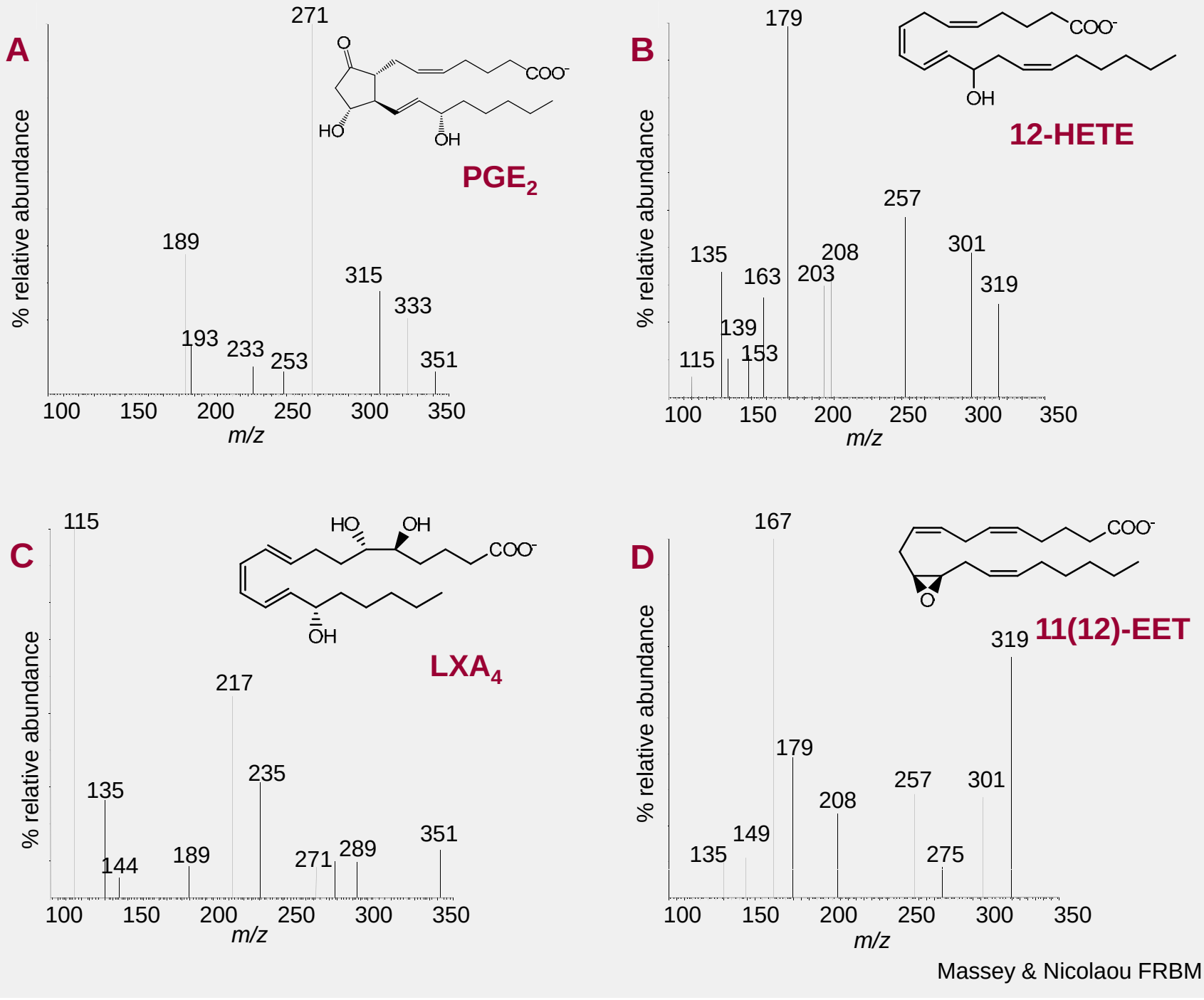
2. **Load extract**:

3. **Wash**: Remove weakly bound impurities, then wash with hexane to remove water → Reduce matrix effects

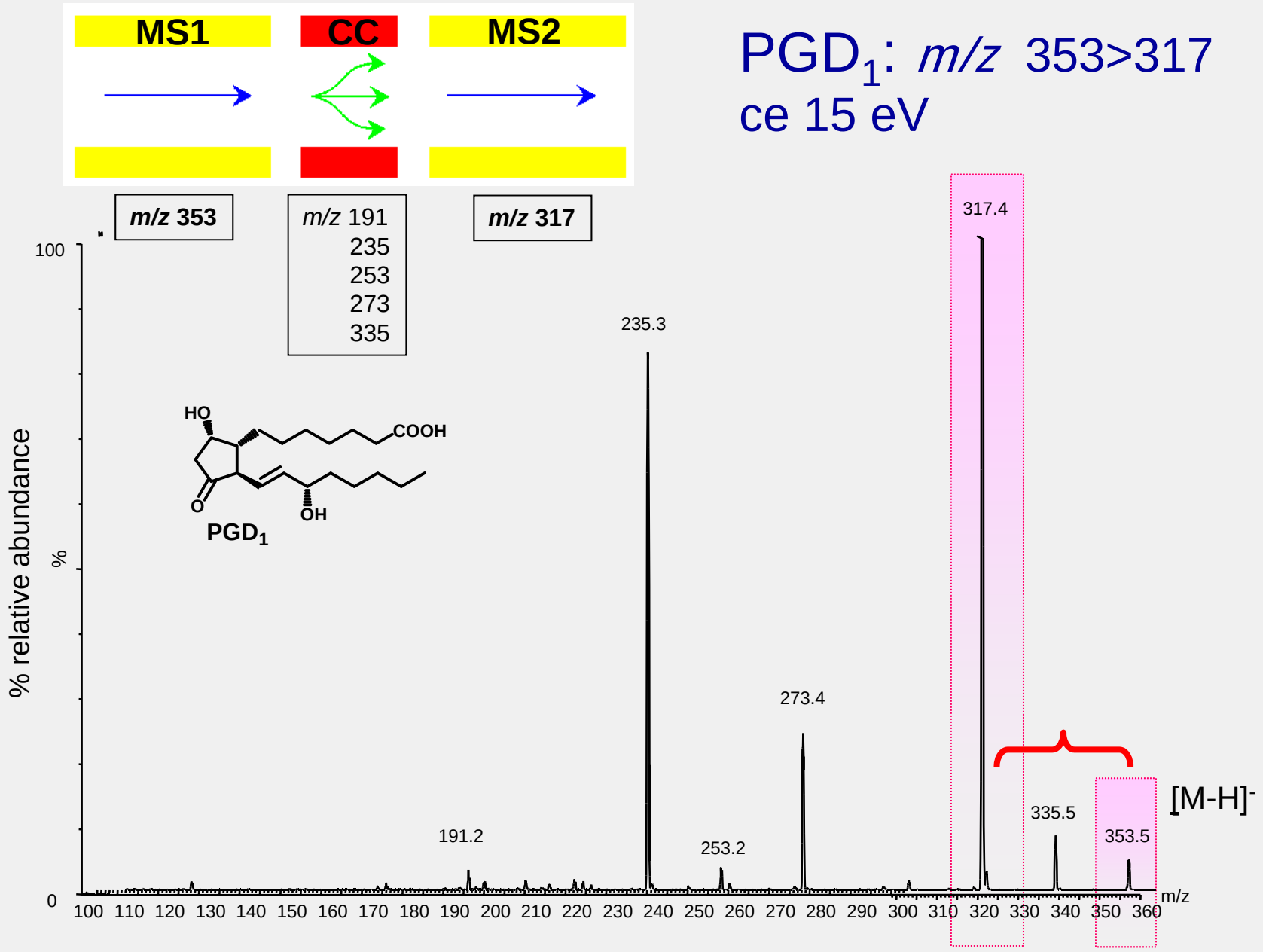
4. **Elute**: Selectively desorb lipid mediators

tandem mass spectrometer
typical lipidomics platform

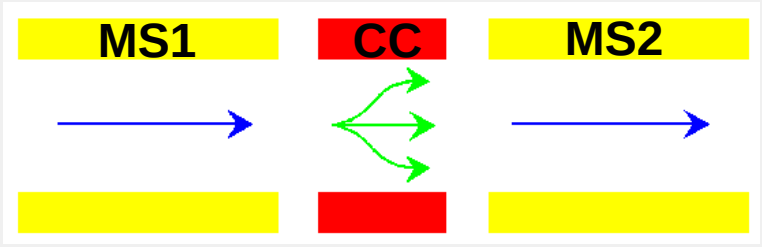


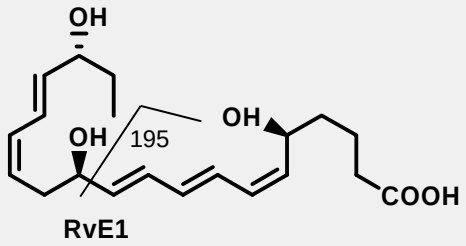


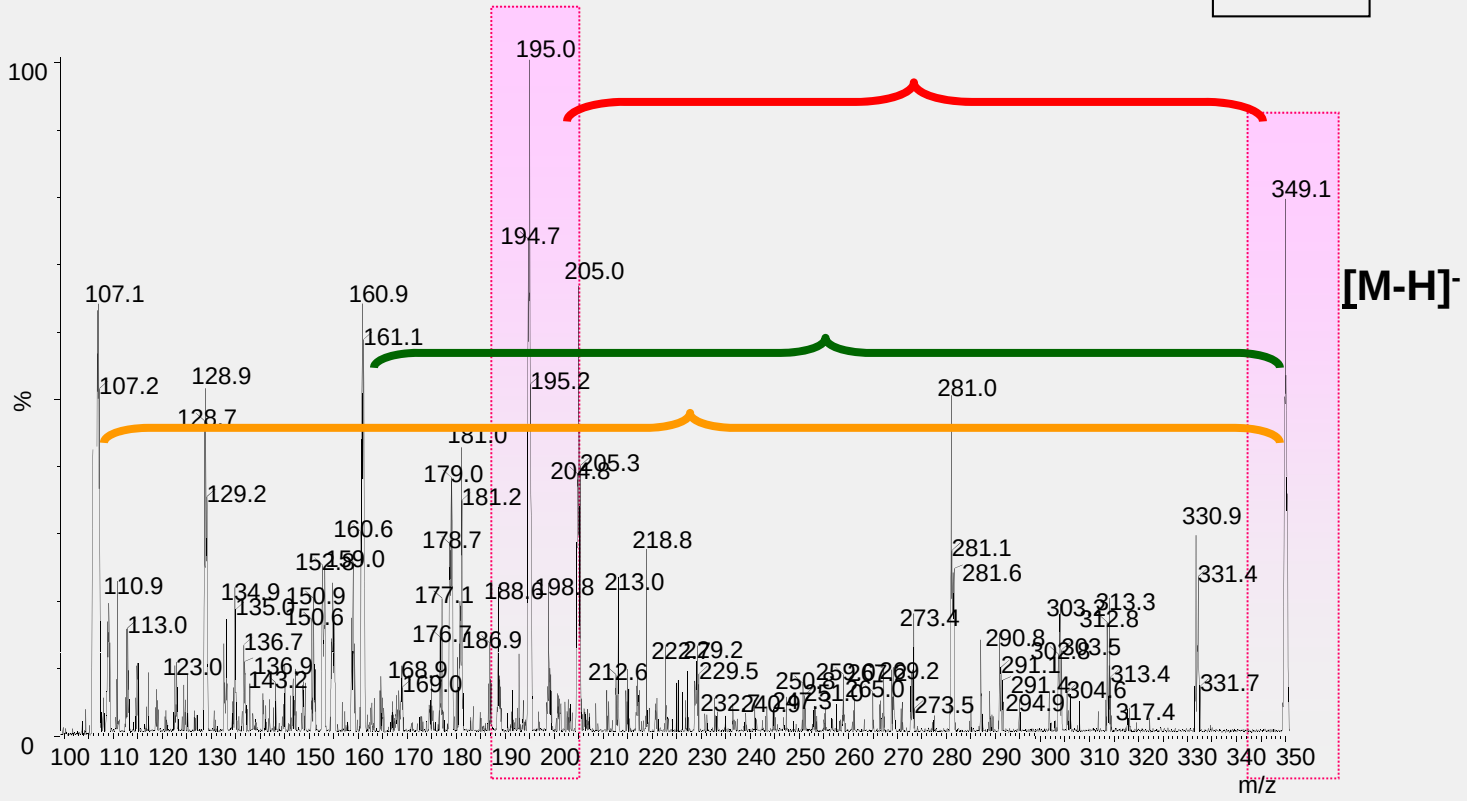
Massey & Nicolaou FRBM 2012


MS1
CC
MS2
m/z 353
m/z 191
235
253
273
335
m/z 317
HO
COOH
O
OH
PGD1
PGD1: m/z 353>317
ce 15 eV
% relative abundance
%
100
0
317.4
235.3
273.4
191.2
253.2
335.5
353.5
[M-H]-
m/z
100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360

RvE1: *m/z* 349>195
ce 17 eV

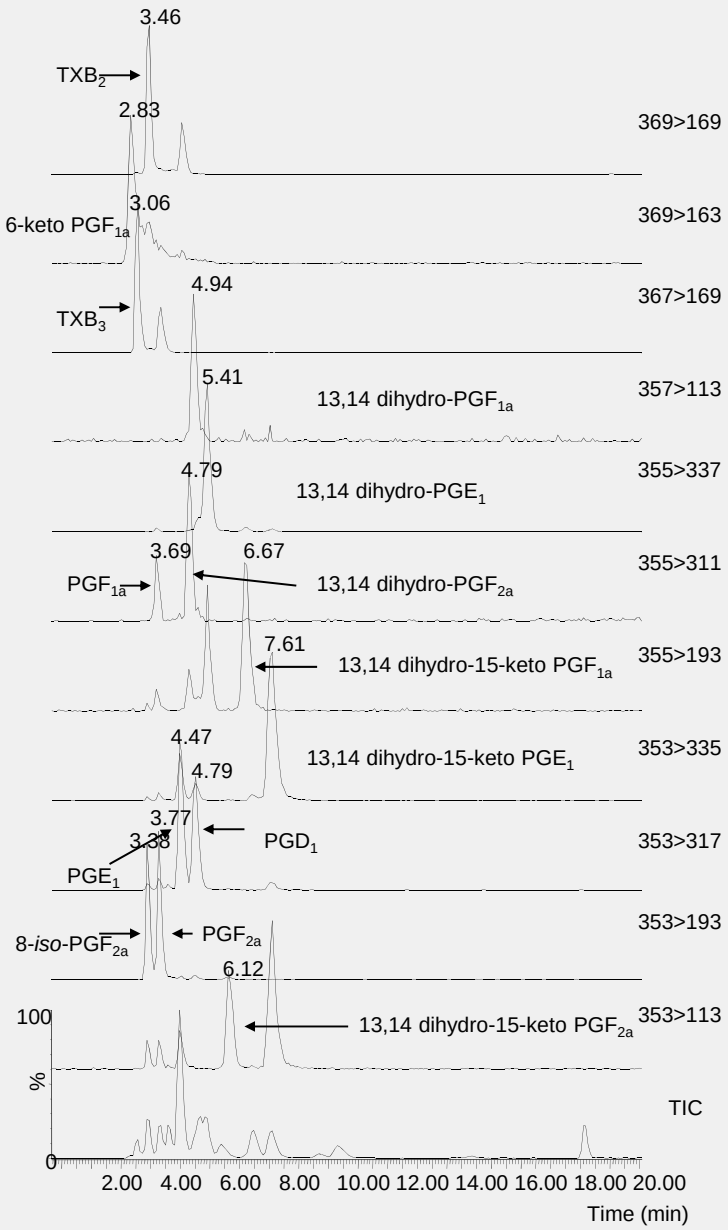






[M-H]⁻

LC/ESI-MS/MS (ESI-)



