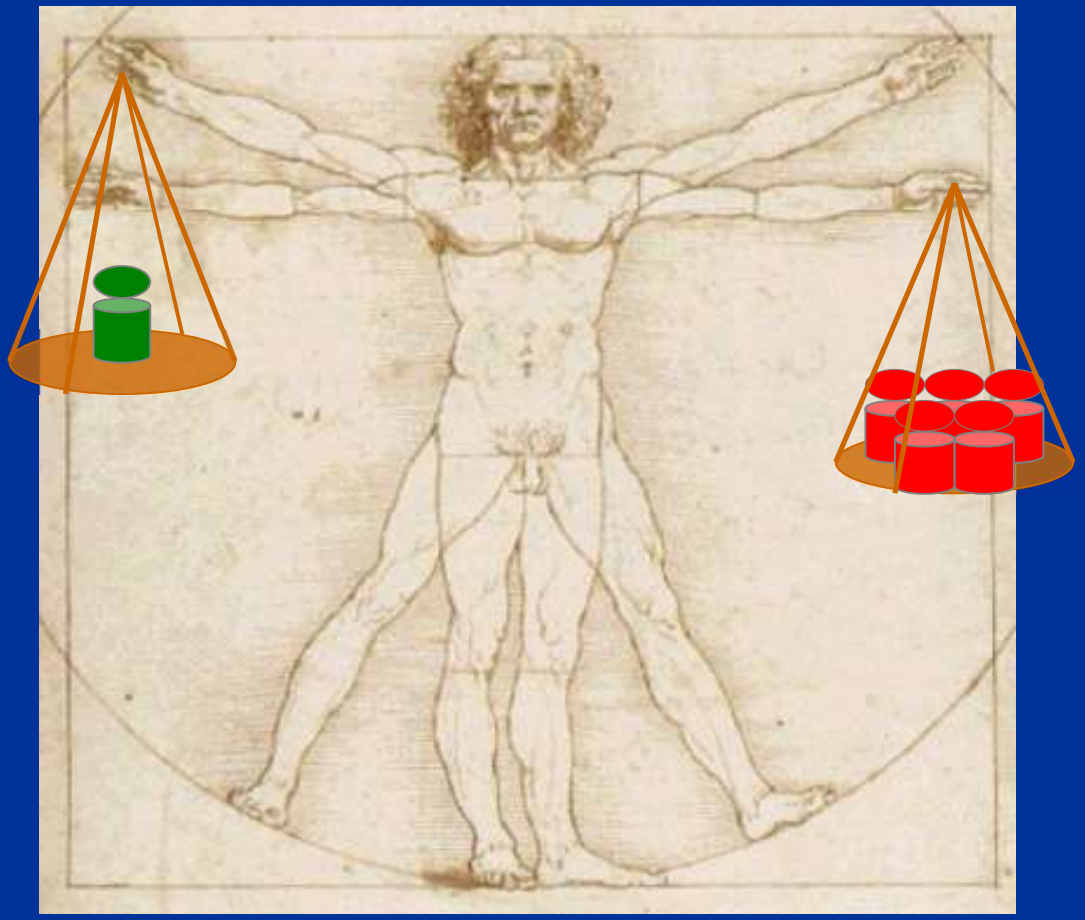
# Oxidative stress :
# From toxicity to cell interactions

# Oxidative stress an umballance between oxidants and antioxidants

Antioxidants:

Is it possible to appreciate defense mechanism ?

Enzymatic or non enzymatic defenses

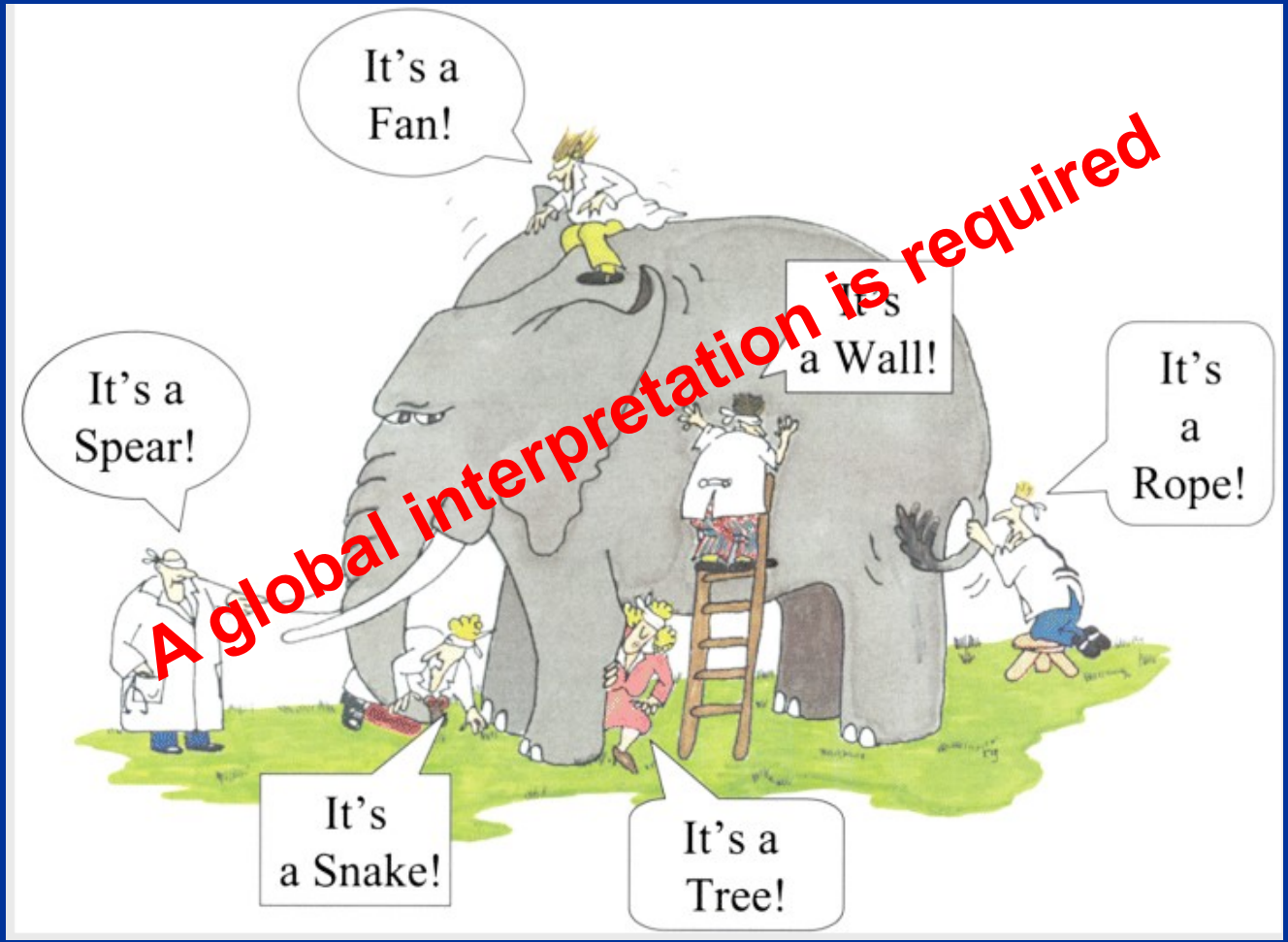


Oxidants:

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*

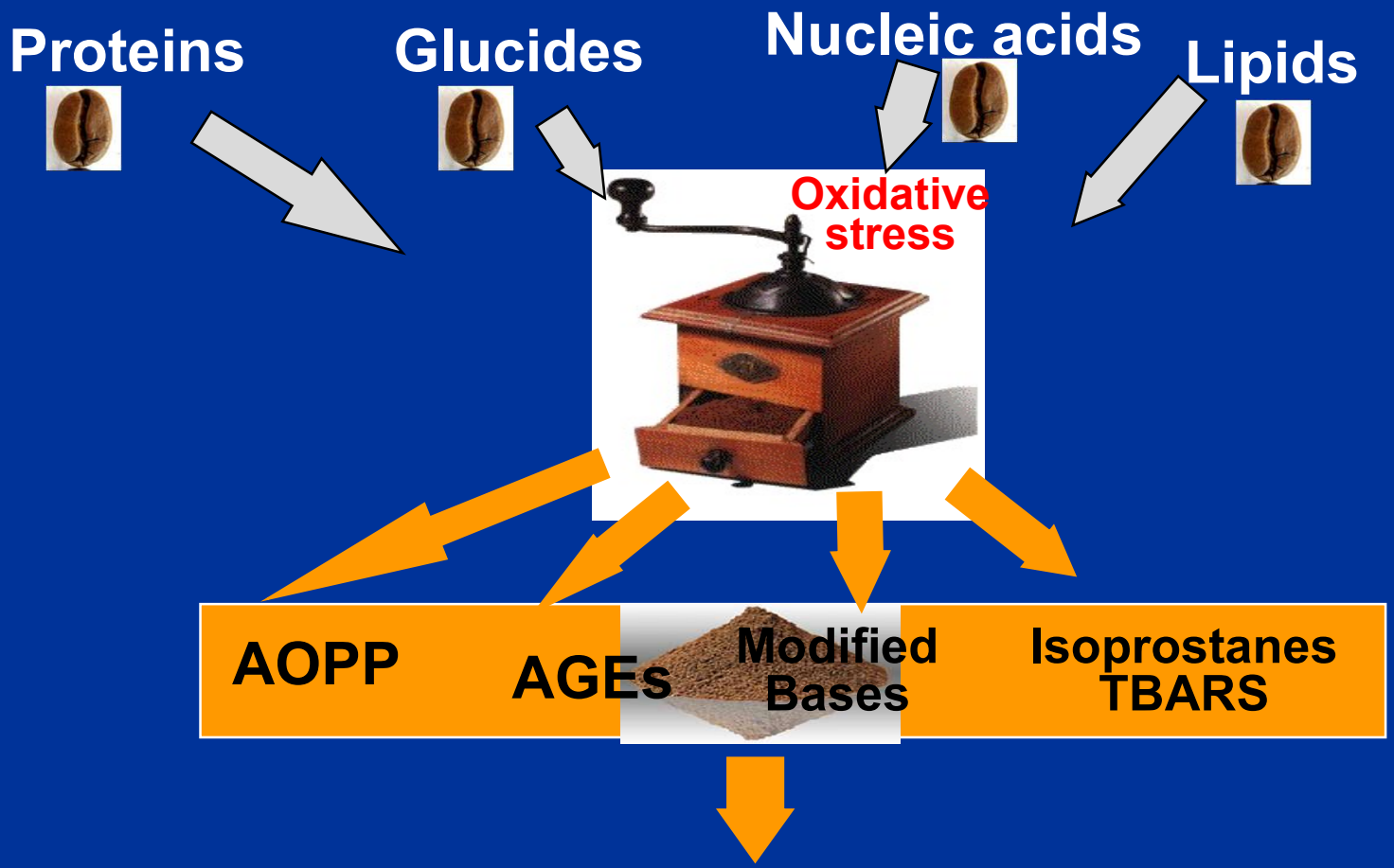
# Exploring Oxidative Stress

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

II) Quantification of Oxidant production ?

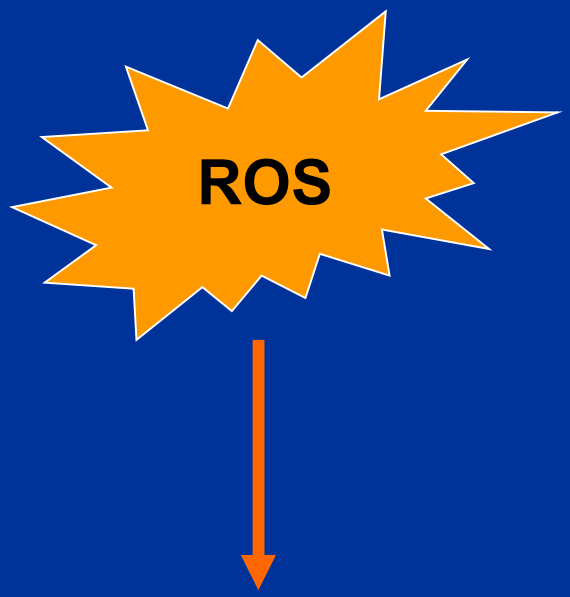
III) Investigation of defense mechanisms ?

# 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

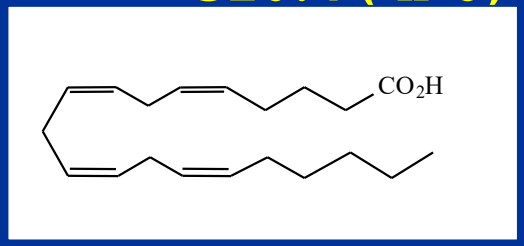
anti-oxidized antibodies

PCOOH (oxydized phosphatidylcholine )

