PHYSIOLOGIE & MEDECINE EXPERIMENTALE COEUR MUSCLES MONTPELLIER

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

cnrs dépasser les frontières

Oxidative stress : From toxicity to cell interactions

FACULTÉ de MÉDECINE Montpellier - Nîmes

CHRU MONTPELLIER CENTRE HOSPITALIER REGIONAL UNIVERSITAIRE

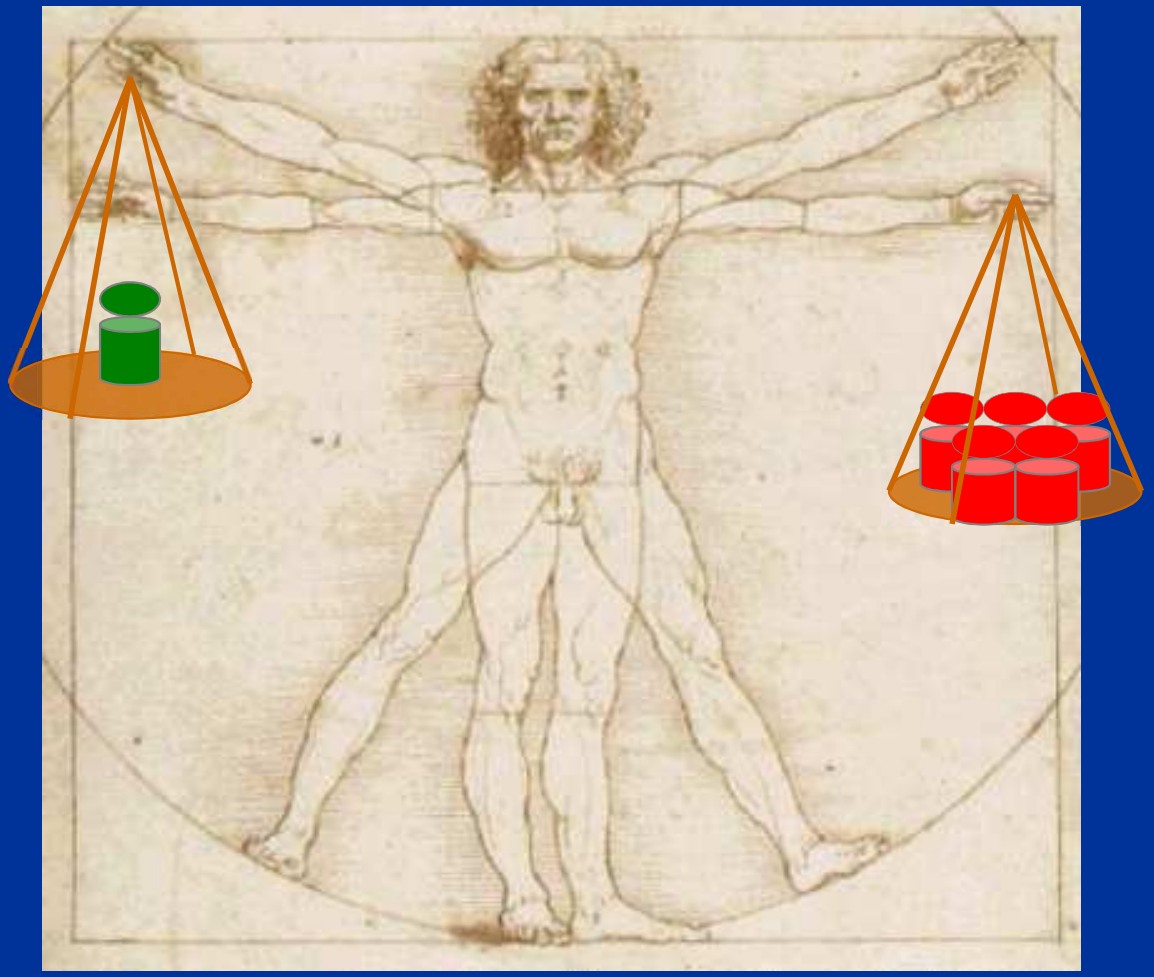
UNIVERSITÉ MONTPELLIER

Oxidative stress an umballance between oxidants and antioxidants

Antioxidants:

Is it possible to appreciate defense mechanism ?

Enzymatic or non enzymatic defenses

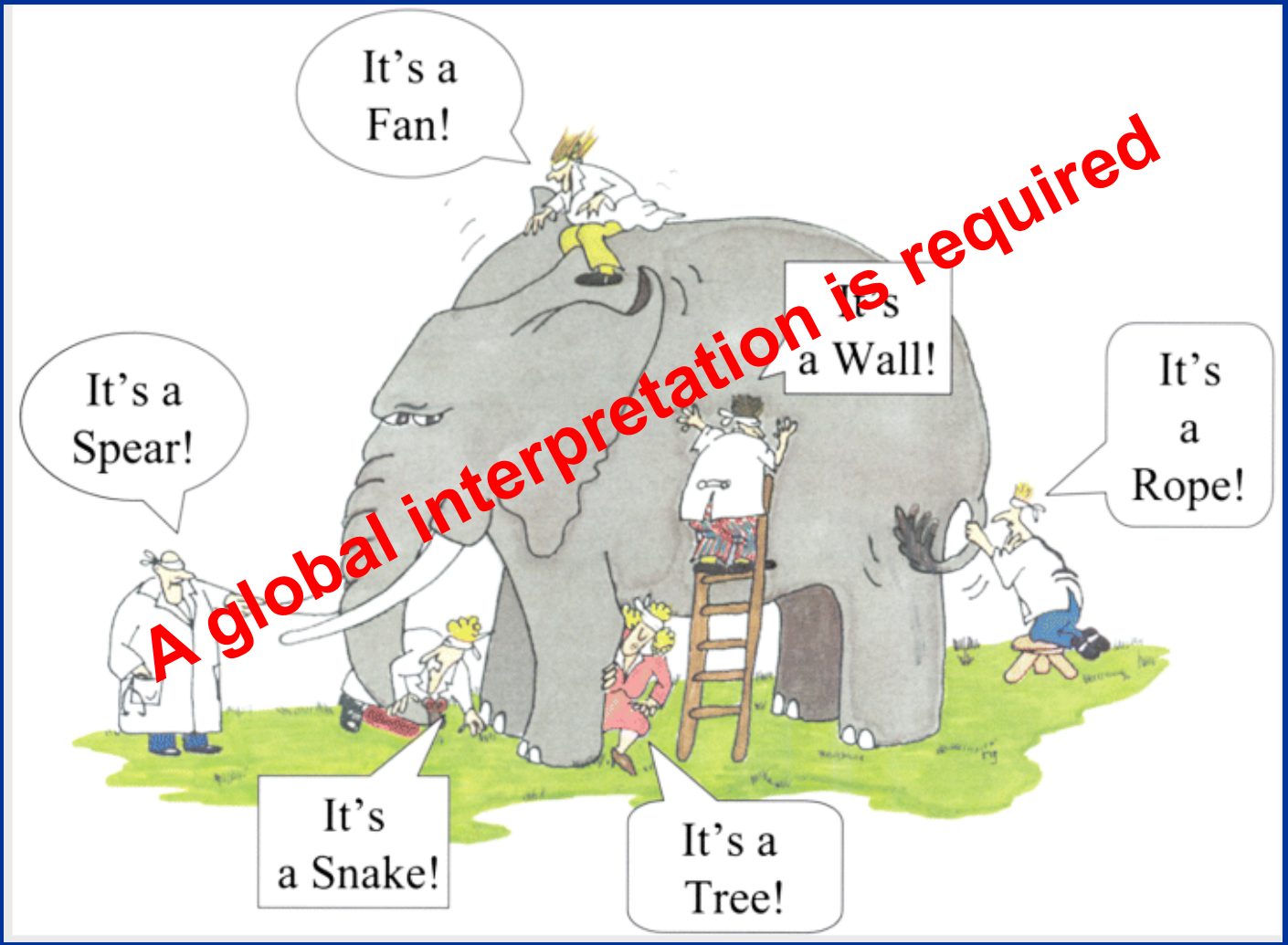


Oxidants:

Are biomarkers of oxidant production available ?

Which place for oxidative stress biomarkers ?

Exploring oxidative stress : a difficult chalenge



Jonathan Himmelfarb, Peter Stenvinkel, T Alp Ikizler and Raymond M Hakim, Kidney International (2002) 62, 1524–1538

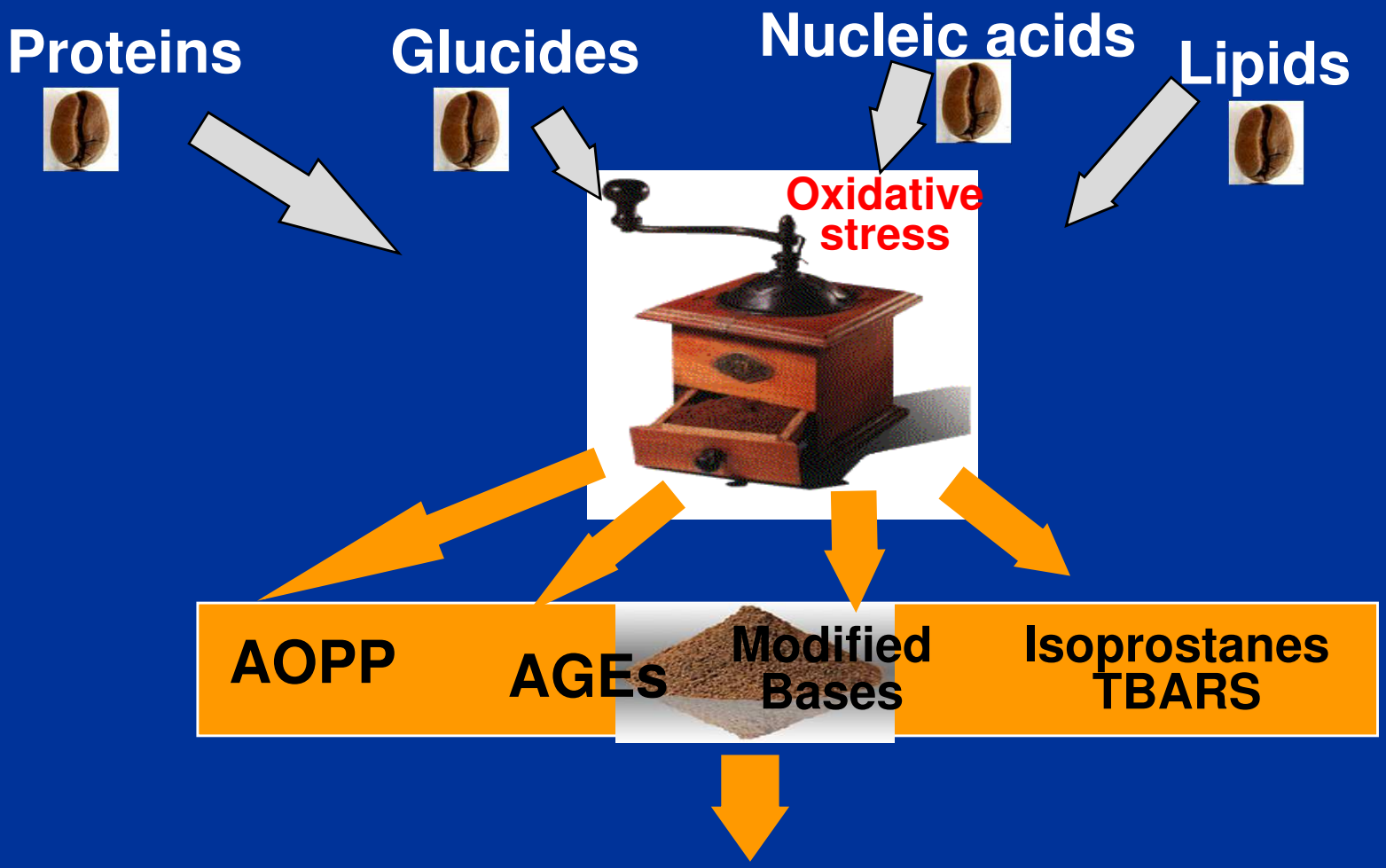
Exploring Oxidative Stress

I) Biomarkers of oxidative stress-related toxicity : « oxidative stress biomarkers »

II) Quantification of Oxidant production ?

III) Investigation of defense mechanisms ?

Oxidative stress : molecular targets



Membrane desorganisation
Loss of protein fonctions
Impairement of genetic information

Lipid peroxidation compounds as biomarkers of oxidative stress

Malonedialdehyde (MDA - TBARS)

4-hydroxynonénal (HNE)

Aldehydes

F2-isoprostanes

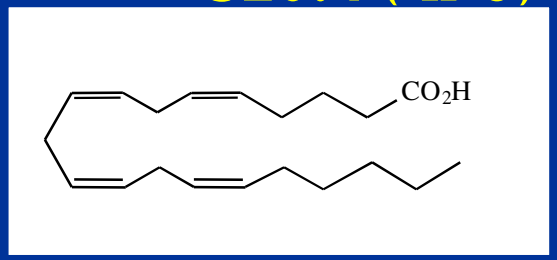
anti-oxidized antibodies

PCOOH (oxydized phosphatidylcholine)

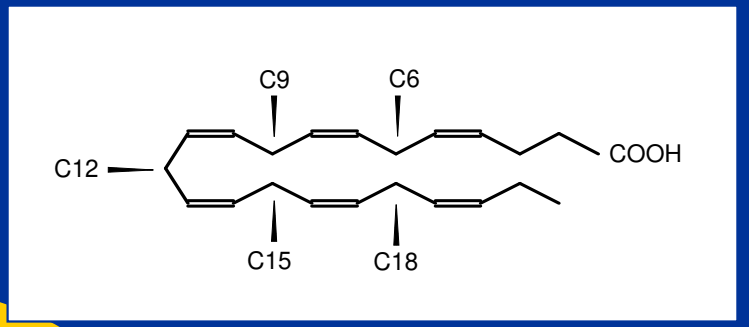
Lipid peroxidation

Isoprostanes : biomarkers of oxidative stress

Arachidonic Acid AA
C20:4 (n-6)



Docosahexaenoïc acid DHA
(C22/6(n-3)



Oxidative stress

Isoprostanes

iPF_{2a}-III
(8-iso-PGF_{2a})