Liquid chromatography: reverse phase

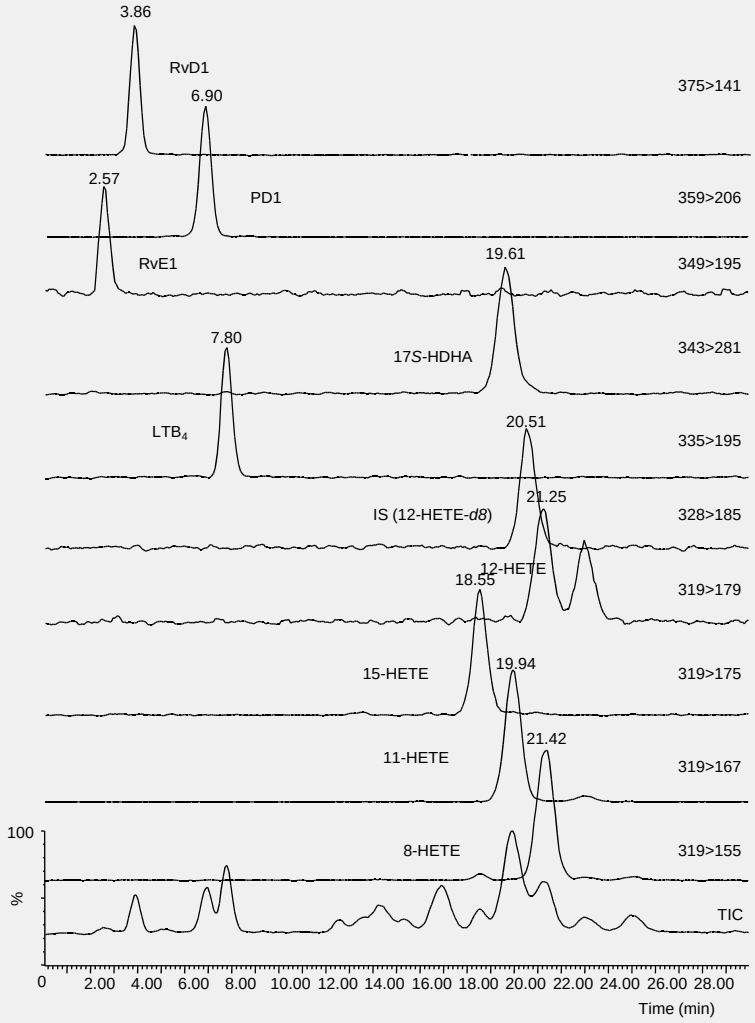
Lipid mediators typically separated by hydrophobic moiety (C18, e.g. Luna ®)

Prostanoids:
isobaric species e.g. PGE and PGD
optimal separation: acetonitrile-based gradient elution system

Masoodi & Nicolaou RCM 2006; Masoodi *et al* RCM 2008

LC/ESI-MS/MS (ESI-)

Liquid chromatography: reverse phase



Lipid mediators typically separated by hydrophobic moiety (C18, e.g. Luna ®)

Hydroxy fatty acids:
poor resolution with acetonitrile
strong interaction with C18 column
improved elution with methanol

Core shell columns: behave like UPLC columns (pore size 2.5µm)
improved peak resolution and better sensitivity

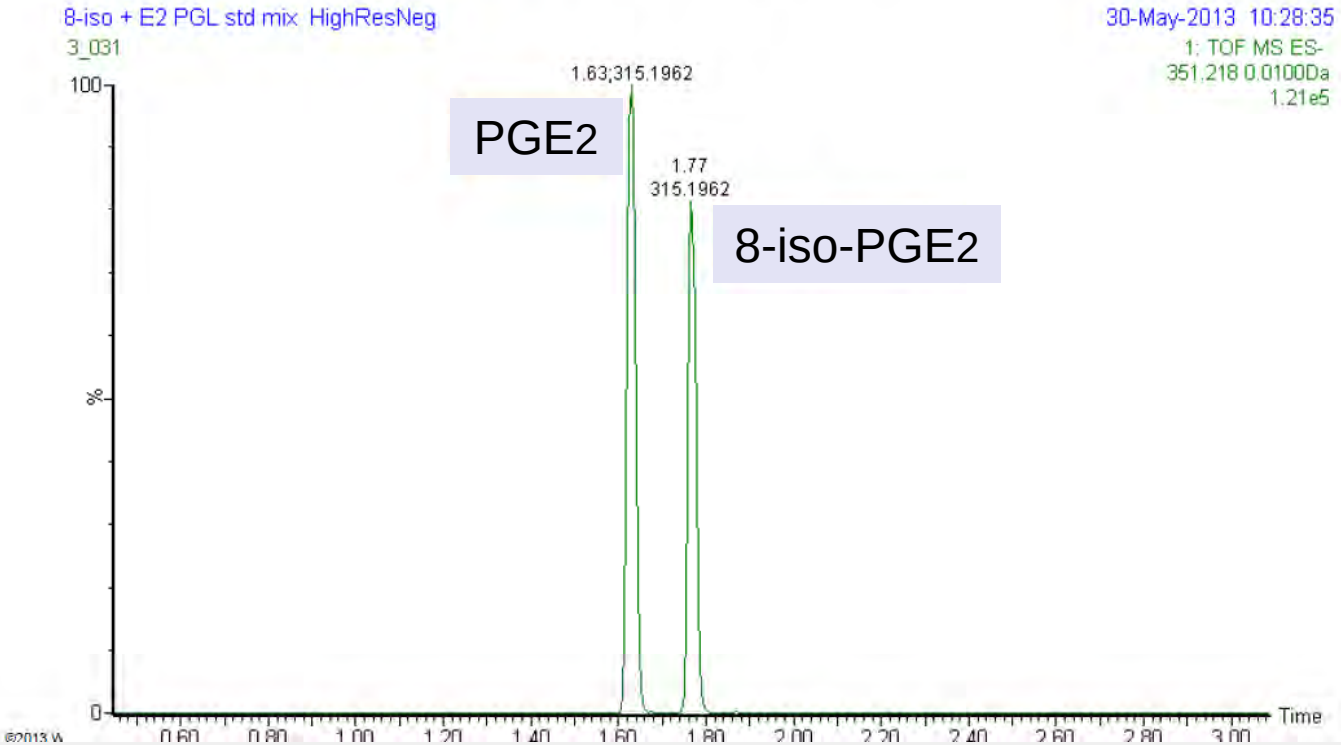
Masoodi & Nicolaou RCM 2006; Masoodi *et al* RCM 2008; Massey & Nicolaou FRBM 2012

Very fast even better separation of isobaric compounds with different RP UPC2 column

PGL-E2 Chromatographyc separation BEH-2EP column

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Significantly better chromatographic resolution (1.65 measures as Rs half-height) was achieved on a BEH-2EP column:



Chiral separation by LC

Stationary phases:

Amylose: 18(*S*)-E Resolvins (Oh et al., J Clin Invest. 2011;121)

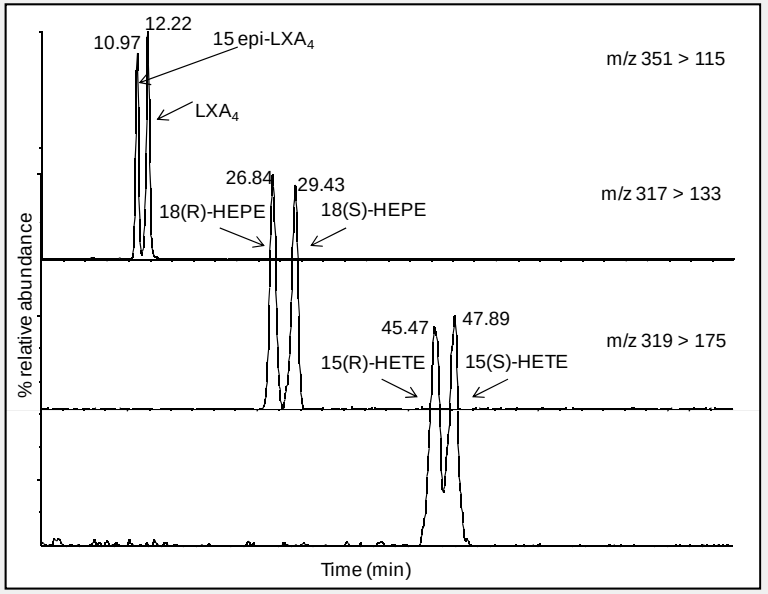
Cellulose:12(*S*)-HETE in blister fluid (Massey and Nicolaou, FRBM. 2012)

Reverse or normal phase solvents

Cellulose (Lux-1)

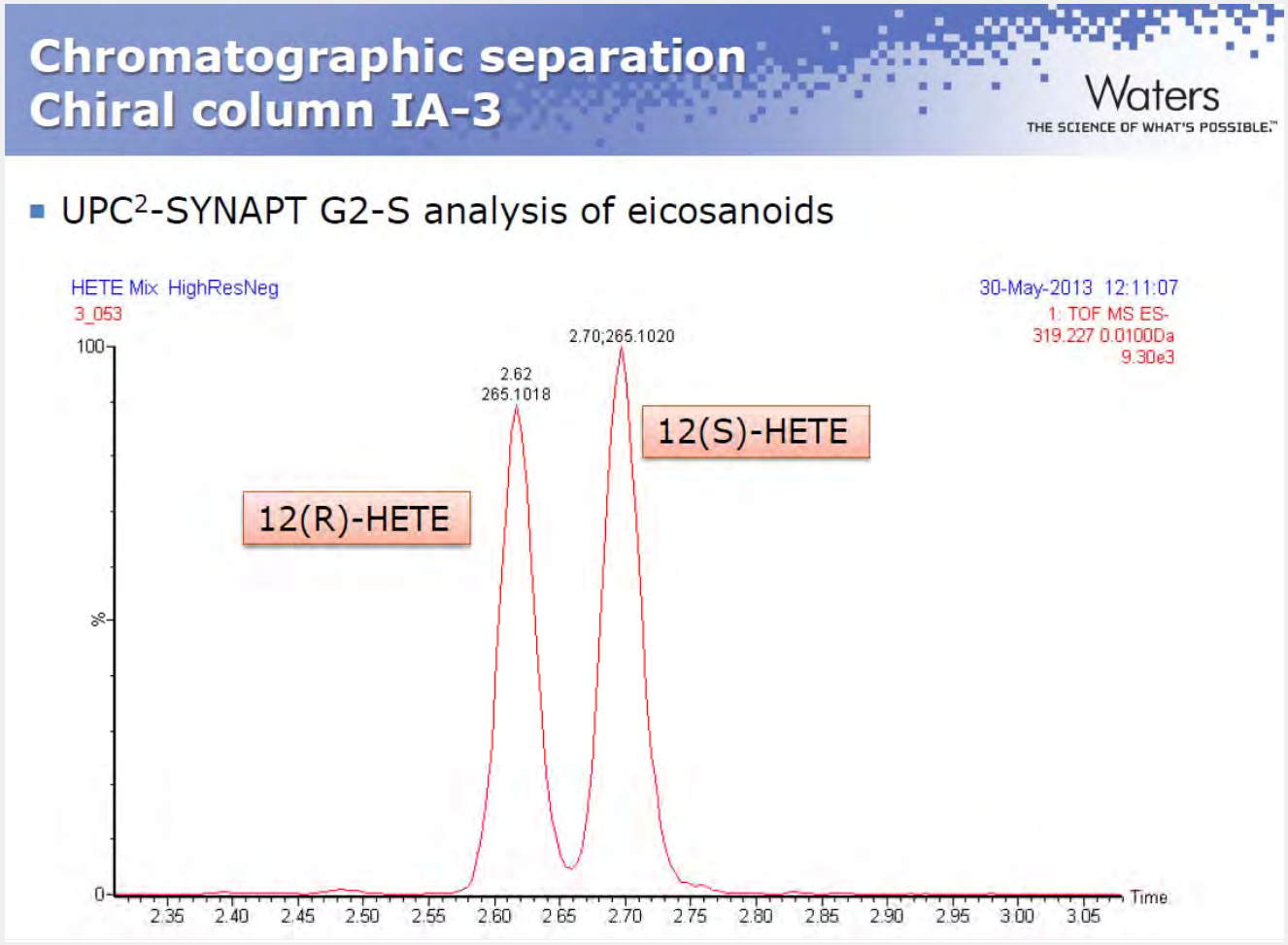
more stable stationary phase

improved separation of enantiomers



Massey& Nicolaou FRBM 2012

Very fast separation of isobaric compounds with chiral UPC2 column



mediator lipidomics protocol

- Solid phase extraction clean-up step (matrix effects).
- Multiple Reaction Monitoring (MRM) assays.

  for > 80 lipid mediators; LoD/LoQ 1-10 pg.
- LC/ESI-MS/MS (Q^3); calibration lines; *d*-internal standards.

Biological material

- Solids: skin, tumours, liver, brain, uterine, ocular, nerve tissues, cells, etc.
- Liquids: plasma, urine, seminal plasma, follicular fluid, blister fluid, cell culture media, etc.
- Samples snap-frozen; extracted/run within days; dark/cold.