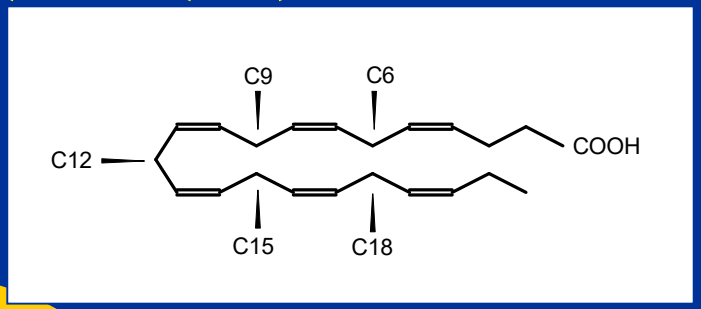
Lipid peroxidation

# 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

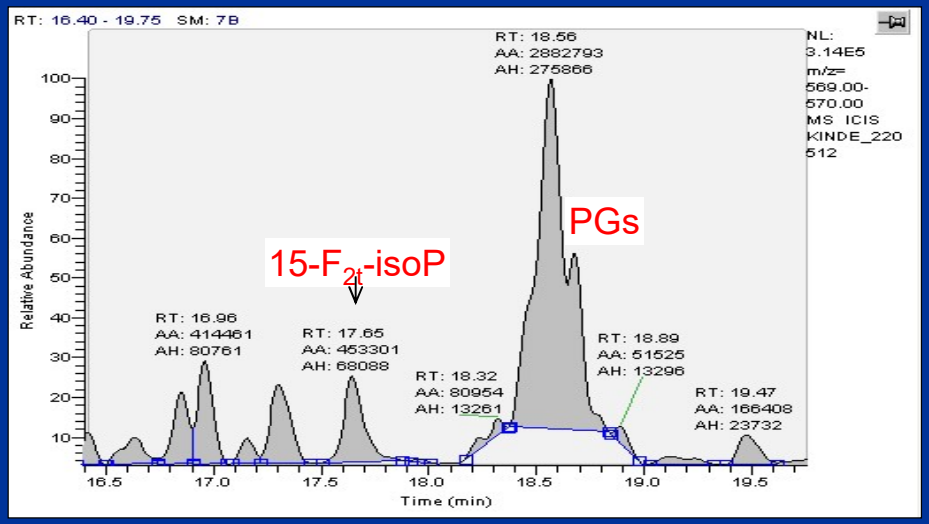
$iPF_{2a}$-V

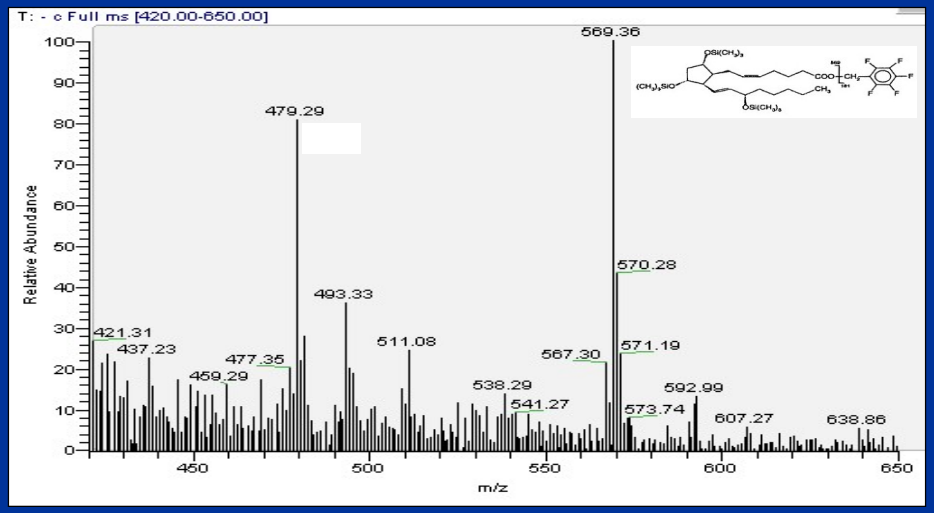
+ de 64 possibles isomers

**Neuroprostanes**

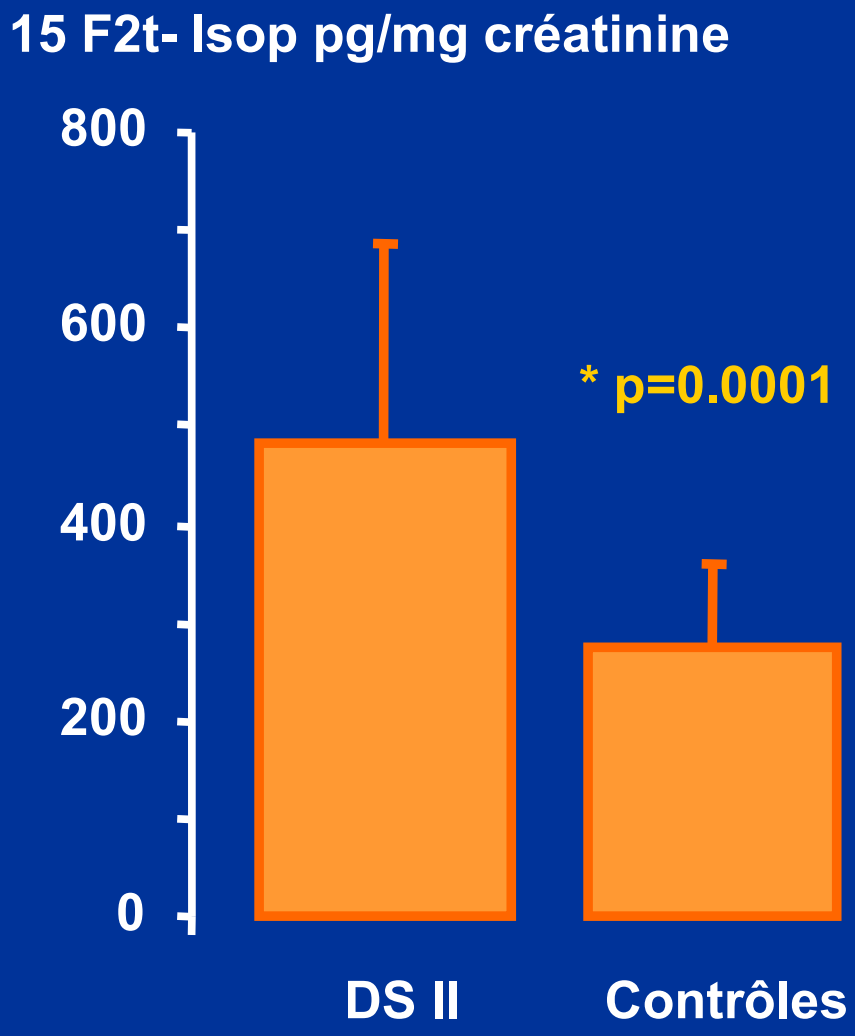
$F_4$-NPs

# Detection of Isoprostanes using GC-MS



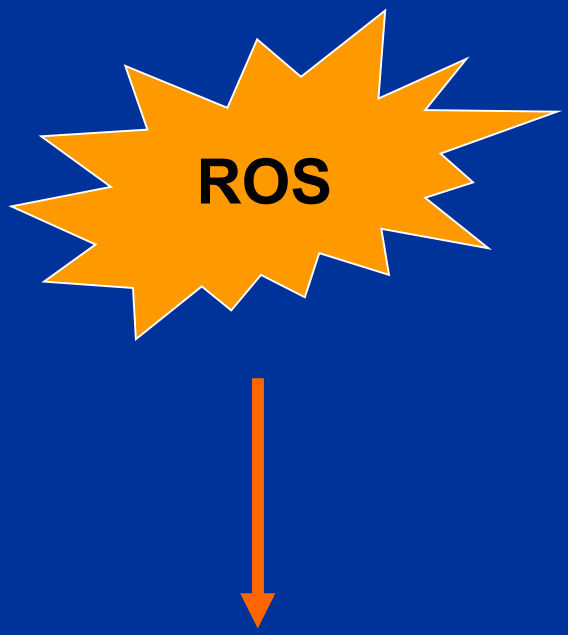

# Isoprostane as a biomarker of oxidative stress in clinical situations




*Monnier et al, JAMA, Avril 2006.*


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

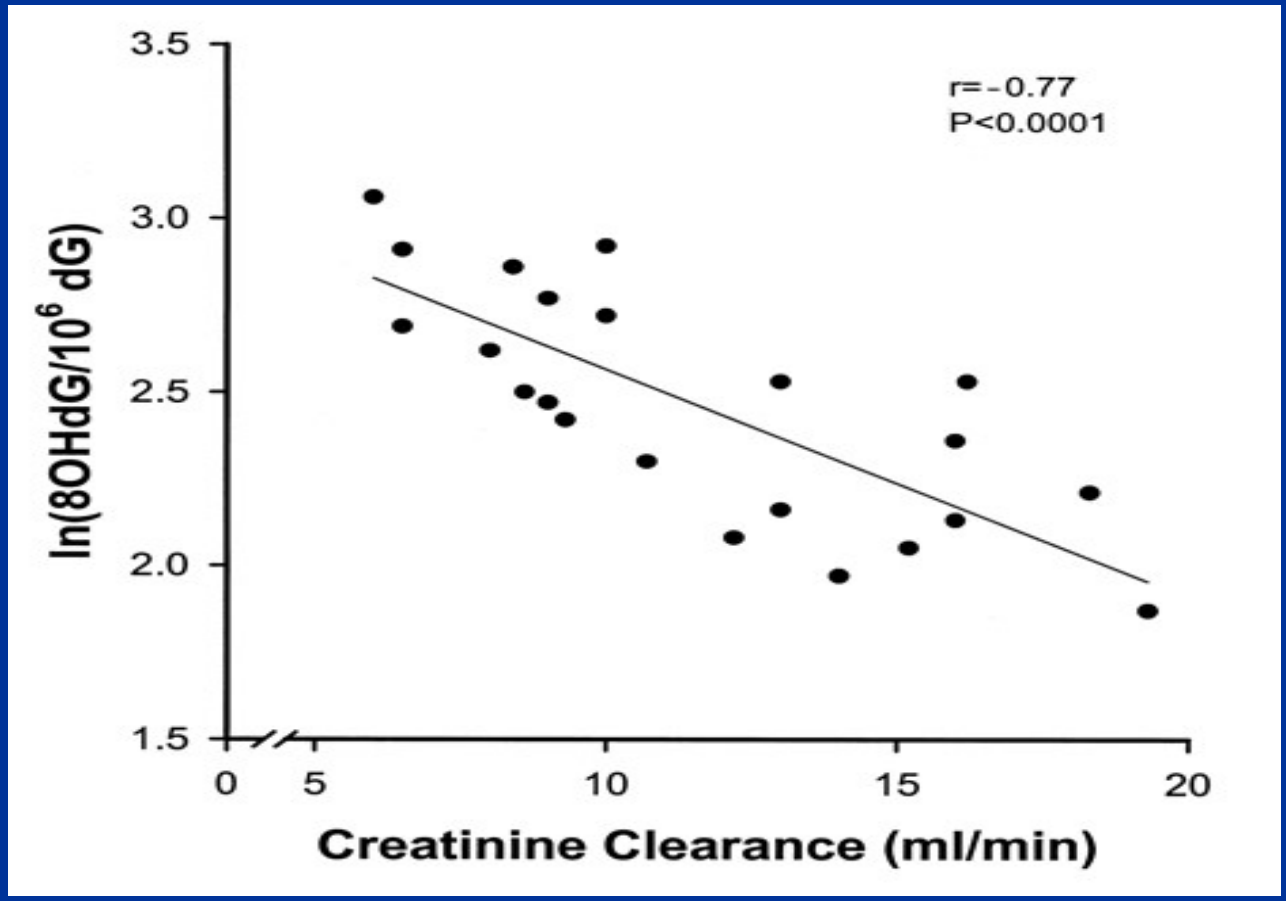
# Oxidative stress Biomarkers: Nucleic acids




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

**Test « COMET »**
Detection of breakdown of DNA using electrophoresis

# Increase in Oxydative stress in CKD




Tarng et coll. JASN, 2002

# Protein oxidative products


**Carbonyl Proteins**

**AGEs, pentosidine, Carboxymethyl Lysine**

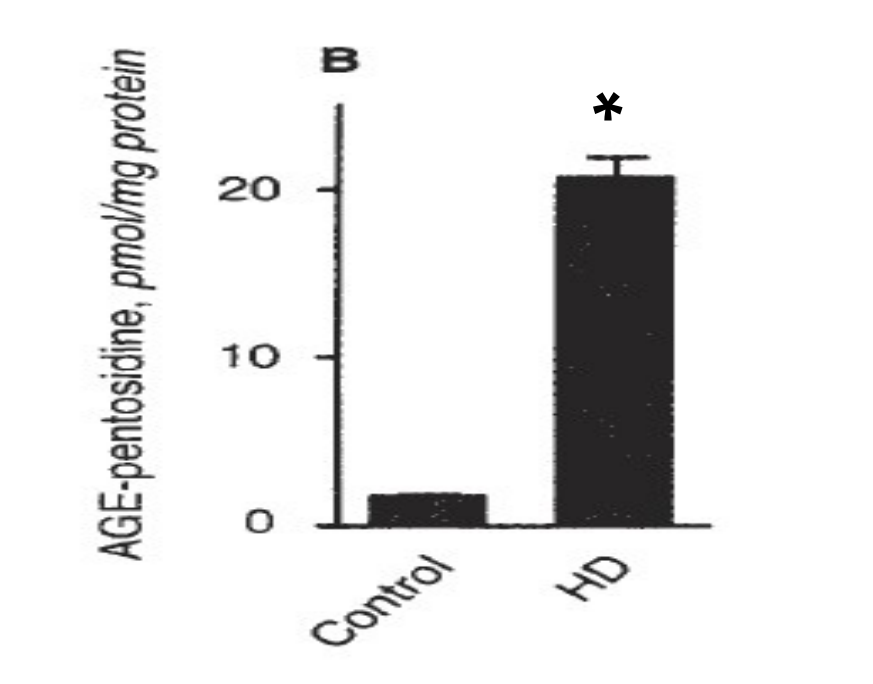
**Protein Oxidative products**

**AOPP advanced oxydation protein products**

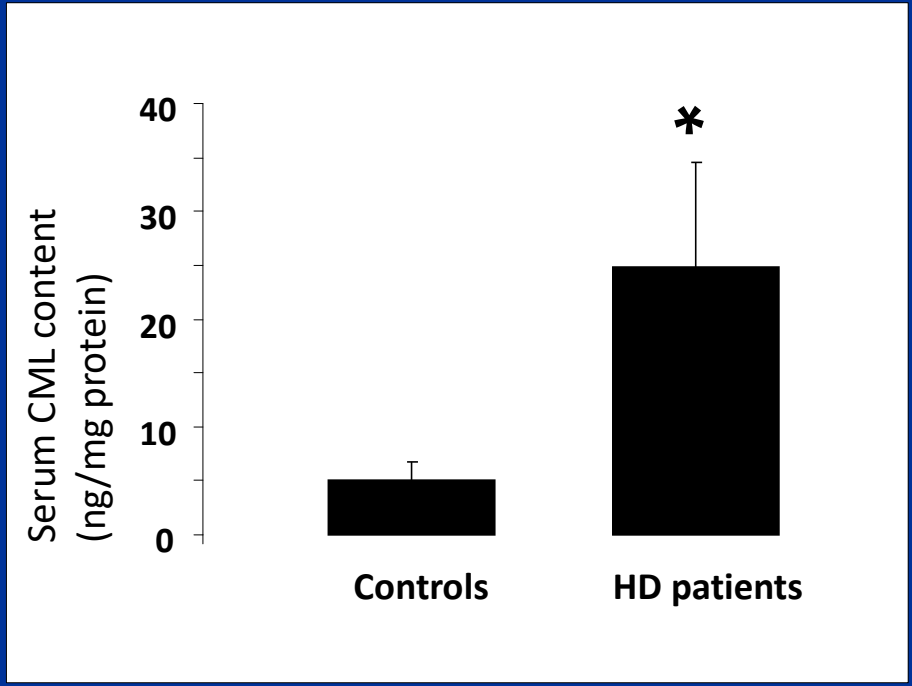
**Dityrosine, chlorotyrosine, nitrotyrosine**
Oxyd. de la tyrosine

**Thiols oxidation : equilibrium GSH/GSSG**

# Carboxymethyllysine and pentosidine : specific biomarkers

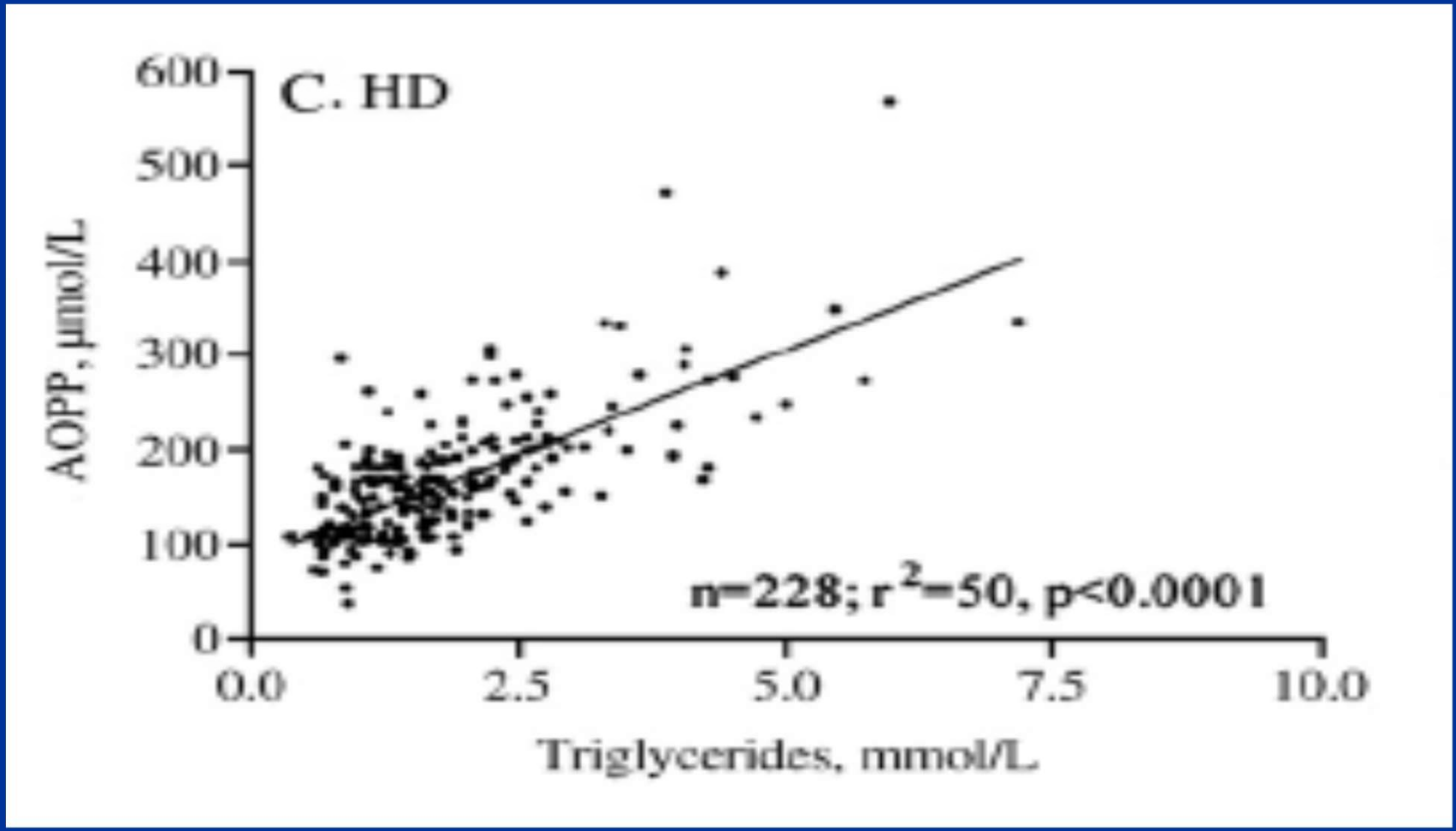



*Witko-Sarsat et al., Kidney Int. 1996*




*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

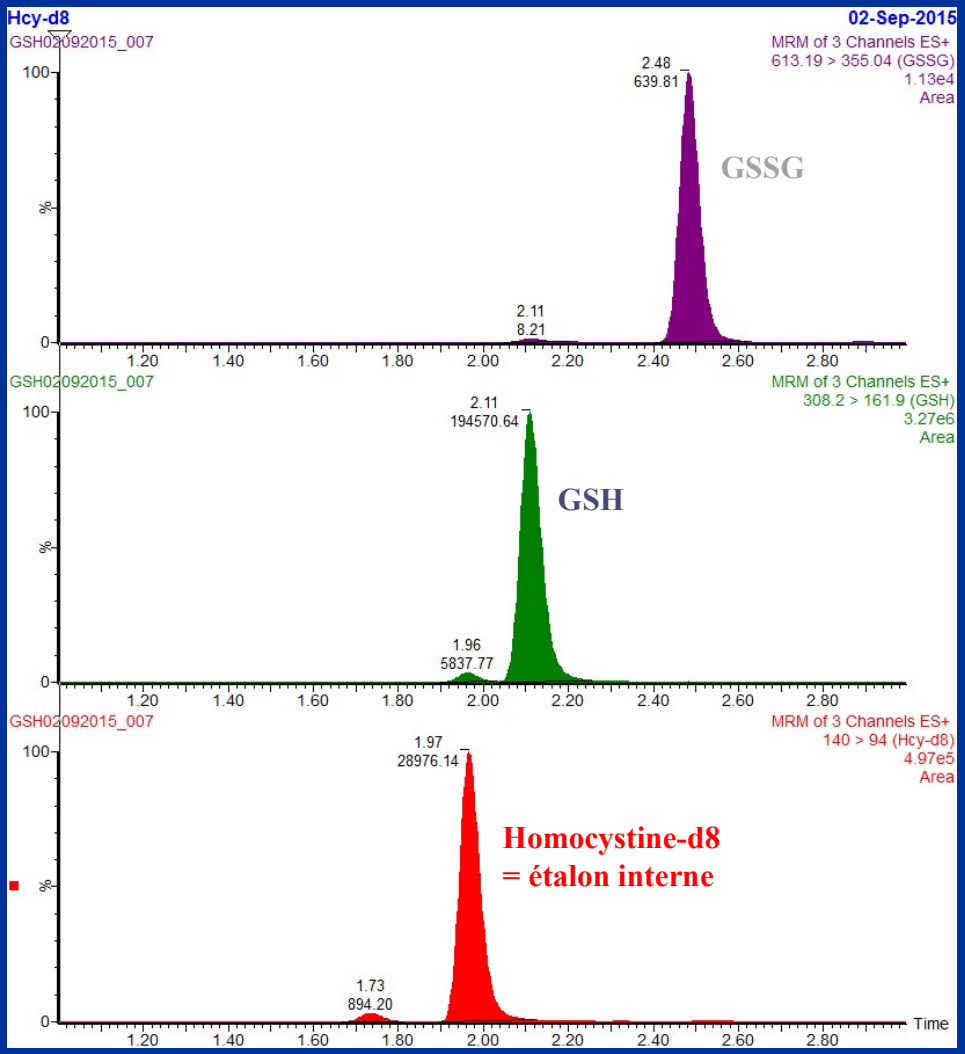
- **Méthode de dosage**

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 :

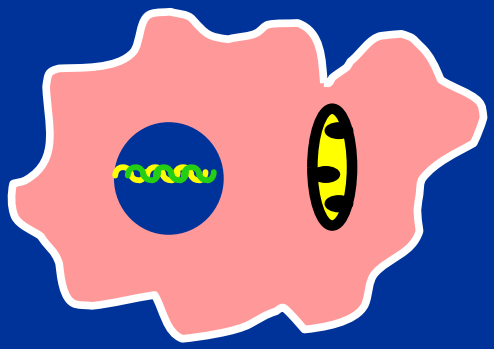
One lipid biomarker (IsoPs), 1 protein biomarker (CML or pentosidine) or GSH/GSSG ratio, 1 nucleic acid biomarker : 8-OH-desoxyguanosine

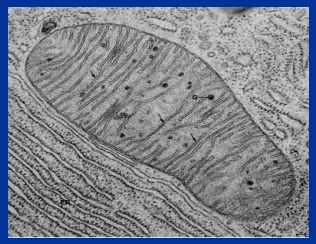
## II) Quantification of Oxidant production ?