iPF_{2a}-IV

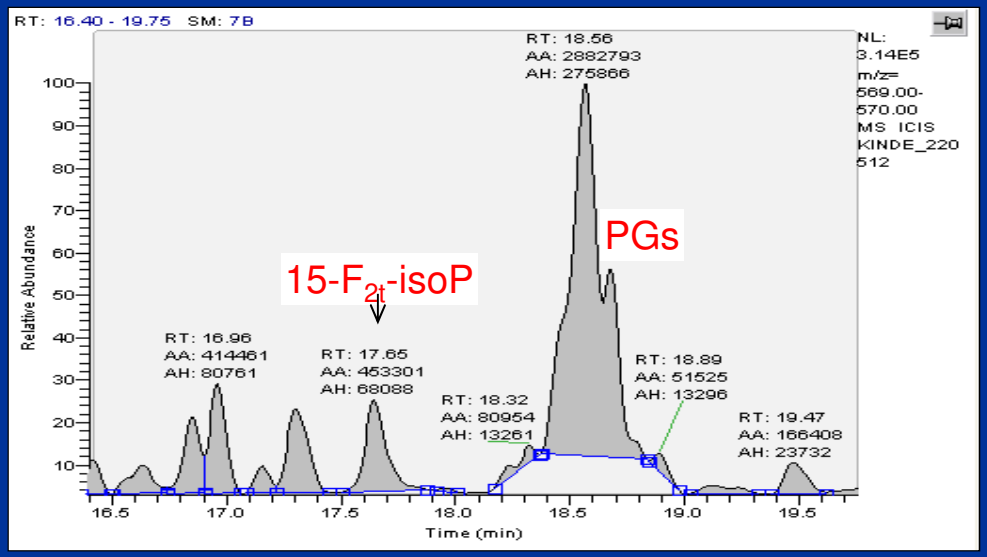
iPF_{2a}-V

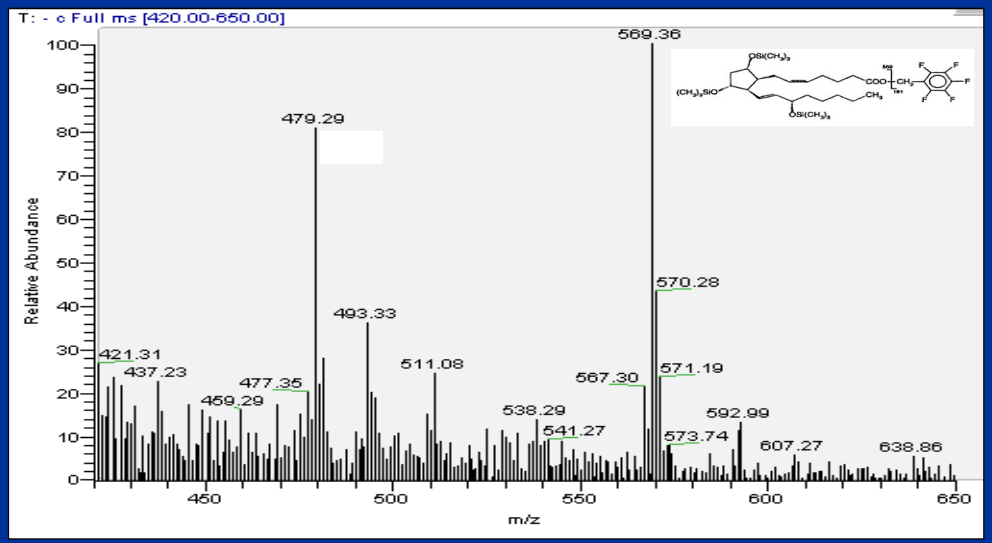
+ de 64 possibles isomers

Neuroprostanes

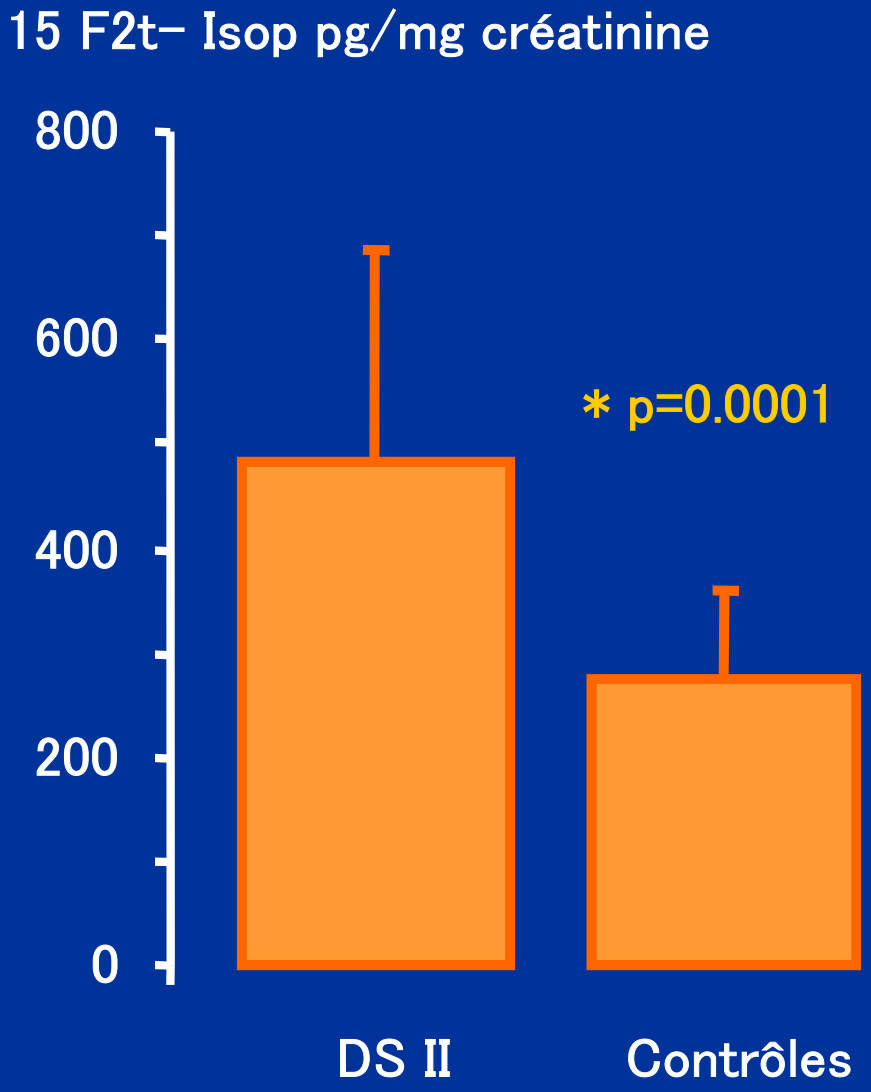
F_4-NPs

Detection of Isoprostanes using GC-MS

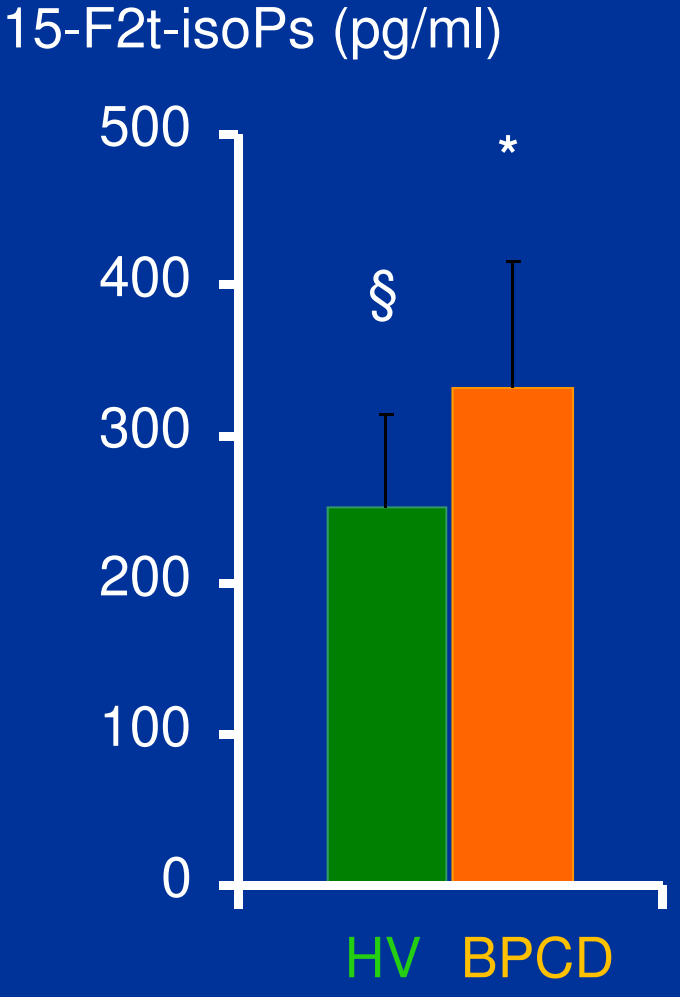




Isoprostane as a biomarker of oxidative stress in clinical situations



Monnier et al, JAMA, Avril 2006.



F. Gouzi et al., J Appl Physiol. 2013;115(12):1796-805

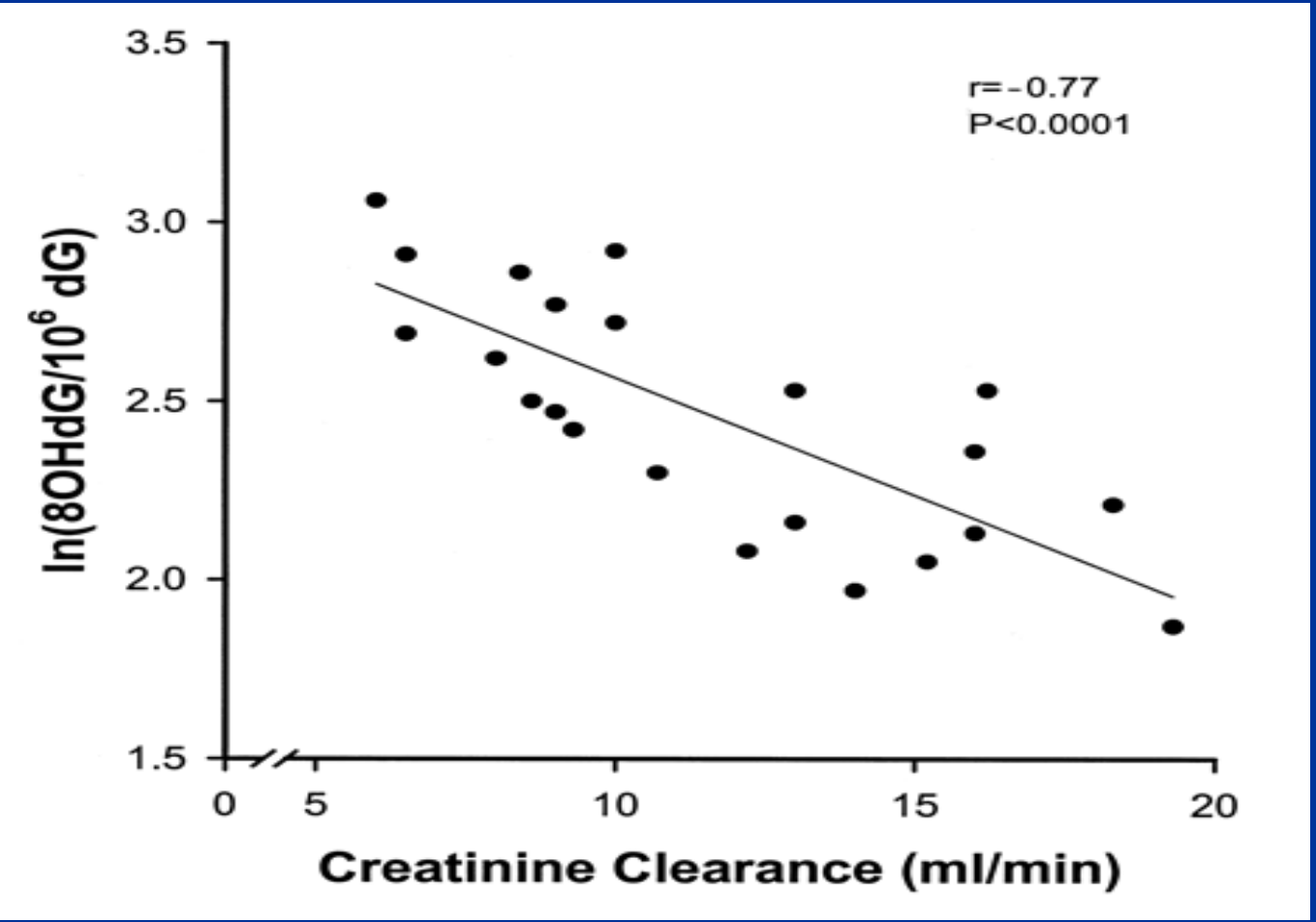
Oxidative stress Biomarkers: Nucleic acids

8-hydroxy-2'deoxyguanosine (8-OHdG)
Guanine oxidation

Test « COMET »
Detection of breakdown of DNA using electrophoresis

Modified Nucleic Acid

Increase in Oxydative stress in CKD



Tarng et coll. JASN, 2002

Protein oxidative products

Carbonyl Proteins

AGEs, pentosidine, Carboxymethyl Lysine

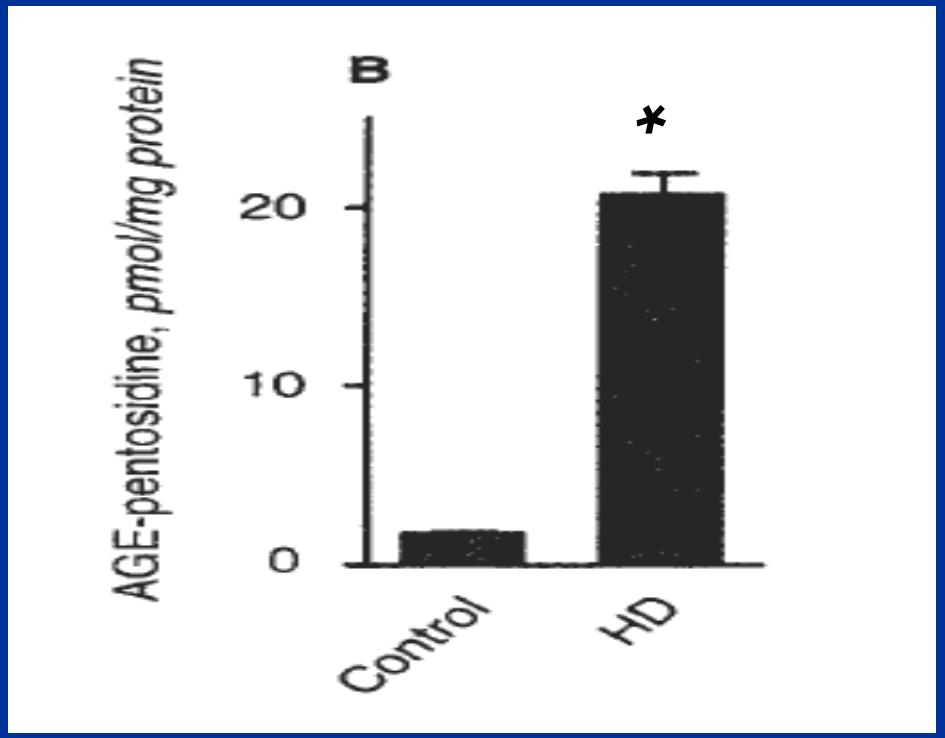
Protein Oxidative products

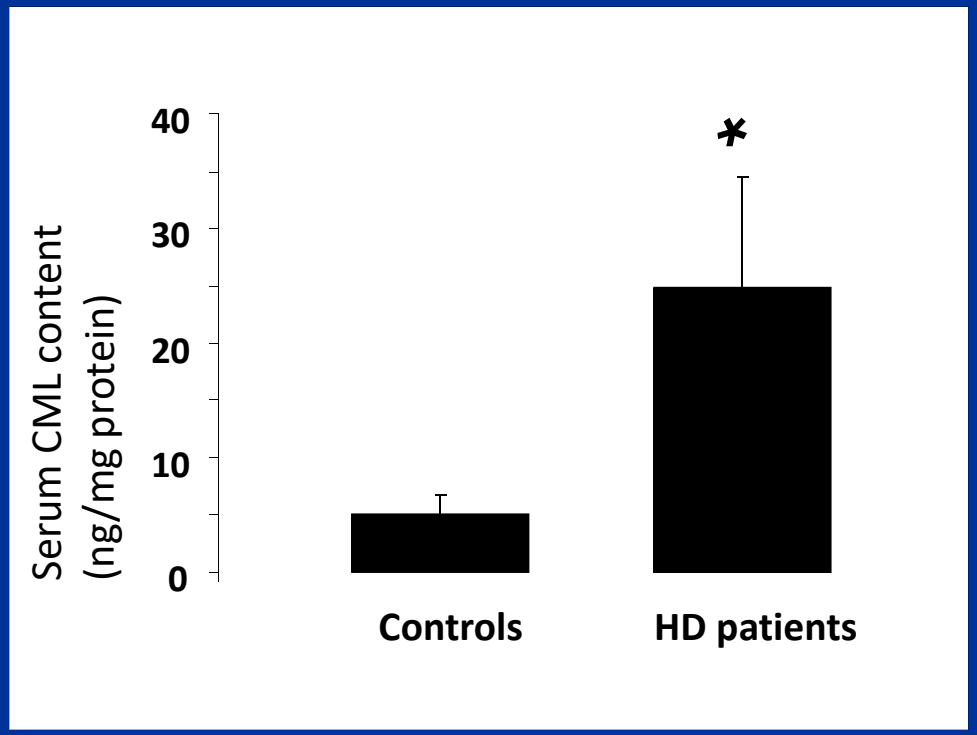
AOPP advanced oxydation protein products