Masoodi & Nicolaou RCM 2006; Masoodi *et al* RCM 2008; Nicolaou MMB 2010; Massey & Nicolaou FRBM 2012

lipid mediators in skin inflammation: the sunburn response

inflammation in cutaneous disease

psoriasis

photoageing

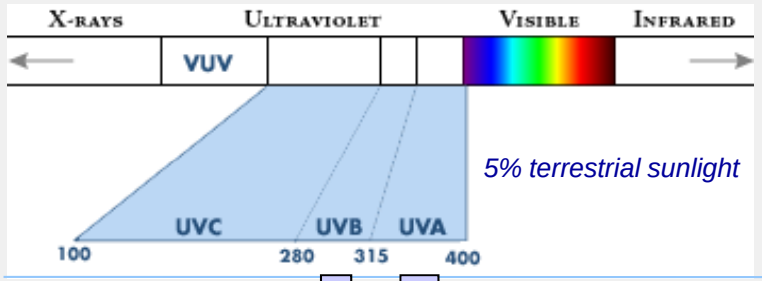
sunburn

atopic dermatitis

wound healing

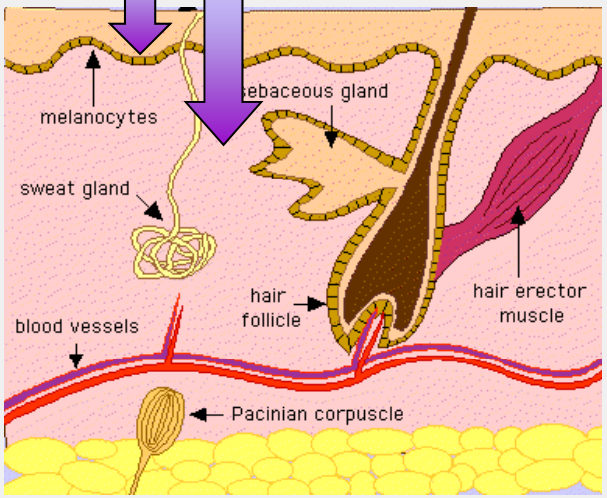
squamous cell
skin cancer

UV radiation and human skin



UVA: 320-400 nm
dermis

UVB: 280-320 nm
epidermis: ***inflammation***



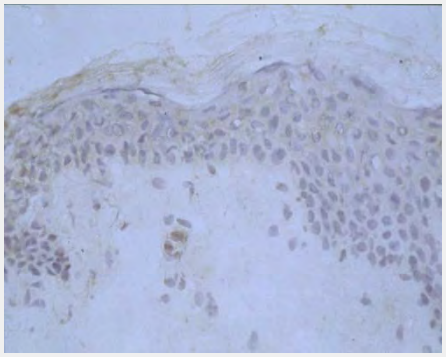
epidermis
protection: radiation, xenobiotics, trauma

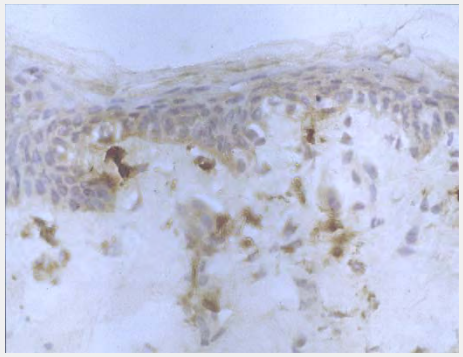
dermis
elasticity; immuno- & biochemical support

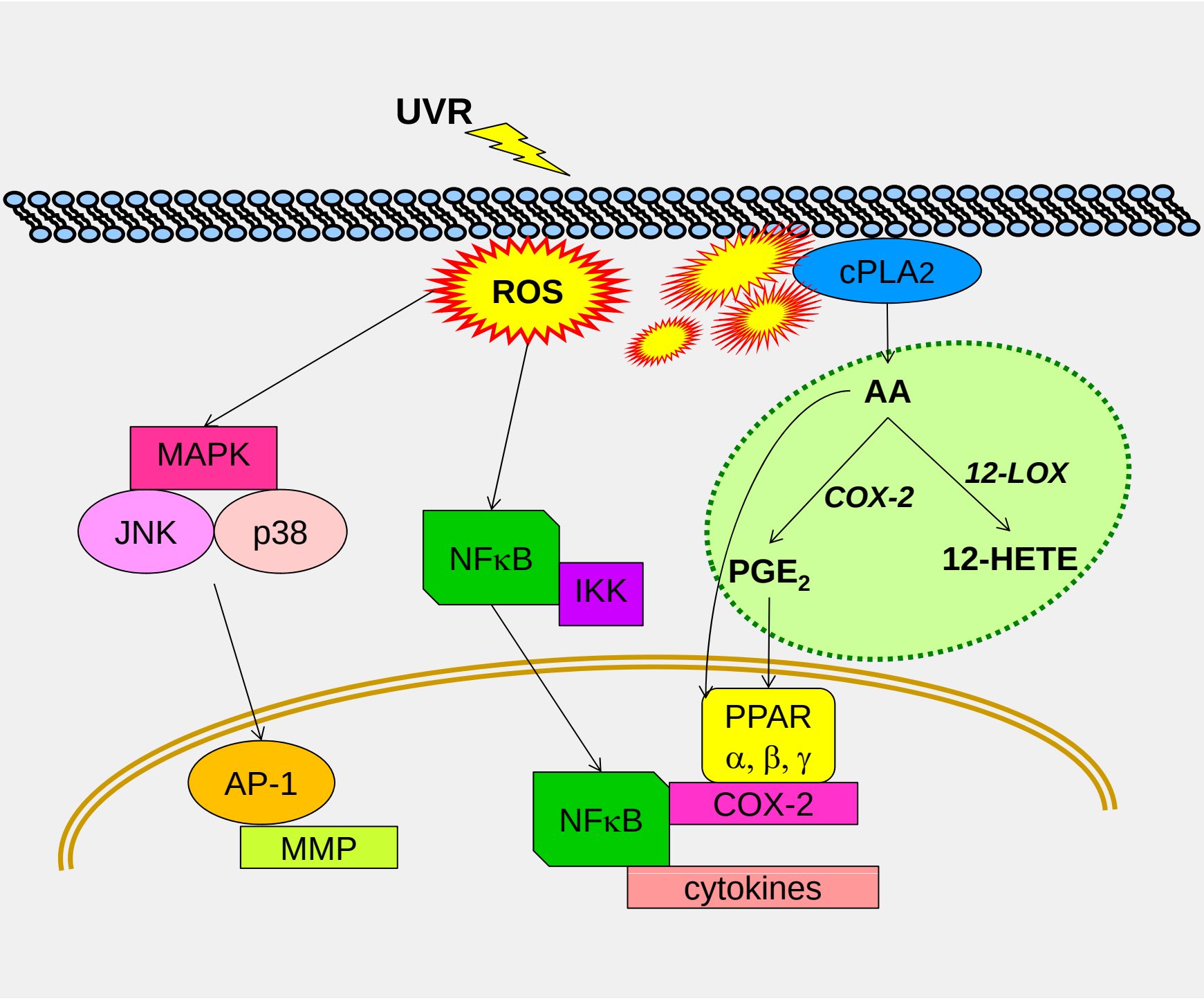
UVR: immunosuppression; photosensitivity; photoageing; photocarcinogenesis

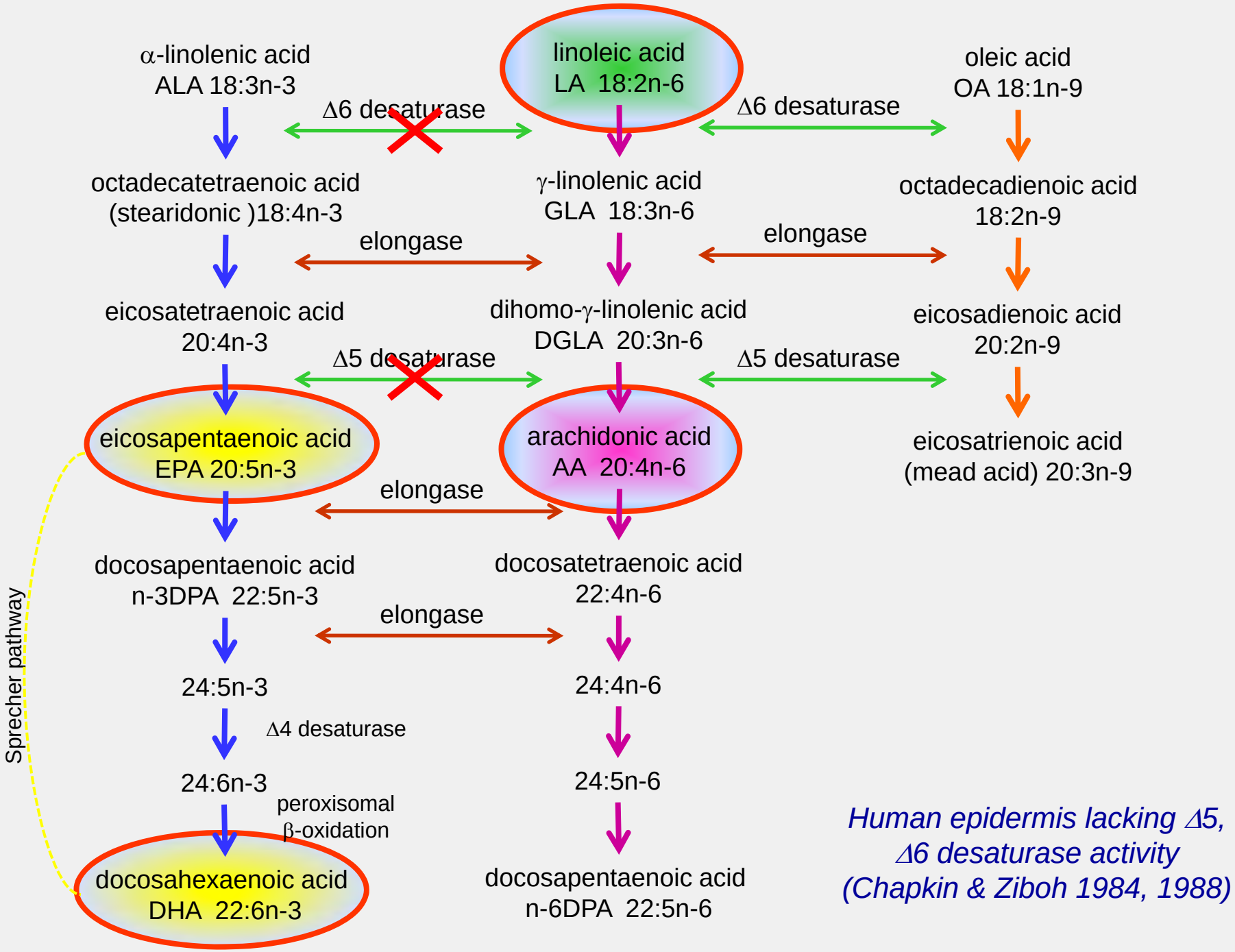
UVR-induced skin inflammation (sunburn)

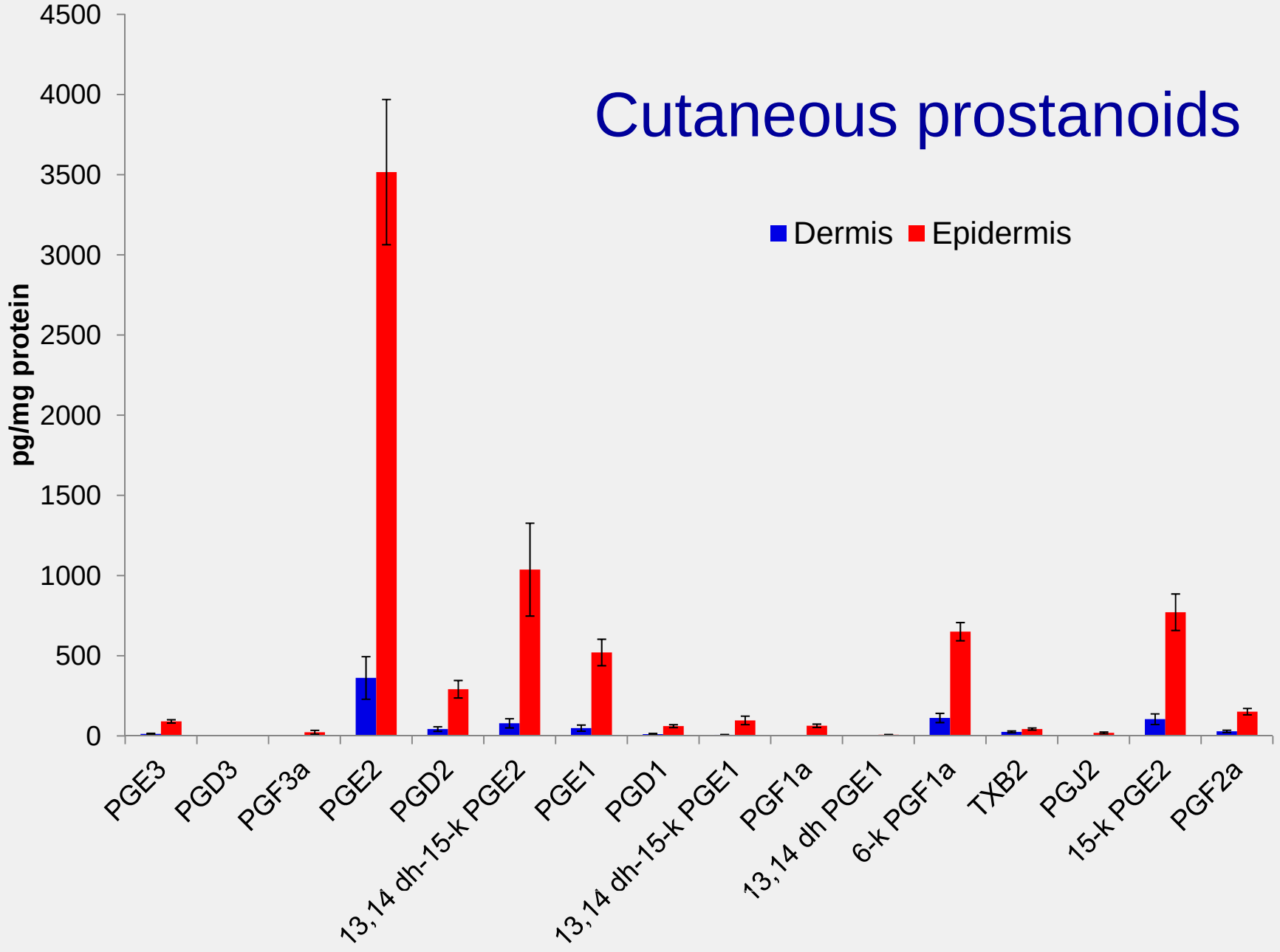
- Acute inflammatory response
- Erythema, pain, oedema (vasodilatation)
- Leukocytic infiltration
- Sunburn (apoptotic) cells





