Functional Redoxomics and Aging.

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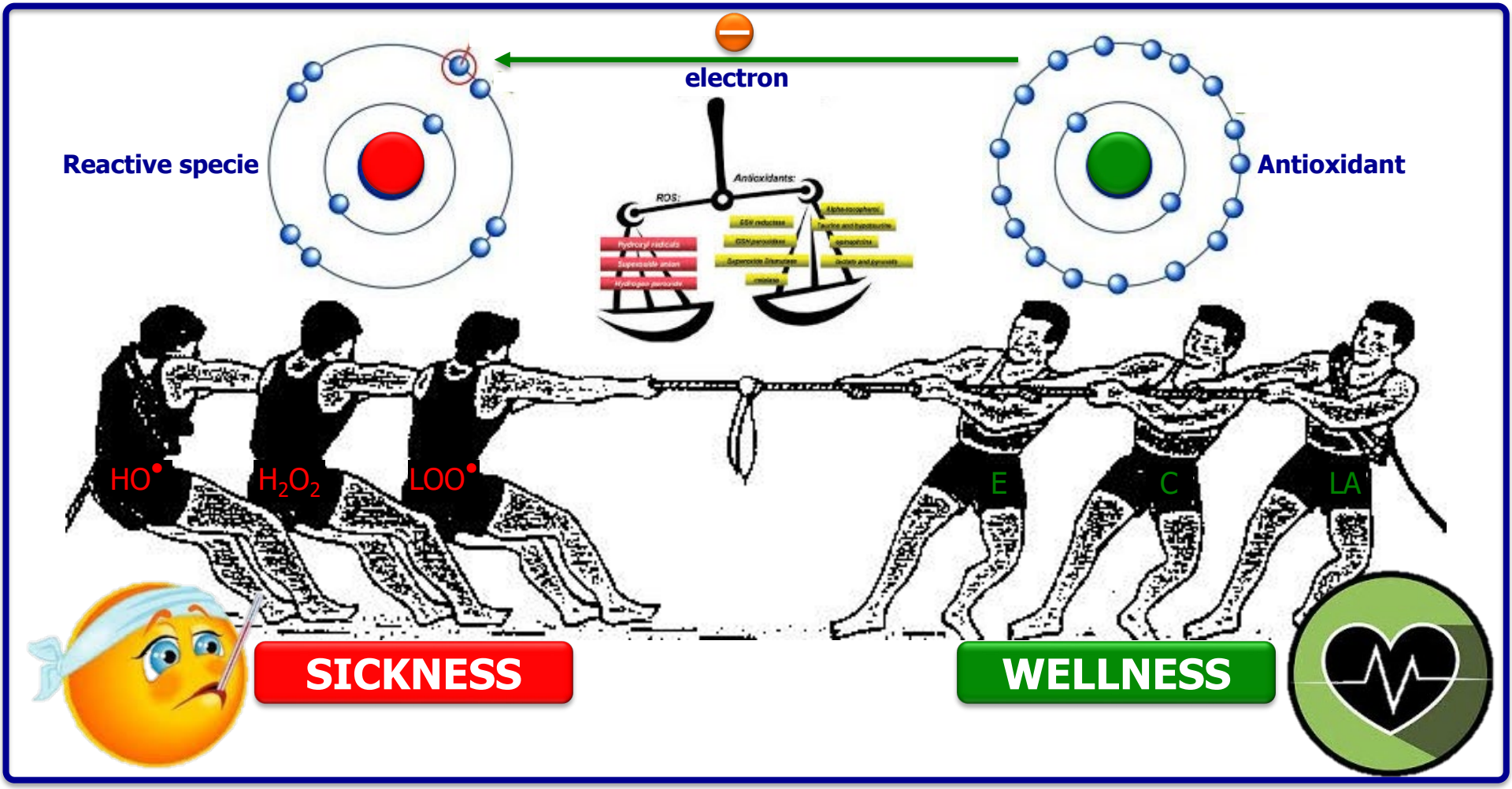
Italian Society of Lifestyle Medicine

London (UK). October 11th, 2018.