Dityrosine, chlorotyrosine, nitrotyrosine
Oxyd. de la tyrosine

Thiols oxidation : equilibrium GSH/GSSG

Carboxymethyllysine and pentosidine : specific biomarkers

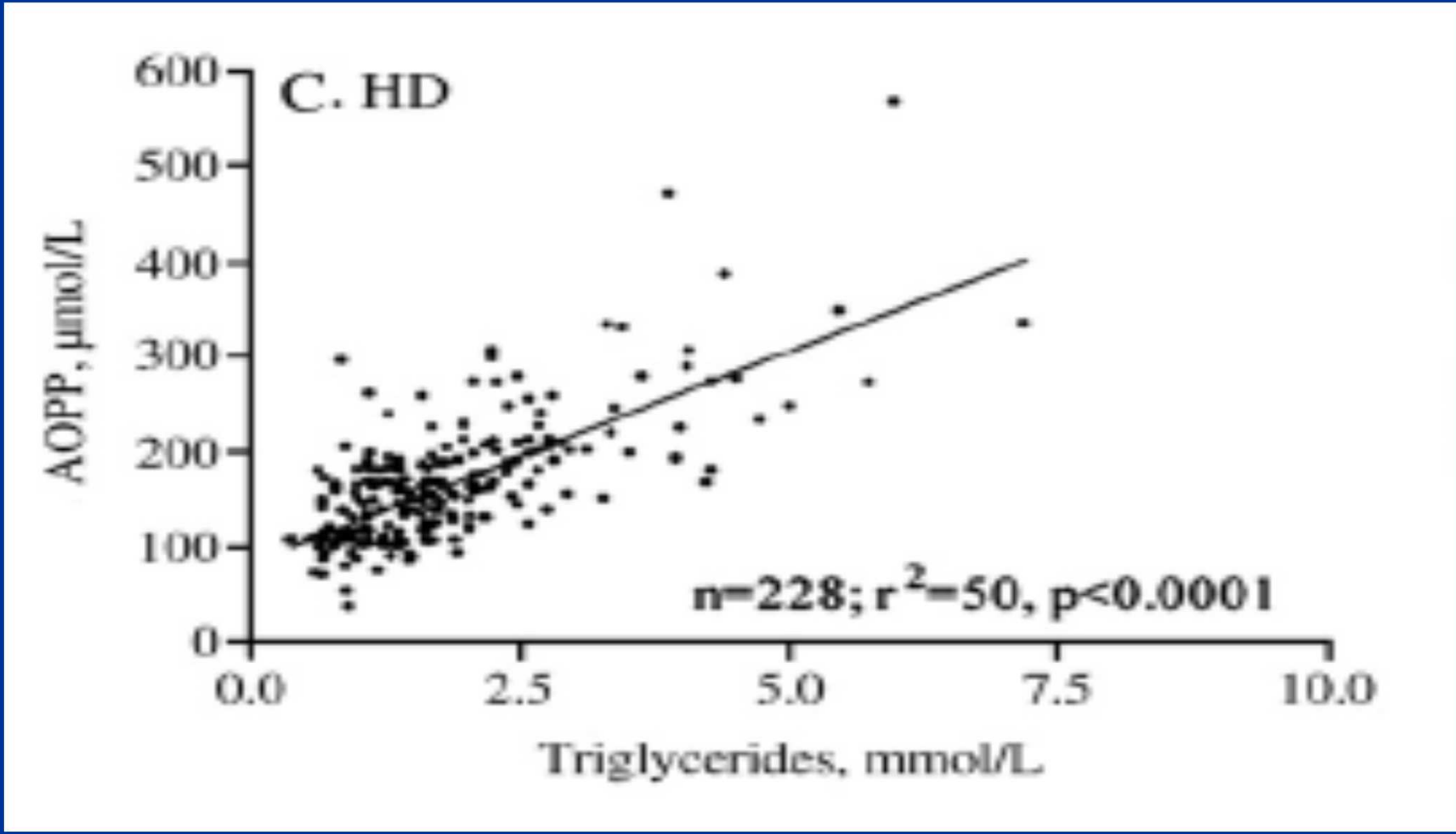




Witko-Sarsat et al., Kidney Int. 1996

Wagner et al., Am J Kidney Dis. 2006

AOPP : analytical limitations



Valli et al Clinica Chimica Acta 379 (2007) 87–94

GSH/GSSG ratio a clear indicator of redox status

- **Valeurs sanguines**

GSH libre: 800 – 1500µM
GSSG: 1 – 10µM

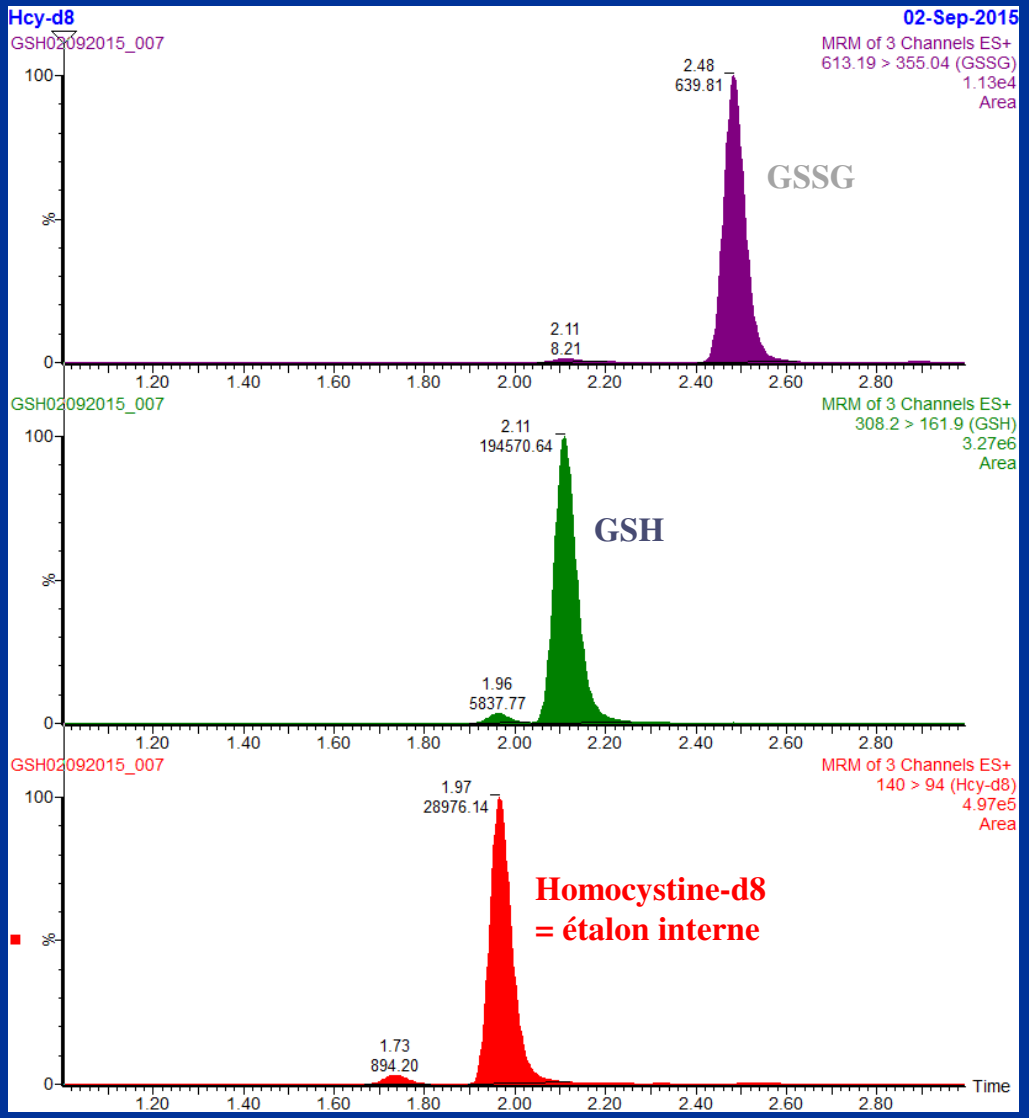
- **Méthode de dosage**

Mesure du GSH sanguin difficile: peu stable

Variabilité intra-individuelle élevée

Important de maîtriser les étapes pré-analytiques

Robustesse de la méthode de dosage= **LCMSMS**



Exploring Oxidative Stress

I) Oxidative stress biomarkers :

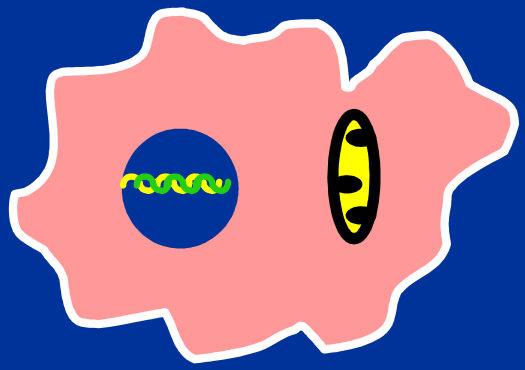
One lipid biomarker (IsoPs), 1 protein biomarker (CML or pentosidine) or GSH/GSSG ratio, 1 nucleic acid biomarker : 8-OH-desoxyguanosine

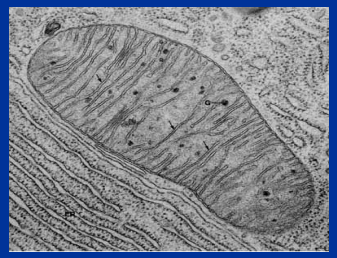
II) Quantification of Oxidant production ?

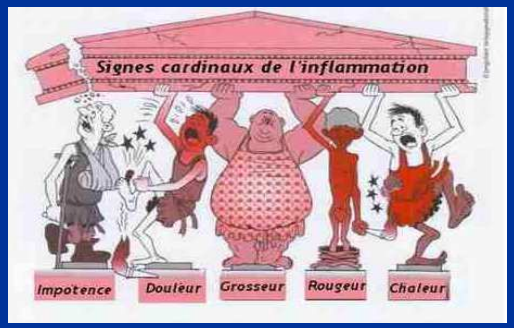
How are ROS produced?
How to quantify ROS ?
How to moduate ROS ?

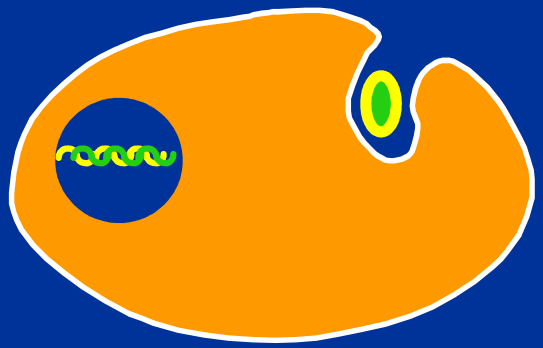
III) Investigation of defense mechanisms ?

ROS are mainly producted as a coproduct of Energy supply or phagocyte activation









Cell metabolism

Phagocyte activation

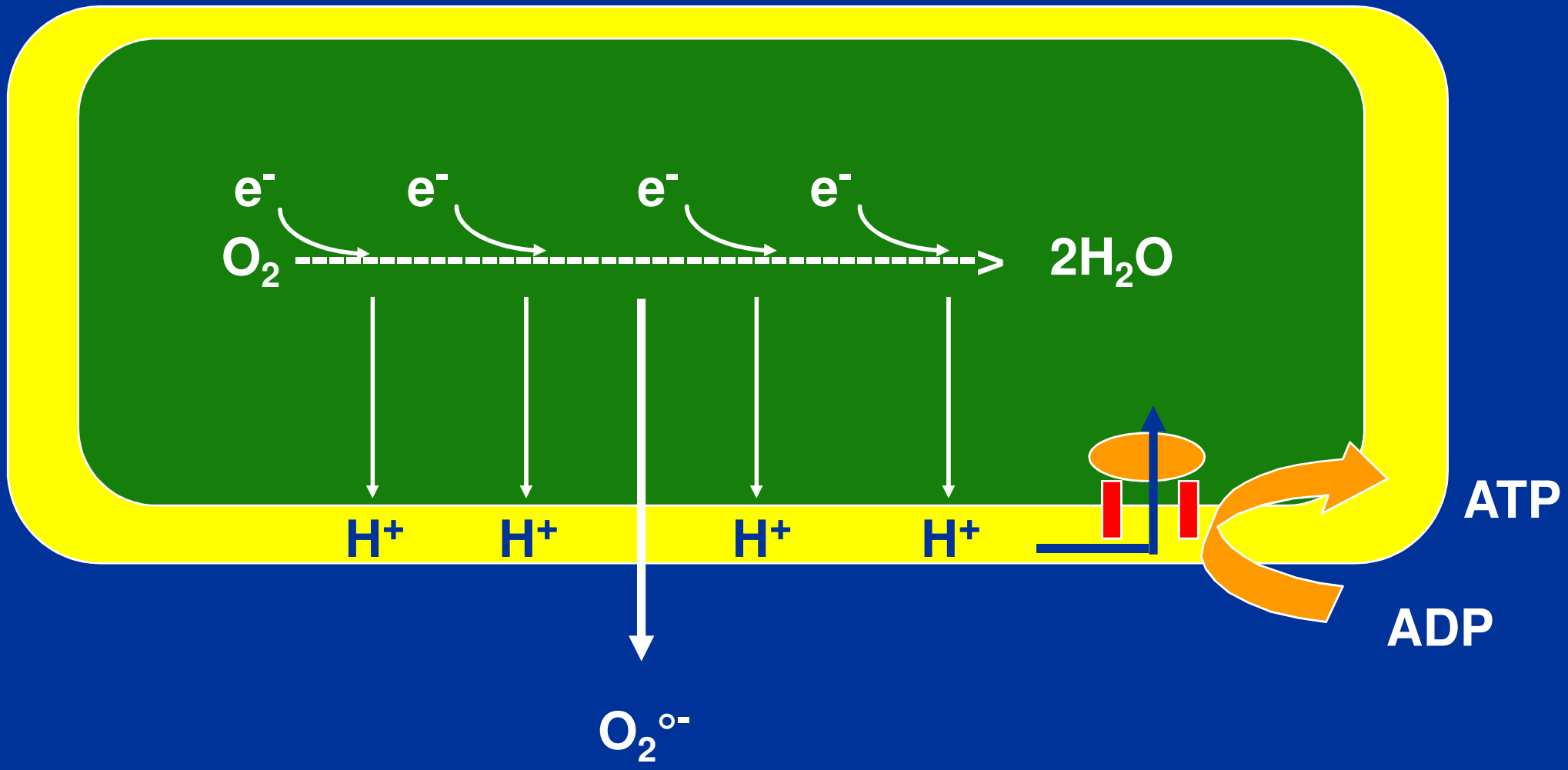
$O_2^{\circ-}$

Chlorinated oxidants

ROS

Nitrated oxidants

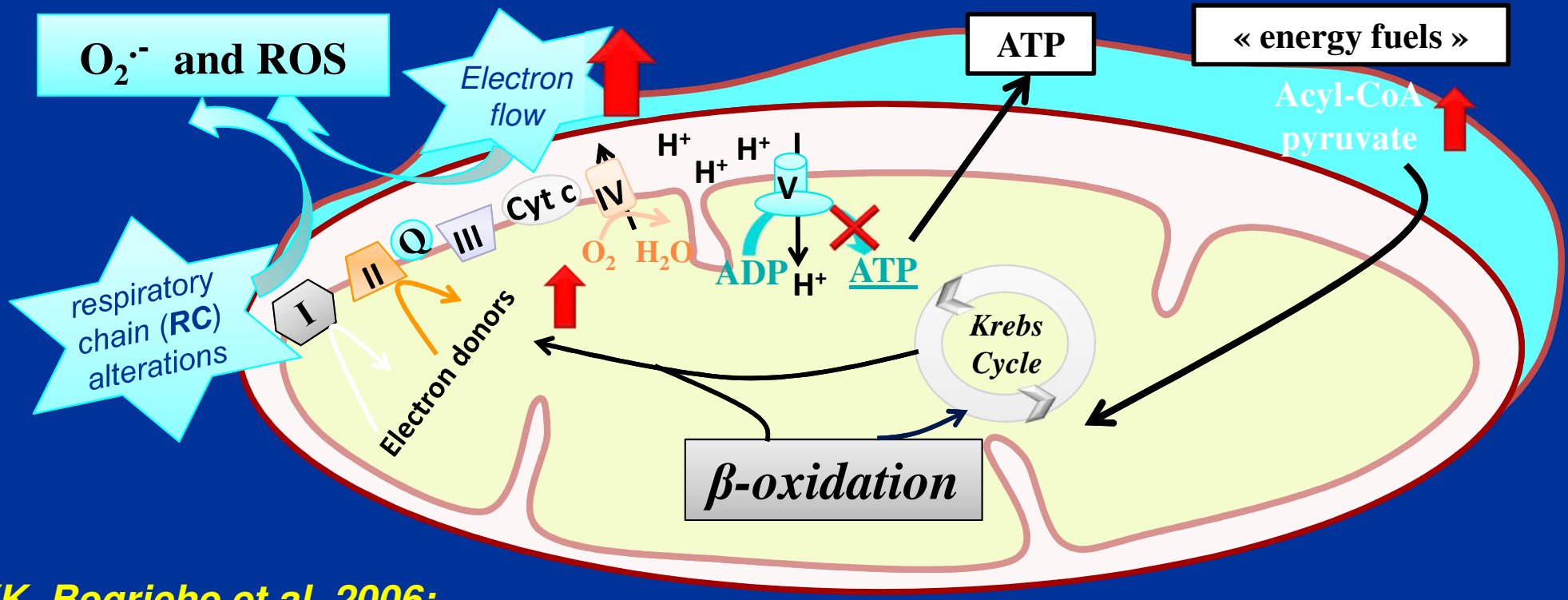
Superoxide anion as a cofactor of mitochondria respiration



Complexes I et III : main source of ROS

ROS production by the respiratory chain

Desequiibrium between ATP consumption and « energy fuel »