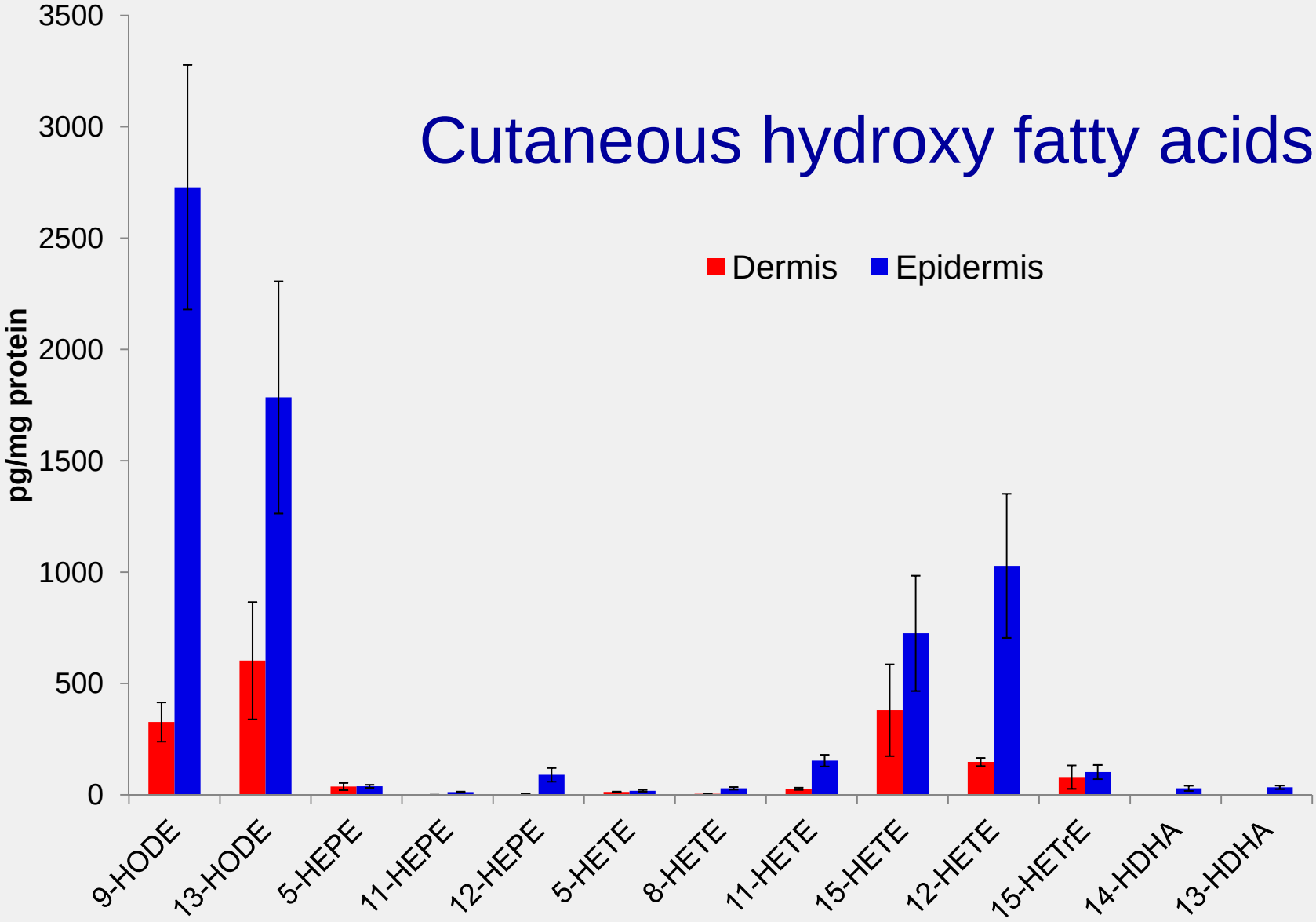

UVR
ROS
cPLA2
AA
MAPK
JNK
p38
NFκB
IKK
COX-2
12-LOX
PGE2
12-HETE
PPAR
α, β, γ
AP-1
MMP
NFκB
COX-2
cytokines


α-linolenic acid
ALA 18:3n-3
linoleic acid
LA 18:2n-6
oleic acid
OA 18:1n-9
Δ6 desaturase
Δ6 desaturase
octadecatetraenoic acid
(stearidonic)18:4n-3
γ-linolenic acid
GLA 18:3n-6
octadecadienoic acid
18:2n-9
elongase
elongase
eicosatetraenoic acid
20:4n-3
dihomo-γ-linolenic acid
DGLA 20:3n-6
eicosadienoic acid
20:2n-9
Δ5 desaturase
Δ5 desaturase
eicosapentaenoic acid
EPA 20:5n-3
arachidonic acid
AA 20:4n-6
eicosatrienoic acid
(mead acid) 20:3n-9
elongase
docosapentaenoic acid
n-3DPA 22:5n-3
docosatetraenoic acid
22:4n-6
elongase
24:5n-3
24:4n-6
Δ4 desaturase
24:6n-3
24:5n-6
peroxisomal
β-oxidation
docosahexaenoic acid
DHA 22:6n-3
docosapentaenoic acid
n-6DPA 22:5n-6
Sprecher pathway
Human epidermis lacking Δ5,
Δ6 desaturase activity
(Chapkin & Ziboh 1984, 1988)



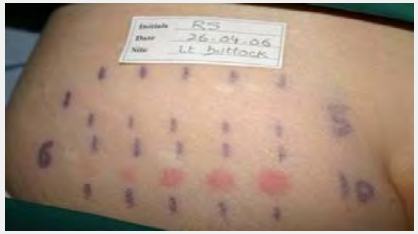
human skin samples: ethical tissue; 3 mm punch biopsies; ~20 mg; n=8; LC/ESI-MS/MS

Cutaneous hydroxy fatty acids



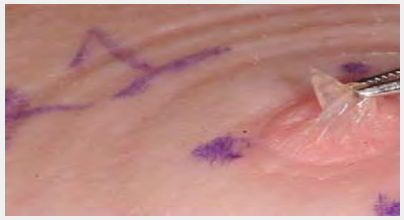
human skin samples: ethical tissue; 3 mm punch biopsies;~20 mg; n=8; LC/ESI-MS/MS

sunburn: experimental model



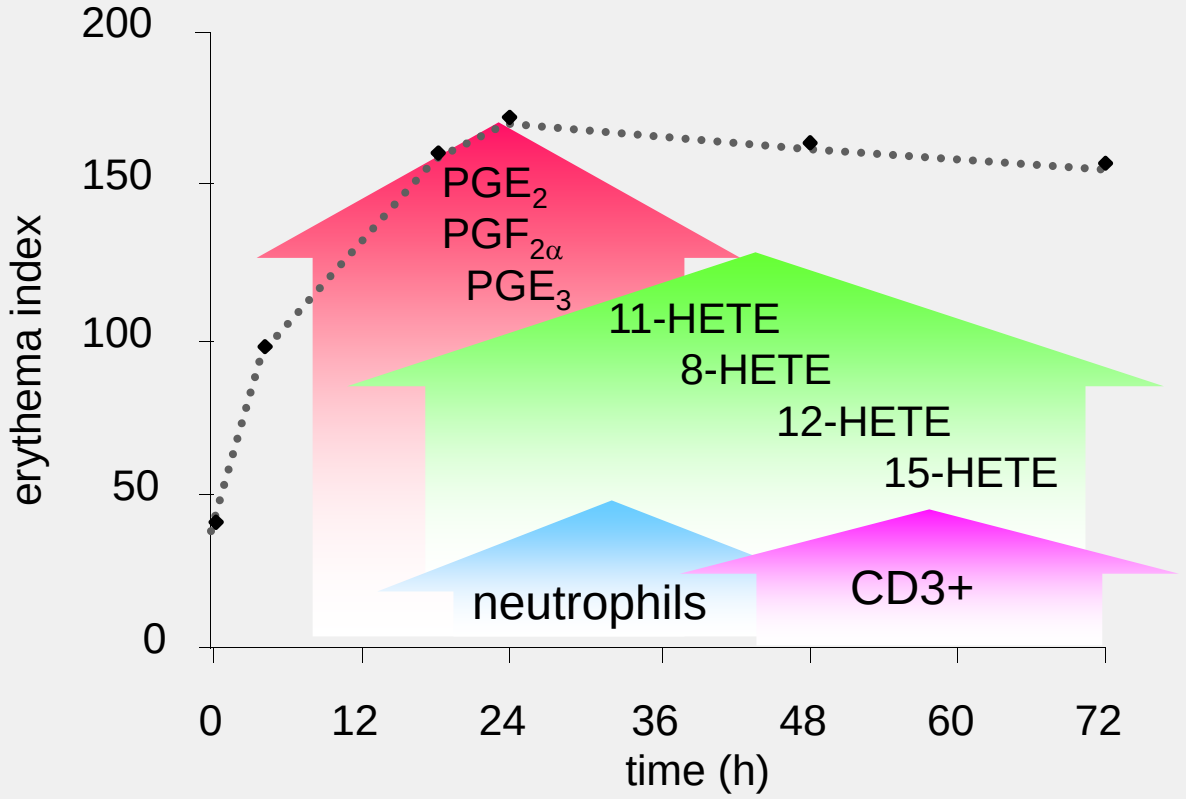
- healthy adult volunteers, skin type I-IV
- skin exposed to UVR

  (UV6; 280-400 nm; 23% UVB :77% UVA)
- 3-4 minimal erythema doses (MED)



Suction blisters and skin sections (0 -72 h post UVR)

Overlapping sequential eicosanoid profiles may mediate the early and late phases of sunburn response

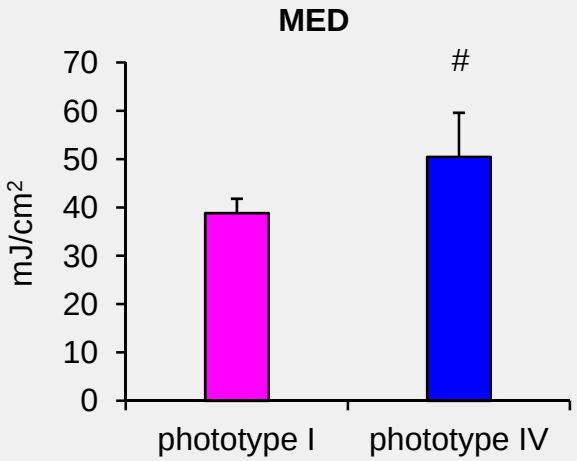


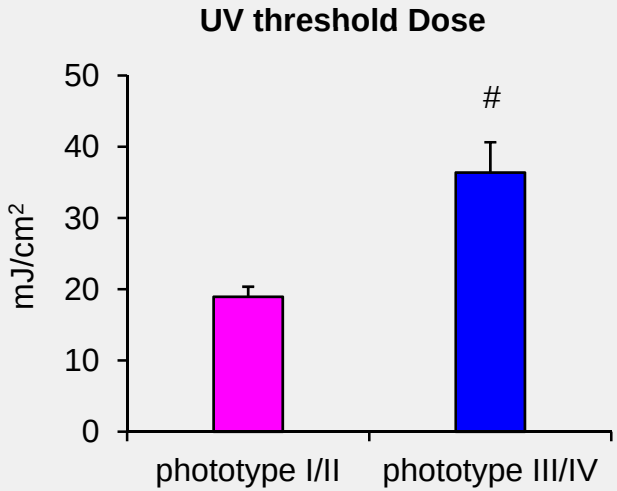
early: inflammation
vasodilatation, chemotaxis

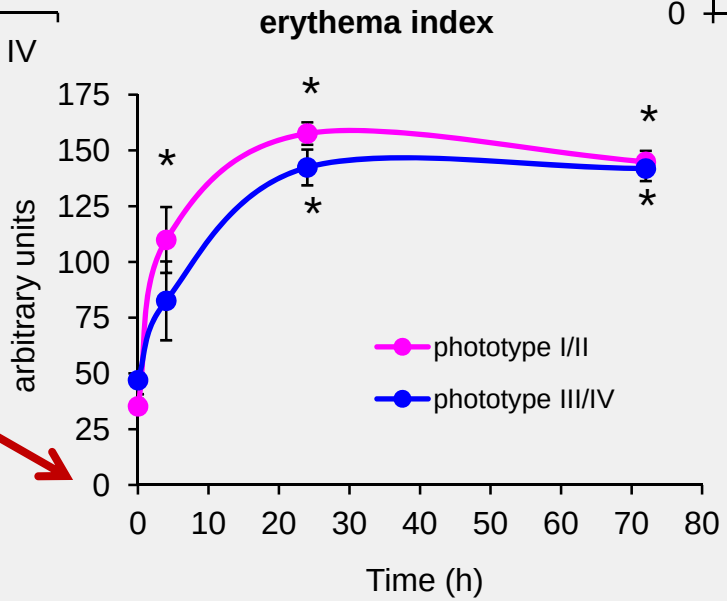
late: resolution
repair

Rhodes, Nicolaou *et al* FASEB J 2009

erythema in skin types I/II and III/IV post single high UVR dose (12 SED)