A classical equation

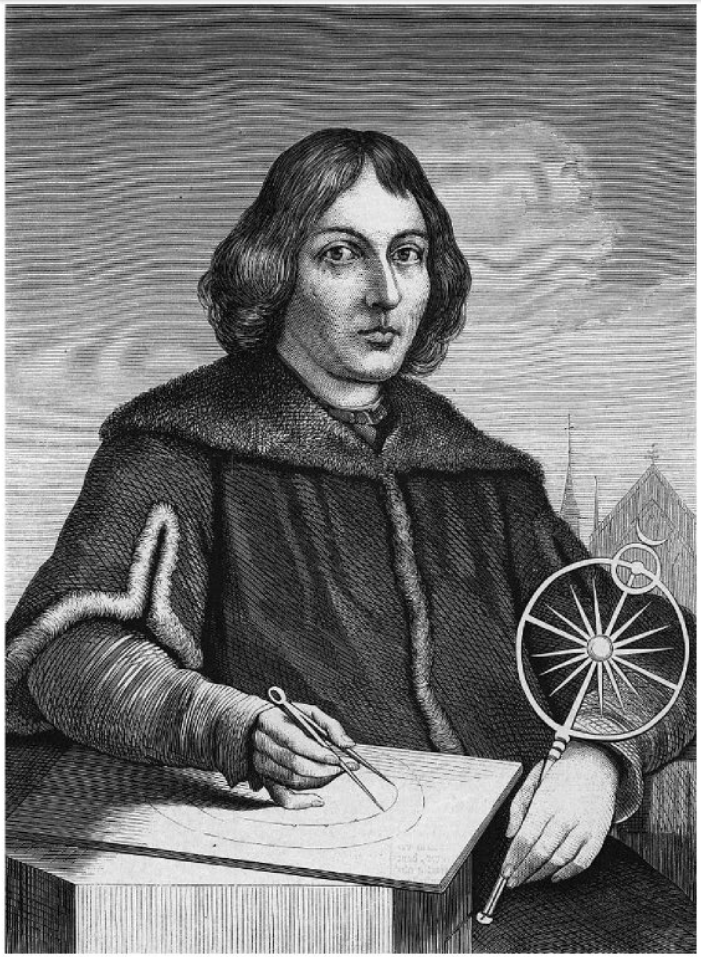
OXIDATION = DAMAGE

The concept of oxidative stress becomes relative because of the antioxidants.



The breakdown of balance between radical/ /non-radical reactive species and antioxidants

The Copernicus Revolution



NICOLAI
COPERNICI TO-
RINENSIS DE REVOLVTIONI-
bus orbium cœleſtium,
Libri VI.