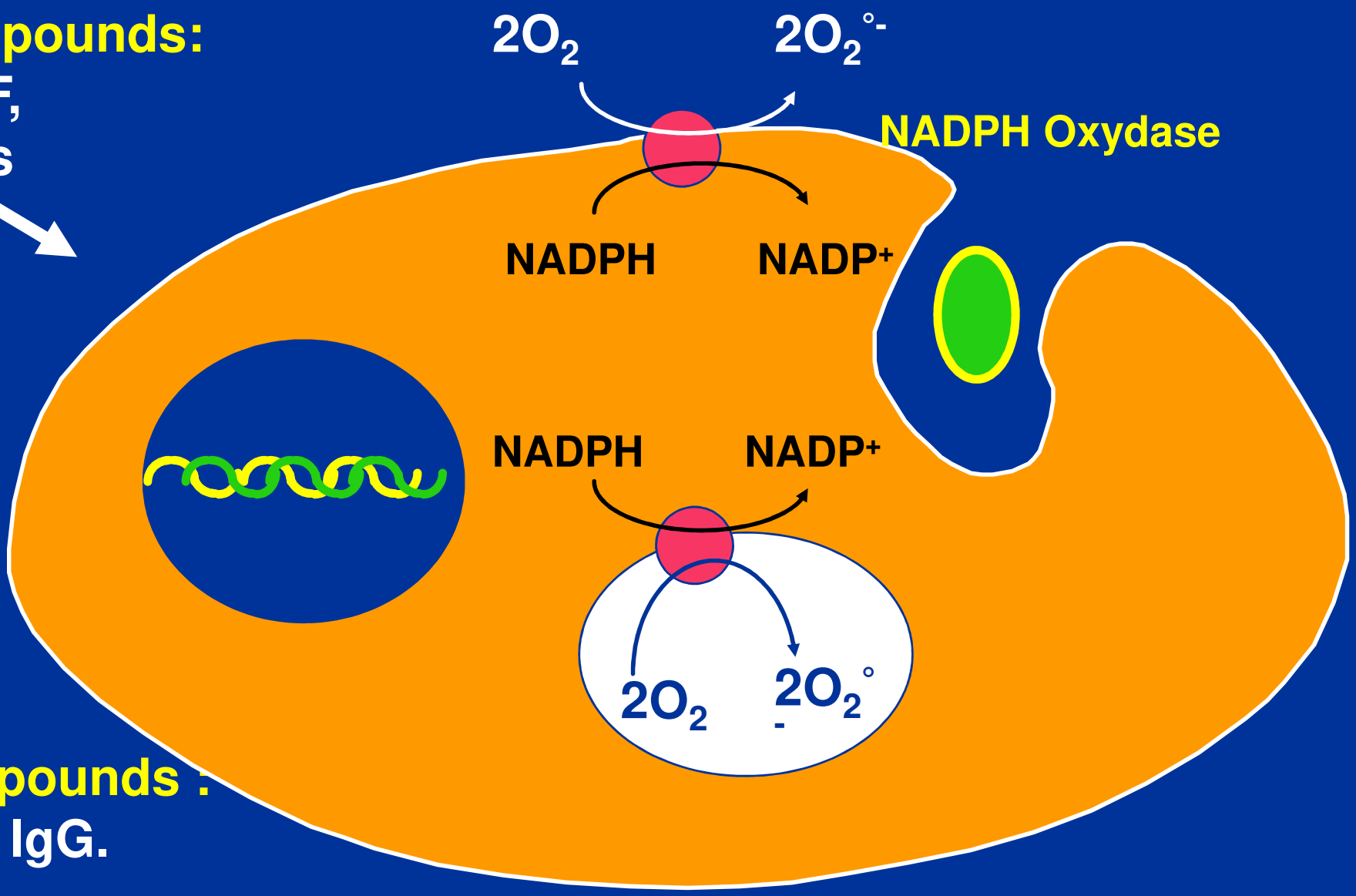


(K. Begriche et al. 2006; Wallace et al. 2010)

Murphy MP 2009

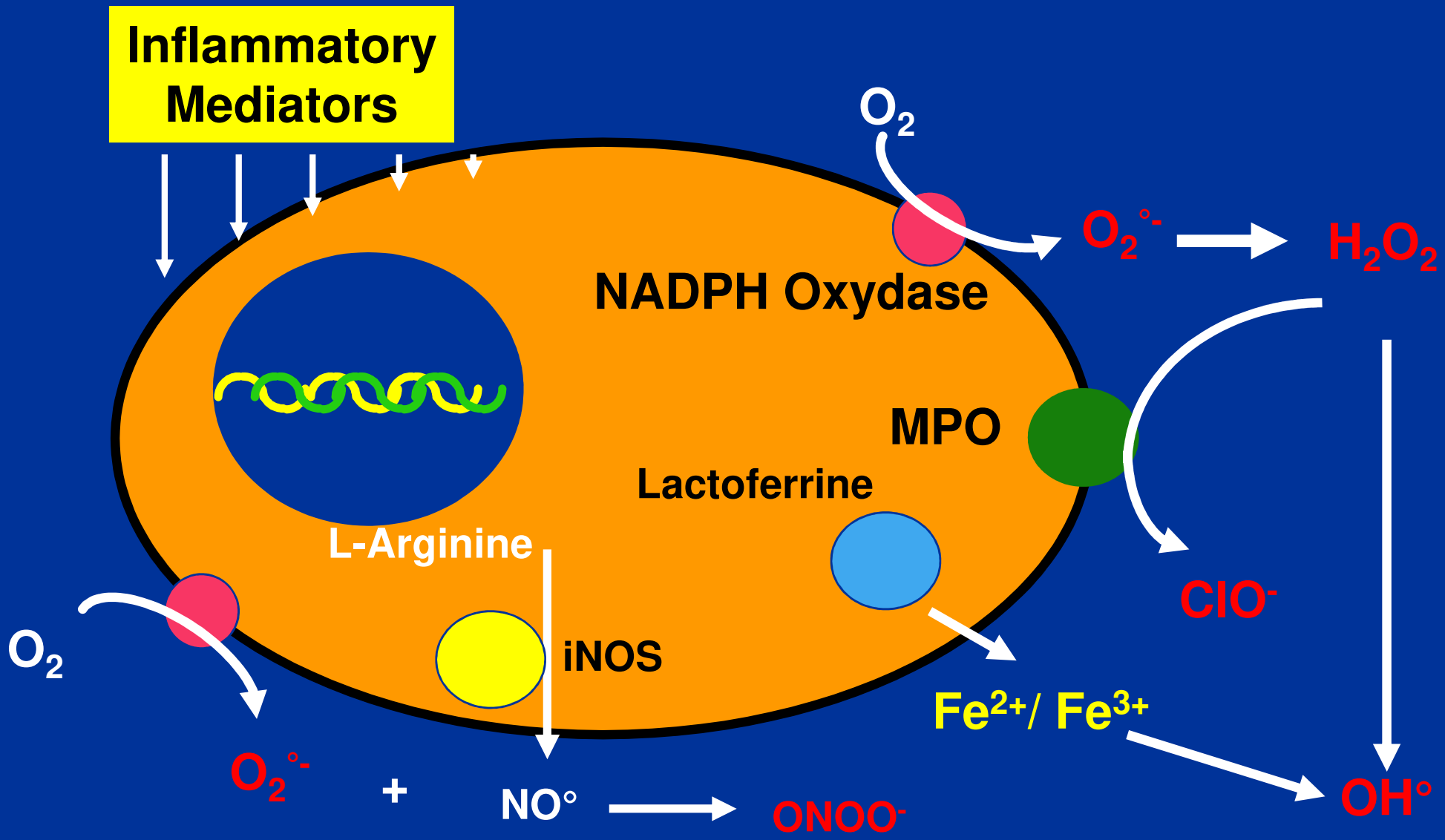
The oxidative burst : an ehanced production of superoxide anion

Soluble Compounds:
C5a, IL1, TNF,
Prostanoïdes
AgII, ET1



Insoluble compounds :
Bacteria, LPS, IgG.

Inflammation and ROS poduction



NAD(P)H oxydase : a NOX superfamilly

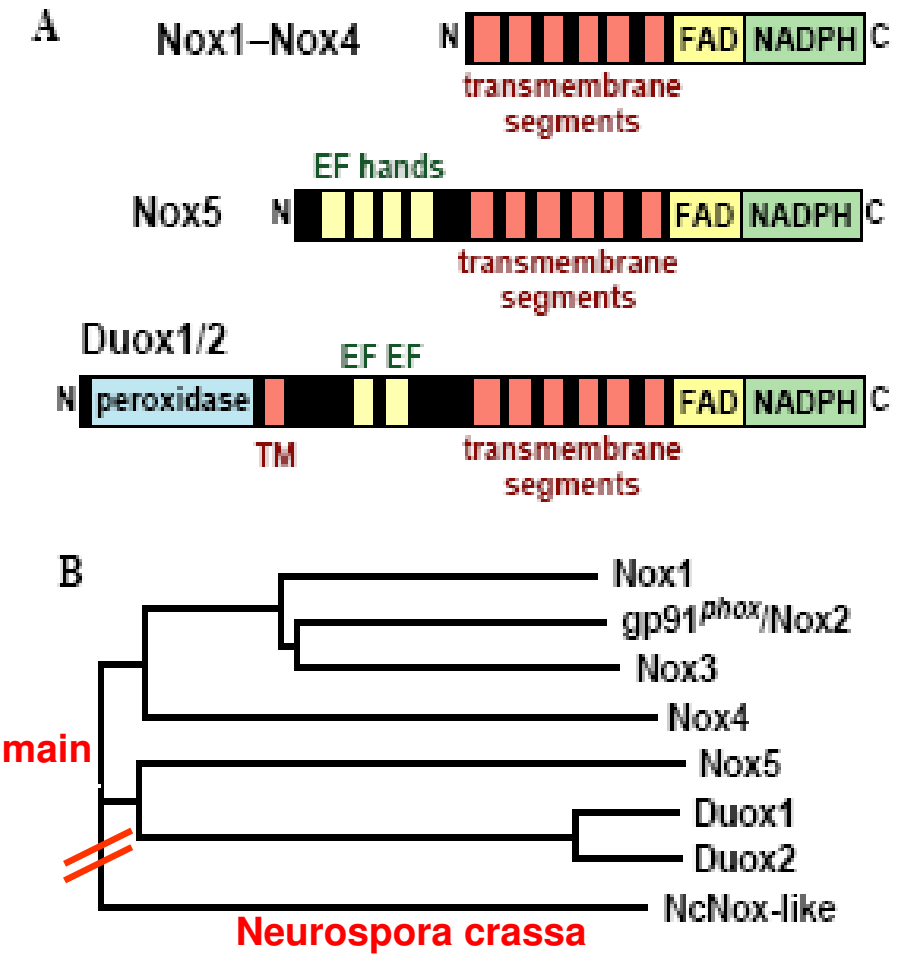


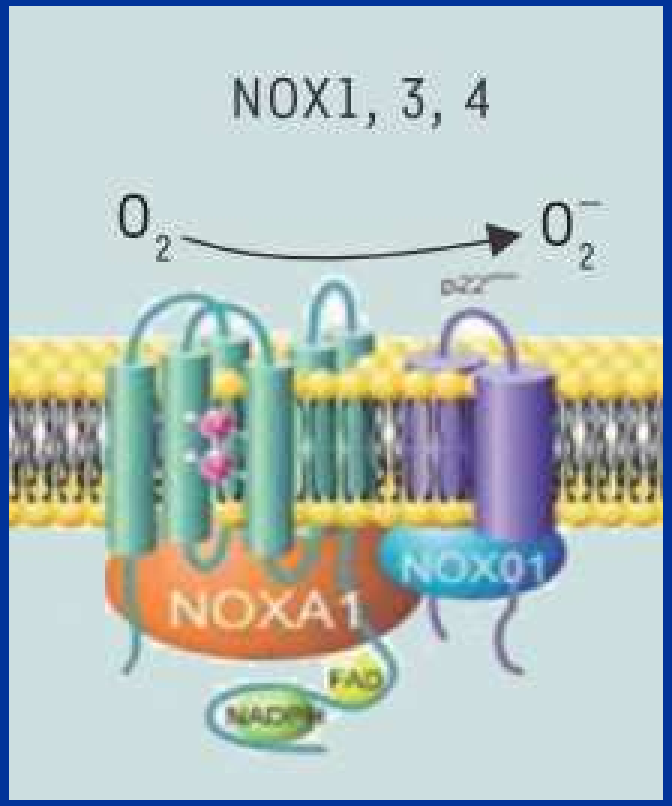
TABLE 2. *Tissue distribution of NOX enzymes*

	High-Level Expression	Intermediate- to Low-Level Expression
NOX1	Colon	Smooth muscle, endothelium, uterus, placenta, prostate, osteoclasts, retinal pericytes
NOX2	Phagocytes	B lymphocytes, neurons, cardiomyocytes, skeletal muscle, hepatocytes, endothelium, hematopoietic stem cells, smooth muscle
NOX3	Inner ear	Fetal kidney, fetal spleen, skull bone, brain
NOX4	Kidney, blood vessels	Osteoclasts, endothelium, smooth muscle, hematopoietic stem cells, fibroblasts, keratinocytes, melanoma cells, neurons
NOX5	Lymphoid tissue, testis	Endothelium, smooth muscle, pancreas, placenta, ovary, uterus, stomach, various fetal tissues
DUOX1	Thyroid	Airway epithelia, tongue epithelium, cerebellum, testis
DUOX2	Thyroid	Salivary and rectal glands, gastrointestinal epithelia, airway epithelia, uterus, gall bladder, pancreatic islets

Bengtsson et al., 2005

Bedard. Physio Rev 2007 ; 87 : 245-313

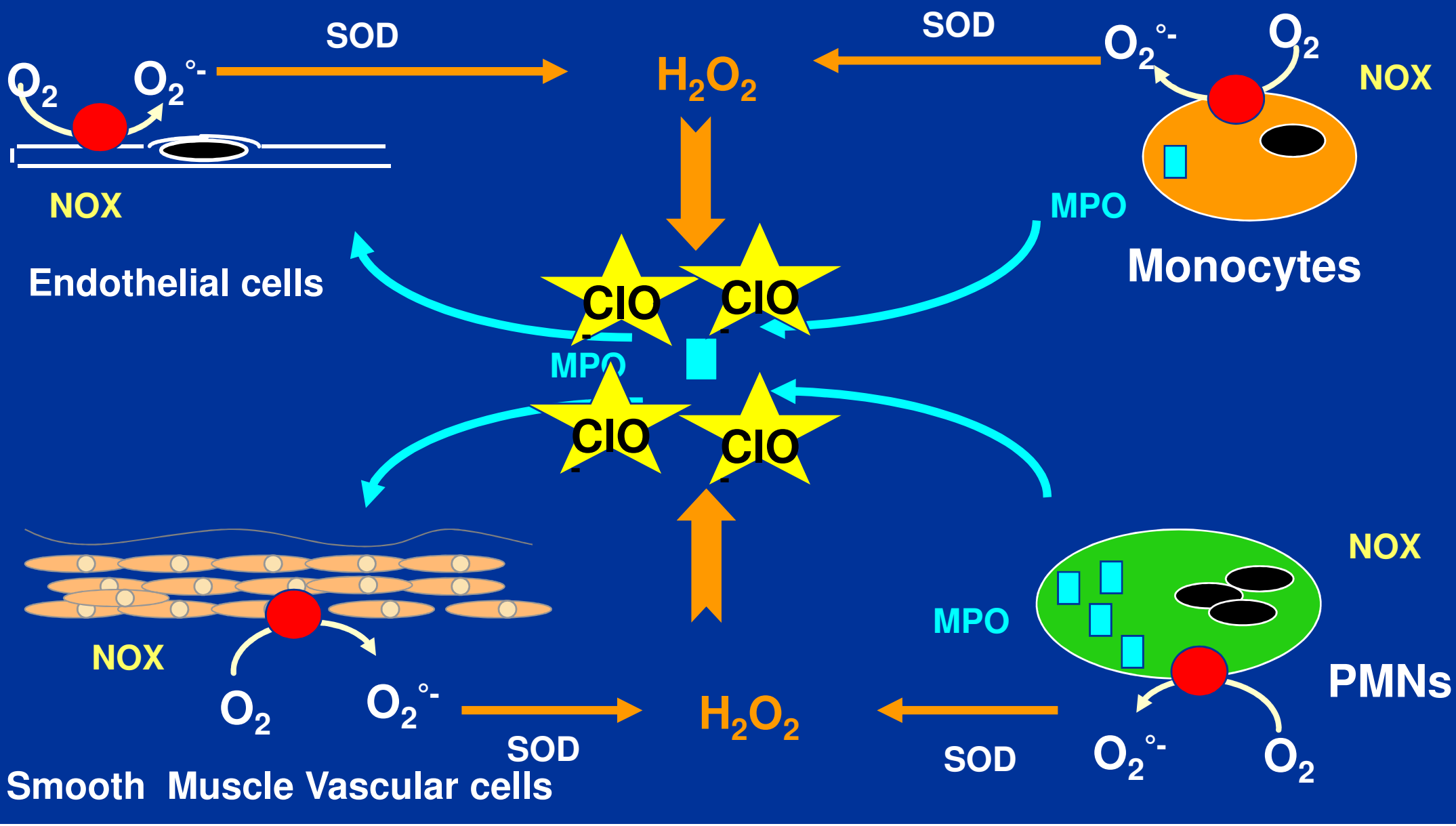
NOX 1, 3 and 4 stimuli and fonctions



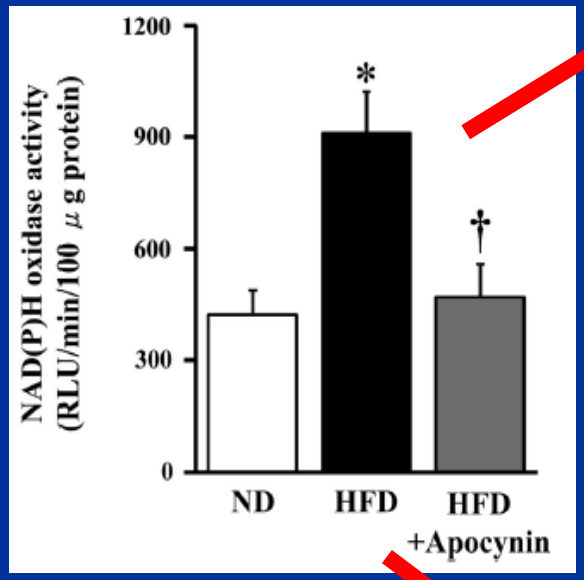
NOX isoformes	Stimulus	Fonction(s)	Type(s) cellulaire(s) localisation
NOX1	H_2O_2	Prolifération	Fibroblastes Cellules épithéliales pulmonaires
	Thrombine		Cellules musculaires lisses vasculaires
	Angiotensine II	Hypertrophie	Cellules musculaires lisses vasculaires
	PDGF	Migration	
	FGF		
	TNF-α	Nécrose	Cellules épithéliales du côlon
	LPS – Flagelline	Défense de l'hôte	
NOX3	cisplatine	Ototoxicité	Oreille interne
NOX4	IGF-1	Migration	Cellules musculaires lisses vasculaires
	PDGF		
	Angiotensine II	Survie et croissance	Cellules mésangiales
	TGF-β		Cellules musculaires lisses des voies aériennes
	Insuline	Différenciation	Adipocytes