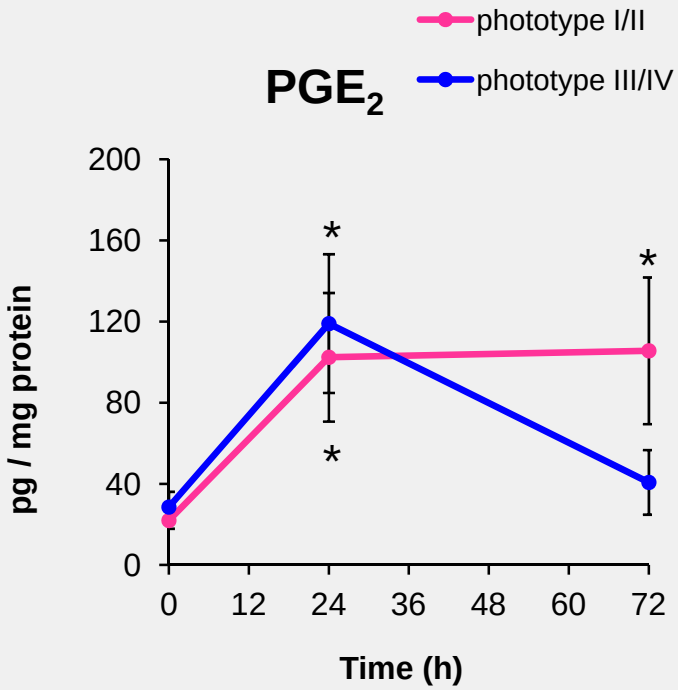


Single UVR dose (12 SED) 120 mJ/cm^2

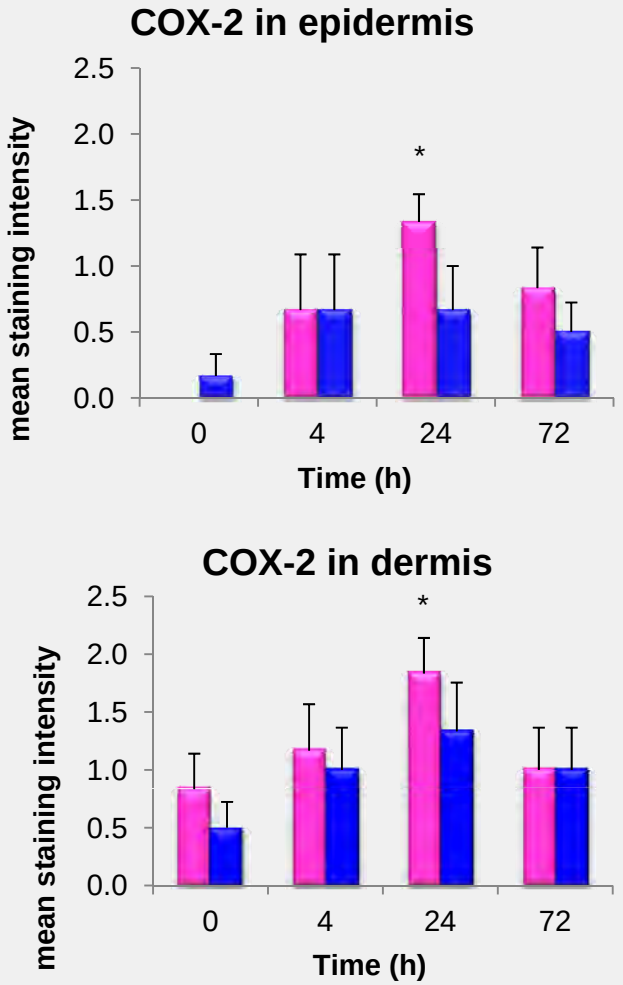
n=16; $^{\#}p<0.05$; *$p<0.01$

Nicolaou et al *Photochem Photobiol Sci 2012*

PGE_2 higher in subjects prone to sunburn



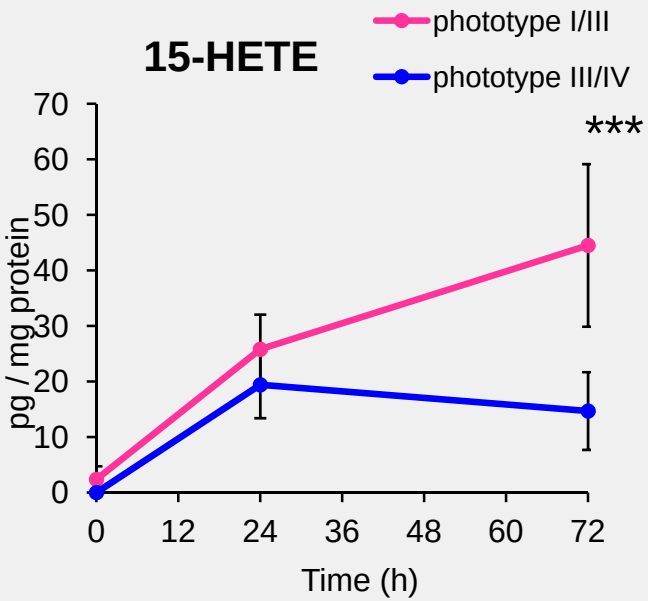
pro-inflammatory vasodilatation



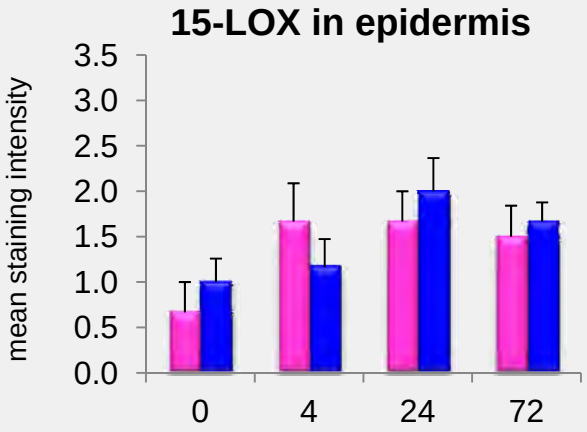
n=9; p<0.05

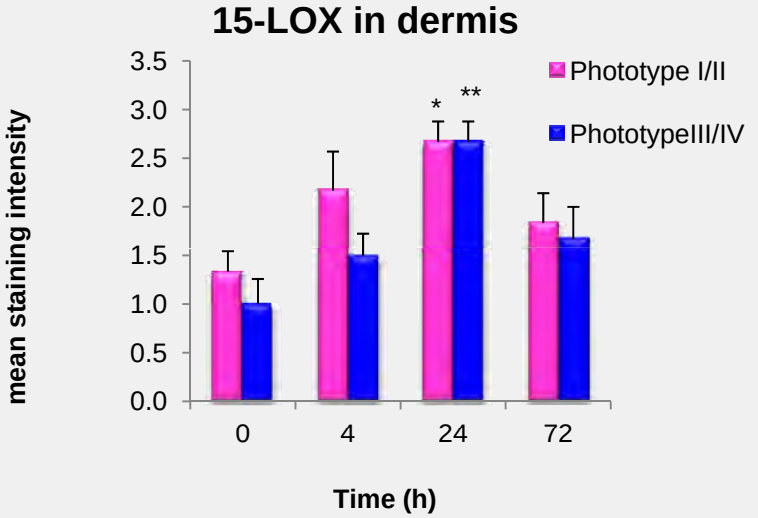
Nicolaou et al *Photochem Photobiol Sci 2012*

15-HETE higher in subjects prone to sunburn



anti-inflammatory precursor to lipoxins

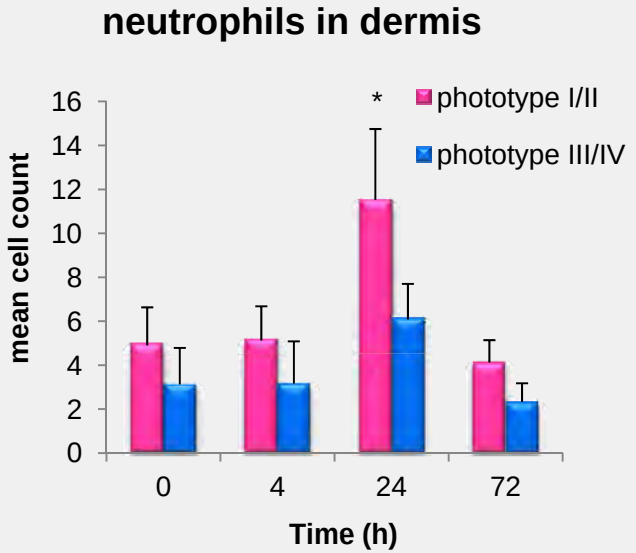


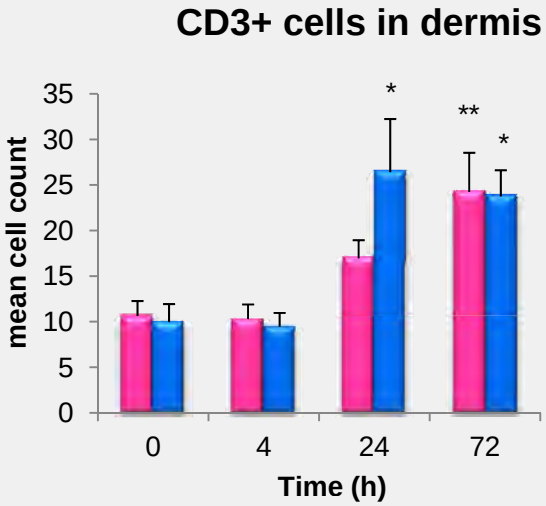


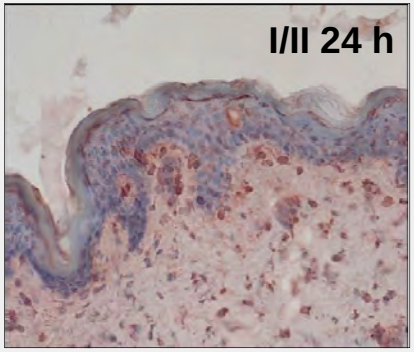
n=9; *p<0.05, ***p<0.001

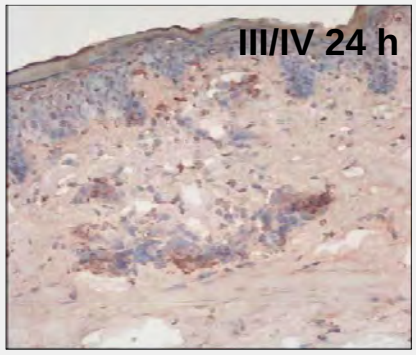
Nicolaou et al *Photochem Photobiol Sci 2012*

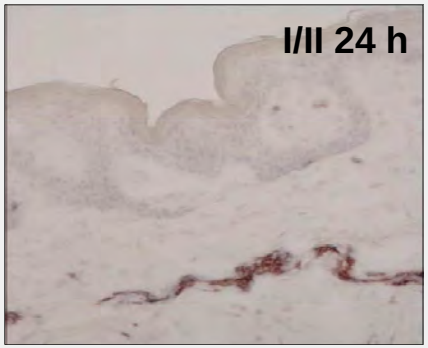
higher neutrophil infiltrate in subjects prone to sunburn

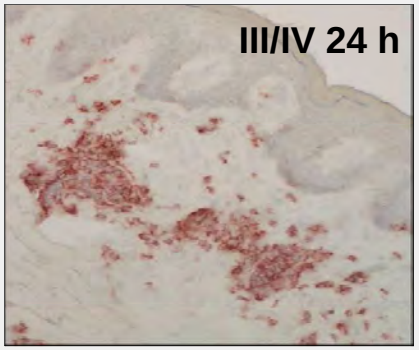








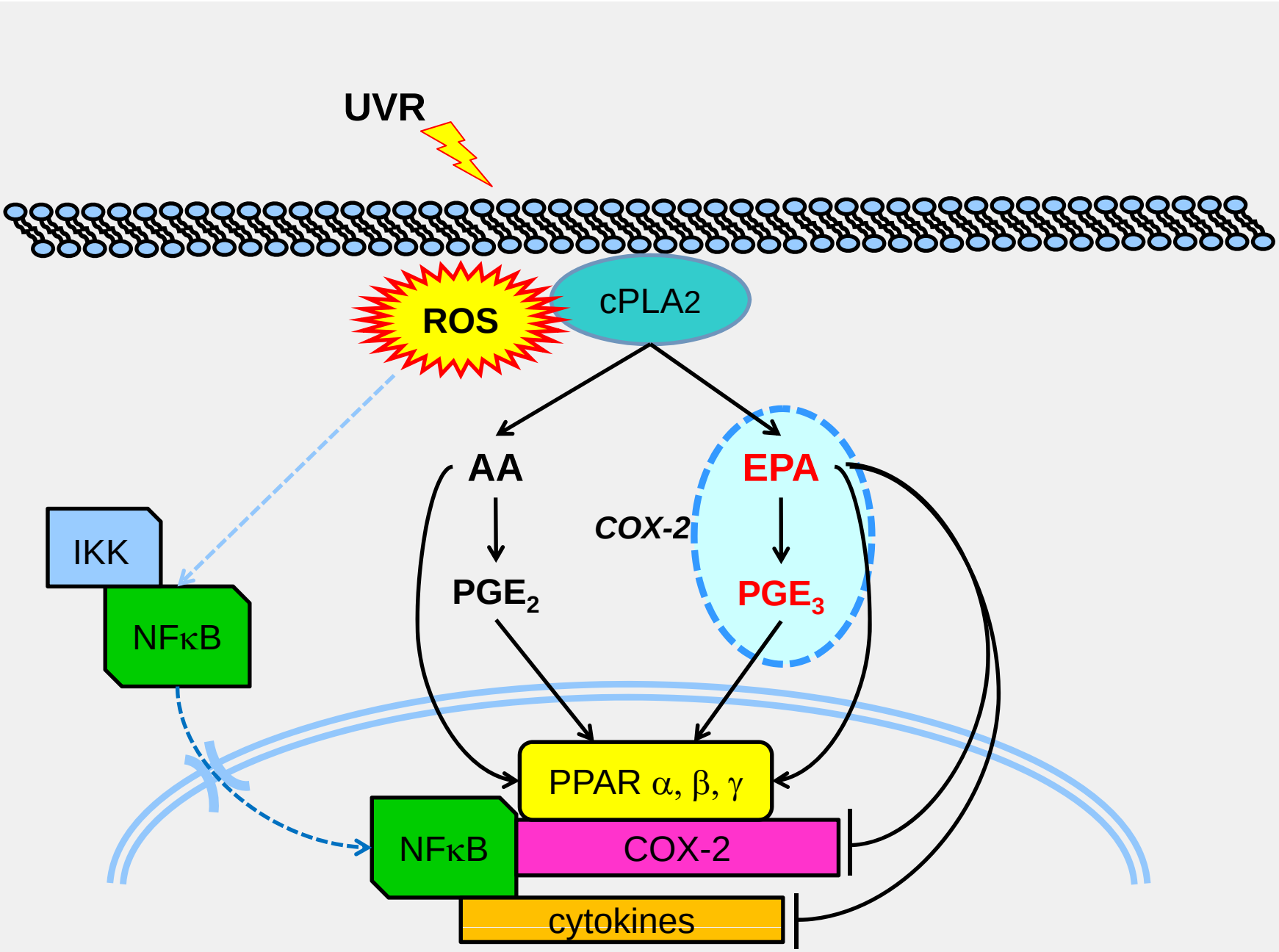




n=6; * p<0.05; ** p<0.01

Nicolaou et al *Photochem Photobiol Sci 2012*

lipid biomarkers of skin inflammation in human nutritional studies



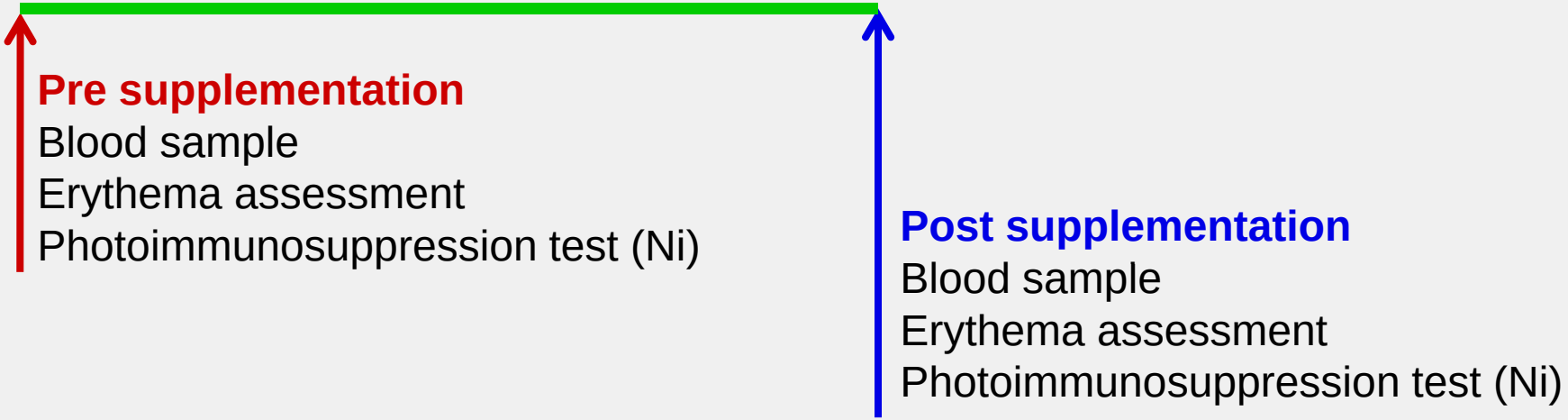
Nicolaou *et al* Chem Phys Lipids 2011

n-3PUFA in skin inflammation and immunity: photoimmunosuppression

Randomised double-blind study (n=79 subjects)