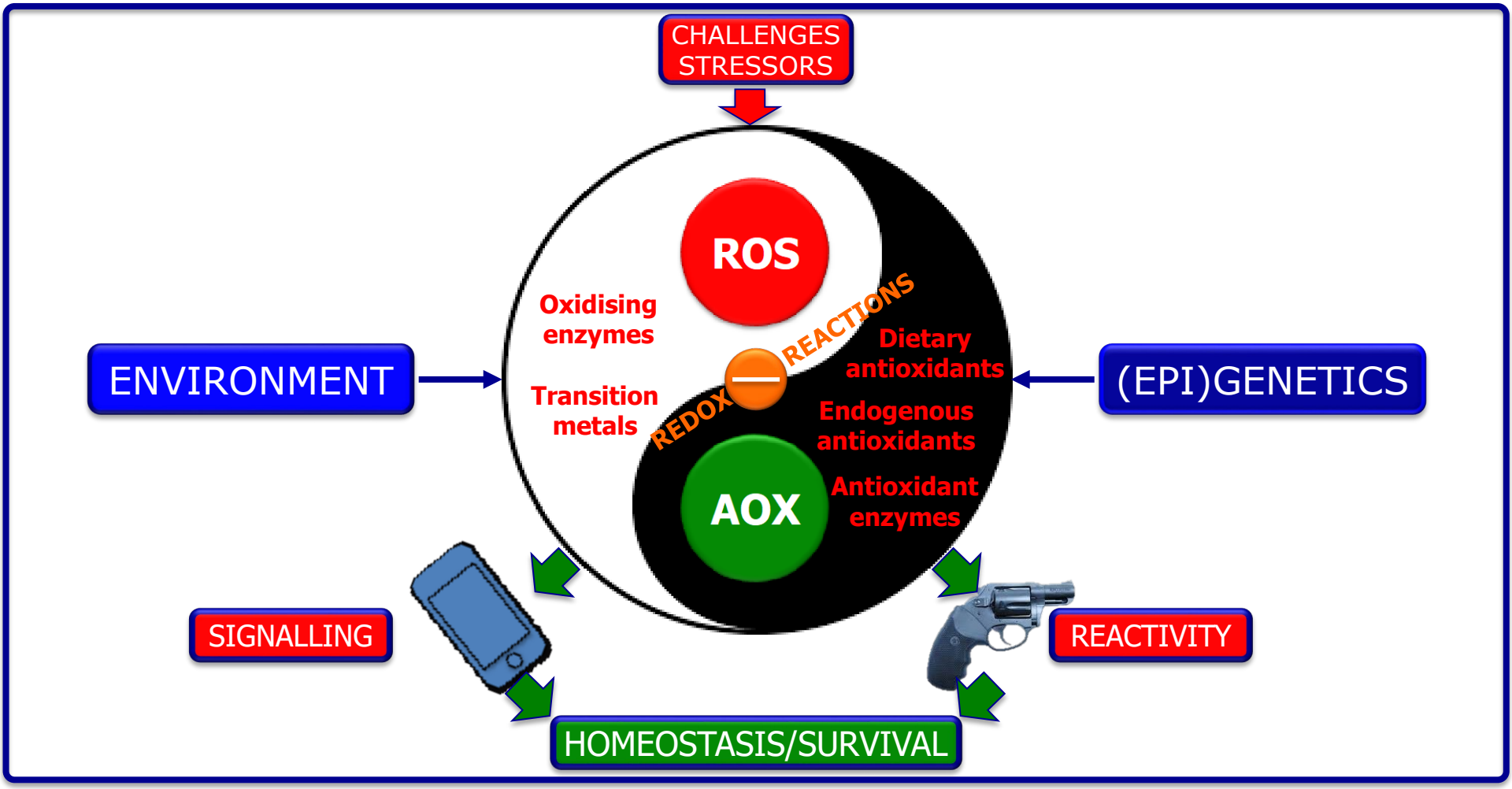
IN QVIBVS STELLARVM ET FI-
XARVM ET ERRATICARVM MOTVS, EX VETE-
ribus atq; recentibus obſeruationibus, reſtituit hic autor.
Præterea tabulas expeditas luculentasq; addidit, ex qui-
bus eoſdem motus ad quoduis tempus Mathe-
matum ſtudioſus facillime calcu-
lare poterit.

ITEM, DE LIBRIS REVOLVTIONVM NICOLAI
Copernici Narratio prima, per M. Georgium Ioachi-
mum Rheticum ad D. Ioan. Schone-
rum ſcripta.

Cum Gratia & Priuilegio Cæſ. Maieſt.
BASILEAE, EX OFFICINA
HENRICPETRINA.

Change the game!

The REDOX system TAO provides the basis for OXIDATIVE STRESS and functional REDOXOMICS



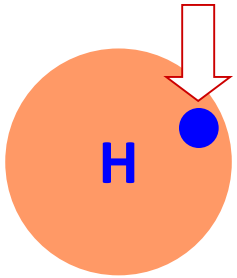
An integrated biochemical system that modulates defence and signalling responses for cell homeostasis/survival