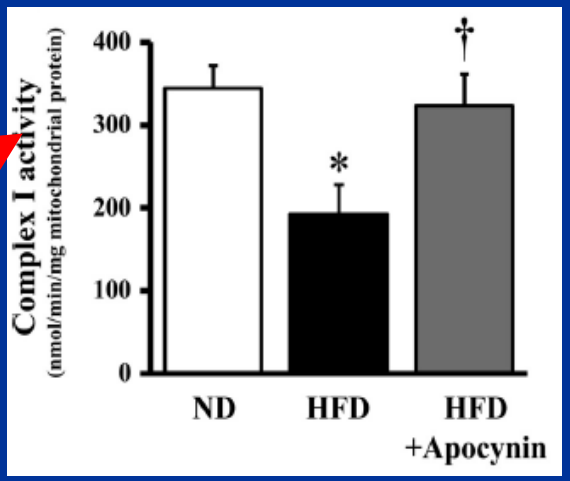
Migdal. Médecine sciences 2011; 27 : 405 412

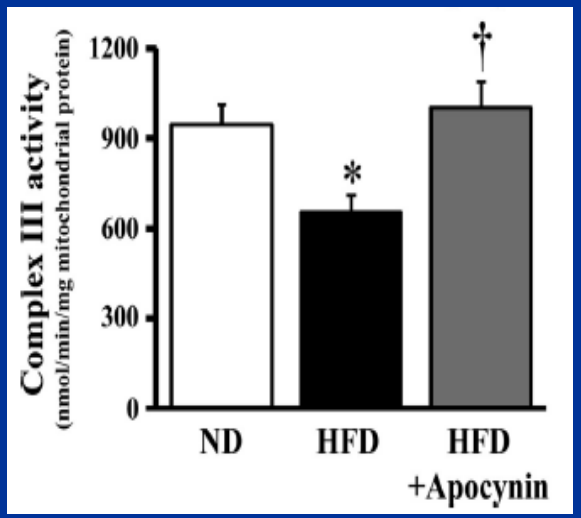
The synergistic action of NADPH oxidase and MPO

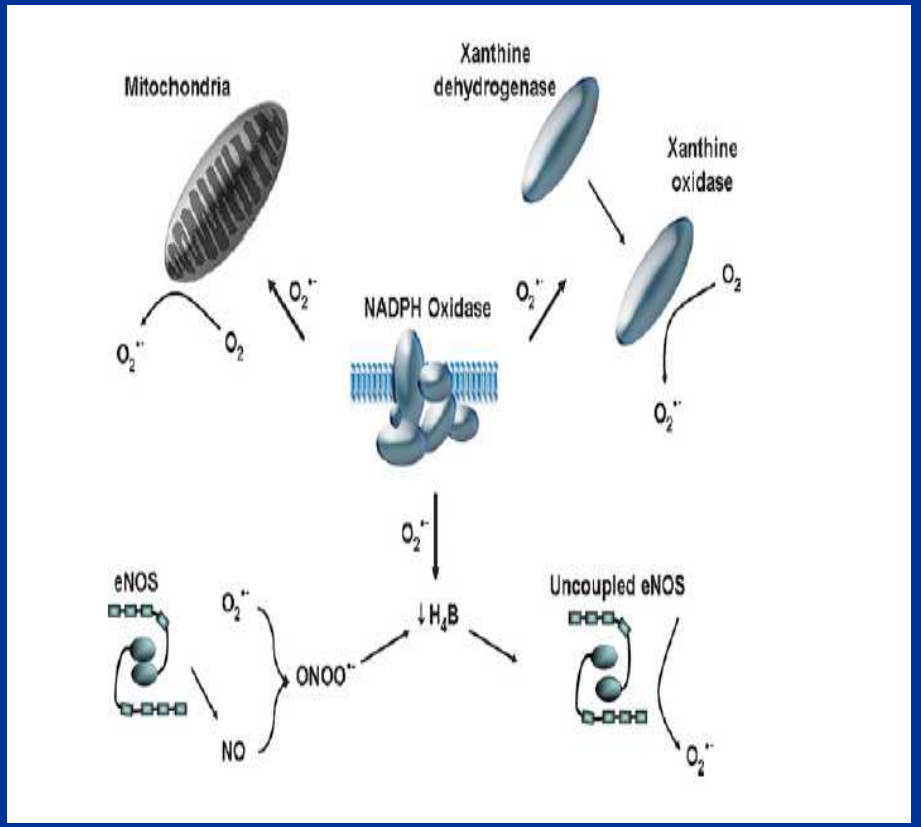


Interactions between NADPH Oxydase - MPO - Mitochondria









(Ray et Shah 2005)

Yokota T. et al., Am J Physiol Heart Circ Physiol 297:H1069-H1077, 2009

Exploring Oxidative Stress

I) Oxidative stress biomarkers :

One lipid biomarker (IsoPs), 1 protein biomarker (CML or pentosidine) or GSH/GSSG ratio, 1 nucleic acid biomarker : 8-OH-desoxyguanosine

II) Quantification of Oxidant production ?

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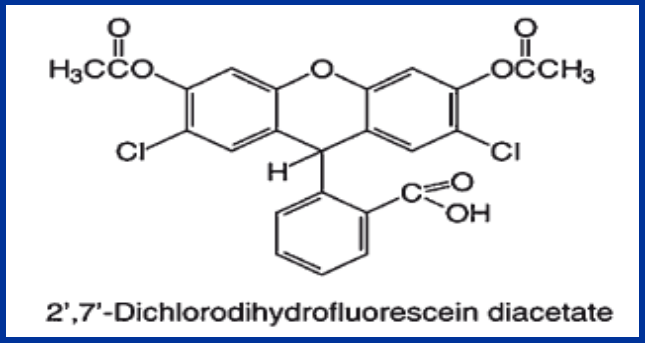
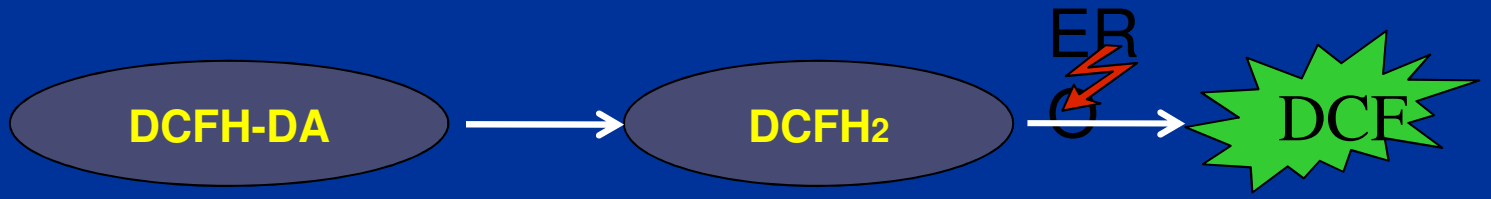
ROS determination could be determined using DCFH-DA (dichlorodihydrofluorescein diacétate)

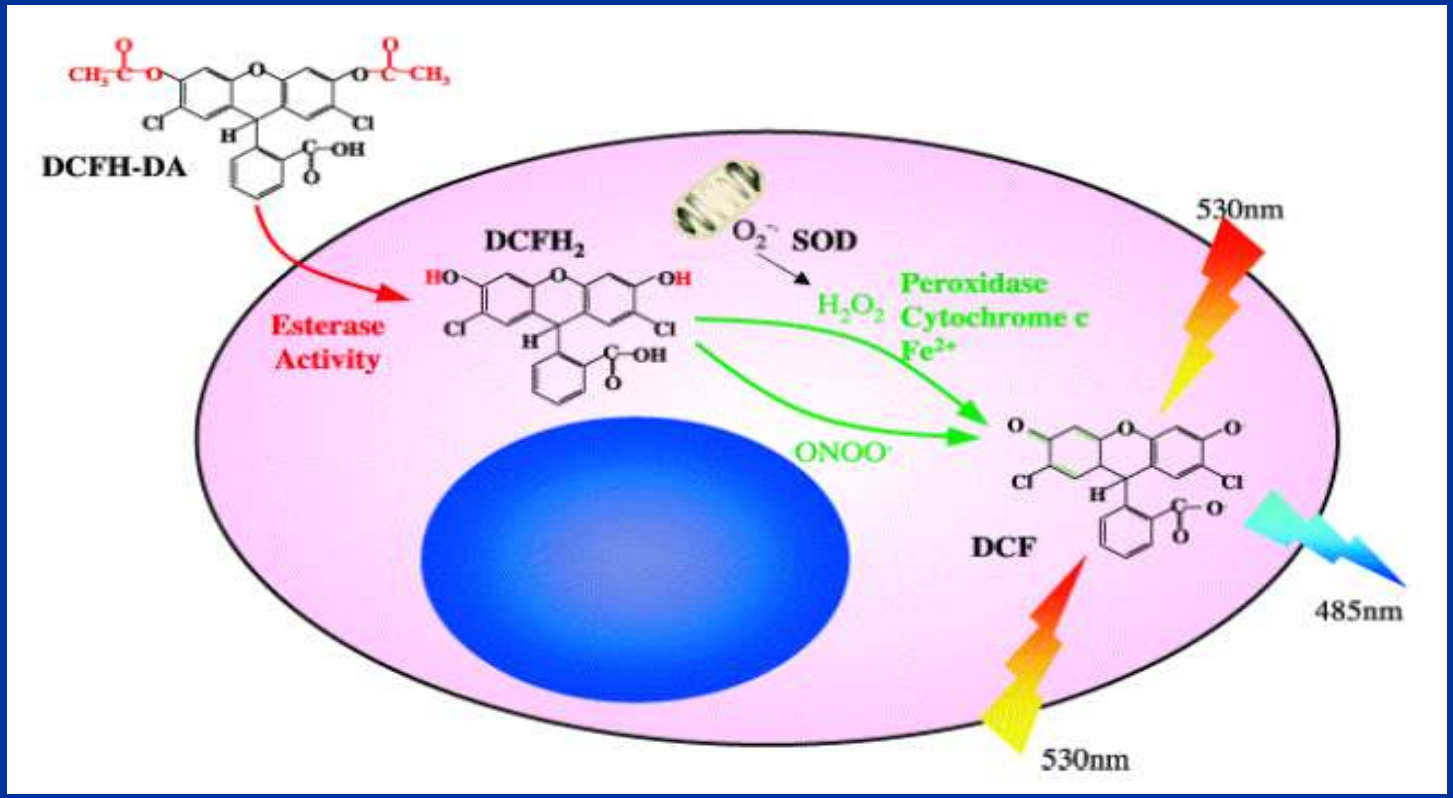




Non fluorescent

Non fluorescent

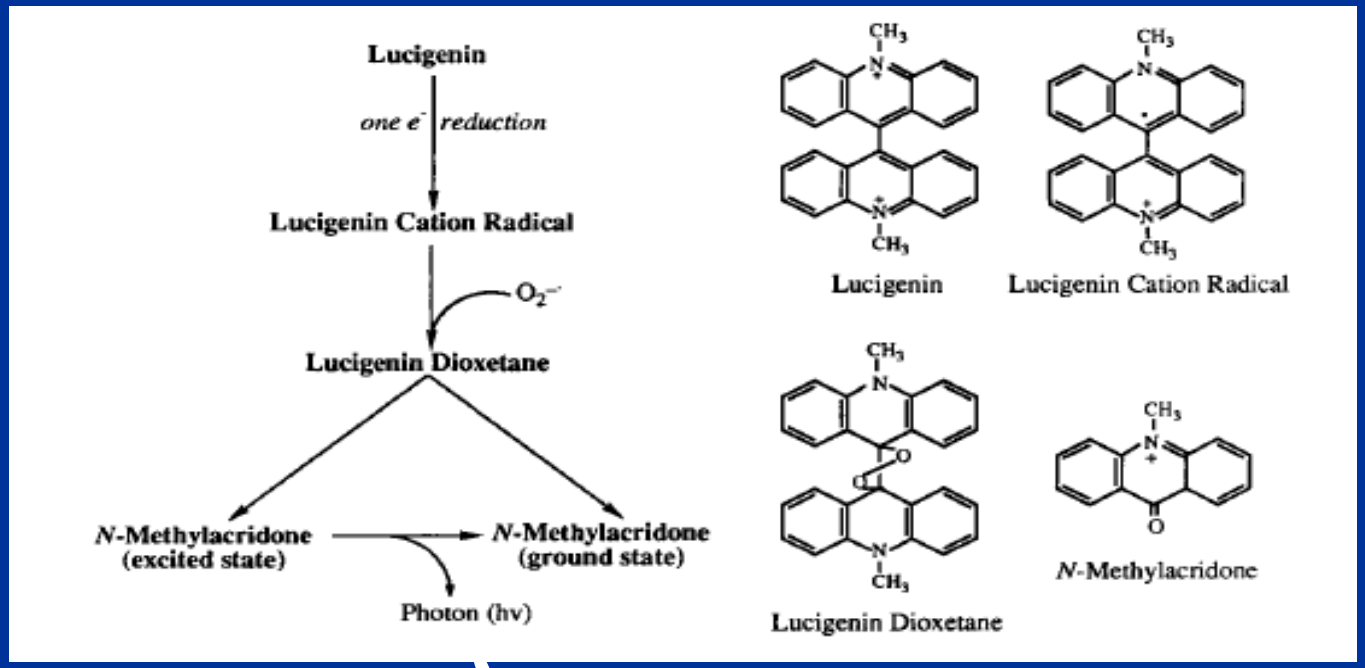
fluorescent



DCFH-DA →DCFH2 piégé à l'intérieur de la cellule

DCFH2 piégé subit l'oxydation → DCF fluorescent

La production d'anion superoxyde par la lucigénine : principe

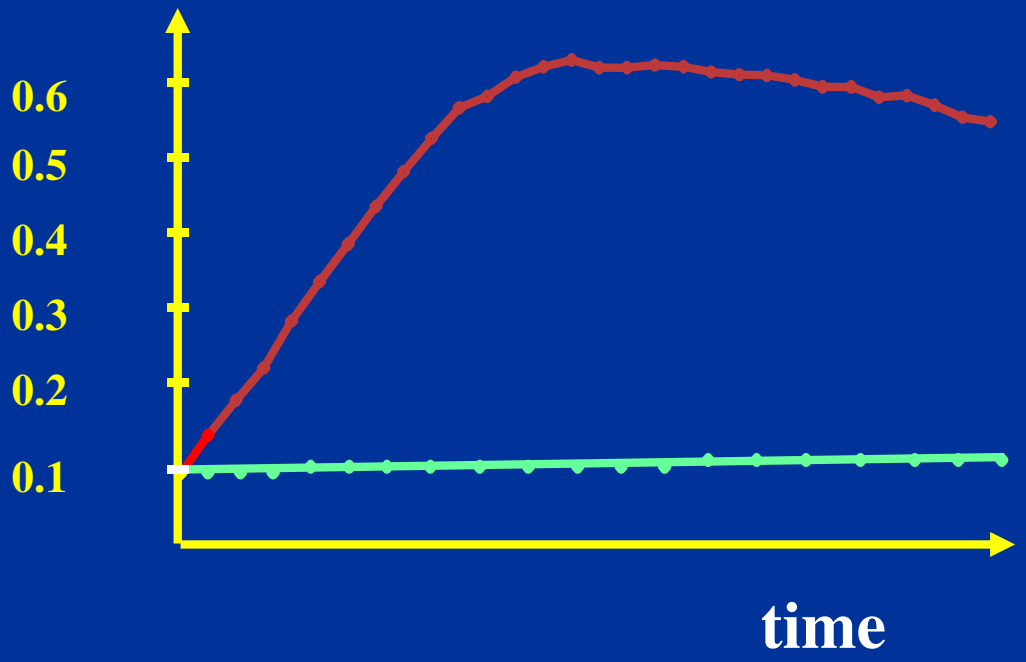


Li Y. The Journal of Biological Chemistry Vol. 273(4): 2015–2023, 1998

Activity and expression of NADPH Oxydase : cell models

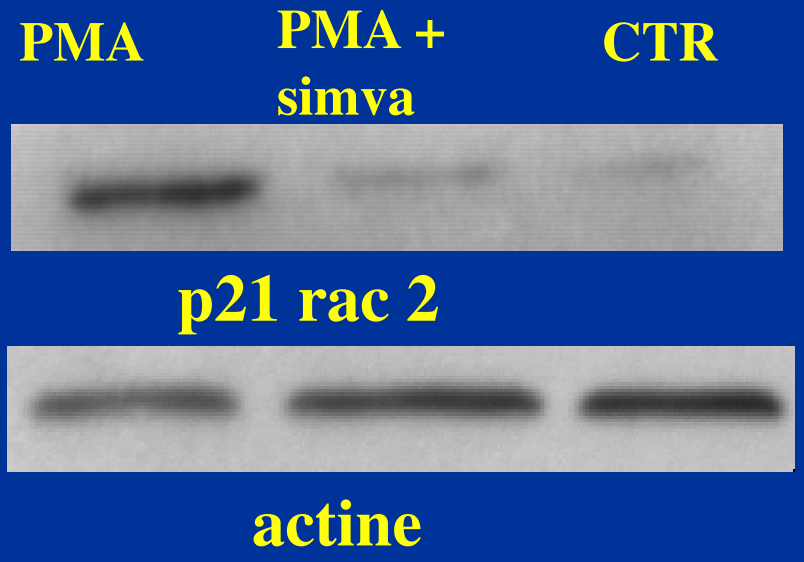
- Activité cellulaire :