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

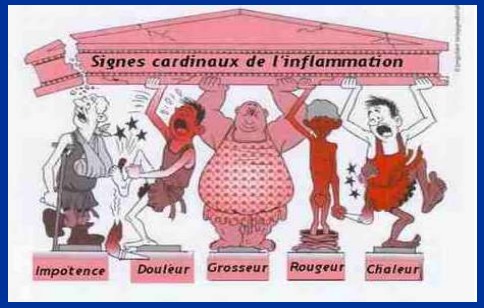
## III) Investigation of defense mechanisms ?

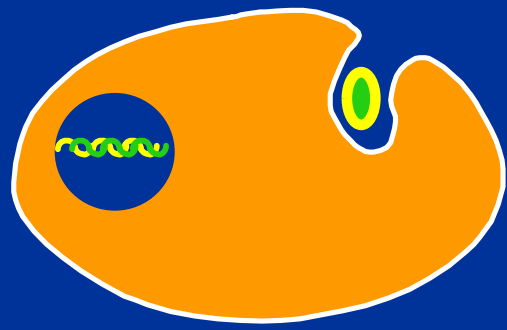
# ROS are mainly producted as a coproduct of Energy supply or phagocyte activation





Cell metabolism



Phagocyte activation

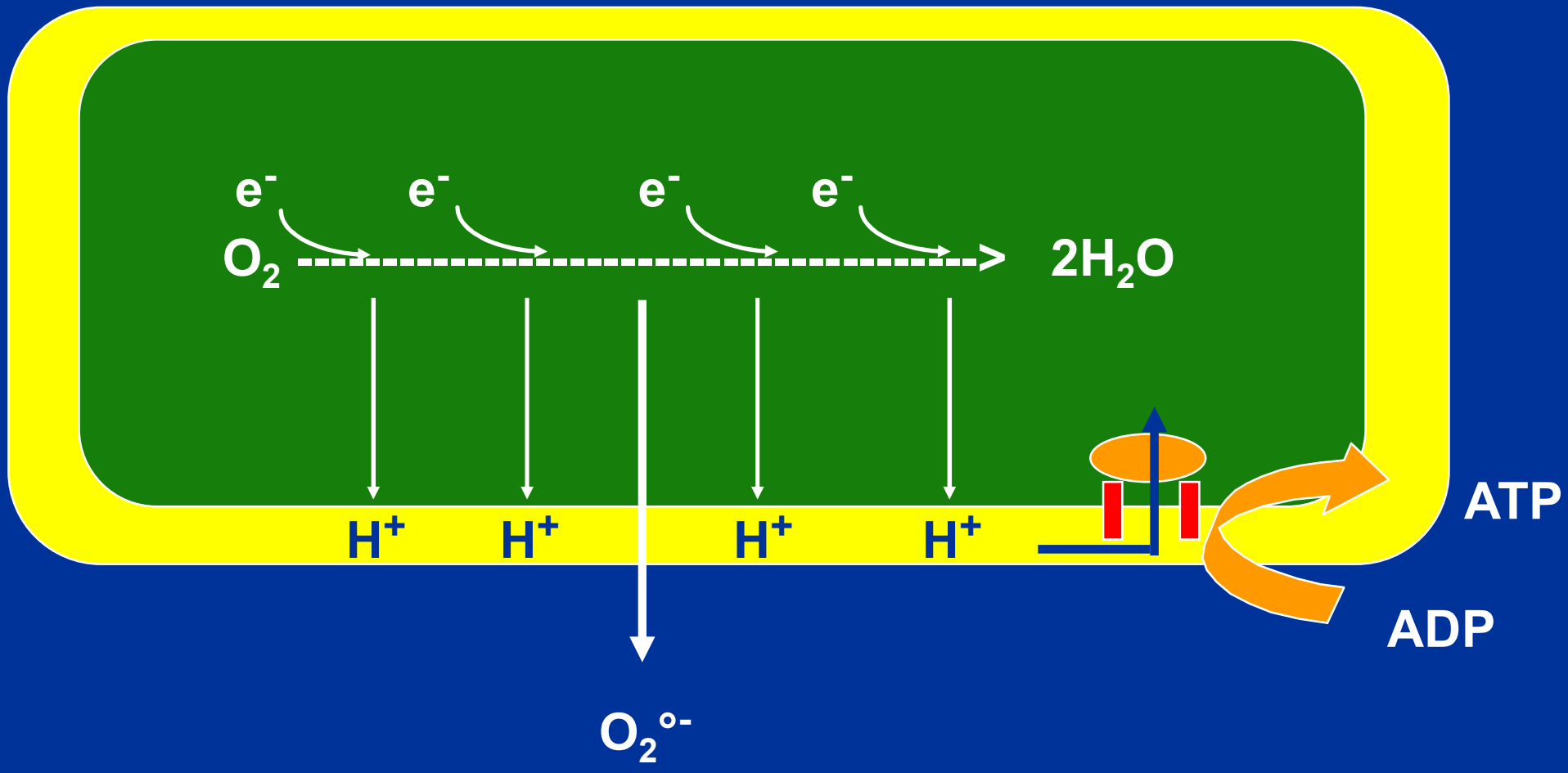
$O_2^{\circ-}$

Chlorinated oxidants

ROS

Nitrated oxidants

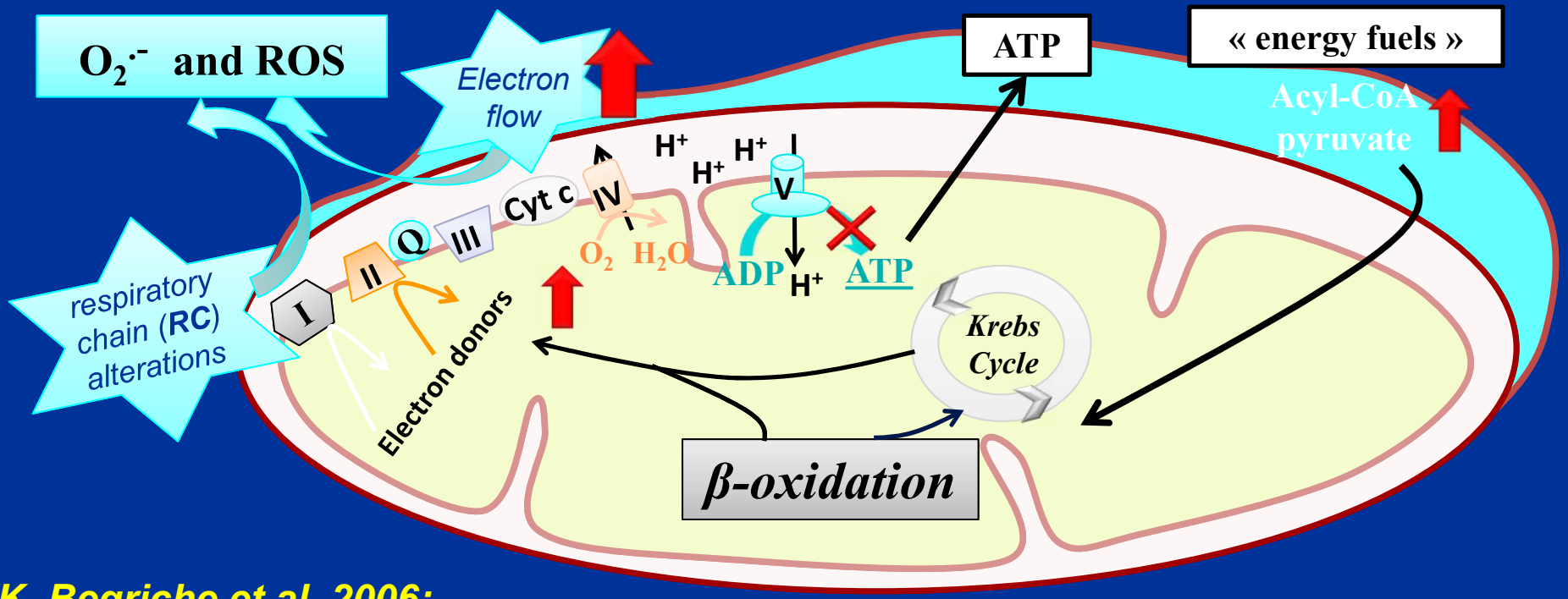
# Superoxide anion as a cofactor of mitochondria respiration



# Complexes I et III : main source of ROS

**ROS production by the respiratory chain**

**Desequiibrium between ATP consumption and « energy fuel »**

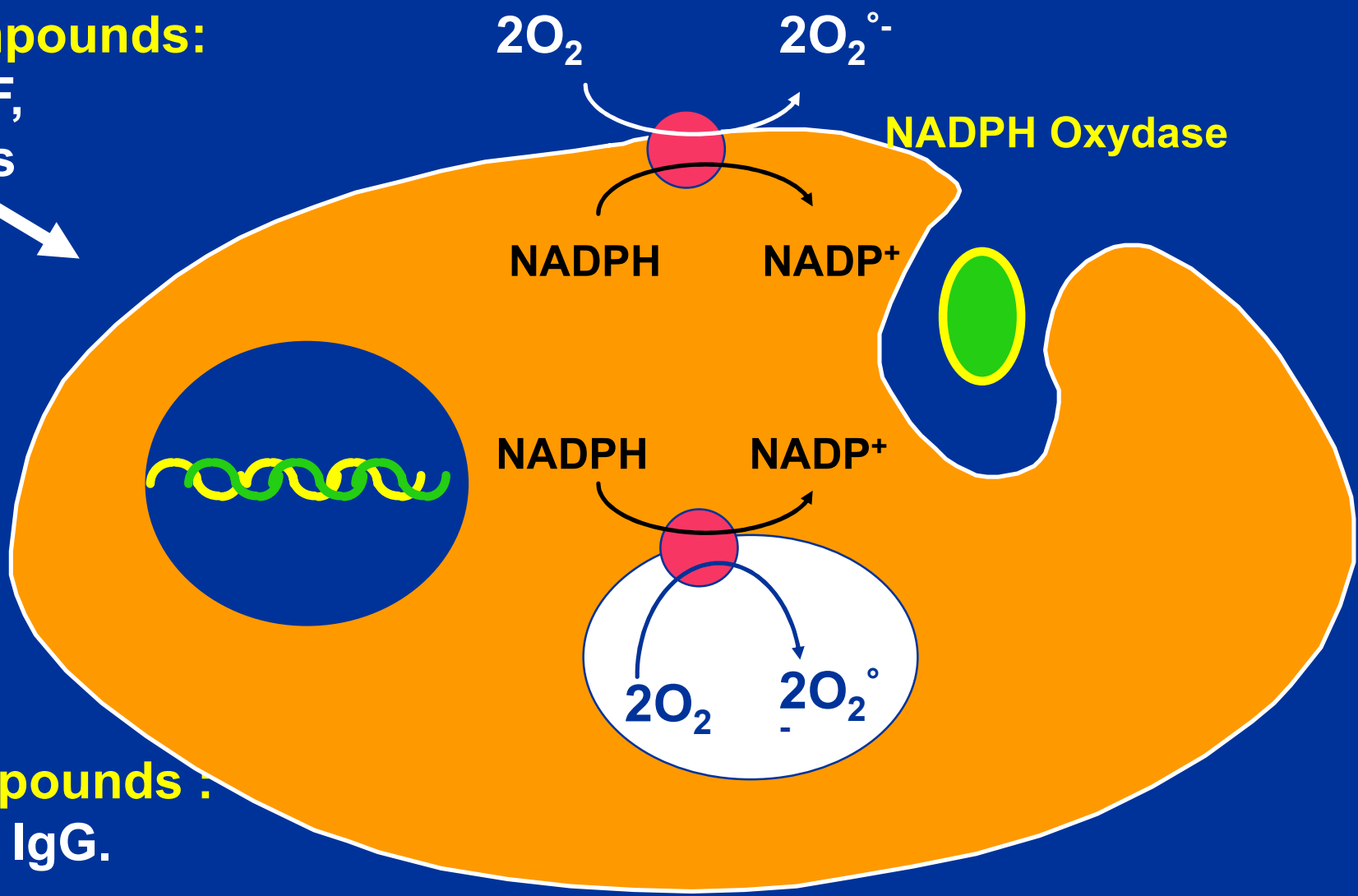



*(K. Begriche et al. 2006; Wallace et al. 2010)*

*Murphy MP 2009*

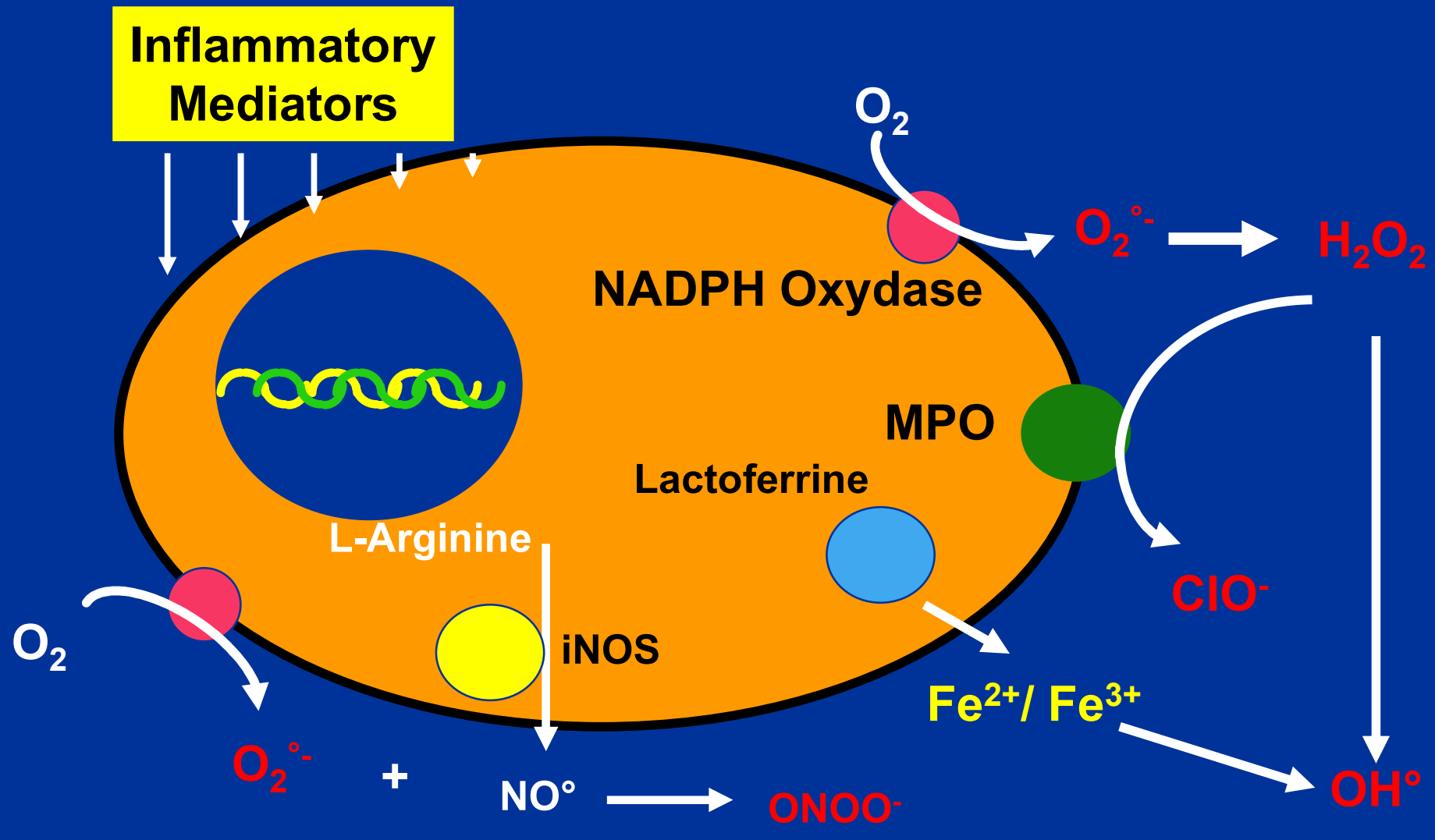
# The oxidative burst : an ehanced production of superoxide anion