The "YIN" face of the REDOX system

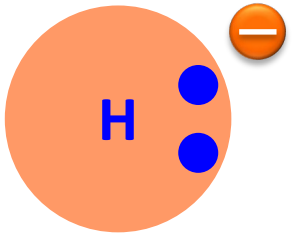
Chemical species	Formula	Class	Chemical species	Formula	Class
Singlet oxygen	$^1O_2^*$	Radical (?)	Nitric oxide	$NO^\bullet$	Radical
Superoxide anion	$O_2^{-\bullet}$	Radical	Nitrous acid	HNO_2	Non radical
Ozone	O_3	Non radical	Nitric tetroxide	N_2O_4	Non radical
Hydroxyl radical	$HO^\bullet$	Non radical	Nitric trioxide	N_2O_3	Non radical
Hydrogen peroxide	H_2O_2	Radical	Peroxynitrite	$ONOO^-$	Non radical
Alkyl radical	$R^\bullet$	Radical	Peroxynitrous acid	ONOOH	Non radical
(Alkyl-)peroxyl radical	$ROO^\bullet$	Radical	Nitronium cation	NO_2^+	Non radical
(Alkyl)hydroperoxide	ROOH	Non radical	(Alkyll)peroxynitrite	$ROONO^-$	Non radical
Semiquinone (from Co Q_{10})	$Q^\bullet$	Radical	Hypochlorous acid	HOClO	Non radical
Tocopheryl (from vitamin E)	$E\text{-}O^\bullet$	Radical	Thyil radical	$-S^\bullet$	Radical

Radical and non-radical reactive oxidant species

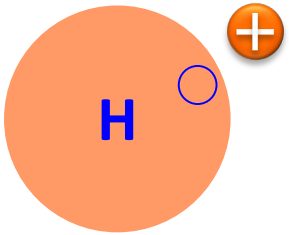
Atoms, ions, radicals and molecules.



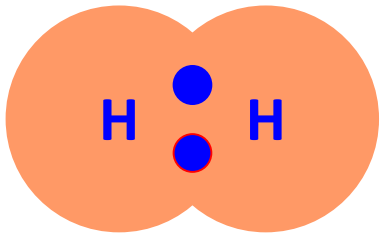
Hydrogen atom (radical)