control: GTCC

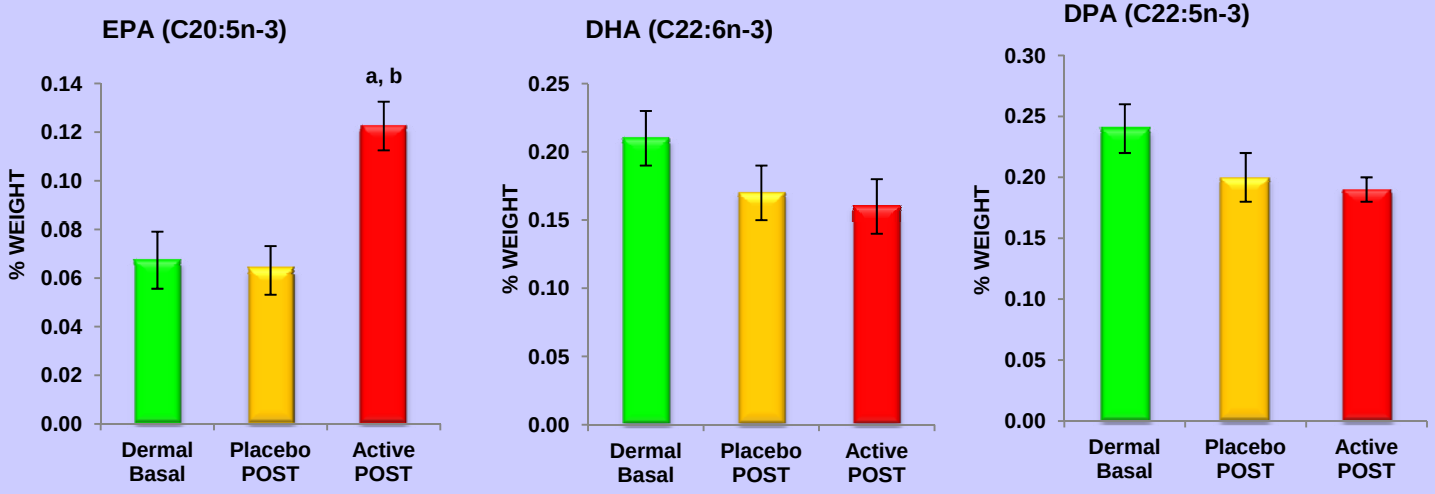
active: 1g capsule~70% EPA&10% DHA; 5 cps/day; 12 weeks



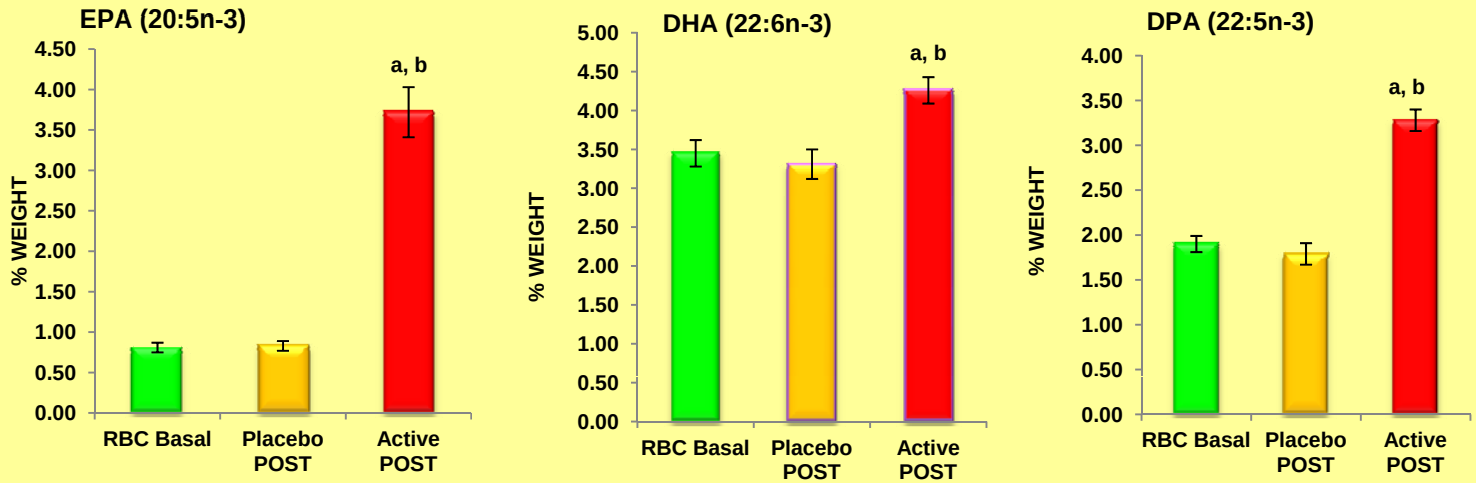
Pilkington *et al* AMCN *in press*

EPA supplementation did not increase skin DPA or DHA levels

skin



r.b.c.

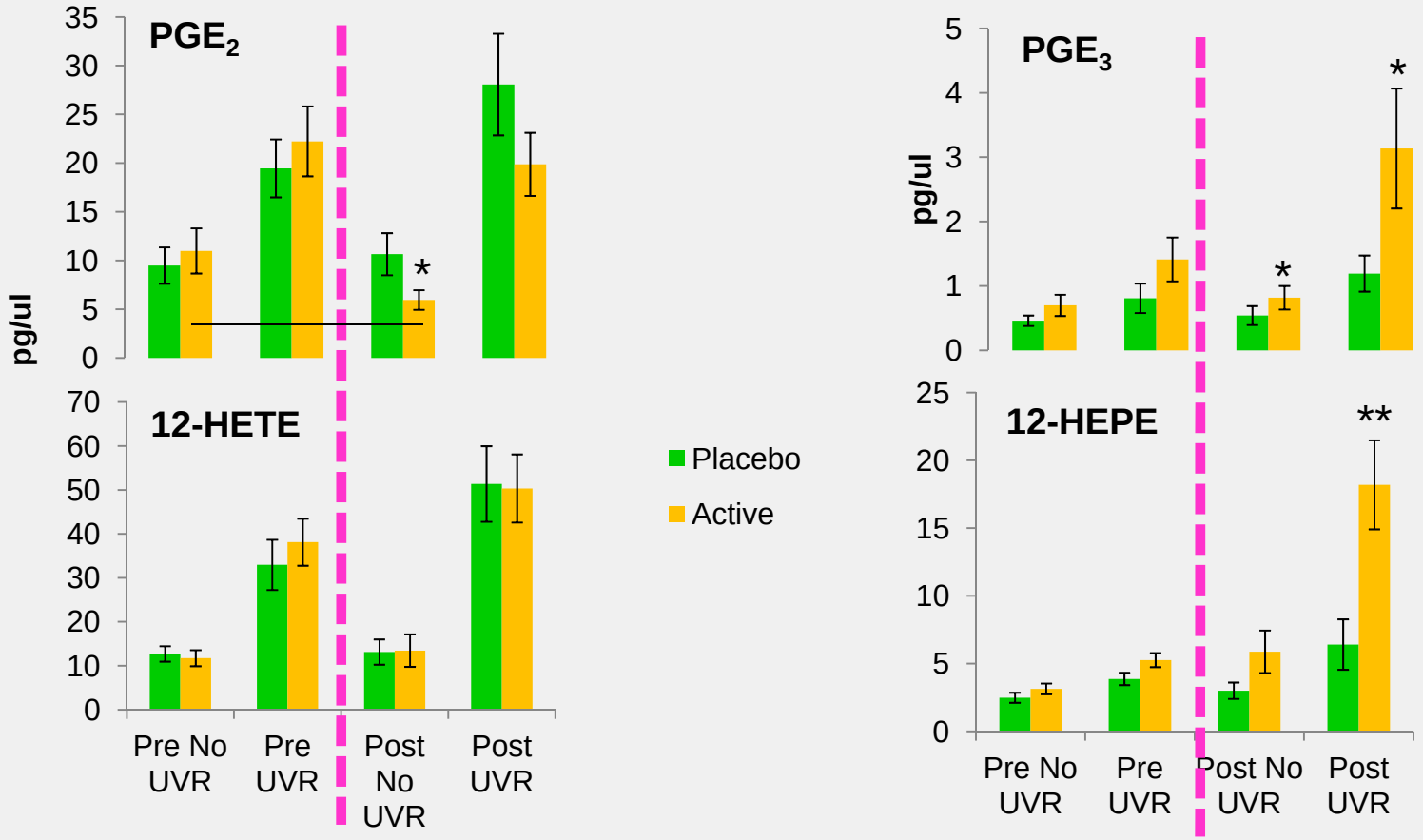


a: p<0.001 comparing to basal; **b**:p<0.001 comparing to placebo

AA, EPA, OA mediators in cutaneous blister fluid

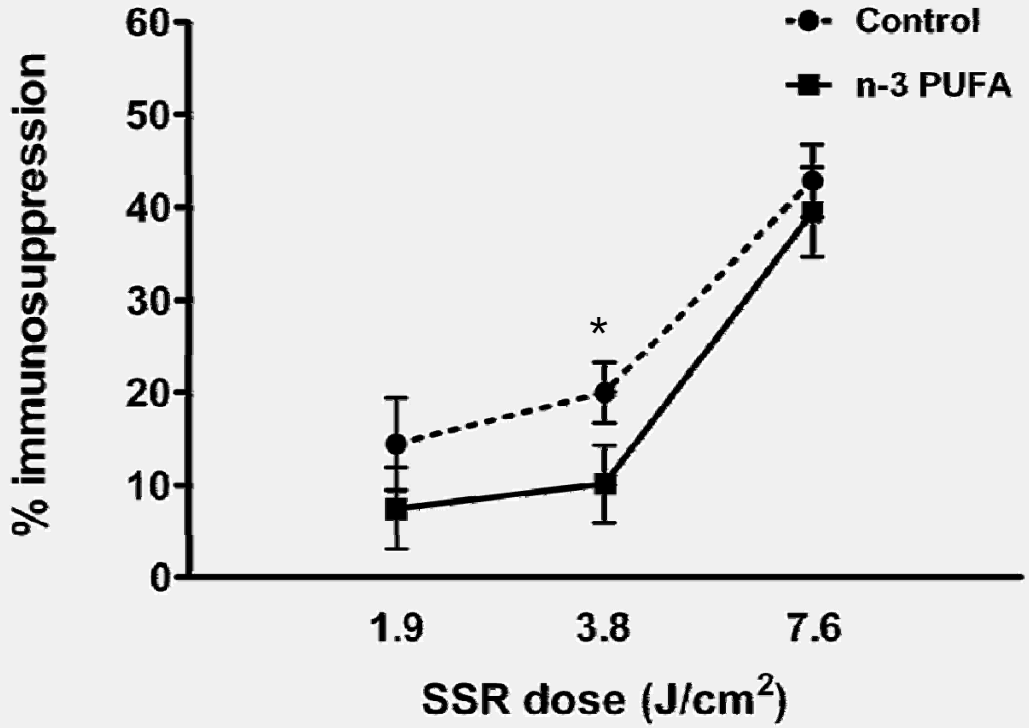
	Mean (SEM) (pg/µl)							
	Baseline				12 weeks			
	Control (n=19)		**EPA (n=17)**		**Control (n=19)**		**EPA (n=17)**	
	Unexposed	UVR-exposed	Unexposed	UVR-exposed	Unexposed	UVR-exposed	Unexposed	UVR-exposed
PGE_2	9.5 (1.9)	19.5 (3.1)†††	11.0 (2.4)	22.2 (3.8)†	10.7 (2.2)	28.1 (5.4)††	6.0 (1.1)*	19.9 (3.4)†††
PGE_3	0.5 (0.1)	0.8 (0.2)	0.7 (0.2)	1.6 (0.4)†	0.6 (0.2)	1.2 (0.3)†	0.8 (0.2)	3.1 (1.0)†
PGE_1	2.7 (0.7)	6.2 (1.2)†††	2.6 (0.6)	7.0 (1.2)††	3.5 (1.4)	8.7 (2.0)††	1.6 (0.4)	6.7 (1.4)†††
13,14 dh-15k-PGE_2	4.6 (1.1)	1.2 (0.4)†††	8.1 (2.2)	1.5 (0.4)†††	4.8 (1.3)	1.4 (0.4)†††	4.9 (1.4)	1.9 (0.4)
12-HETE	12.7 (1.8)	33.0 (5.7)†††	11.7 (1.9)	38.1 (5.7)	13.1 (2.9)	51.4 (8.6)†††	13.4 (3.9)	50.3 (8.2)†††
11-HETE	1.6 (0.2)	3.7 (0.6)†††	1.6 (0.2)	4.3 (0.5)†††	1.4 (0.2)	4.8 (0.5)†††	1.3 (0.3)	4.3 (0.6)†††
15-HETE	3.4 (0.5)	4.6 (0.6)	3.3 (0.5)	6.0 (0.7)††	3.0 (0.5)	6.3 (1.3)††	4.5 (0.9)	6.1 (0.9)†
15-HETrE	0.9 (0.1)	2.4 (0.5)††	1.3 (0.3)	2.2 (0.5)†	0.9 (0.1)	5.4 (2.4)††	0.9 (0.2)	1.9 (0.3)††
12-HEPE	2.5 (0.4)	3.9 (0.5)†	3.1 (0.4)	5.3 (0.5)	3.0 (0.6)	6.4 (1.9)†	5.9 (1.7)	18.2 (3.5)†††**
11-HEPE	ND	0.4 (0.14)a	1.7 (0.9)b	1.7 (0.6)f	7.4 (4.5)b	0.4 (0.05)c	0.6 (0.3)c	4.1 (2.0)g
15-HEPE	ND	ND	ND	ND	ND	ND	3.4 (0.9)d	5.0 (2.2)e
9-HODE	34.3 (5.6)	46.3 (9.6)	45.9 (10.6)	63.7 (7.7)†	26.1 (4.8)	51.1 (9.0)†	32.6 (11.0)	56.1 (9.3)††
13-HODE	36.6 (7.0)	32.6 (5.2)	32.3 (4.4)	55.5 (8.4)†	30.5 (5.9)	33.2 (4.7)	26.3 (5.2)	38.5 (5.9)

systemic EPA alters skin eicosanoids



RCT: n=16-19 volunteers per group; skin type I/II;
n-3 LC-PUFA (EPA: 70%; DHA: 10%) 5 cps/d, 3 months