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

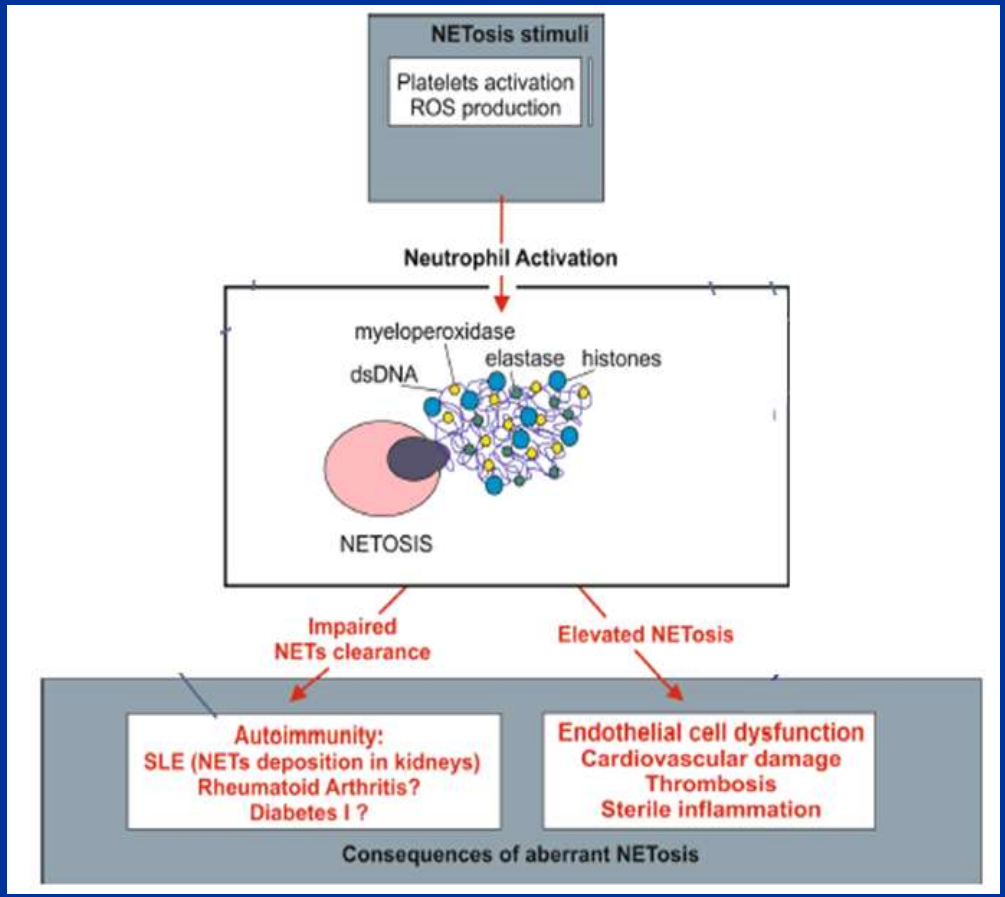



**Insoluble compounds :**
Bacteria, LPS, IgG.

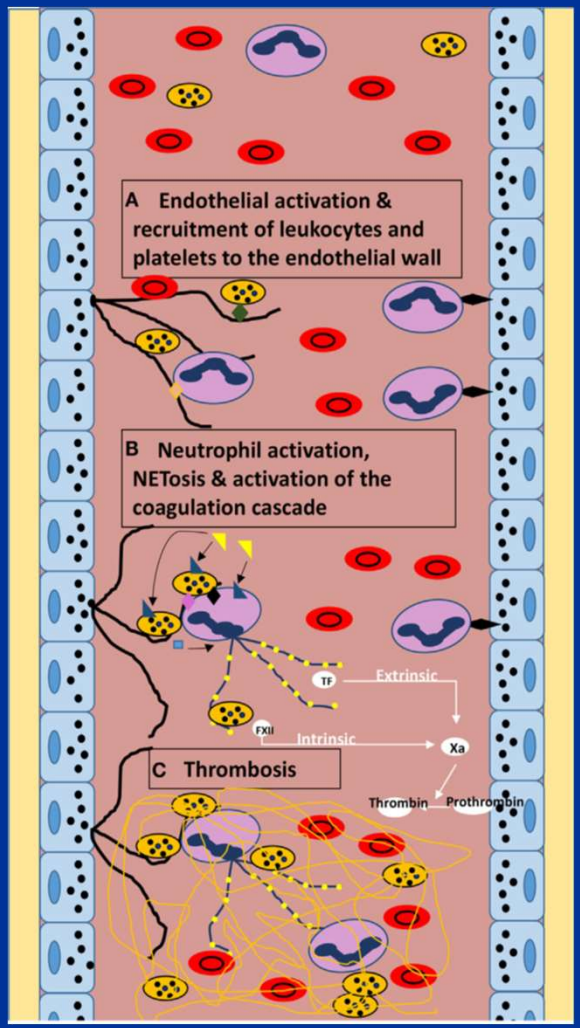
# Inflammation and ROS poduction



# ROS production and NETosis

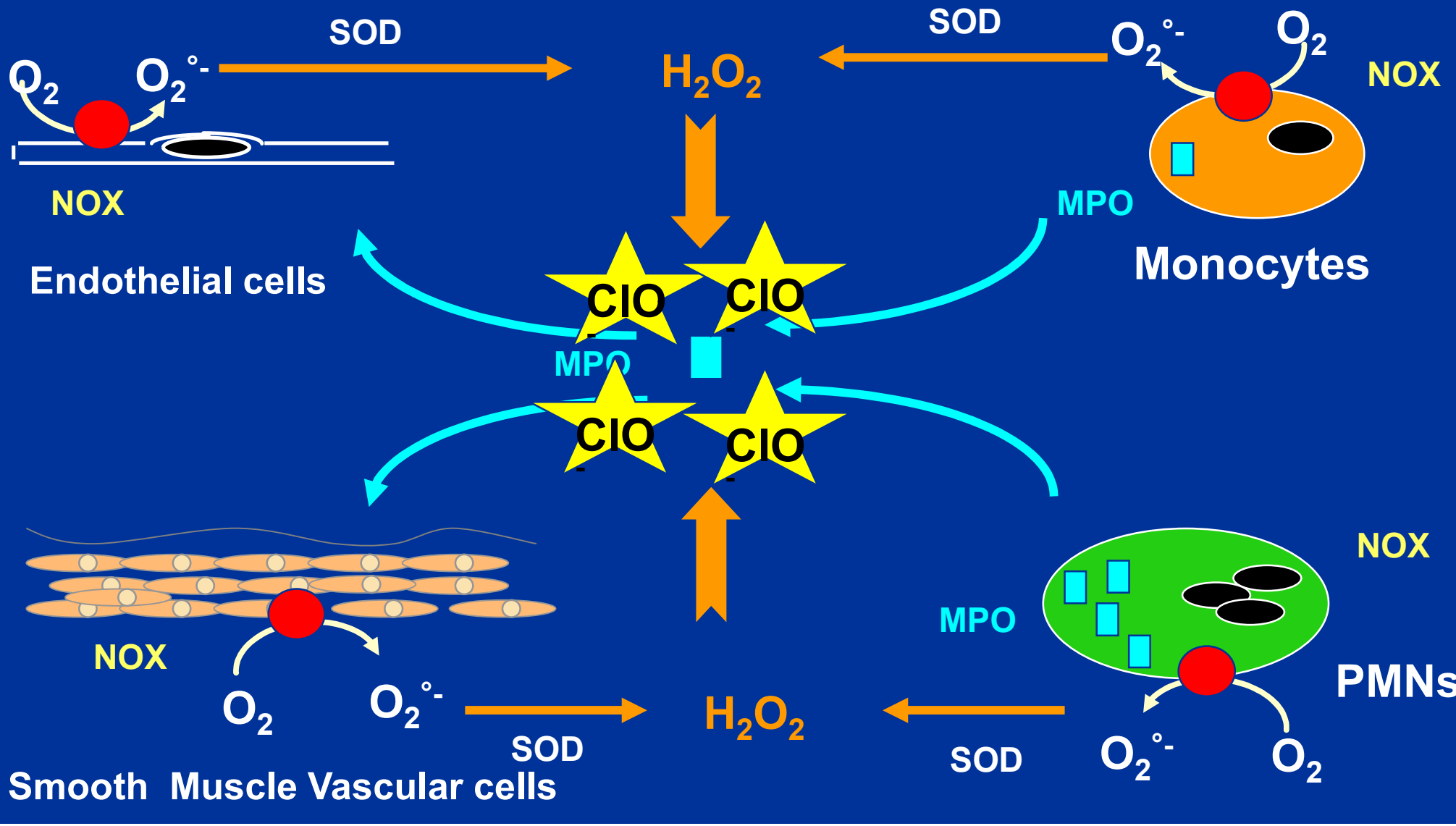



*Marie Korabecna et al.; Inflamm. Res. (2017) 66:369 378*




*Andrew S. Kimball et al.; fimmu.2016.00236*

# The synergistic action of NADPH oxidase and MPO



# NAD(P)H oxydase : a NOX superfamilly

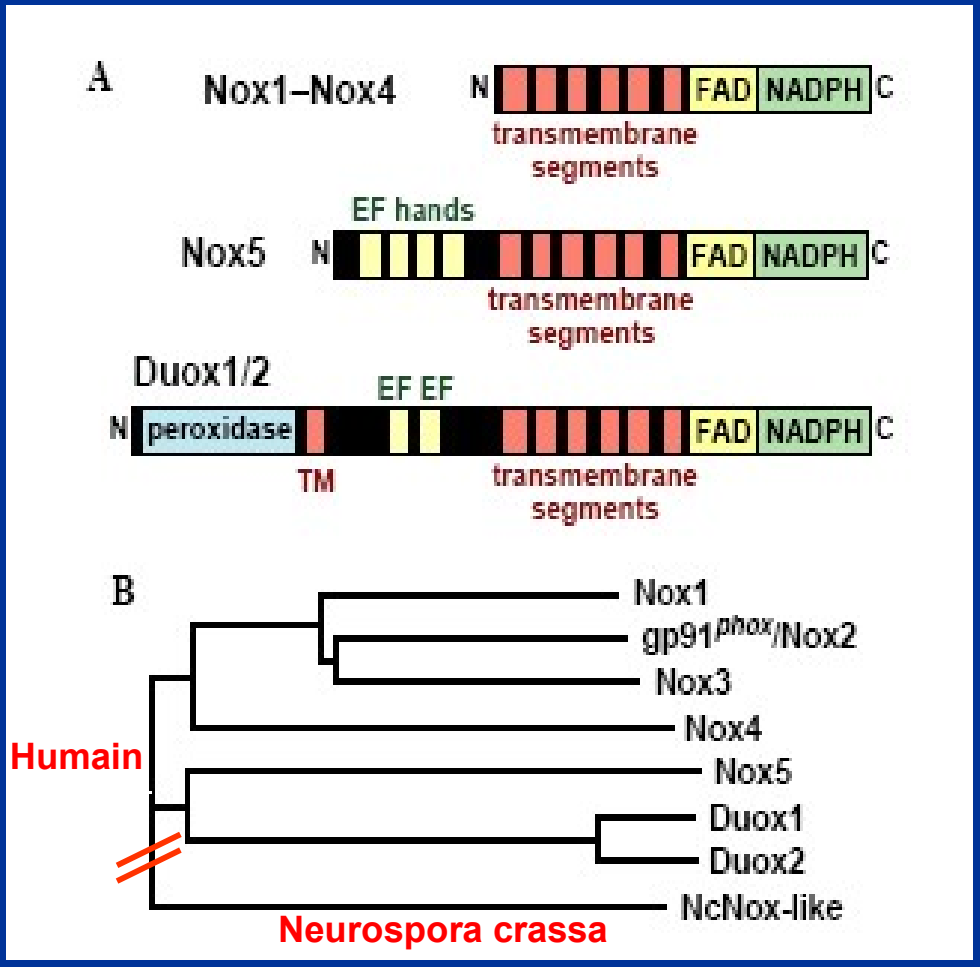


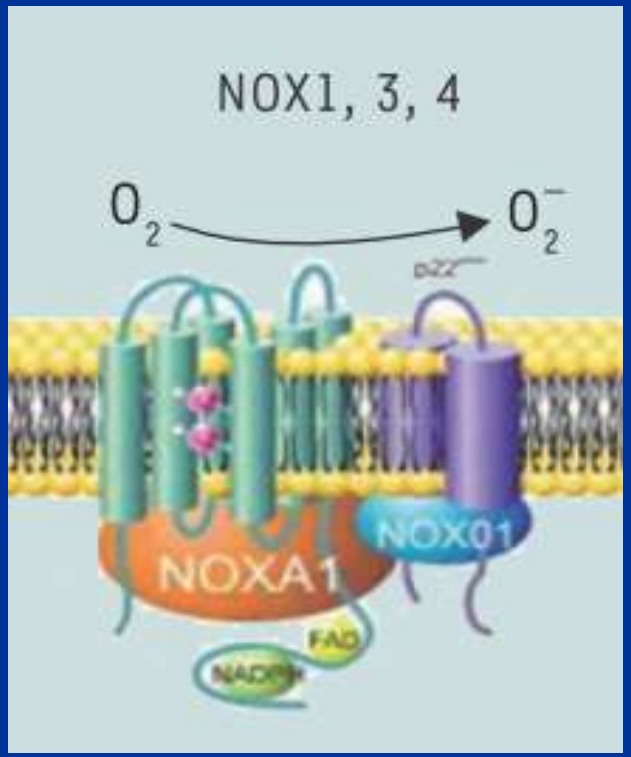

TABLE 2. *Tissue distribution of NOX enzymes*

| | 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*

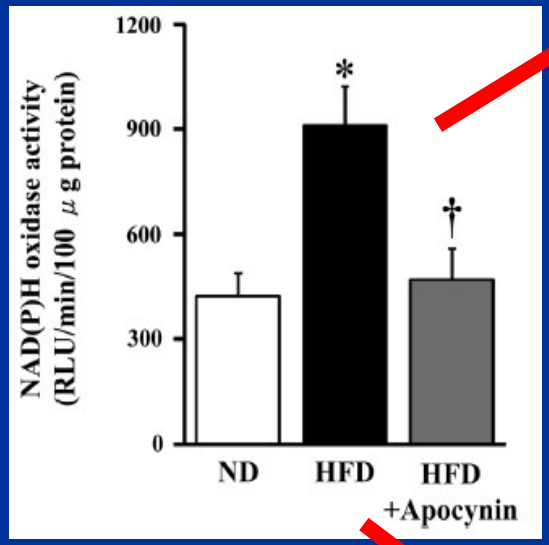
# NOX 1, 3 and 4 stimuli and fonctions

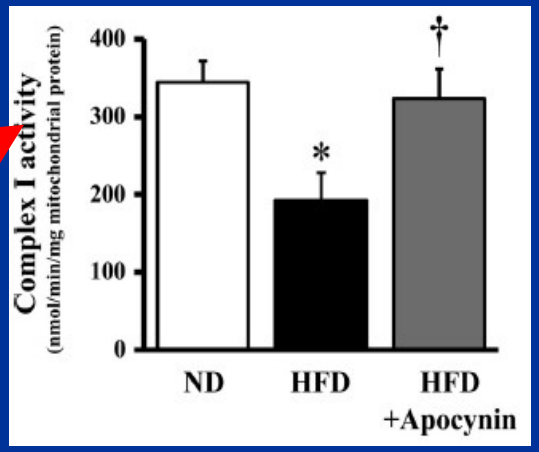


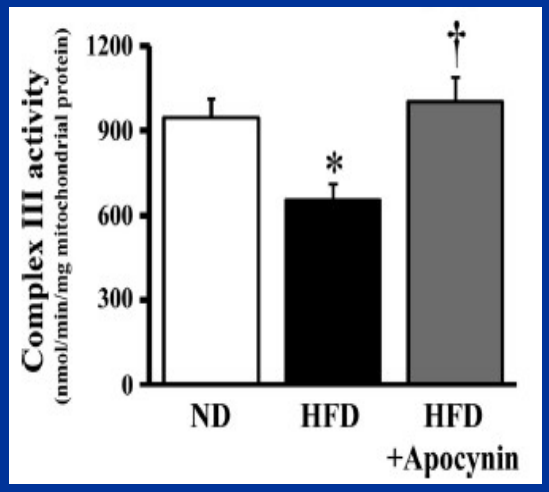

| NOX isoformes | Stimulus | Fonction(s) | Type(s) cellulaire(s) localisation |
|---|---|---|---|
| NOX1 | $H_2O_2$ | Prolifération | Fibroblastes<br>Cellules épithéliales pulmonaires |
| | Thrombine | | Cellules musculaires lisses vasculaires |
| | Angiotensine II | Hypertrophie | Cellules musculaires 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 |

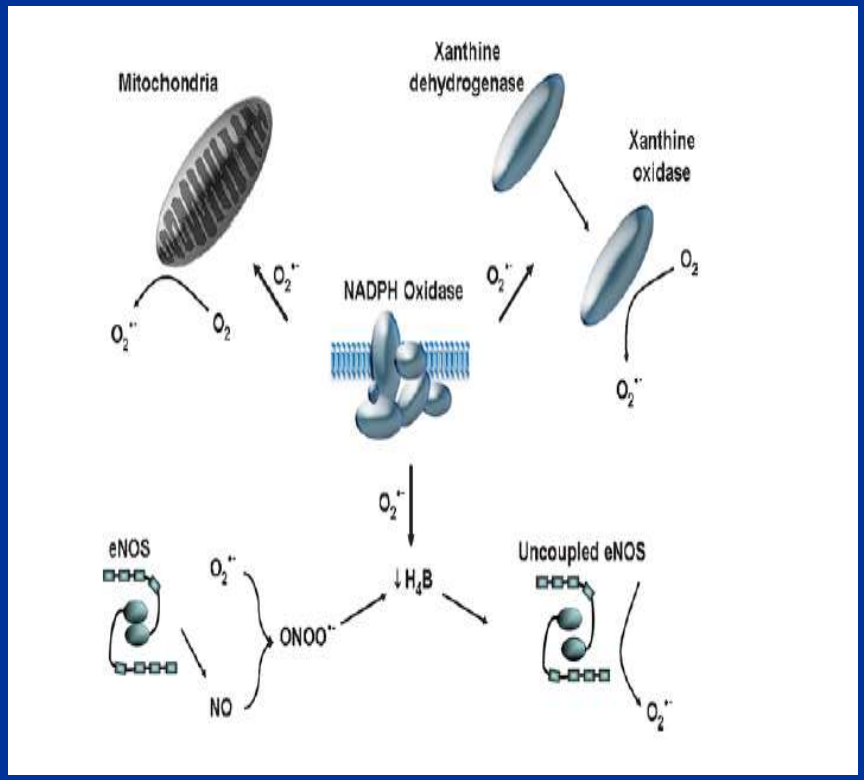
*Migdal. Médecine sciences 2011; 27 : 405 412*

# Interactions between NADPH Oxydase - MPO - Mitochondria




*(Ray et Shah 2005)*

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

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## II) Quantification of Oxidant production ?