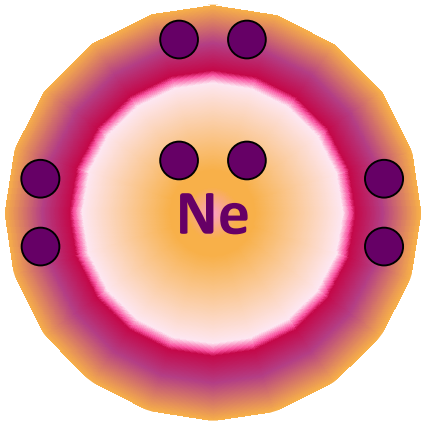
Hydride ion



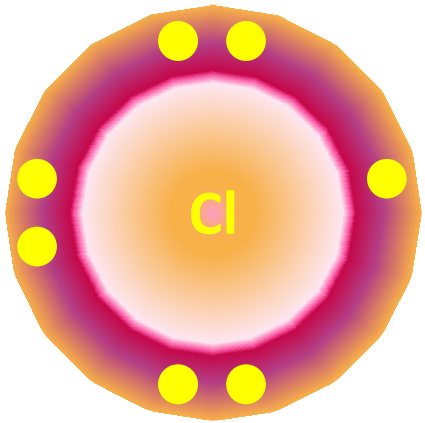
Hydrogen ion



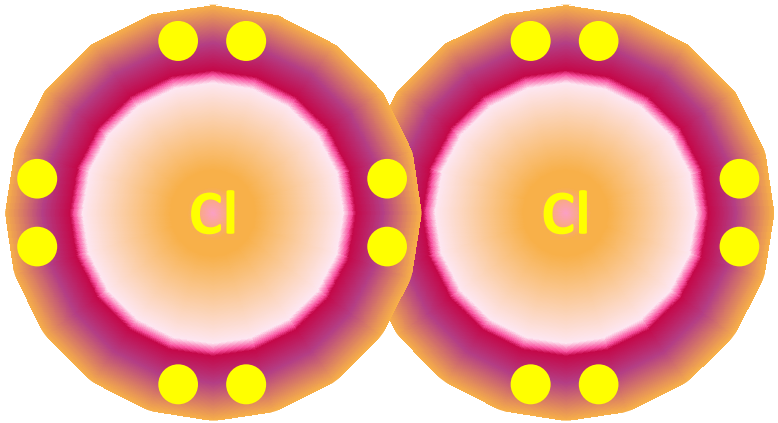
Hydrogen molecule



Neon atom



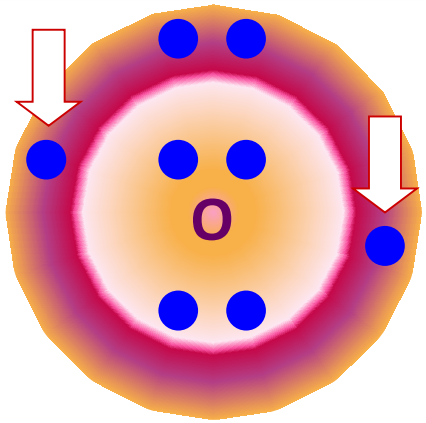
Chlorine atom (radical)



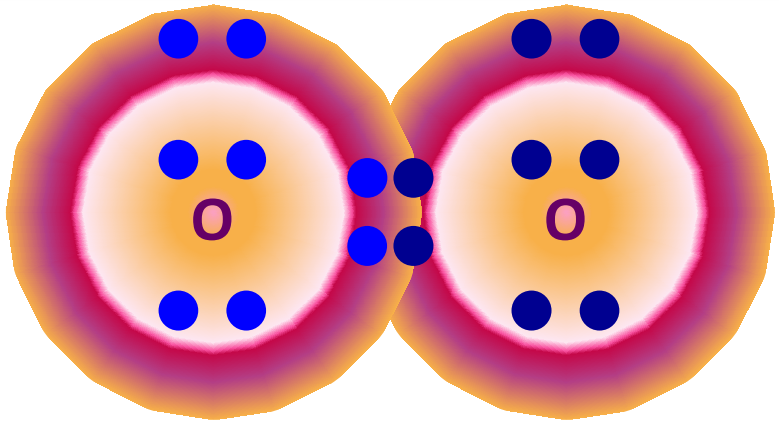
Chlorine molecule

Both hydrogen atom and chlorine atom are radicals

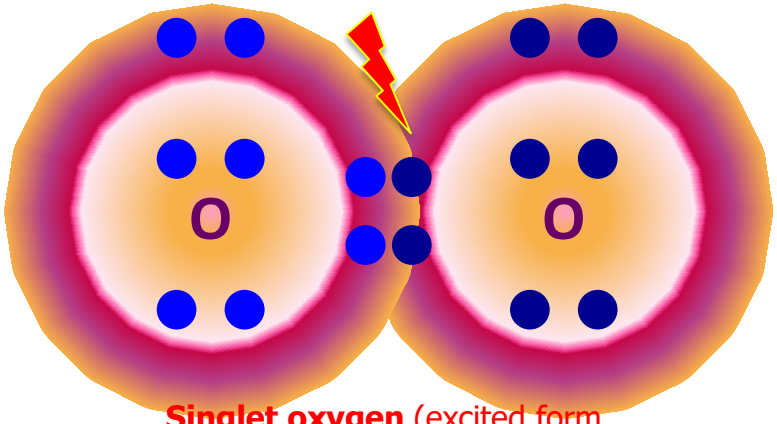
The main species of oxygen



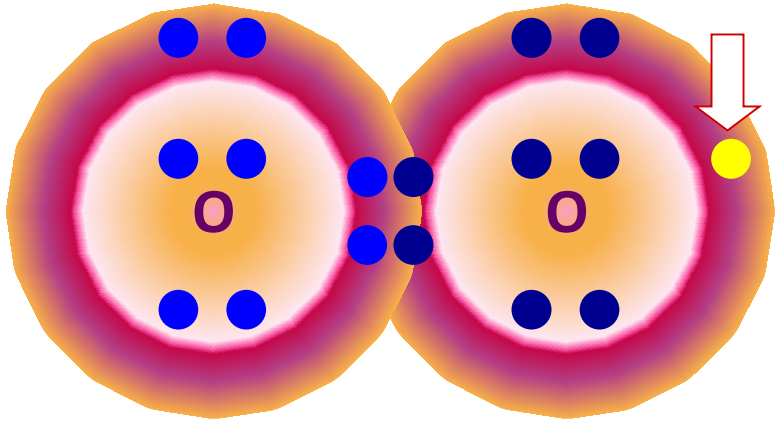
Oxygen's atom (2 unpaired e^-, unstable/reactive)