oral n-3 PUFA supplement protects against UVR-induced immunosuppression



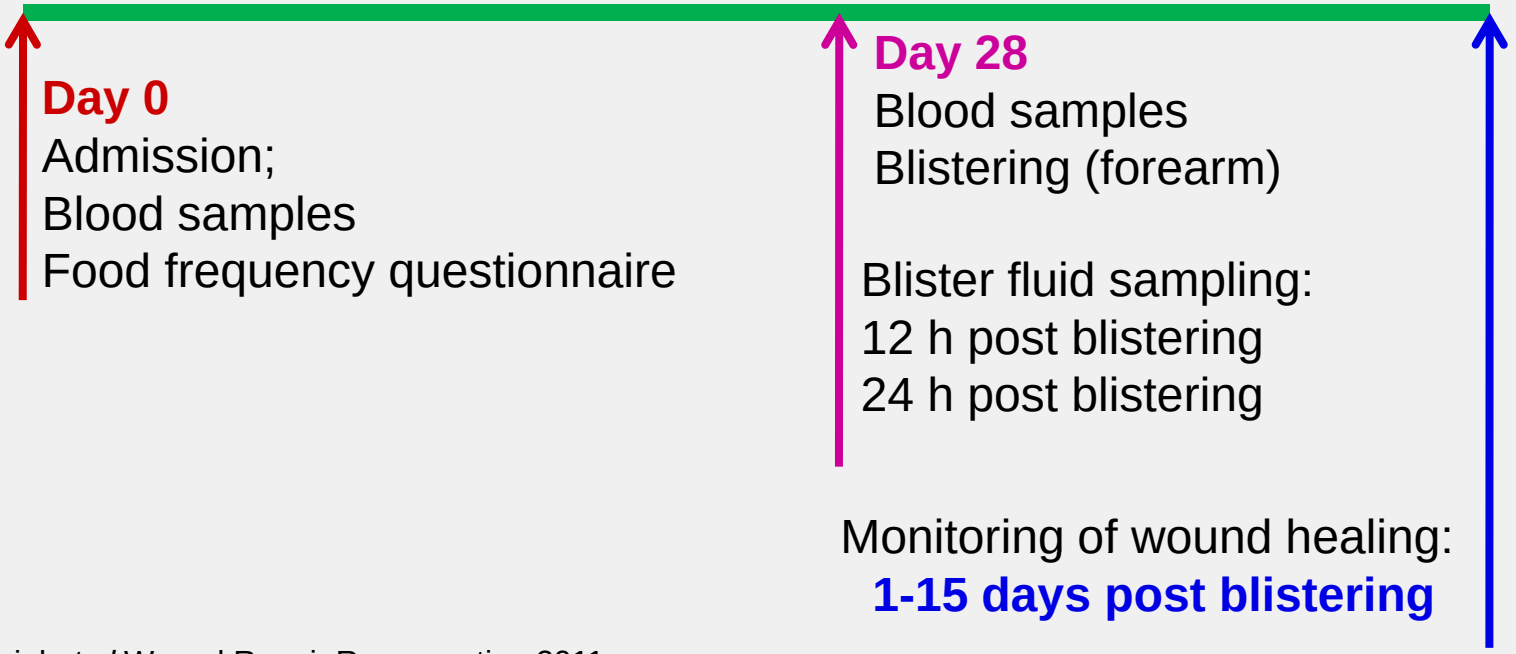
Protection at 3.8 J/cm^2 – 15 min summer midday sun at Manchester

SSR: Solar simulator; nickel allergy; n=33-36 per group; p=0.04

Pilkington *et al* AMCN *in press*

n-3PUFA in wound healing

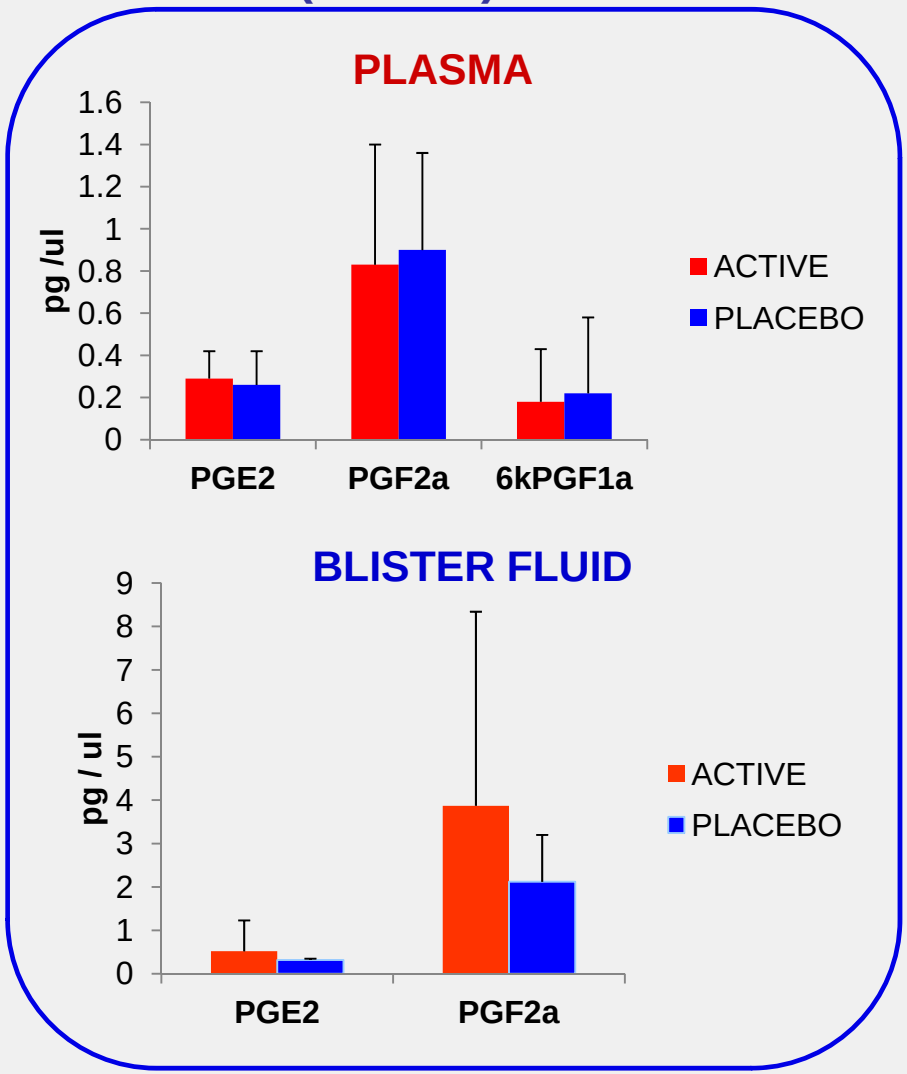
- Randomised double blind study (n=18 subjects)
  - **placebo** (mineral oil) or
  - **active** (1.6 g EPA+1.2 g DHA/day, 81 mg aspirin) 28 days



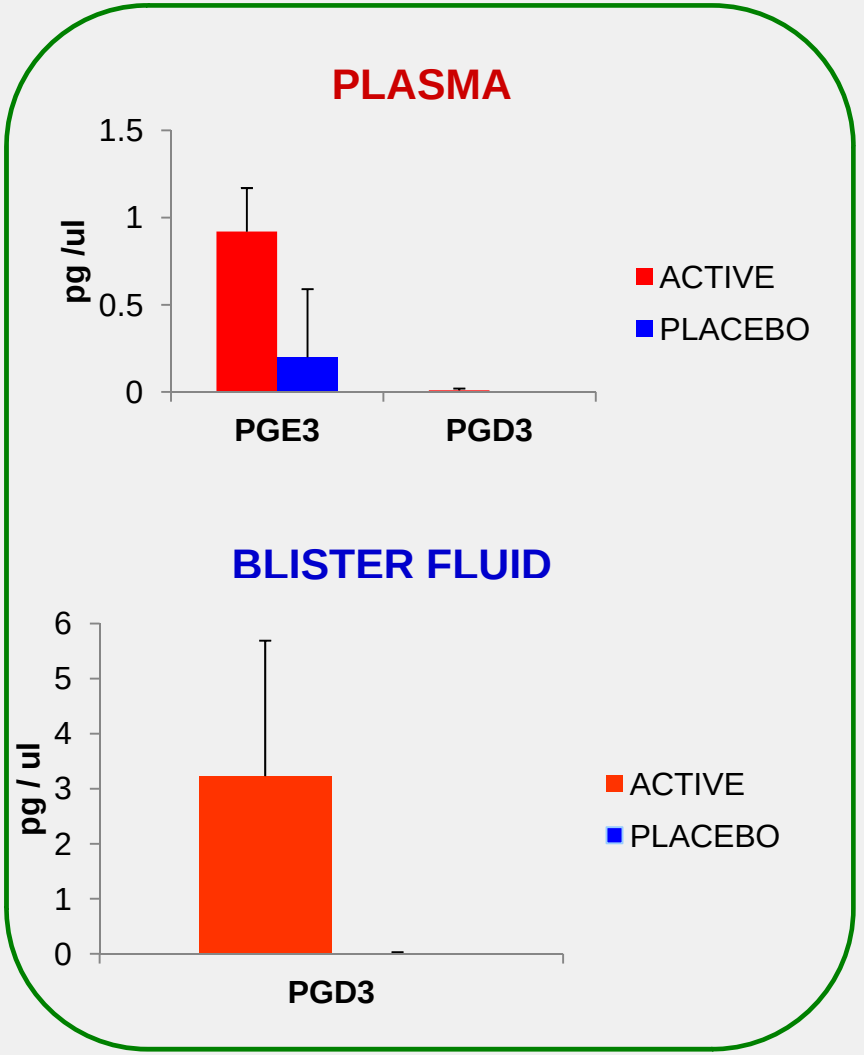
McDaniel *et al* Wound Repair Regeneration 2011

n-3PUFA supplement and COX-mediators

AA (20:4n-6)-derived



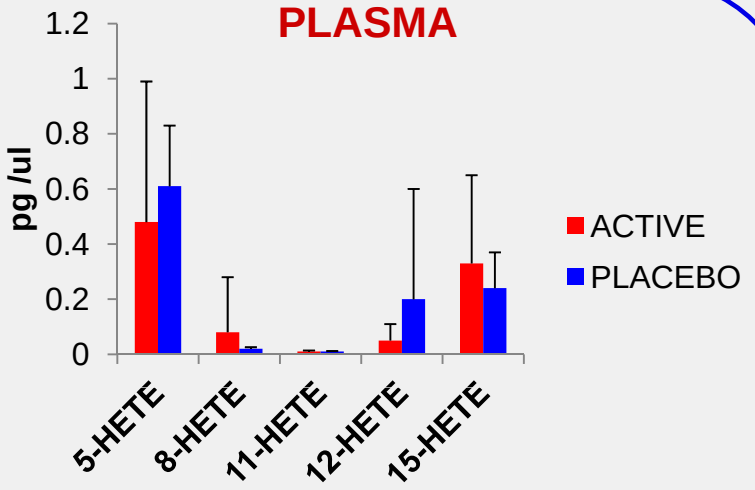
EPA(20:5n-3)-derived

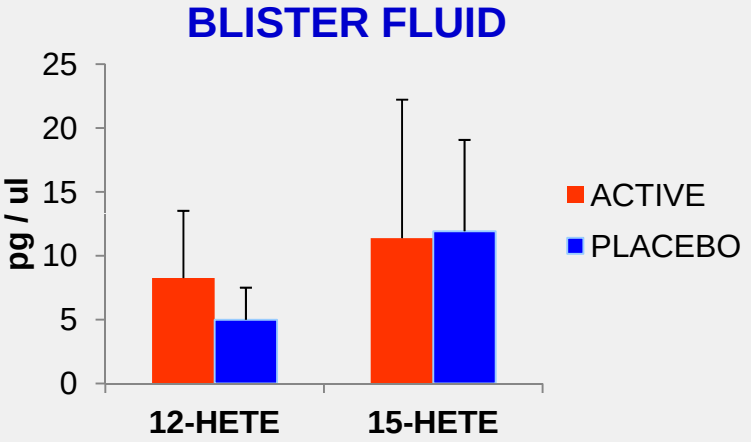


McDaniel *et al* Wound Repair Regeneration 2011

n-3PUFA supplement and LOX-mediators

AA (20:4n-6)-derived





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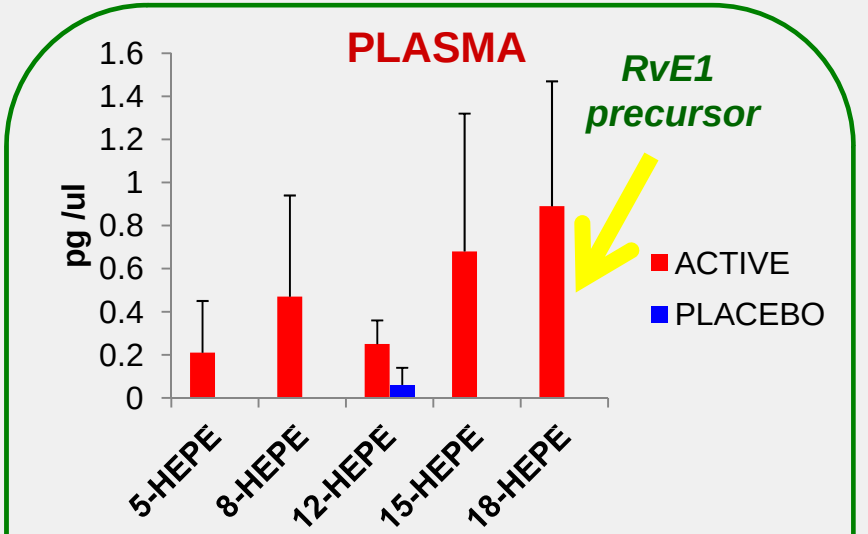
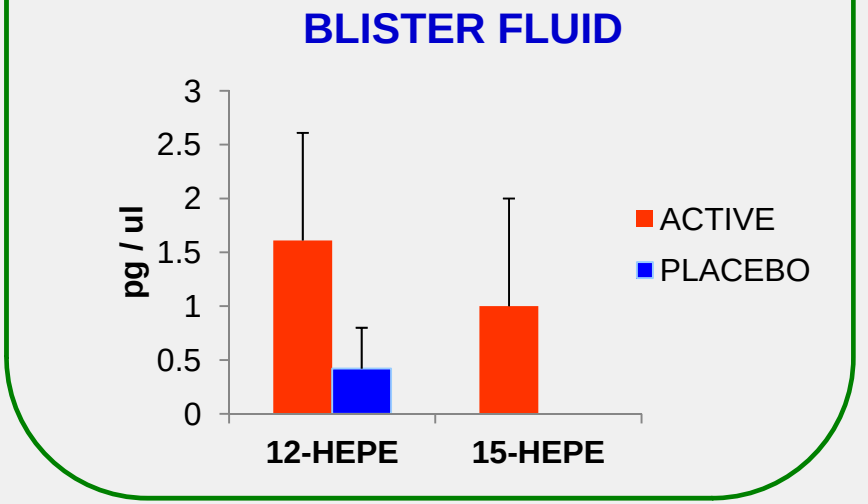
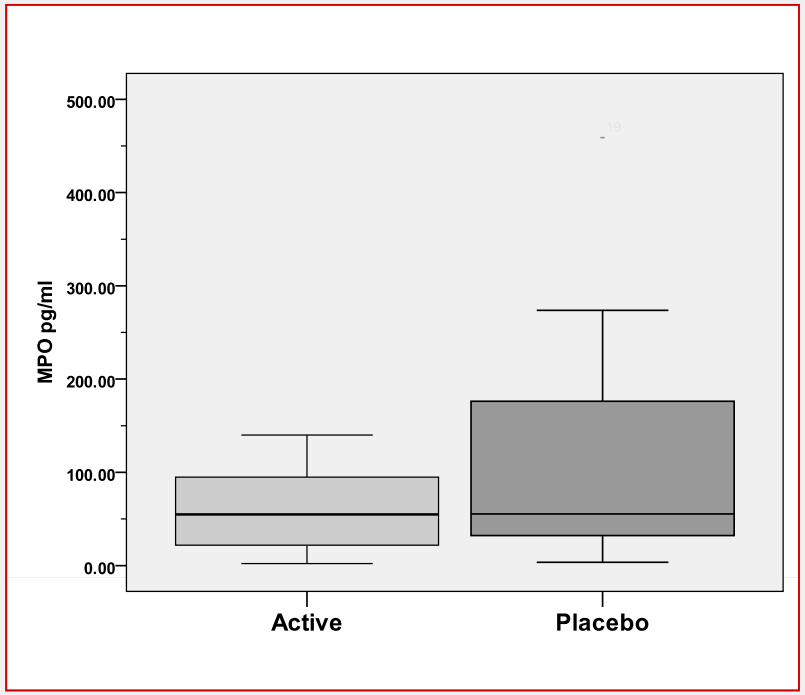