How are ROS produced?

How to quantify ROS ?

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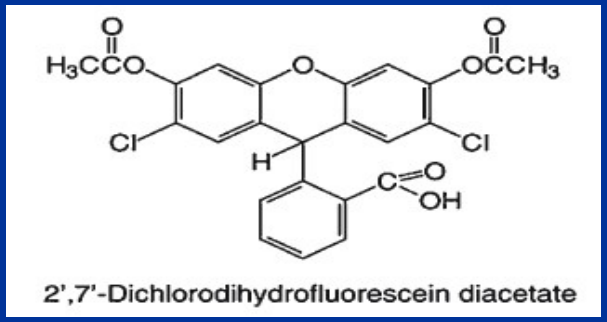
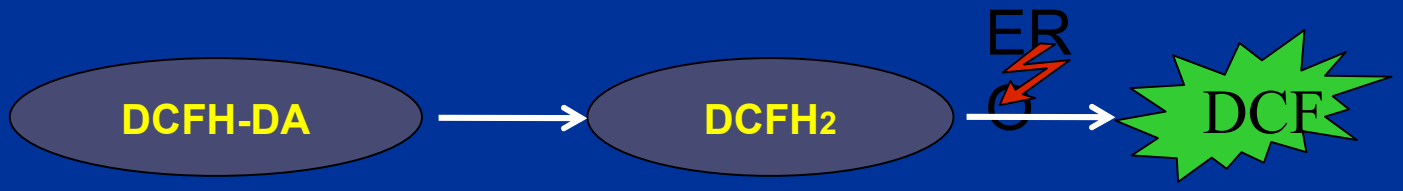
# ROS determination could be determined using DCFH-DA (dichlorodihydrofluorescein diacétate)

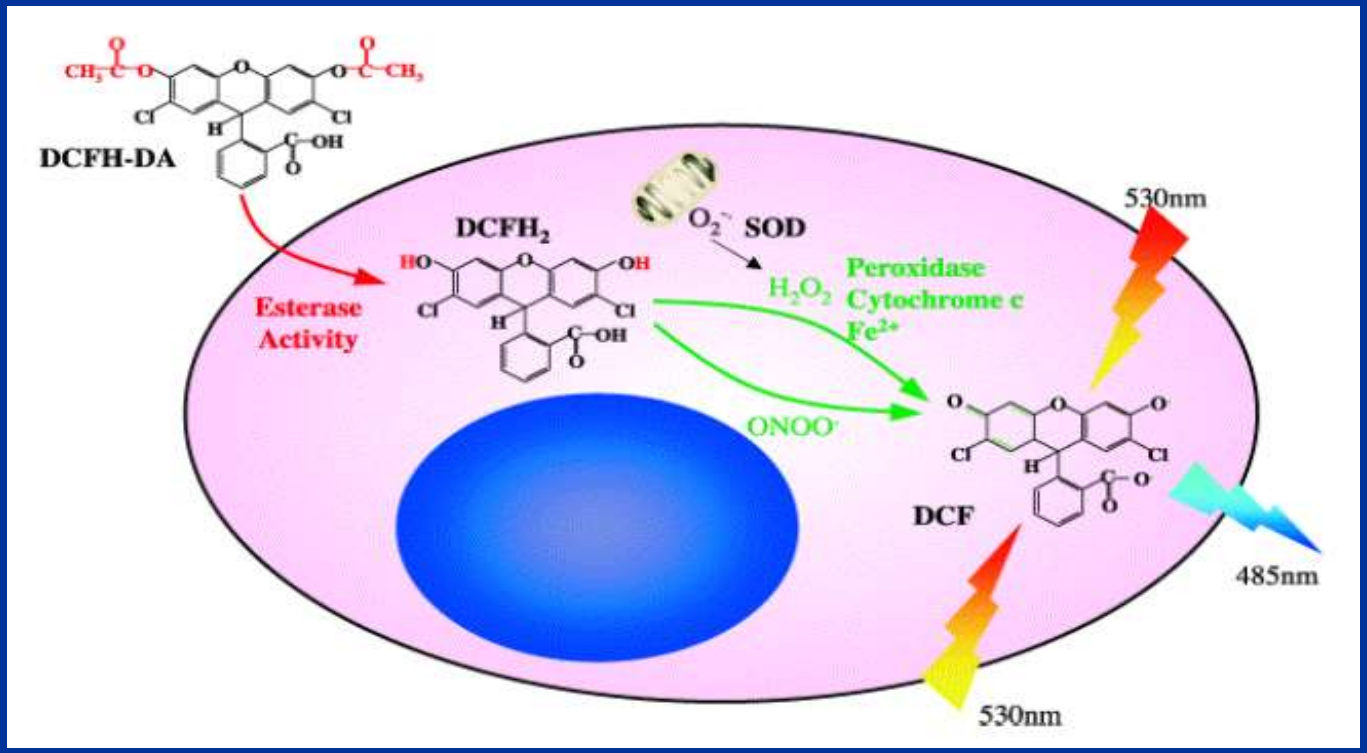



Non fluorescent

Non fluorescent

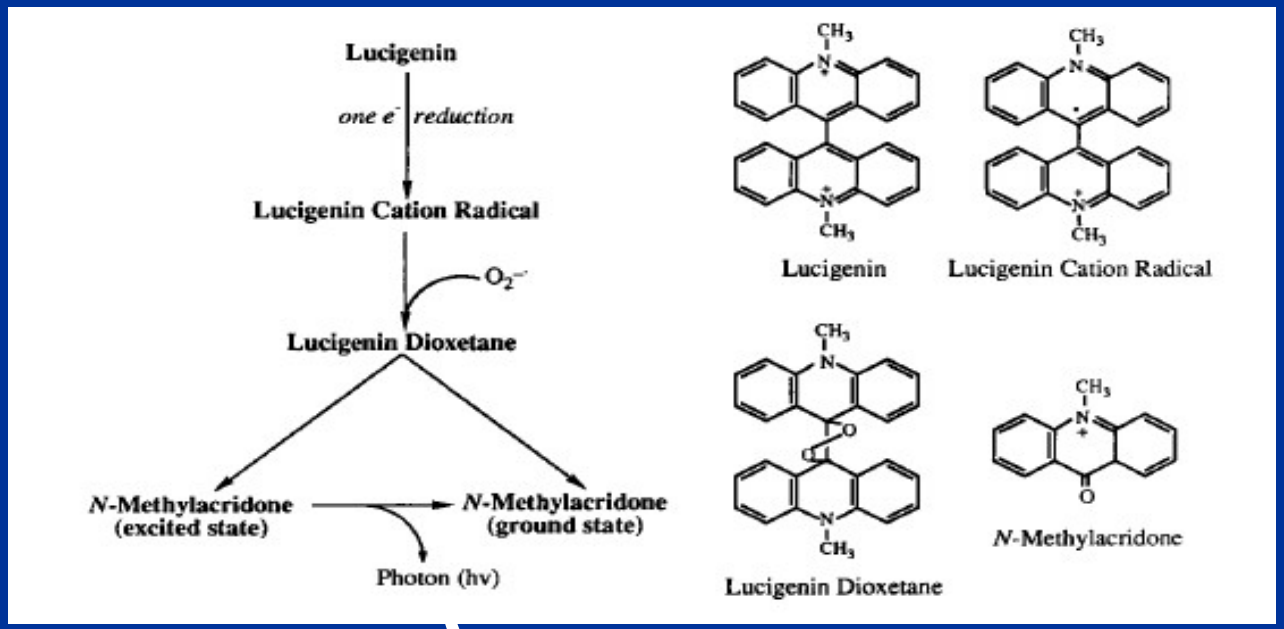
fluorescent




**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

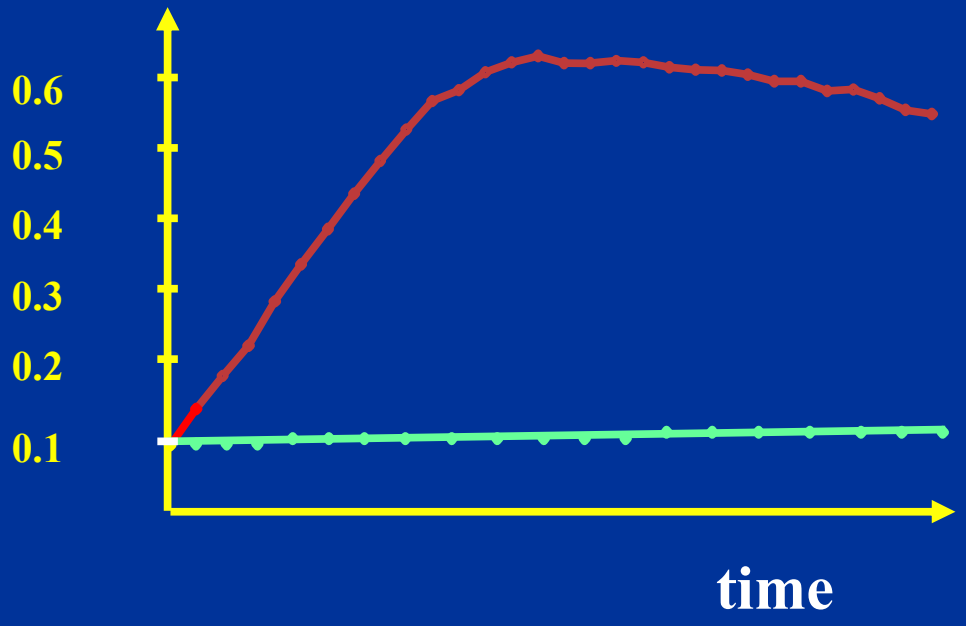



*Li Y. The Journal of Biological Chemistry Vol. 273(4): 2015–2023, 1998*

# Activity and expression of NADPH Oxydase : cell models

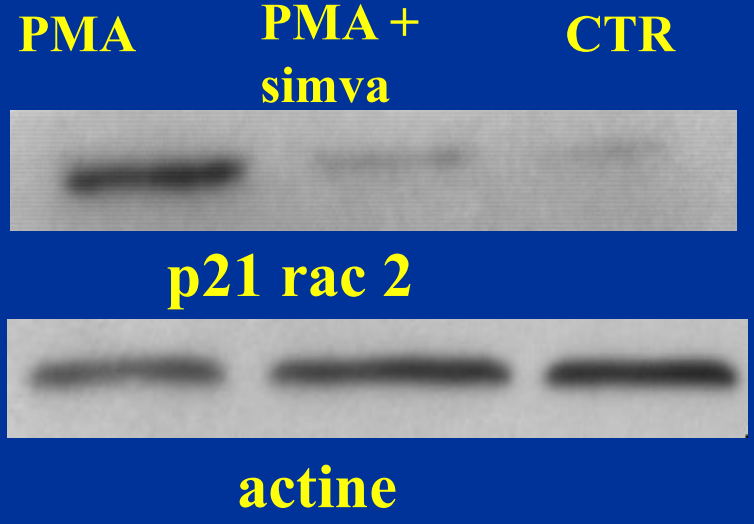
**- Activité cellulaire :**

Lucigénine : anion superoxyde




**- Etude des sous unités :**

Western Blotting



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# Oxidative stress and cell information

Cell components

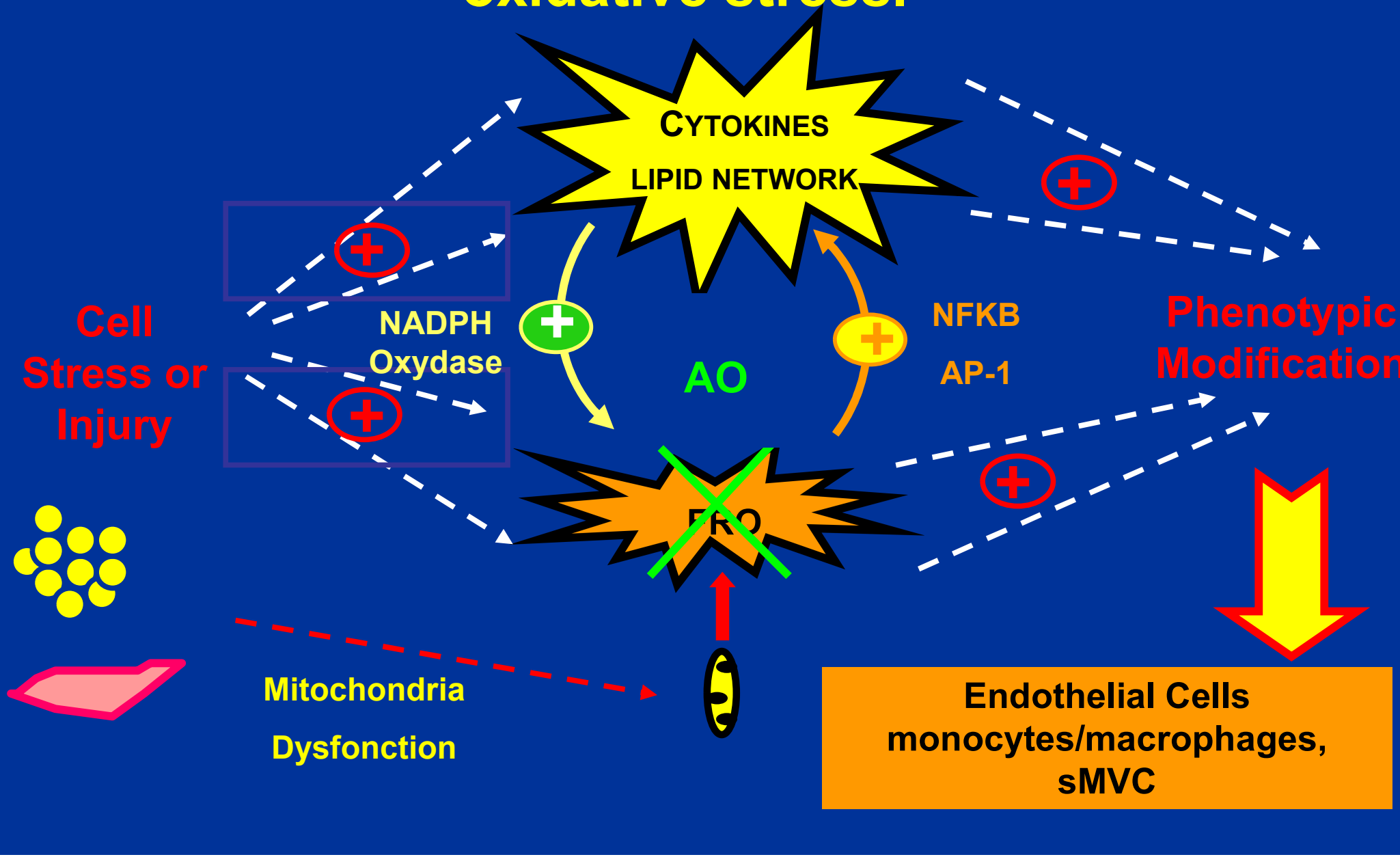

Oxidative stress biomarkers
Cell toxicity

Transcription
factor

de novo protein synthesis

Proinflammatory proteins

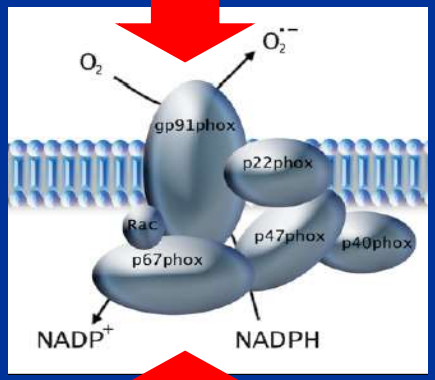
# Amplification loops between inflammation and oxidative stress.