Oxygen's molecule (all paired electrons, stable)



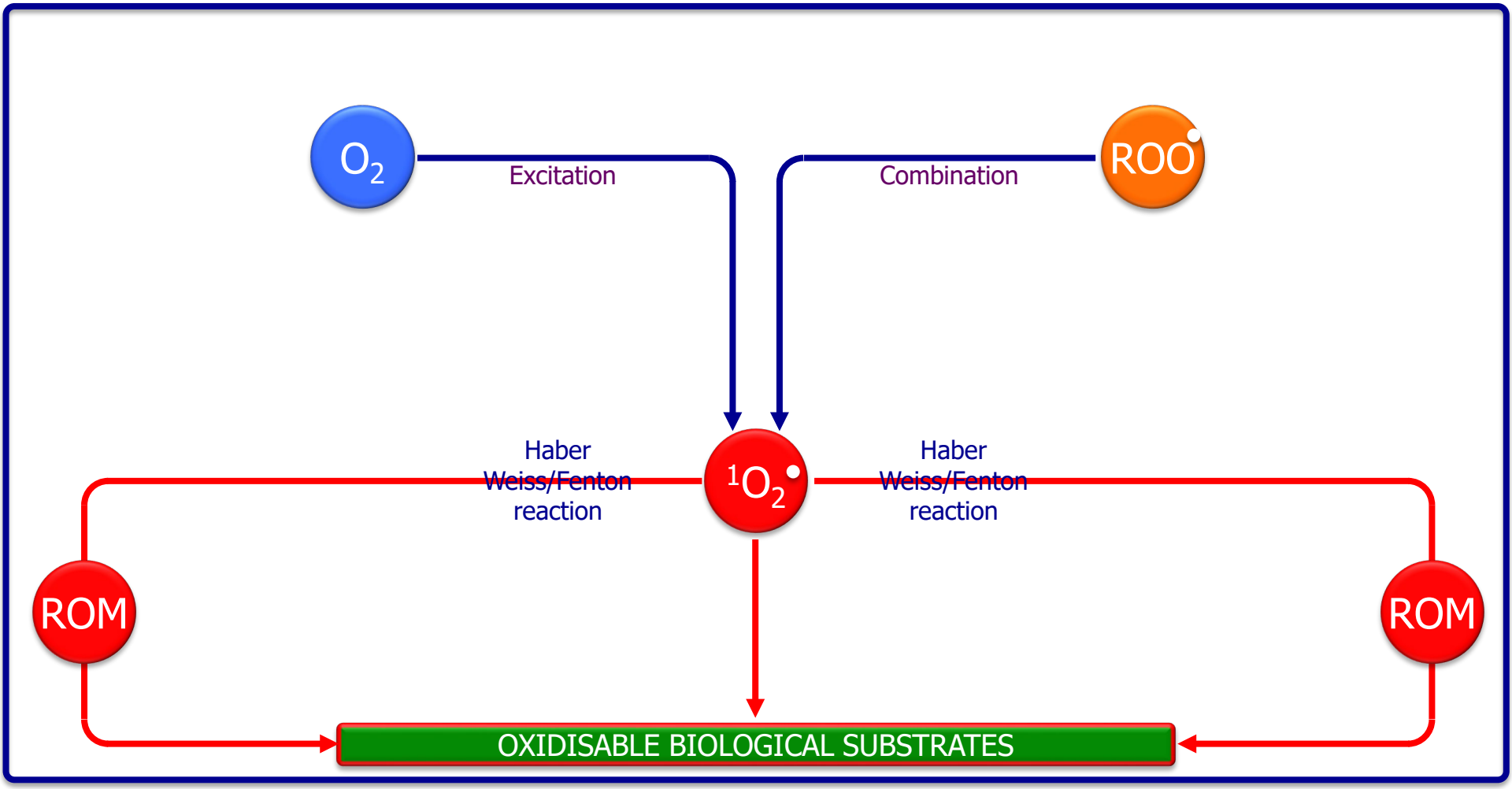
Singlet oxygen (excited form, unstable/reactive)