Lucigénine : anion superoxyde



- Etude des sous unités :

Western Blotting



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Oxidative stress and cell information

Cell components

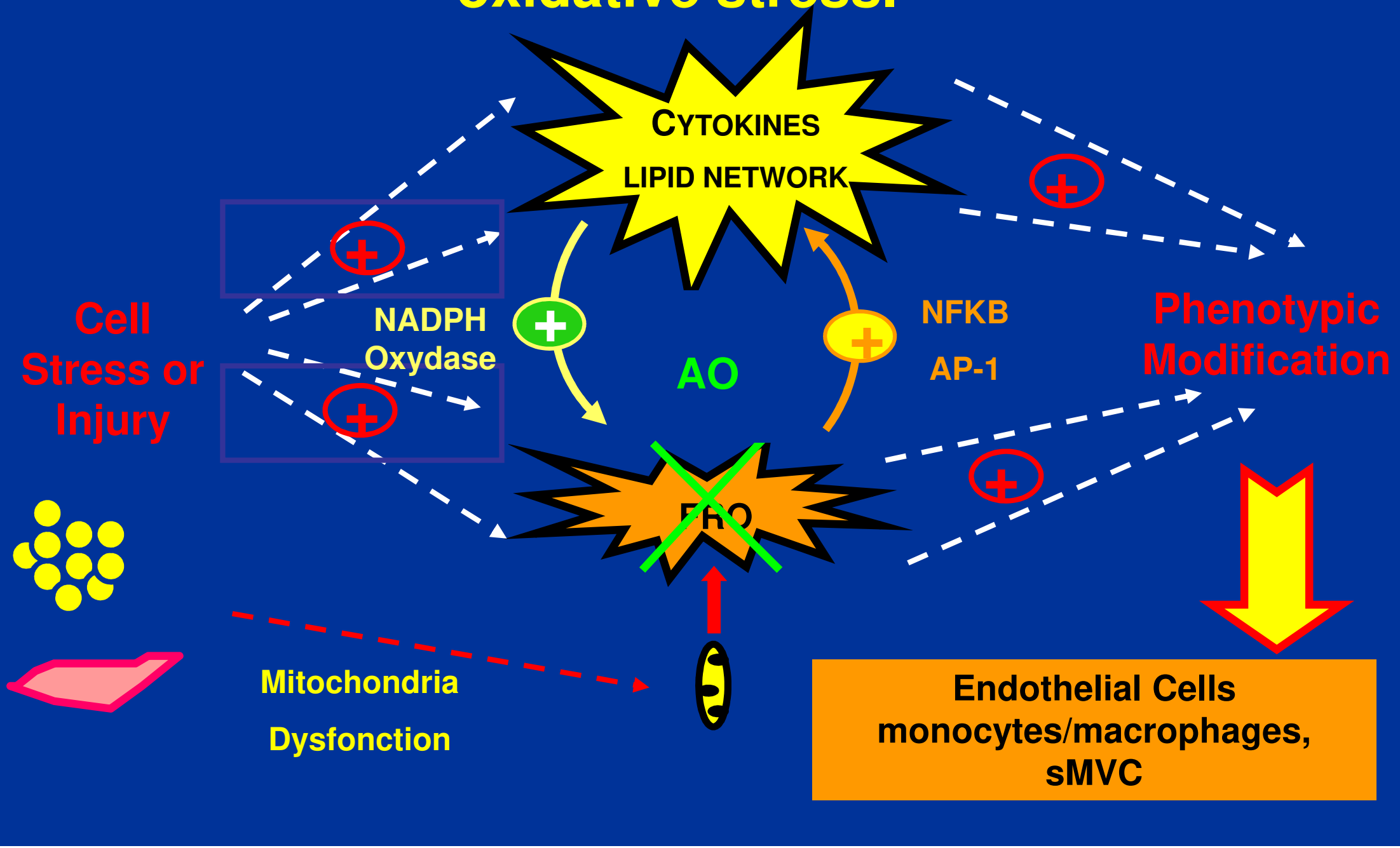
Oxidative stress biomarkers
Cell toxicity

Transcription
factor

de novo protein synthesis

Proinflammatory proteins

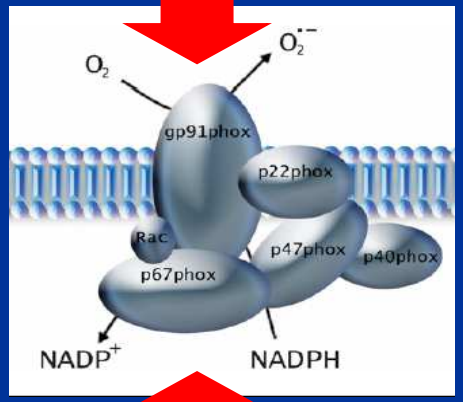
Amplification loops between inflammation and oxidative stress.



Working hypothesis …
A clinical proof in elderly

Analyse Univariée
Hcy(p<0.01), CRP(p<0.01), Fibrinogène (p<0.01), α-1 glycoprotéine acide (p=0.03), Albumine(p=0.01), TG(p=0.02), CT(p=0.05)

n = 478
> 65 ans



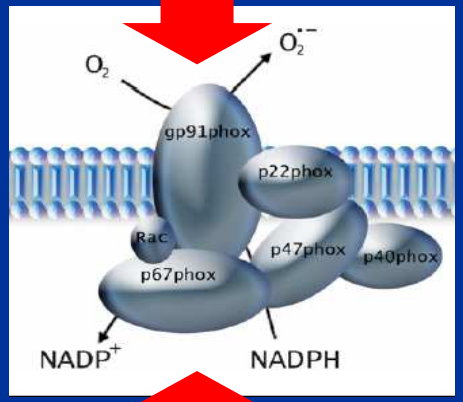
Analyse Multivariée : Déterminants de la production d'$O_2^{\bullet -}$
Homocystéine (p<0.02), CRP (p<0.01)

(Ventura et al, Free Radical Biol Med, 2009)

Working hypothesis …
A clinical proof in CKD

Analyse Univariée
MDRD(p<0.004), Fibrinogène (p<0.02), HDL (p=0.03), PTH (p=0.04), Hémoglobine (p=0.05), HTA (p=0.08)

n = 136
Stades 1-5



Analyse Multivariée : Déterminants de la production d'$O_2^{\bullet -}$
Fibrinogène (p<0.04), HDL (p<0.04), MDRD (p<0.04)

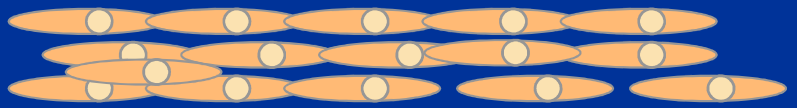
Morena et al., Free Radical Research, June 2011; 45(6): 735–745

Oxidative stress, amplification loops and aterosclerosis

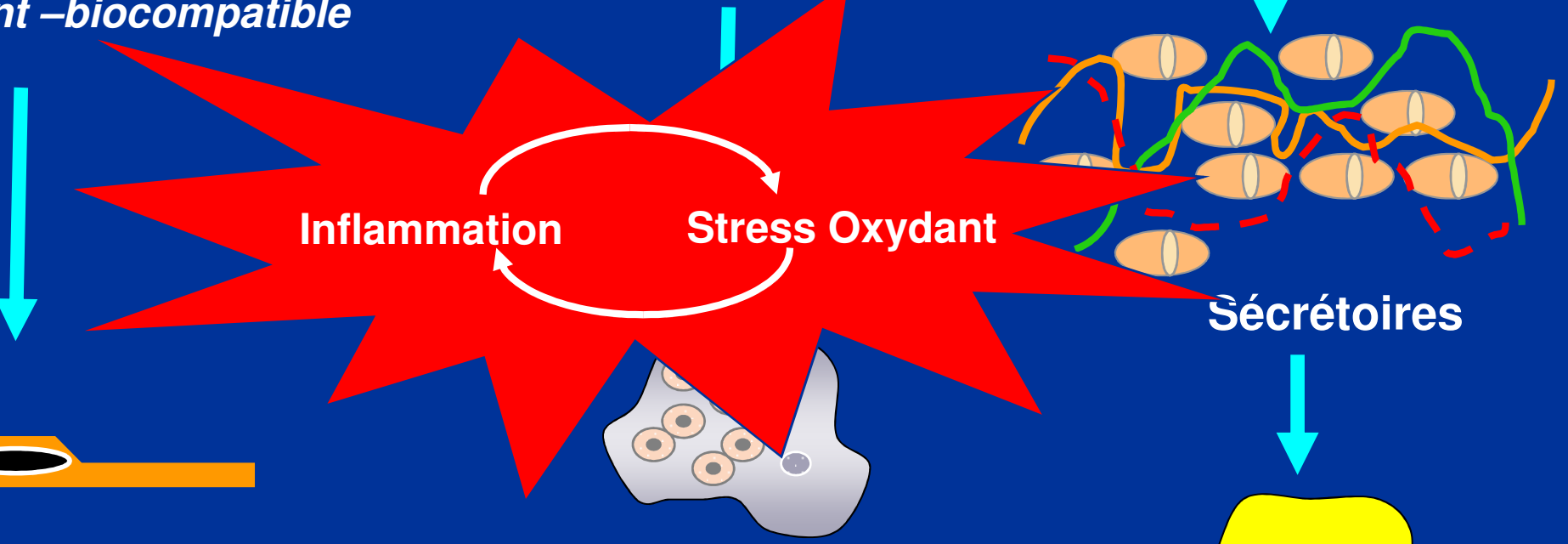
Cellules endothéliales

Monocytes/ Macrophages

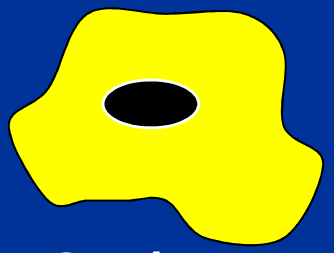
Cellules musculaires lisses Contractiles



Vasorelaxation
Antiagrégant –biocompatible



Sécrétoires



Dysfonction endothéliale :
Vasoconstricton
Adhérence
Prothrombotique

Cellules spumeuses

Ostéoblastiques

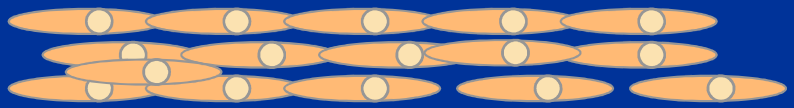
Stress oxydant et transdifférentiation cellulaire

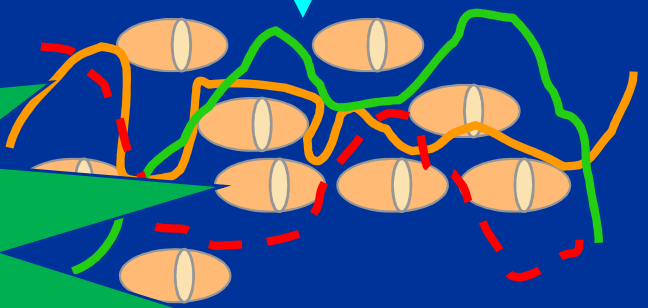
Cellules endothéliales

Vasorelaxation
Antiagrégant –biocompatible

Monocytes/ Macrophages

Cellules musculaires lisses Contractiles

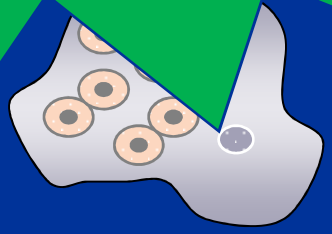




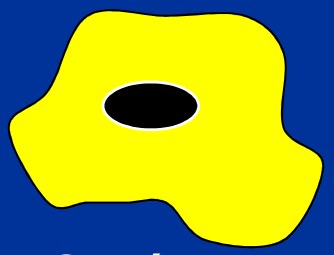
Sécrétoires

Anti-oxidants

Dysfonction endothéliale :
Vasoconstricton
Adhérence
Prothrombotique

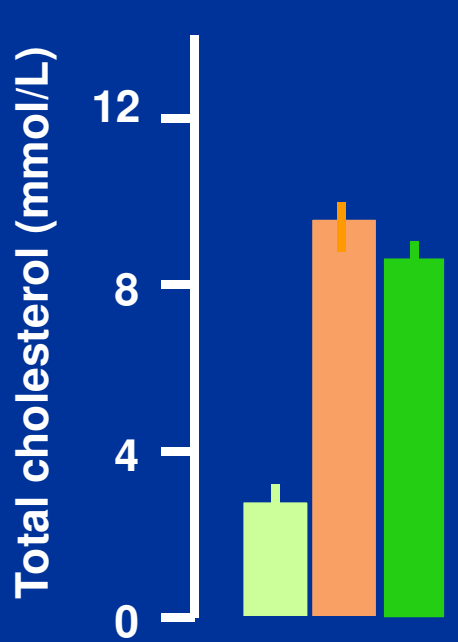


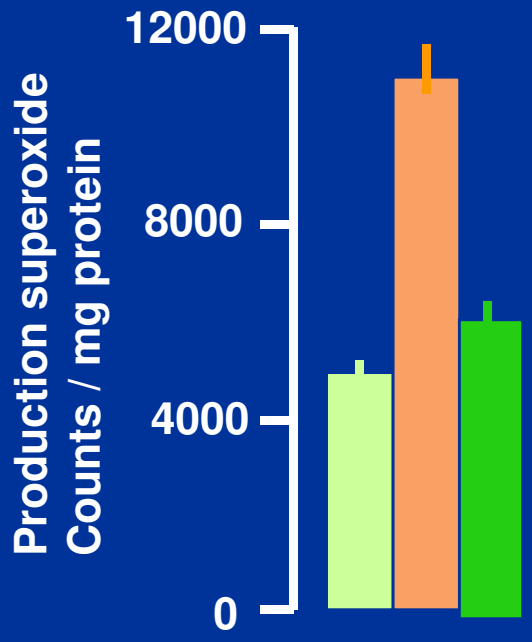
Cellules spumeuses

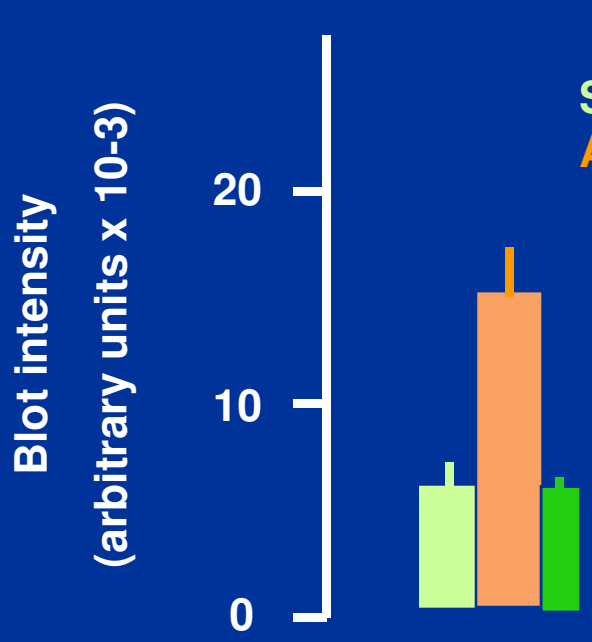


Ostéoblastiques

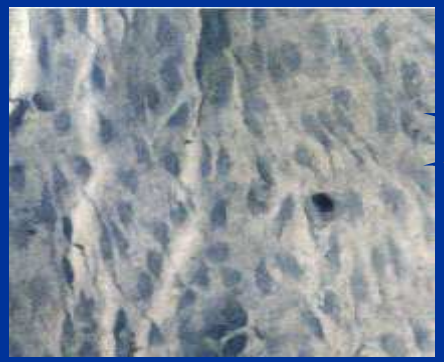
Nutritional preventionof atherosclerosis:

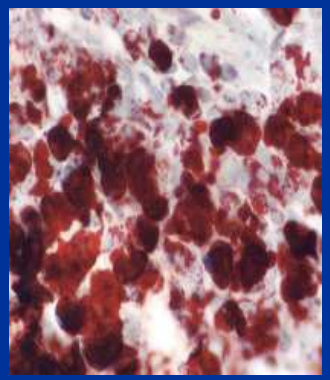






Standard
Athérogène





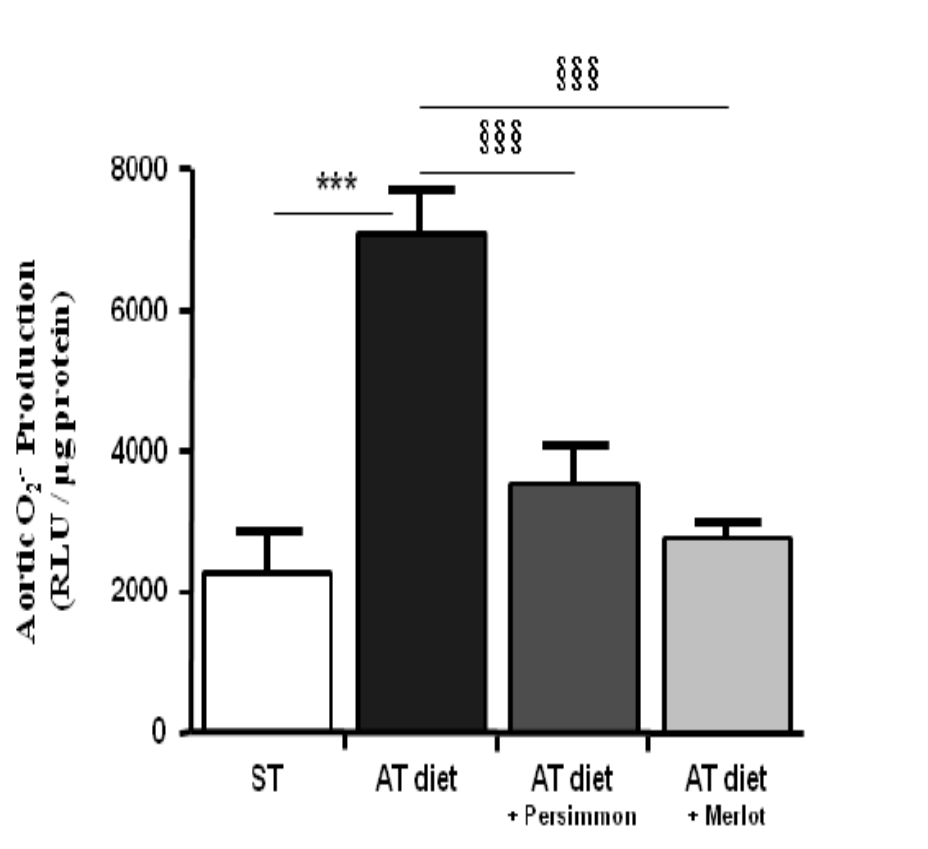
CTR Aorta

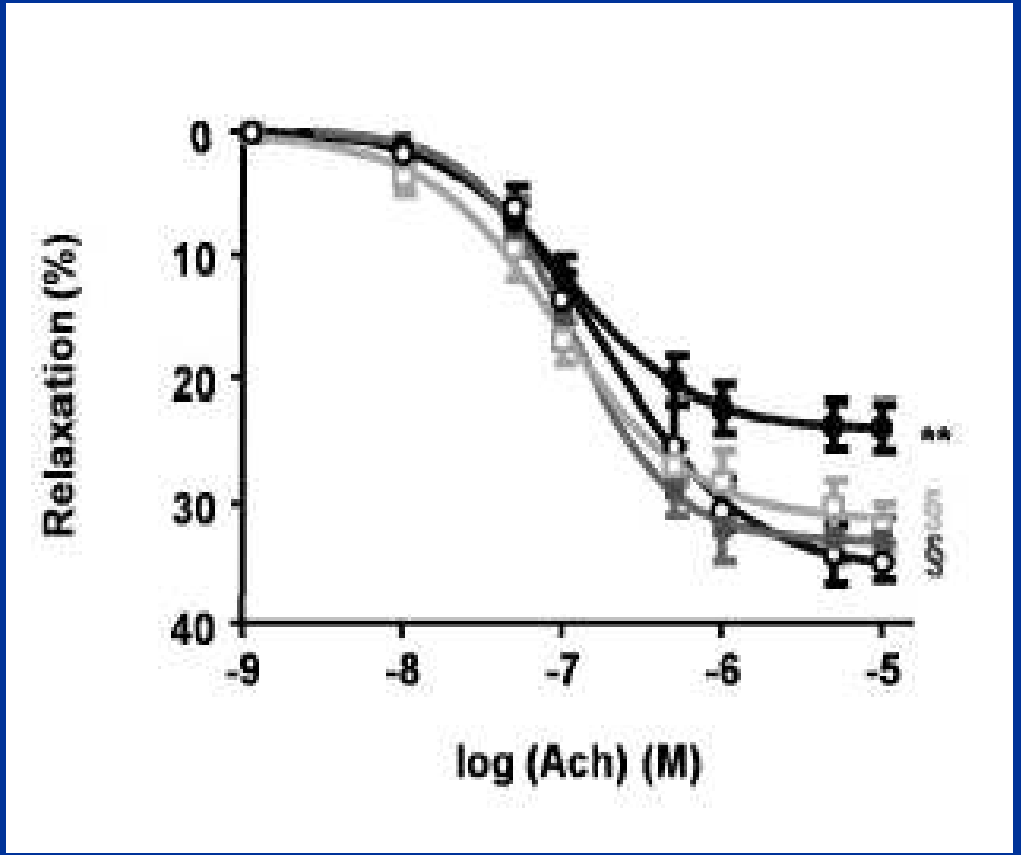
Nutritional
prevention

Vegetal extract

(Sutra.T. et al., 2007)

Les Polyphenols préviennent la dysfonction endothéliale chez les hamsters





Suh JH, et al., Food Funct. 2011

Exploring Oxidative Stress

I) Oxidative stress biomarkers :

One lipid biomarker (IsoPs), 1 protein biomarker (CML or pentosidine) or GSH/GSSG ratio, 1 nucleic acid biomarker : 8-OH-desoxyguanosine

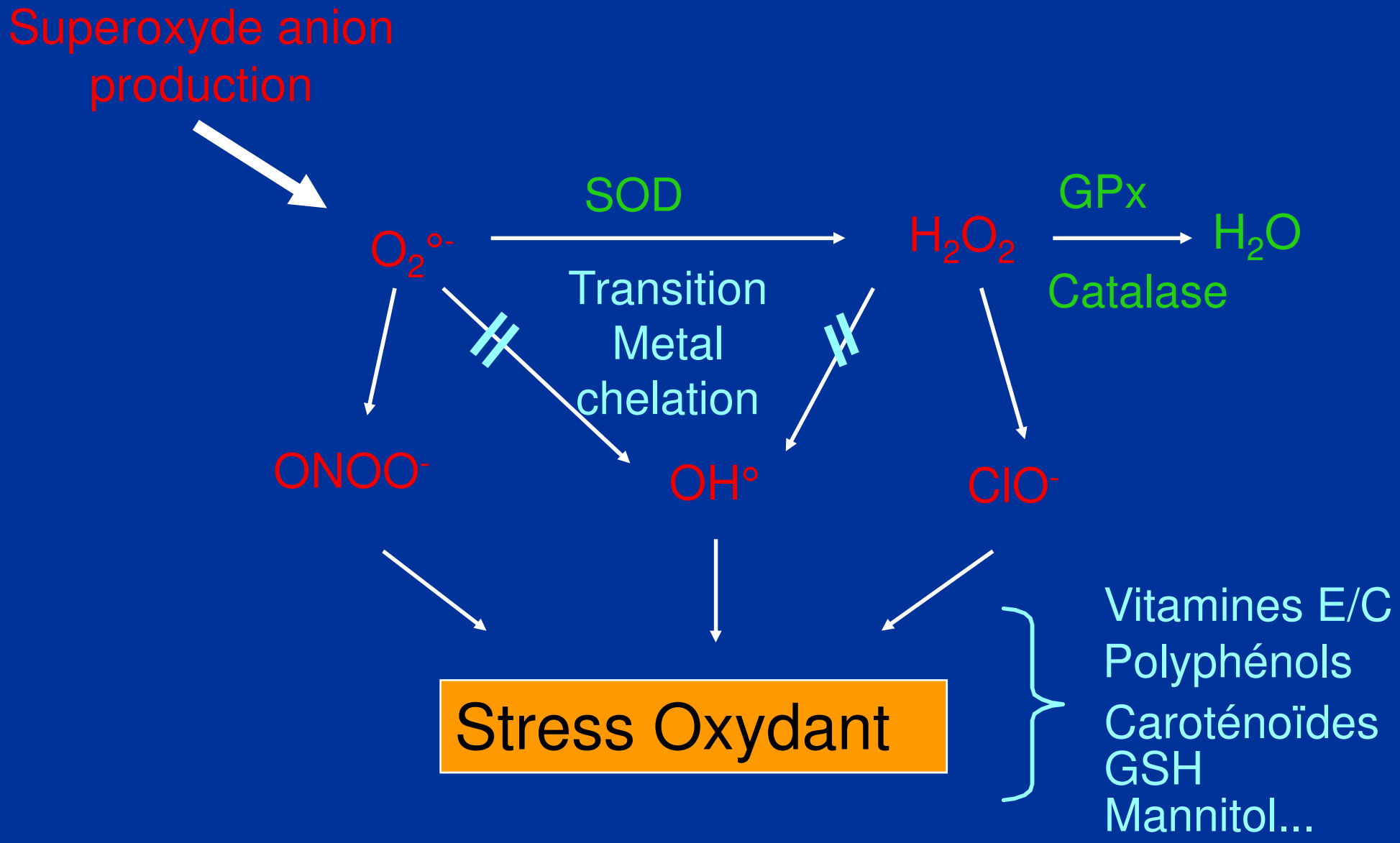
II) Quantification of Oxidant production ?

How are ROS produced?
How to quantify ROS ?
How to moduate ROS ?

III) Investigation of defense mechanisms ?

How to measure the defense mechanism
How to interprete the defense mechanism
How to modulate defense mechanism

Defense mechanisms

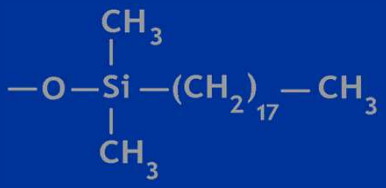


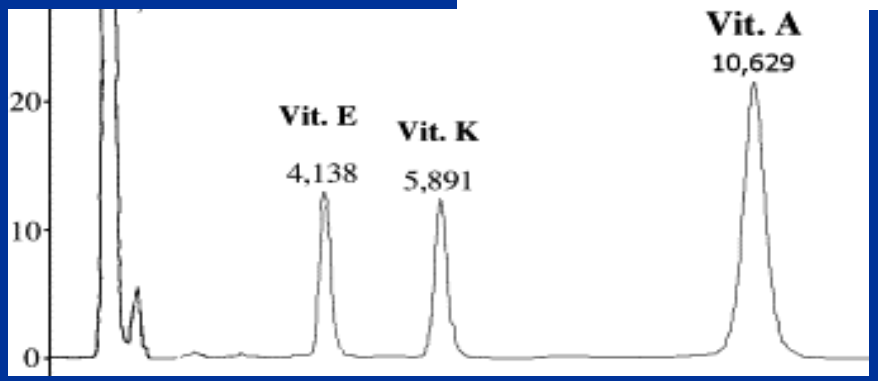
Détection des vitamines : méthodes analytiques

- Les vitamines A, C et E plasmatiques sont dosées par HPLC-UV
- La vitamine E érythrocytaire est dosée par HPLC-Electrochimie

Conditions chromatographiques similaires

Colonne en phase inverse C18





Elution avec un gradient de solvant de polarité croissante

Seul le détecteur change:

- *Le détecteur UV* mesure l'absorption de la lumière par le produit à la sortie de la colonne.

Conditions: Il faut que le produit à détecter absorbe la lumière à une longueur **d'onde accessible à l'appareil et que** la phase mobile n'absorbe pas la lumière à la longueur d'onde choisie par l'opérateur

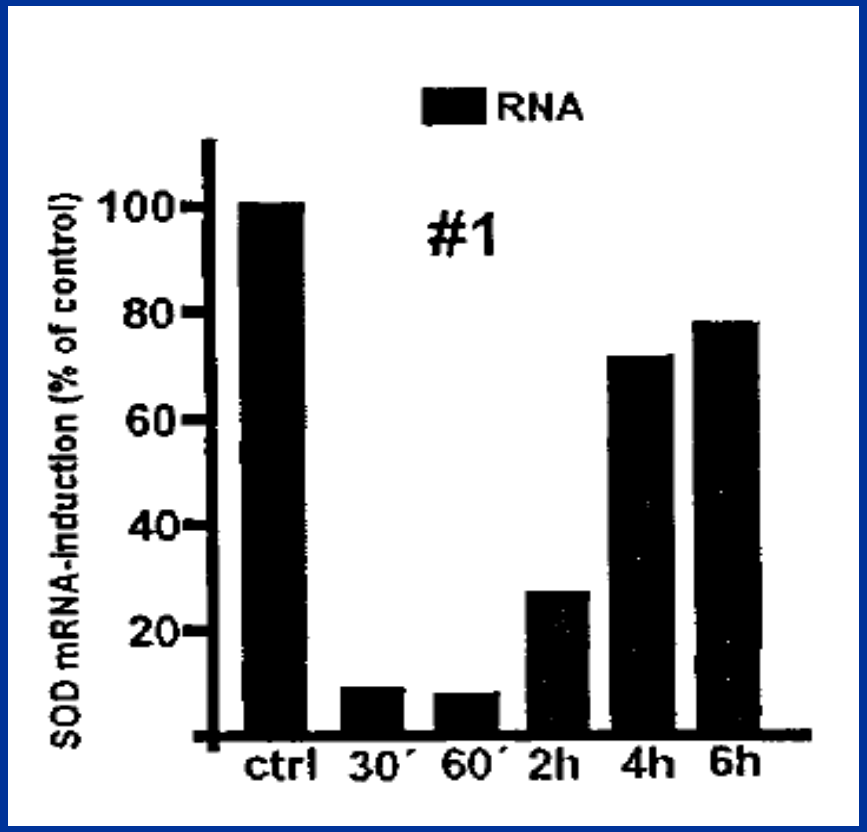
- *Le détecteur électrochimique* mesure les réactions d'oxydoréductions qui produisent un courant proportionnel à la concentration du soluté.

Meilleure sensibilité

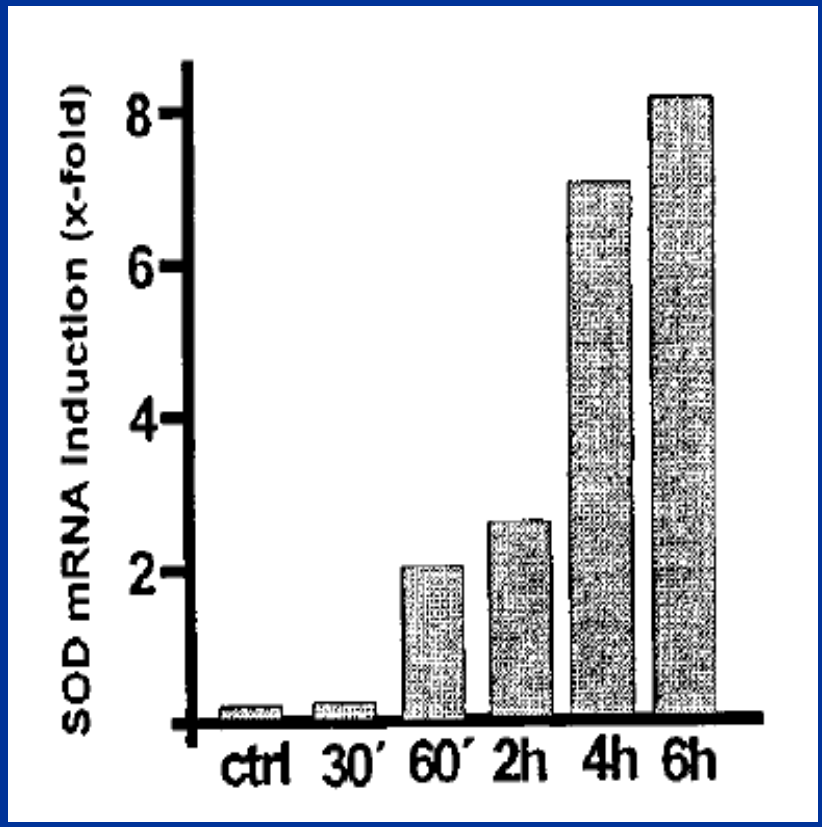
Limitations of antioxidant system determination

- Tissue variability :
- Different origin of antioxidant system :
  - Enzymatic system : synthesis and induction
  - Non enzymatic : strictly dependant of nutritional support
- Duration of the insult :
  - Consomtion
  - Induction
- Comorbidity and nutritional status:
  - age :
  - nutritional status…