# 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***



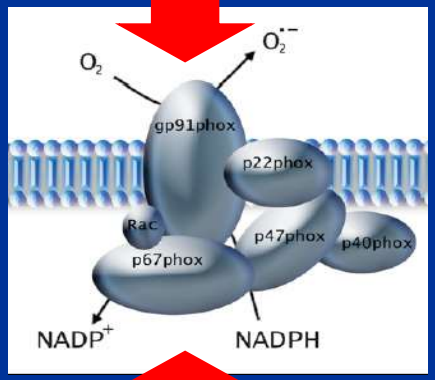

**Analyse Multivariée : Déterminants de la production d'$O_2^{\bullet -}$**
**Homocystéine (p<0.02), CRP (p<0.01)**

*(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)**

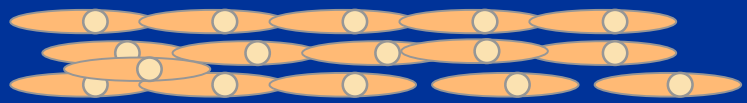
*Morena et al., Free Radical Research,* June 2011; 45(6): 735–745

# Oxidative stress, amplification loops and aterosclerosis

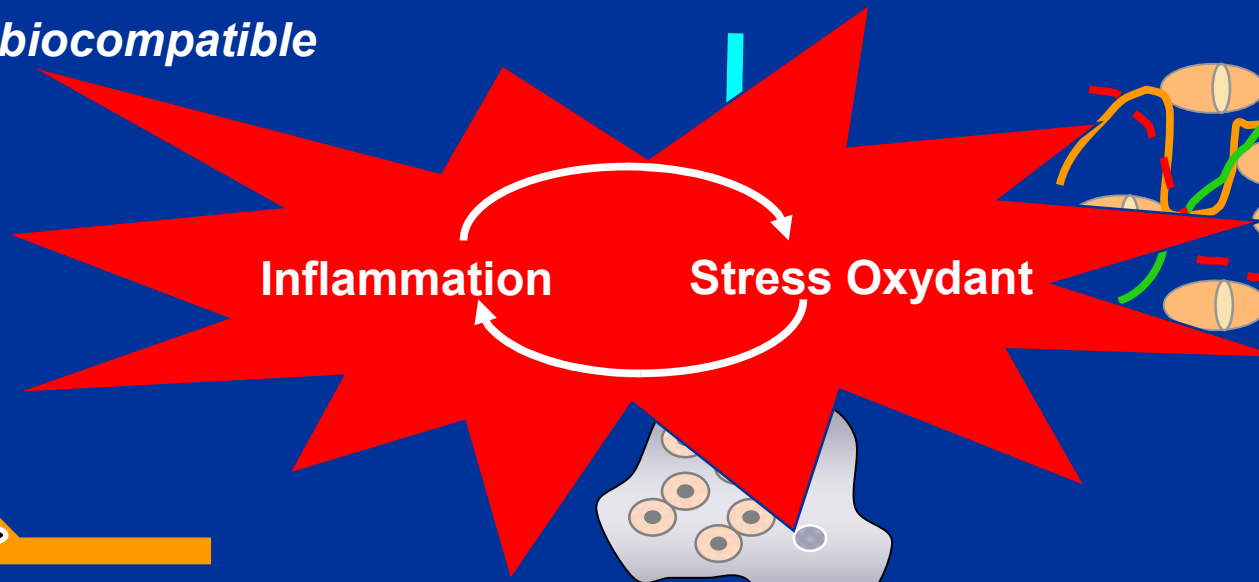
Cellules endothéliales

Monocytes/ Macrophages

Cellules musculaires lisses Contractiles

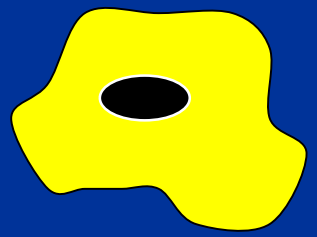


*Vasorelaxation*
*Antiagrégant –biocompatible*




Sécrétoires





**Dysfonction endothéliale :**
*Vasoconstricton*
*Adhérence*
*Prothrombotique*

Cellules spumeuses

Ostéoblastiques

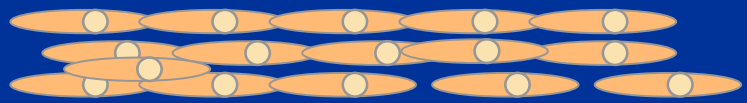
# Stress oxydant et transdifférentiation cellulaire

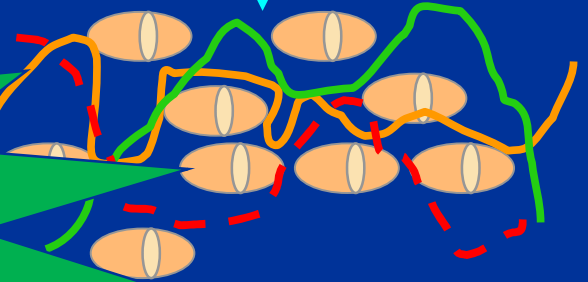
Cellules endothéliales

*Vasorelaxation*
*Antiagrégant –biocompatible*

Monocytes/ Macrophages

Cellules musculaires lisses Contractiles

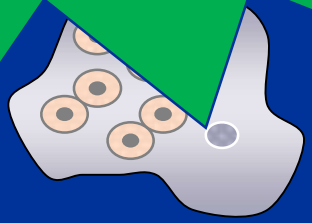




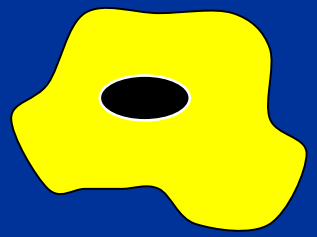
Sécrétoires

Anti-oxidants

**Dysfonction endothéliale :**
*Vasoconstricton*
*Adhérence*
*Prothrombotique*

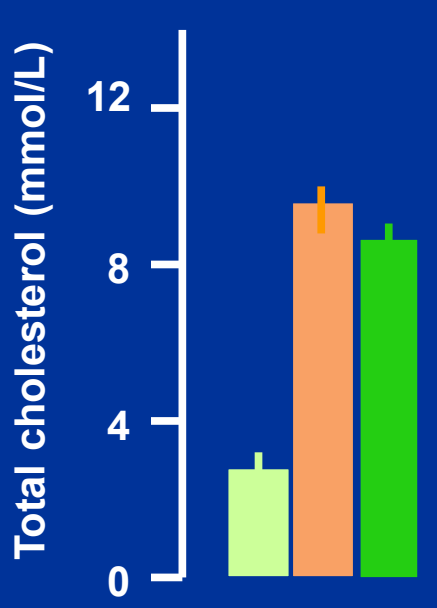


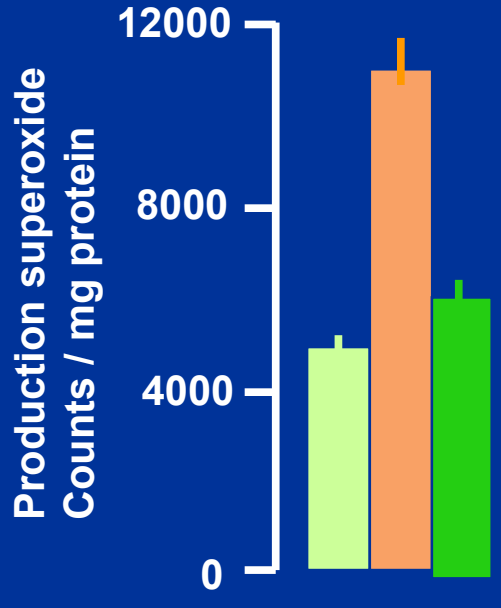
Cellules spumeuses

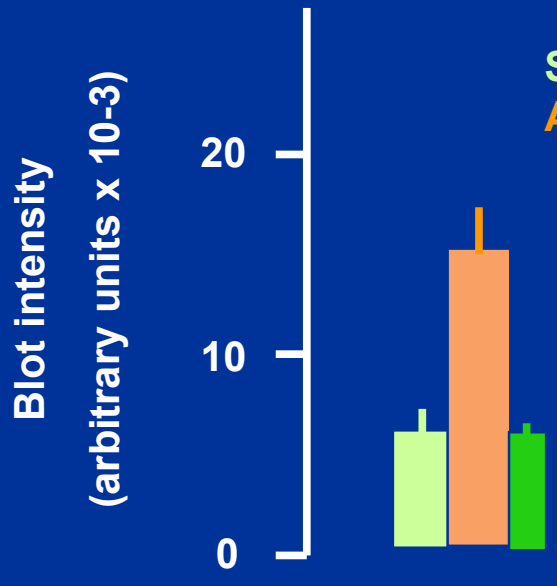


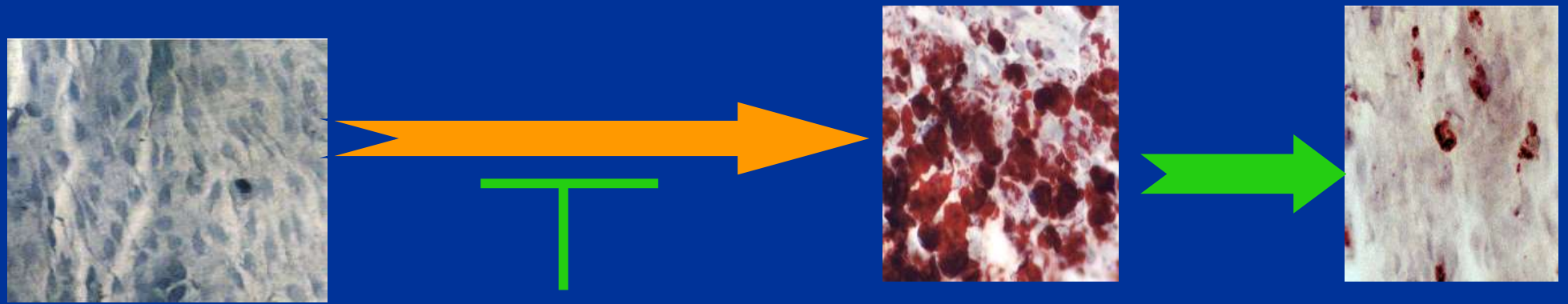
Ostéoblastiques

# Nutritional preventionof atherosclerosis:



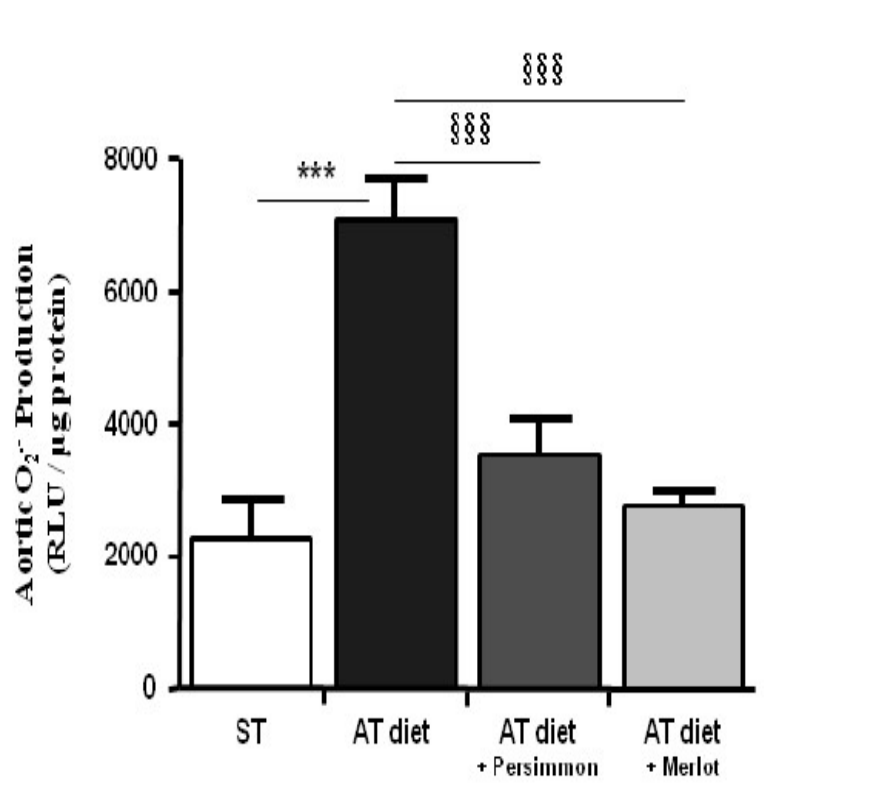
CTR Aorta

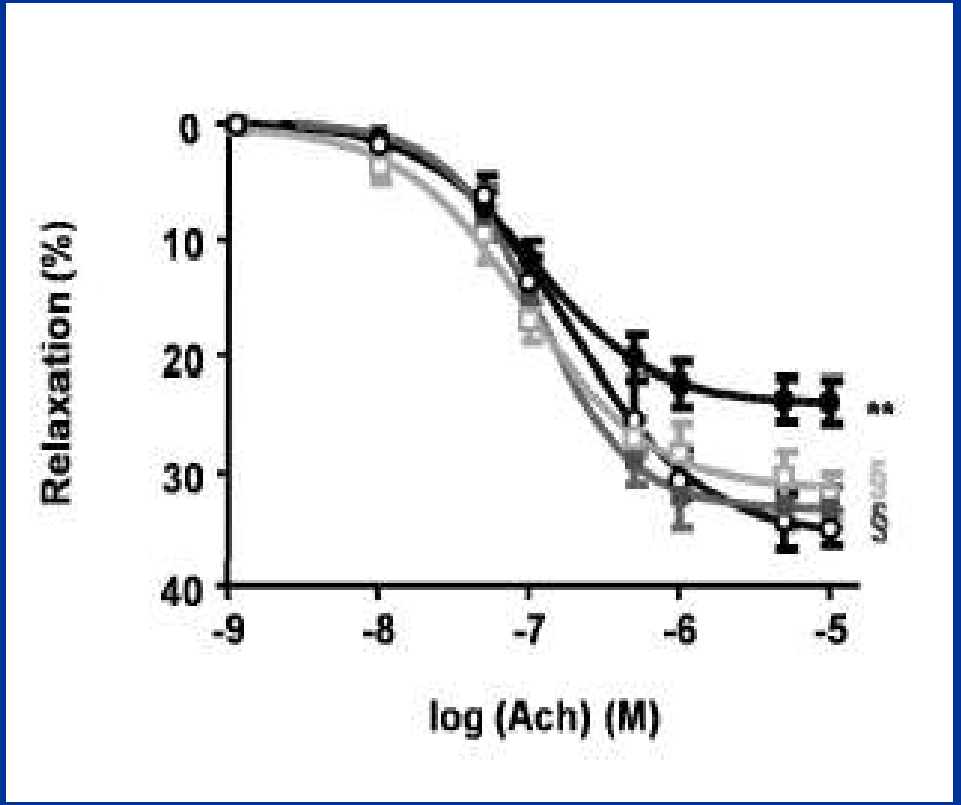
Nutritional prevention

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

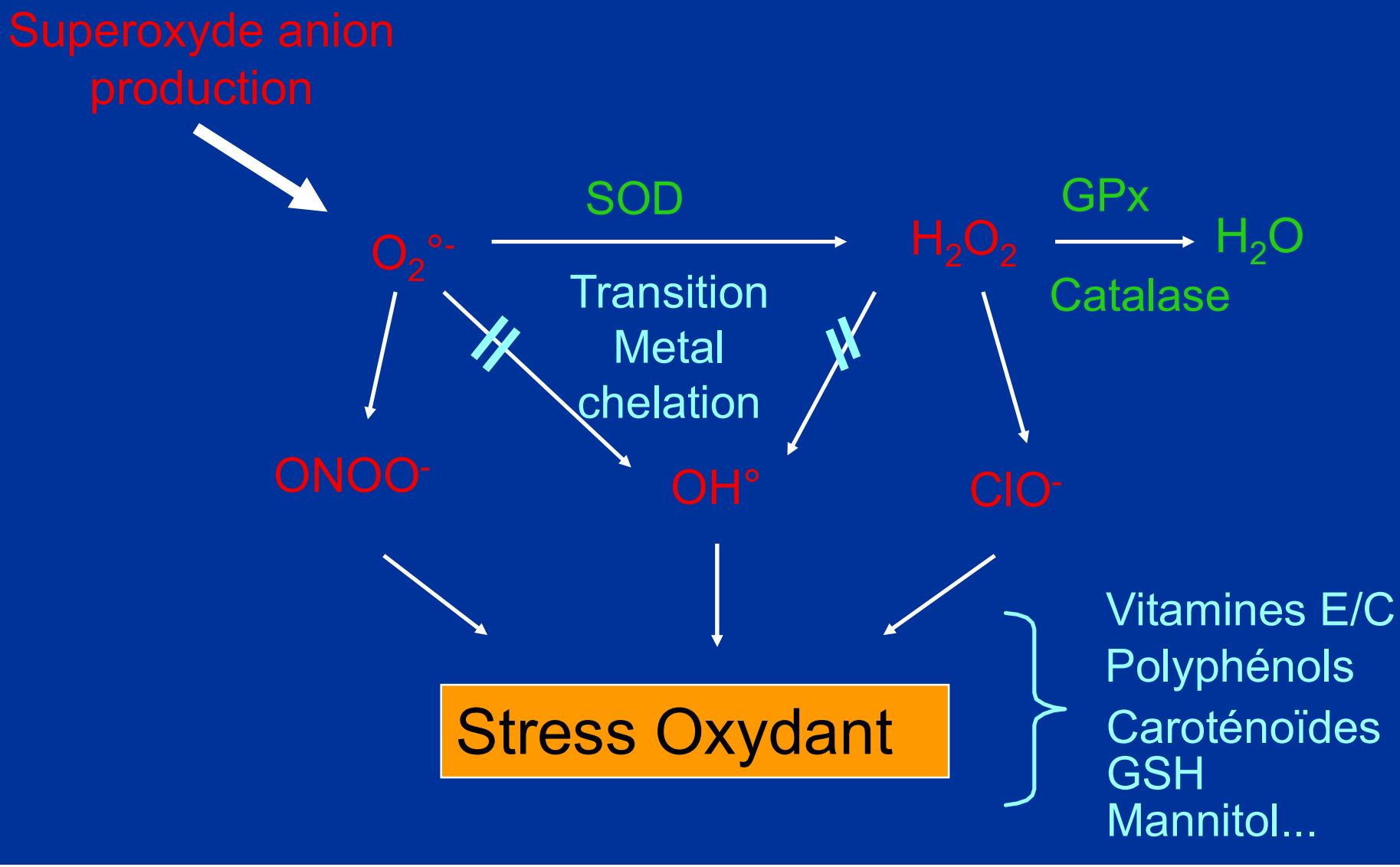
## II) Quantification of Oxidant production ?