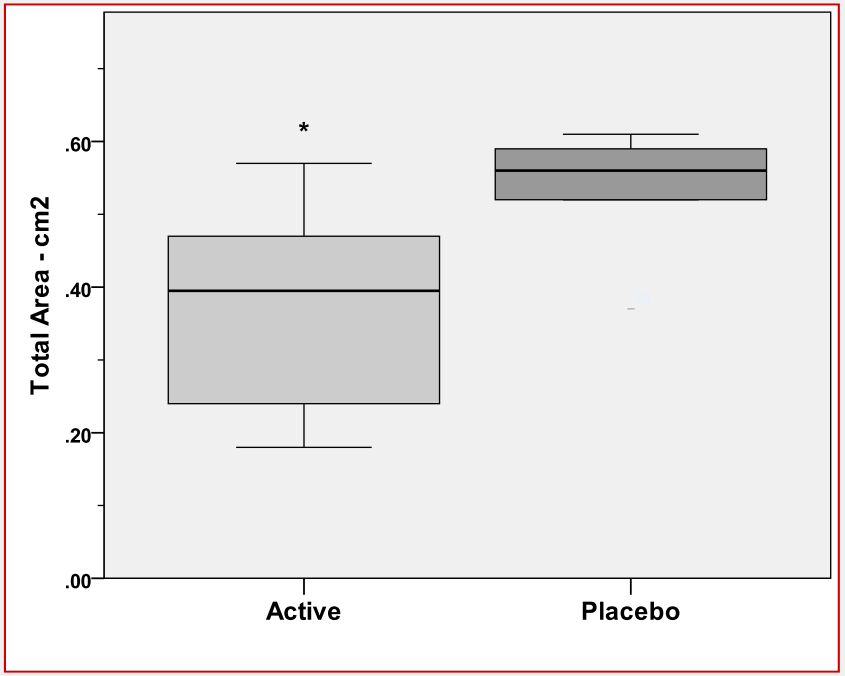
McDaniel *et al* Wound Repair Regeneration 2011

N-3 PUFA reduced wound area (improved healing)

myeloperoxidase (MPO) levels,
(leukocyte marker enzyme)



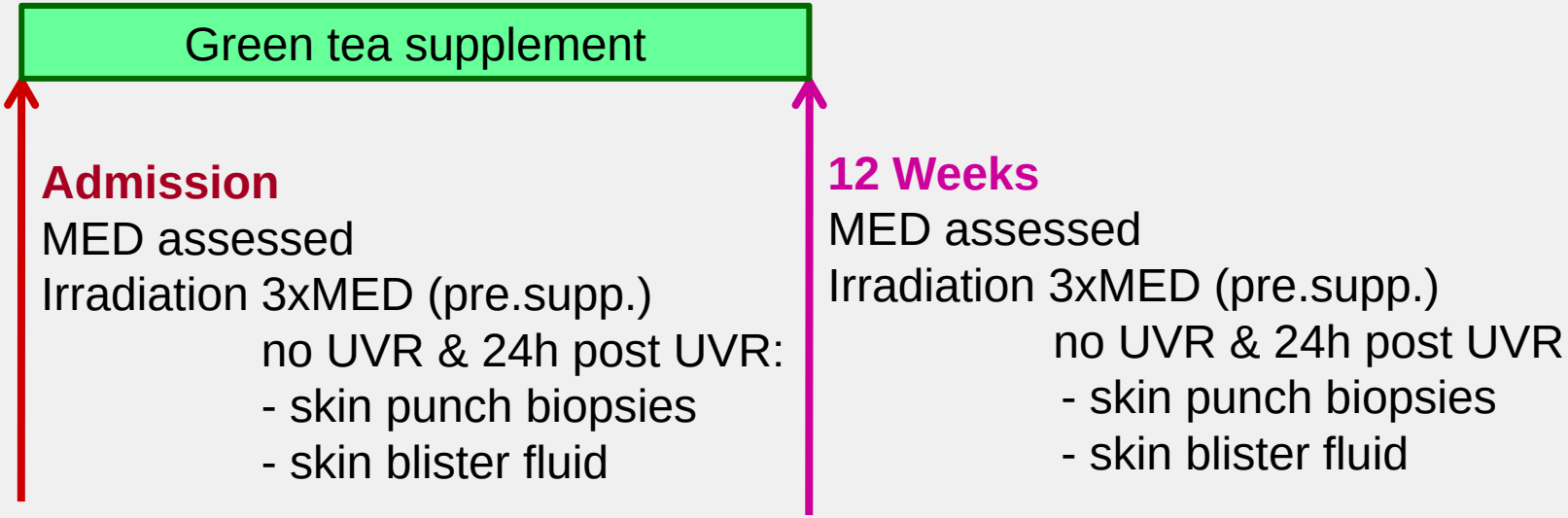
Area of wound (blisters) remaining to re-epithelialize on Day 5



McDaniel *et al* Wound Repair Regeneration 2011

Green Tea Catechins (GTC) and UVR-induced cutaneous inflammation

Healthy human volunteers:	n=14, 27-56 yrs; all female; phototype I/II (tend to burn not tan)
Supplement:	GTC 550 mg/day + 50mg/day vit.C
Study period:	12 weeks



urine samples: compliance

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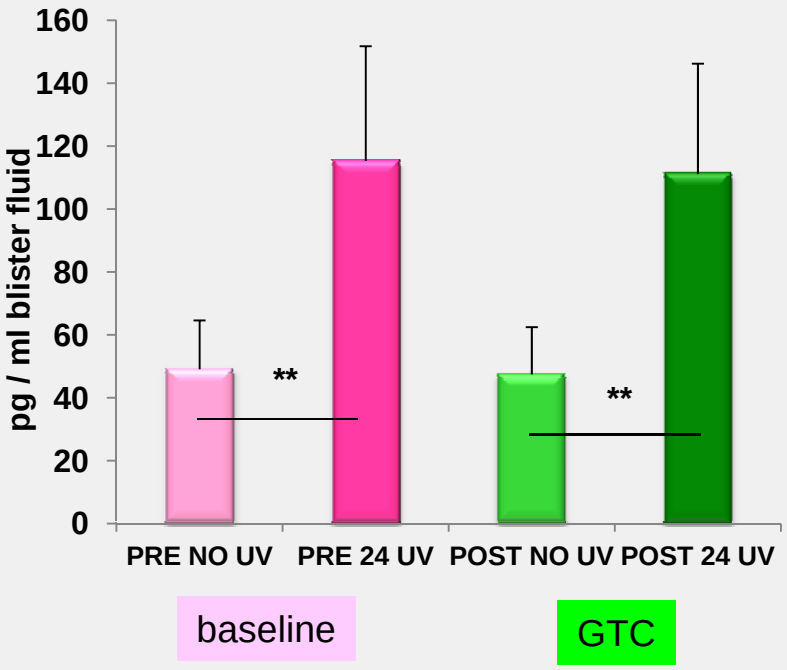
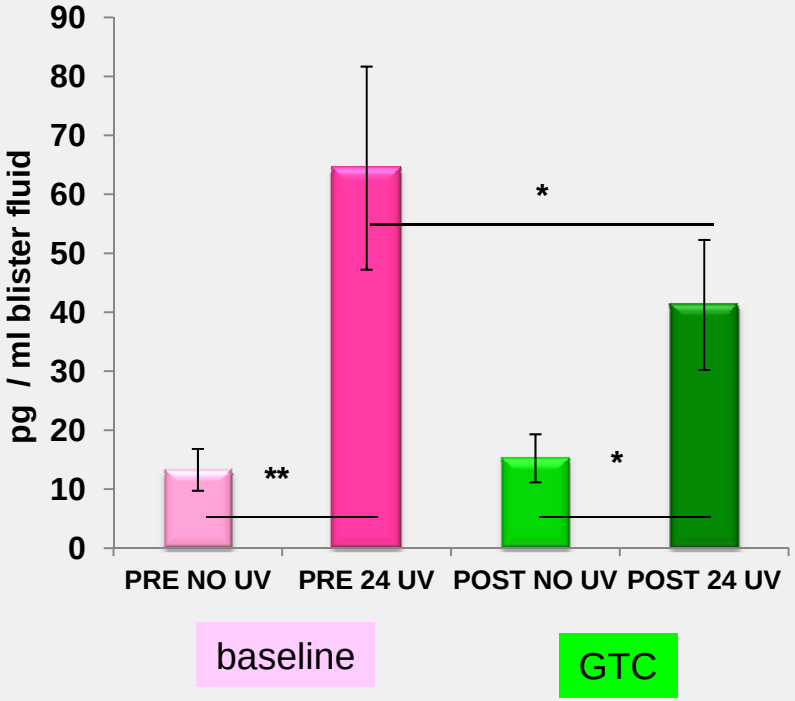
Effect of low dose GTC on cutaneous eicosanoids

PGE_2
pro-inflammatory, vasoactive



12-HETE
leukocyte chemoattractant



* p<0.05** p<0.001

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A Consideration of Biomarkers to be used for Evaluation of Inflammation in Human Nutritional Studies

P.C. Calder[1], N. Ahluwalia[2], R. Albers[3,4], N. Bosco[5], R. Bourdet-Sicard[6], D. Haller[7], S.T. Holgate[1], L.S. Jönsson[8], M.E. Latulippe[8], A. Marcos[9], J. Moreines[10], C. M'Rini[11], M. Müller[12], G. Pawelec[13], R.J.J. van Neerven[14], B. Watzl[15] and J. Zhao[16]

Table 2. Lipid mediators associated with inflammation*

Class	Mediator	Substrate	Receptor(s)
Prostanoids	PGD_2	Arachidonic acid via COX	DP1, DP2
	PGE_2	Arachidonic acid via COX	EP1, EP2, EP3, EP4
	$PGF_{2\alpha}$	Arachidonic acid via COX	FP
	PGI_2	Arachidonic acid via COX	IP
	TXA_2	Arachidonic acid via COX	TP
	PGE_1	Dihomo-γ-linolenic acid via COX	EP1, EP2, EP3, EP4
	PGD_3	EPA via COX	DP1, DP2
	PGE_3	EPA via COX	EP1, EP2, EP3, EP4
Leukotrienes	5-HETE	Arachidonic acid via 5-LOX	BLT2
	5-HPETE	Arachidonic acid via 5-LOX	OXE
	LTB_4	Arachidonic acid via 5-LOX	BLT1, BLT2
	LTC_4, D_4, E_4 (termed cys-LT)	Arachidonic acid via 5-LOX	CysLT1, CysLT2
	15-HETE	Arachidonic acid via 15-LOX	BLT2
	15-HPETE	Arachidonic acid via 15-LOX	BLT2
	12-HETE	Arachidonic acid via 12-LOX	BLT2
	LTB_5	EPA via 5-LOX	BLT1, BLT2
Lipoxins	LXA_4	Arachidonic acid via 15-LOX and 5-LOX or 5-LOX and 12-LOX (transcellular)	FPR2/ALX
Endocannabinoids	2-Arachidonoylglycerol	1,2-Diacylglycerol with arachidonic acid at the *sn*-2 position	CB1, CB2
	Anandamide	*N*-arachidonoylphosphatidylethanolamide via phospholipase D; in turn, *N*-arachidonoylphosphatidylethanolamide is formed from phosphatidylcholine with arachidonic acid at the *sn*-1 position and phosphatidylethanolamine	CB1, CB2
Resolvins, protectins and maresins	RvE1	EPA via acetylated COX-2 and 5-LOX (transcellular)	RvE1 (ChemR23), BLT1
	RvD1	DHA via acetylated COX-2 and 5-LOX or via 15-LOX and 5-LOX (transcellular)	RvD1 (GPR32), ALX/FPR2
	PD1 (NPD1)	DHA via 15-LOX and LOX (transcellular)	Not yet known
	MaR1	DHA via 15-LOX and 12-LOX (transcellular)	Not yet known
Lysolipids	PAF	Phosphatidylcholine with diethyl ether link at the *sn*-1 position	PAF-R
	Lyso-PA	Phosphatidic acid, which in turn is synthesised from phosphatidylcholine	LPA1, LPA2, LPA3, LPA4, LPA5, LPA6
	Sphingosine-1-phosphate	Sphingosine, which in turn is synthesised from ceramide	S1P1, S1P2, S1P3, S1P4, S1P5

Mediator Lipidomics

- LC/ESI-MS/MS mediator lipidomics: versatile, sensitive approach.
- Role of lipid mediators in health and disease.
- Discovery of novel mediators and biomarkers; development of therapeutics.
- Contribution to systems biology.

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Sue Bennett

Gemma Darby

Mags Brownrigg

Gary Williamson (Leeds, UK)

Tristan Dew

Kayleigh Clarke

Jodi McDanie (Ohio, USA)

Robert Tonge (Waters, UK)