Enzymatic system : kinetics analysis



Cu/Zn SOD : rein de rat



Mn SOD : rein de rat

Leach M., B Pharmacol J., 1998

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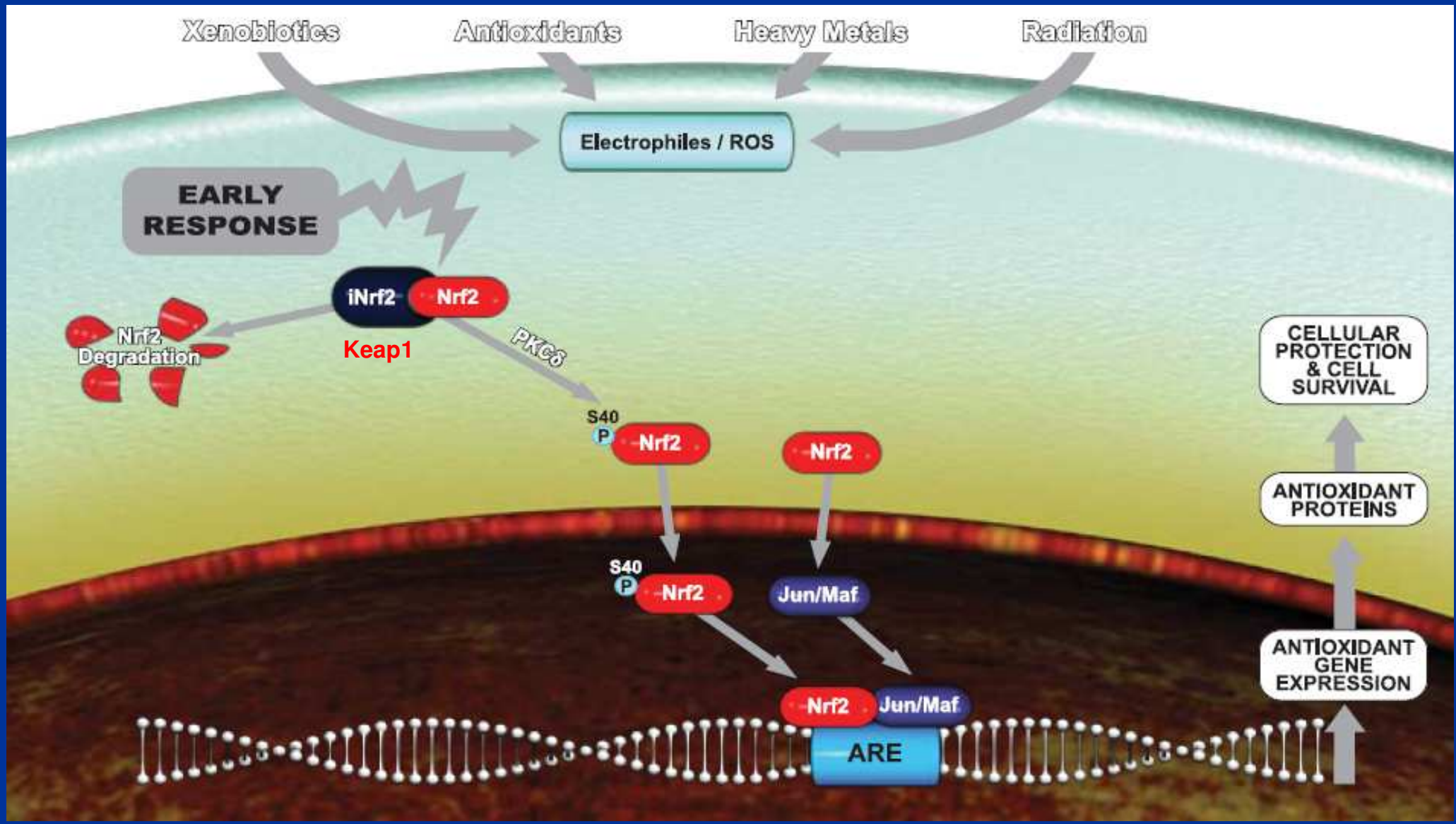
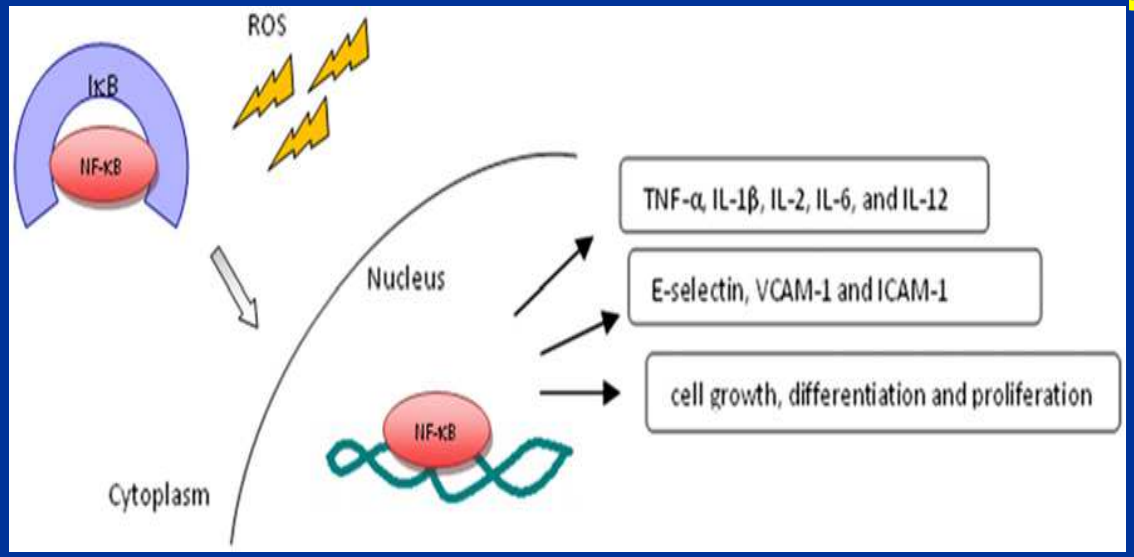
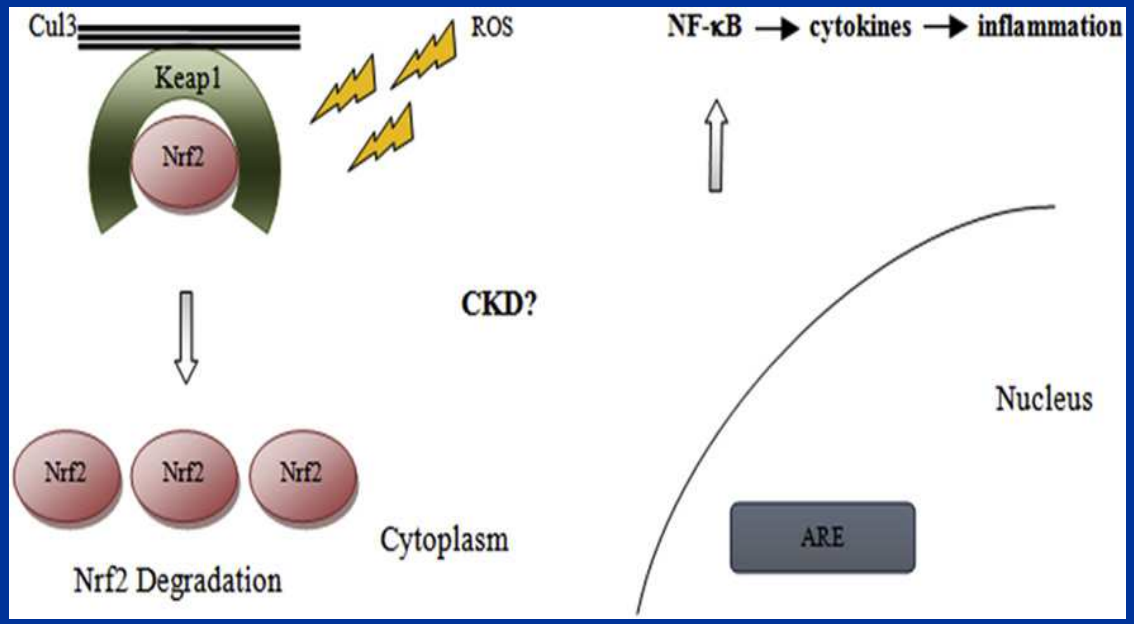
Inducing enzymatic defense: the Nrf2 pathway

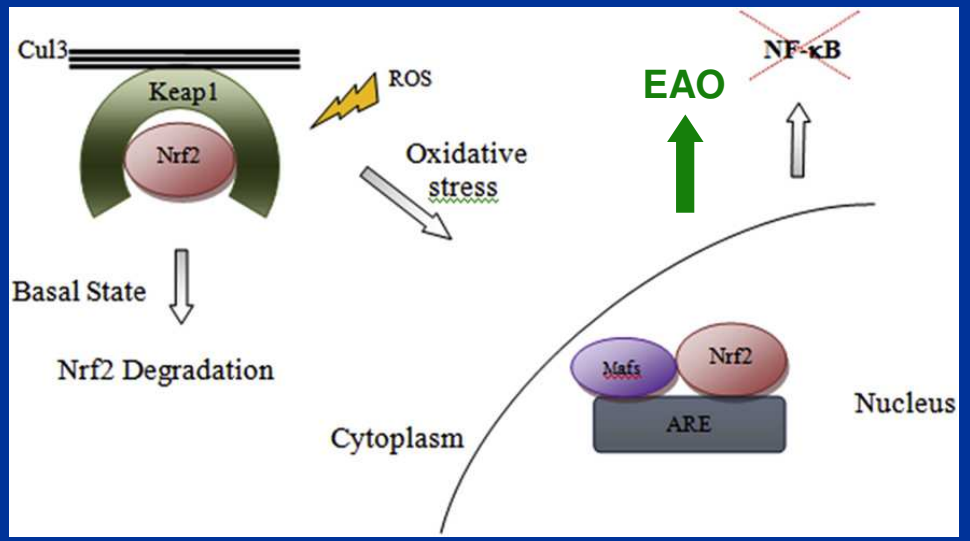


Jaiswal AK, www.SABiosciences.com/support_literature.php

Un équilibre dépendant de l'intensité du stress oxydant ?







L.M. Pedruzzi et al. / Biochimie xxx (2012) 1-6

Les antioxydants (flavonoïdes) stimulent … Les enzymes antioxydantes …. via le Nrf 2 ?

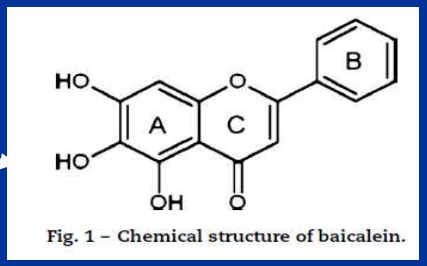
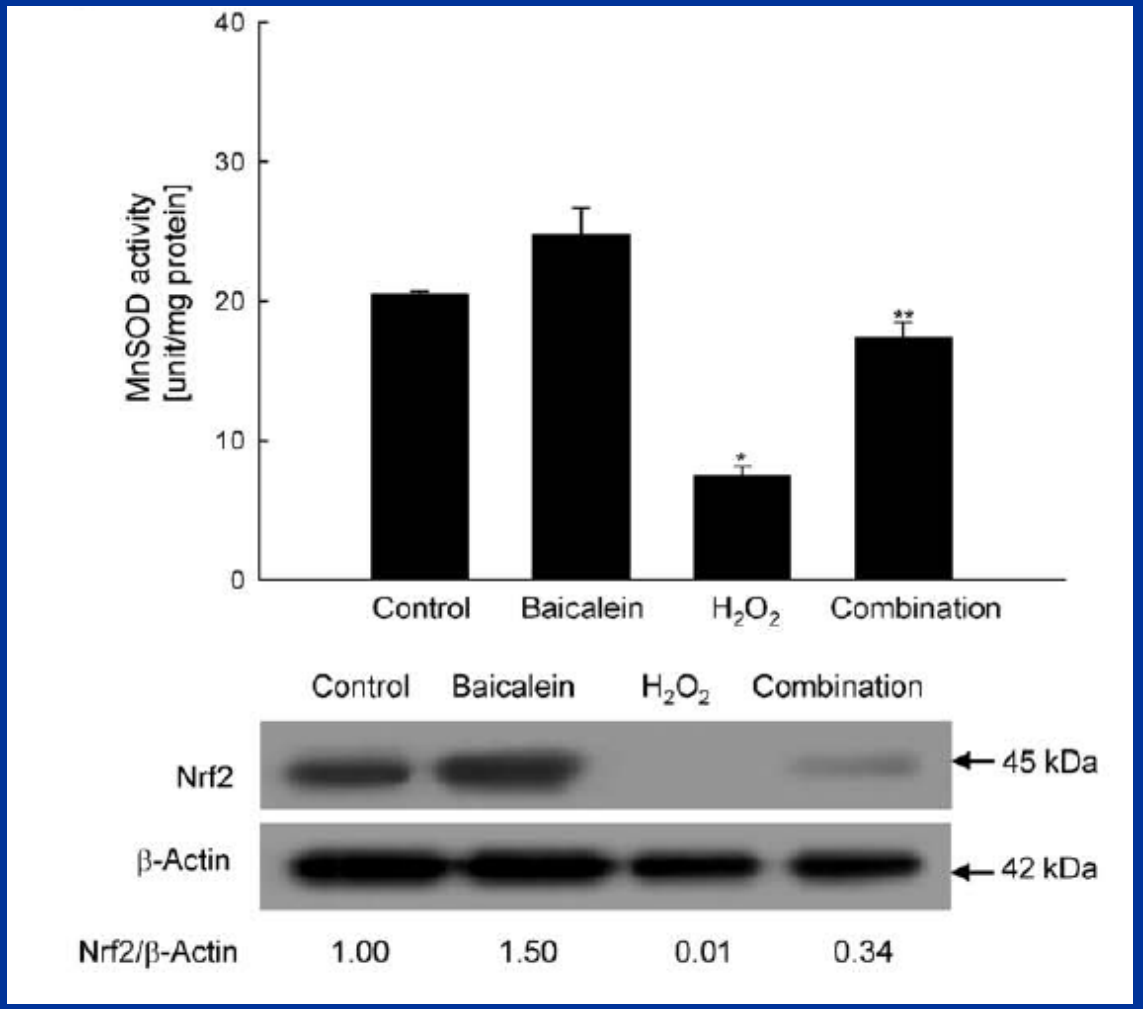
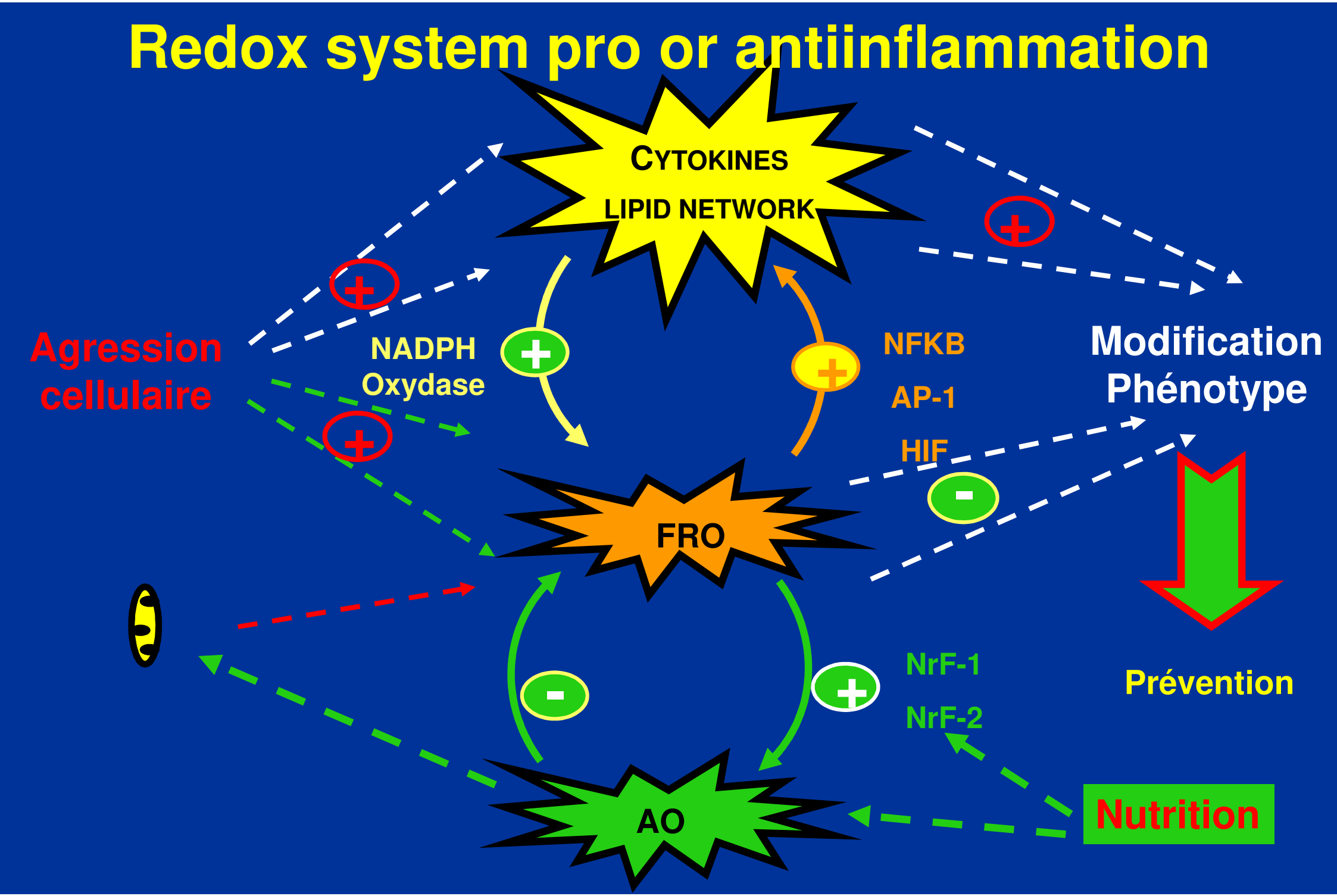


Fig. 1 – Chemical structure of baicalein.



Lee I.K. Environmental toxicology and pharmacology 2 0 1 1

Redox system pro or antiinflammation



Redox system and cardiovascular disease