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



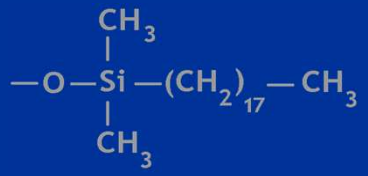
# Détection des vitamines : méthodes analytiques

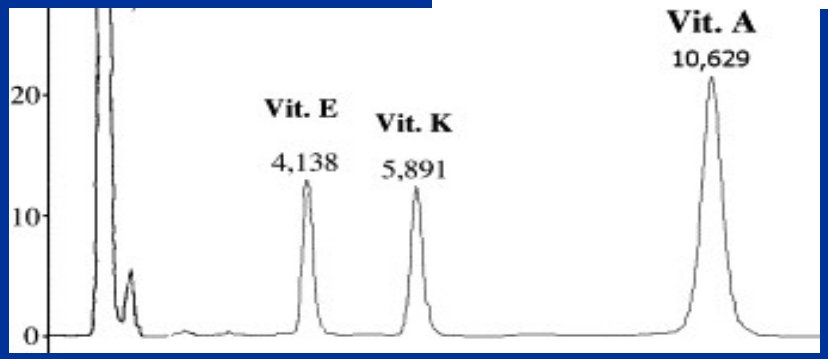
- Les vitamines A, C et E plasmatiques sont dosées par HPLC-UV
- La vitamine E érythrocytaire est dosée par HPLC-Electrochimie

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

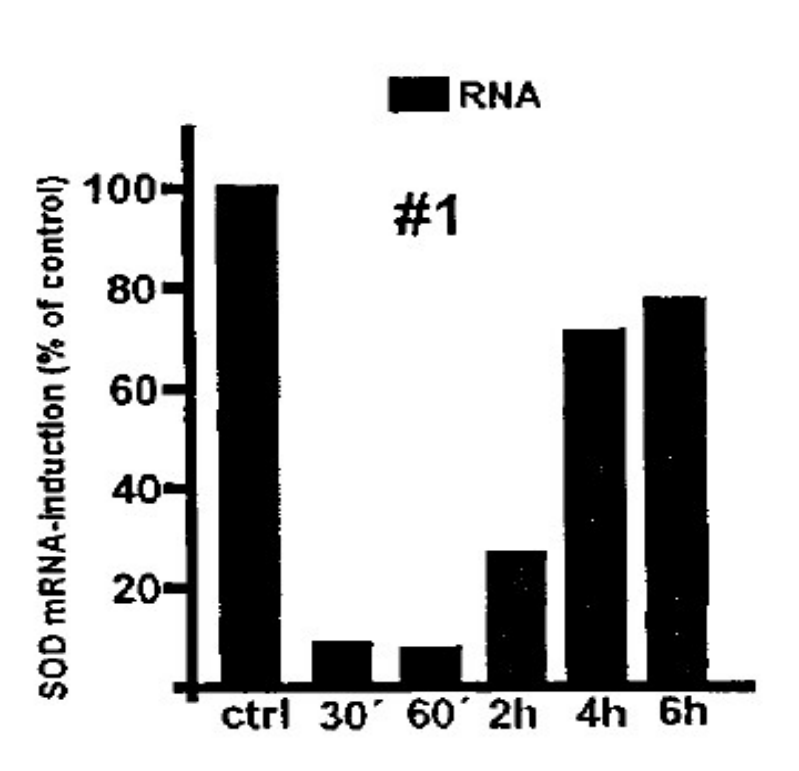
- *Le détecteur électrochimique* mesure les réactions d'oxydoréductions qui produisent un courant proportionnel à la concentration du soluté.

Meilleure sensibilité

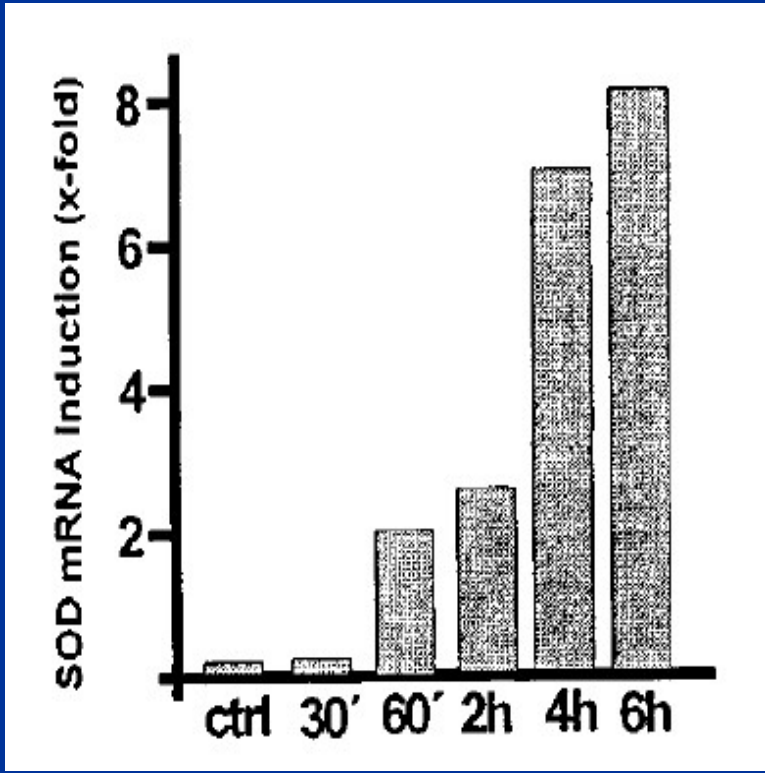
# Limitations of antioxidant system determination

- 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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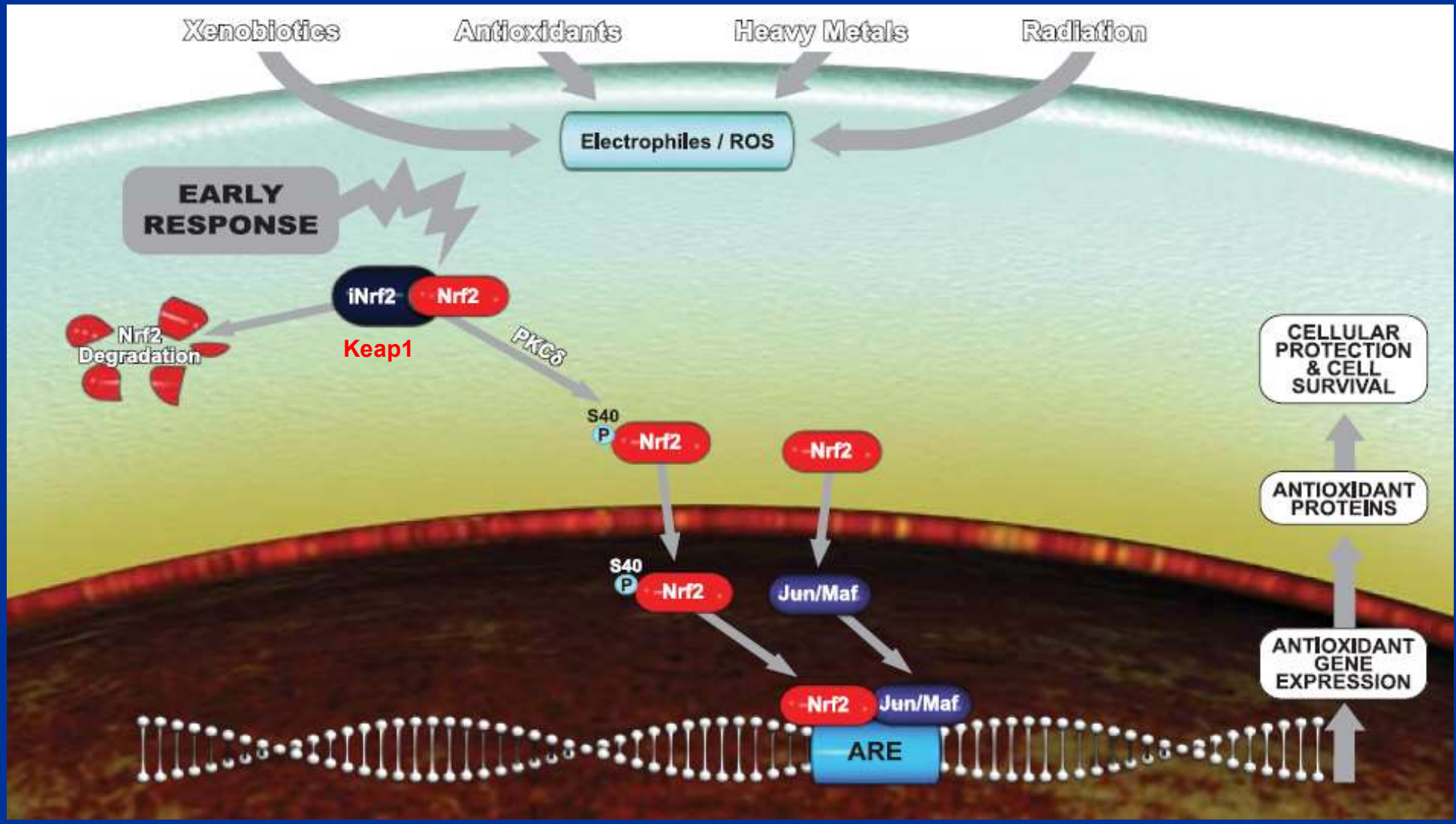
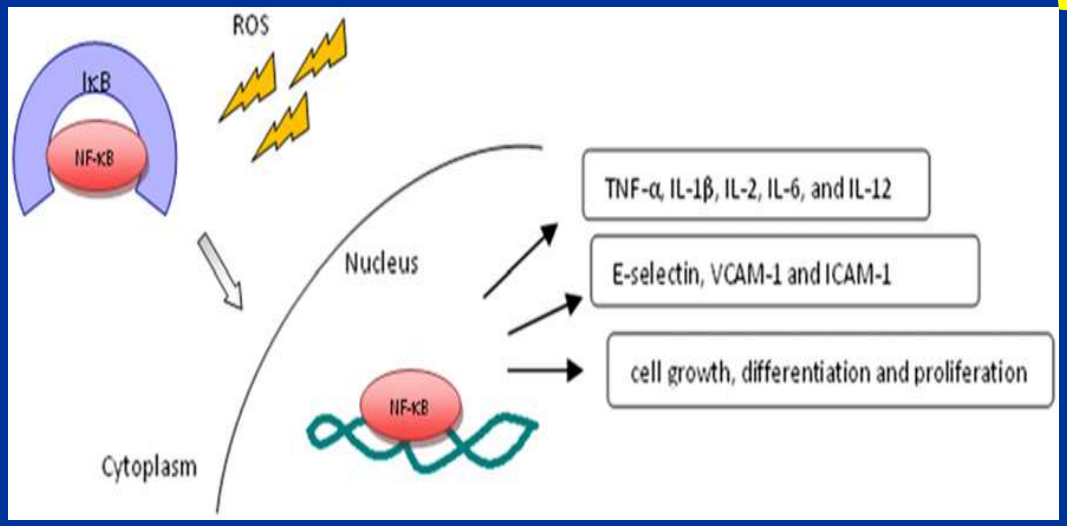
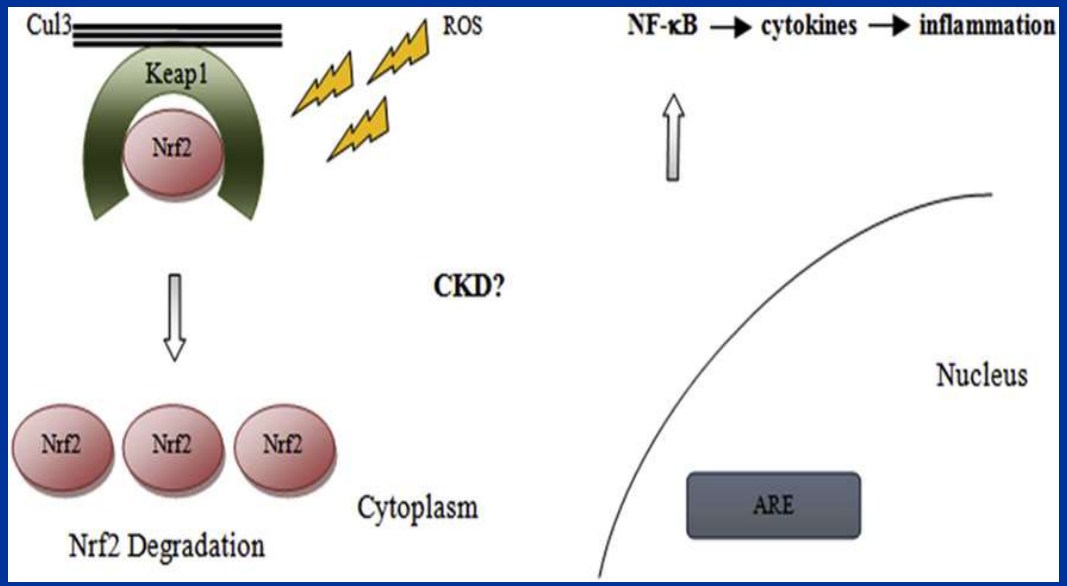
# Inducing enzymatic defense: the Nrf2 pathway

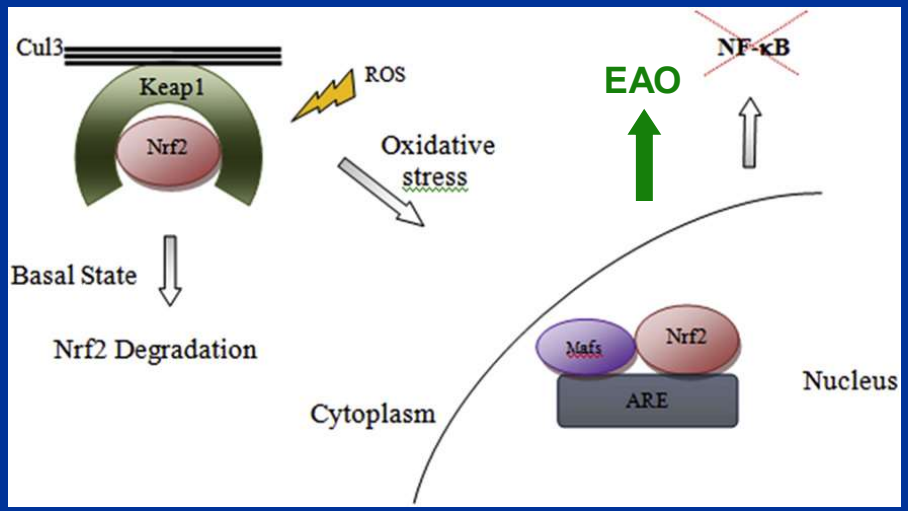



*Jaiswal AK, www.SABiosciences.com/support_literature.php*

# 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 ?

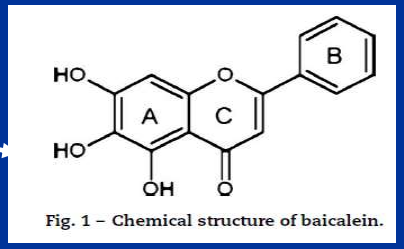
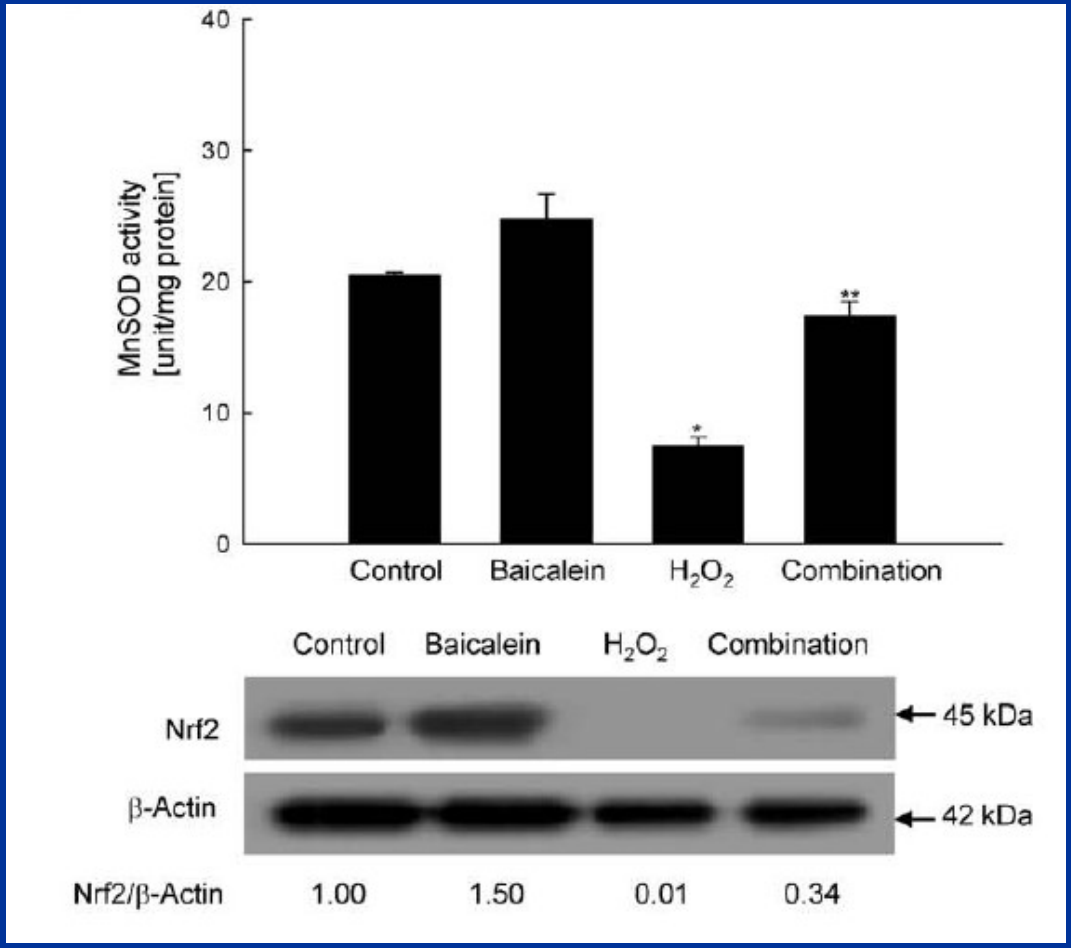
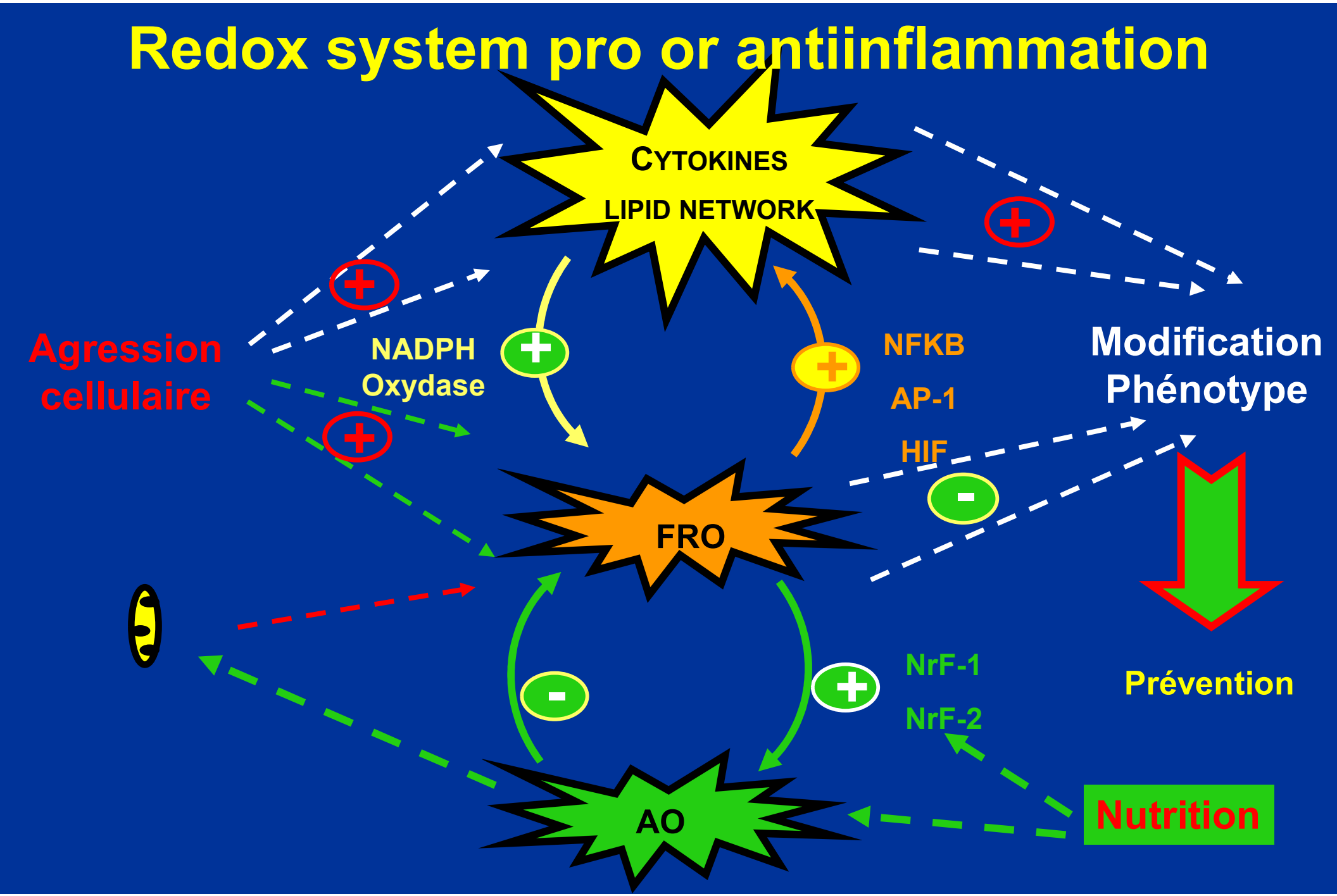


Fig. 1 – Chemical structure of baicalein.

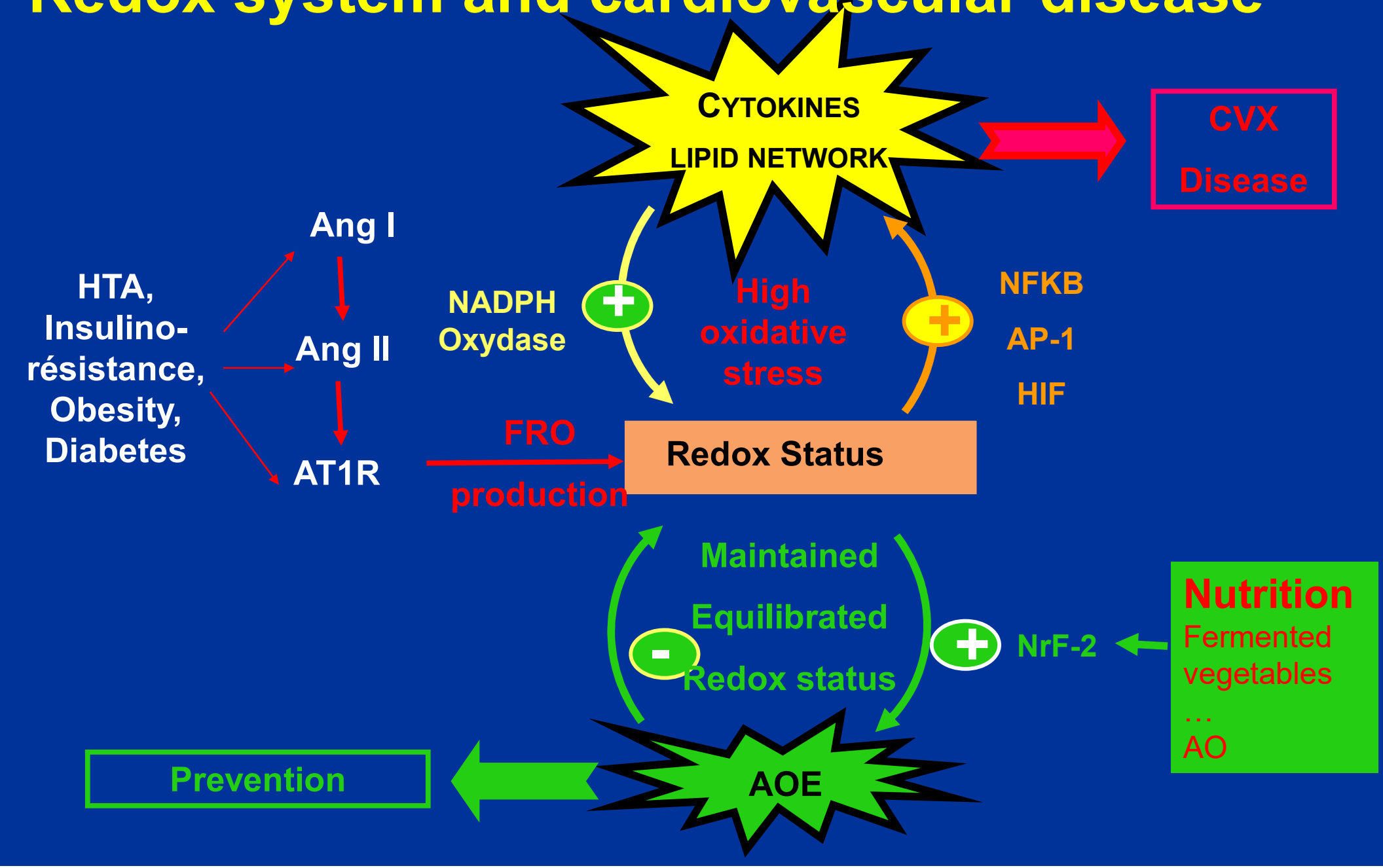



*Lee I.K. Environmental toxicology and pharmacology 2 0 1 1*

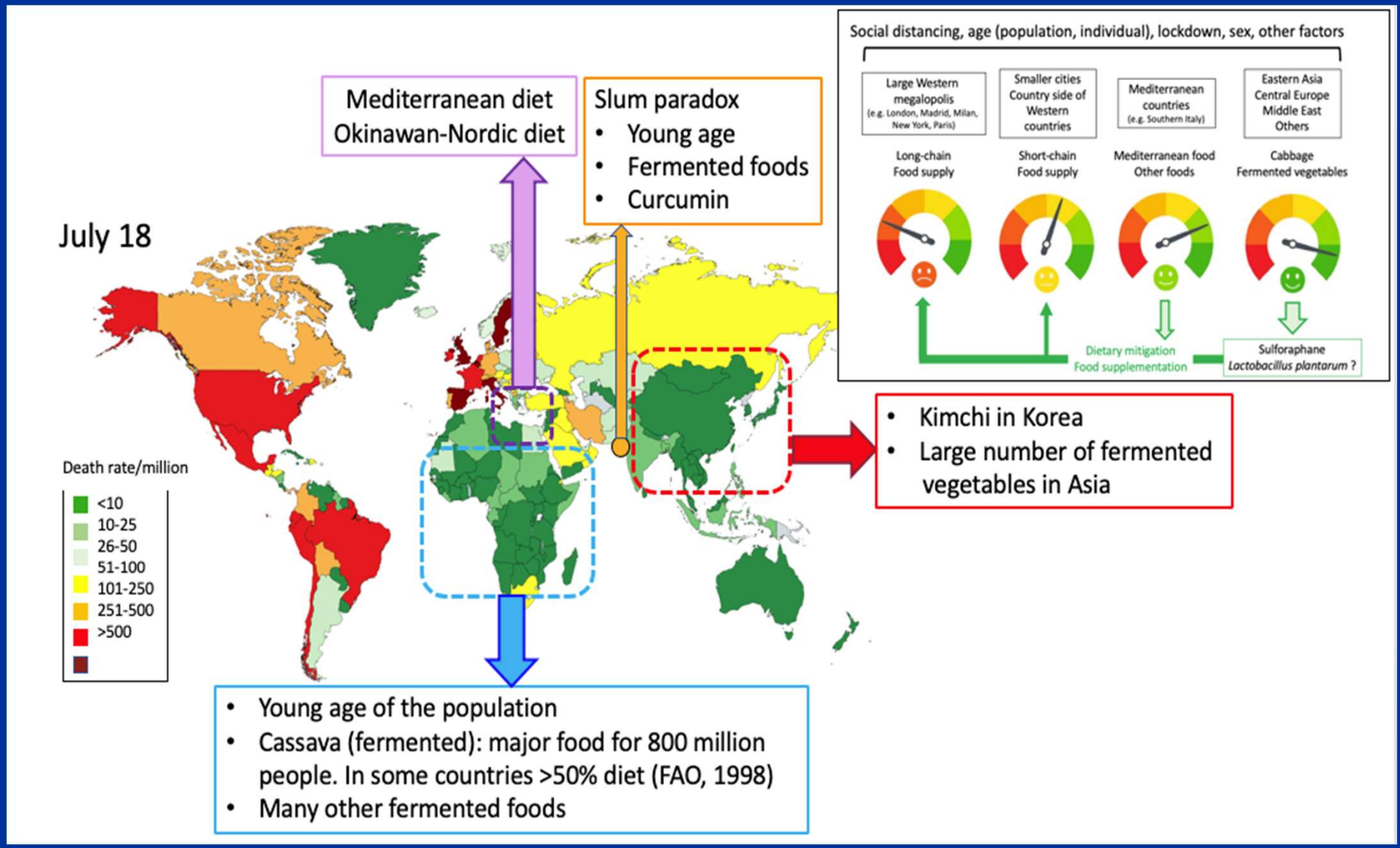
# Redox system pro or antiinflammation



# Redox system and cardiovascular disease

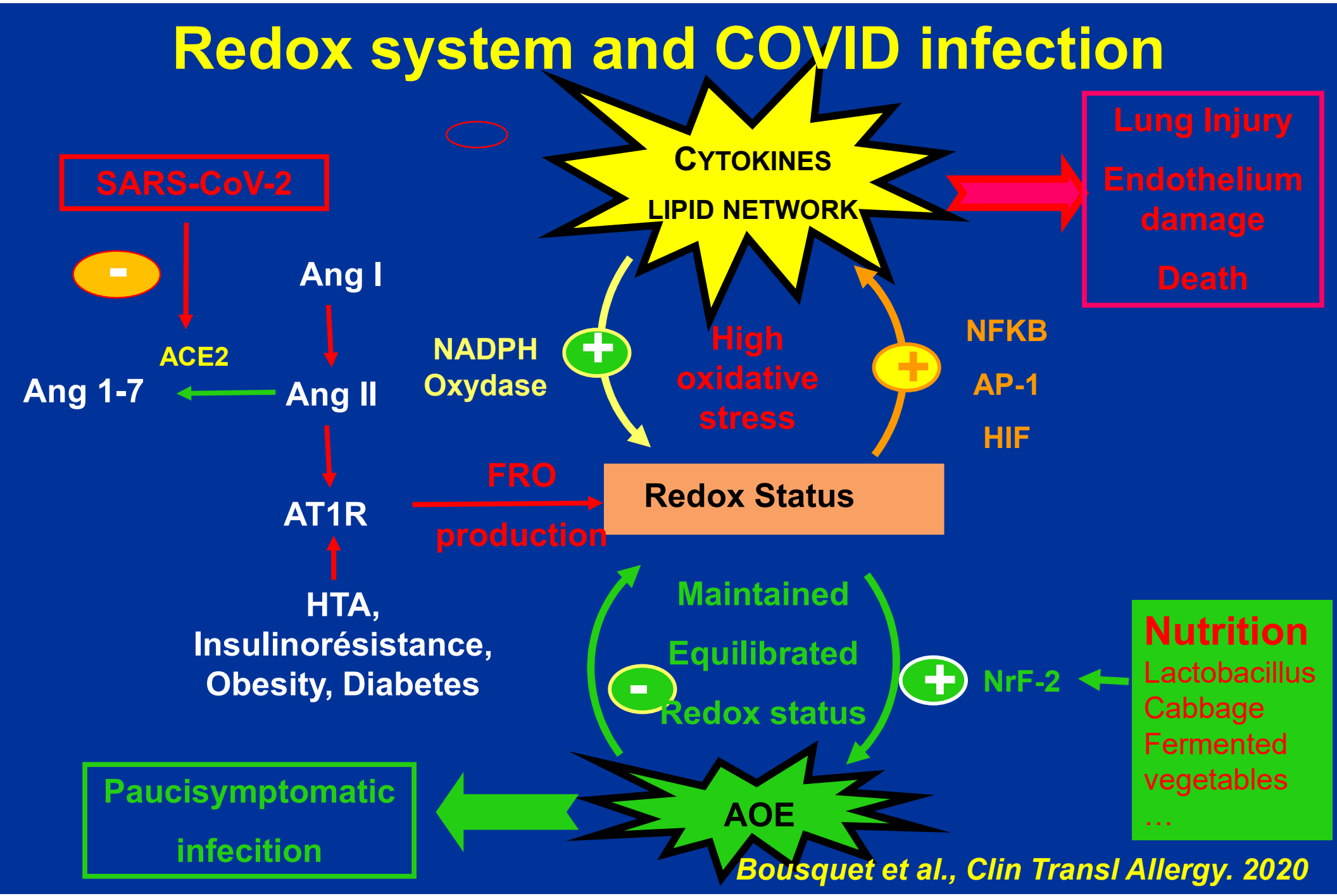


# Nutrition and COVID-19 mortality ?




*Bousquet et al., Allergy. 2020*

# Redox system and COVID infection




*Bousquet et al., Clin Transl Allergy. 2020*