Superoxide anion (one unpaired e^-, unstable/reactive)

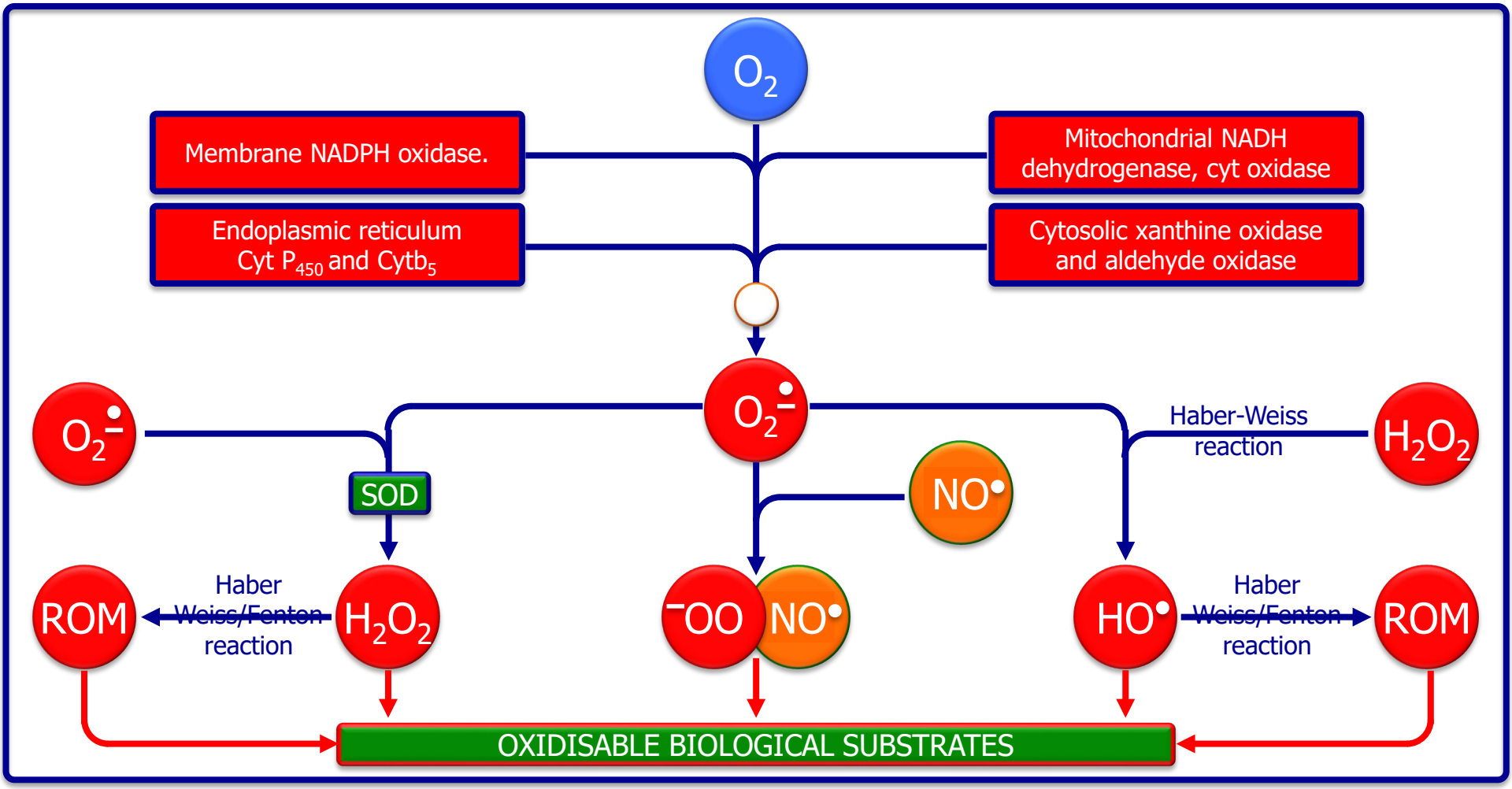
Oxygen atom and superoxide anion are classical radicals with different reactivity and differs form singlet oxygen.

Singlet oxygen metabolism



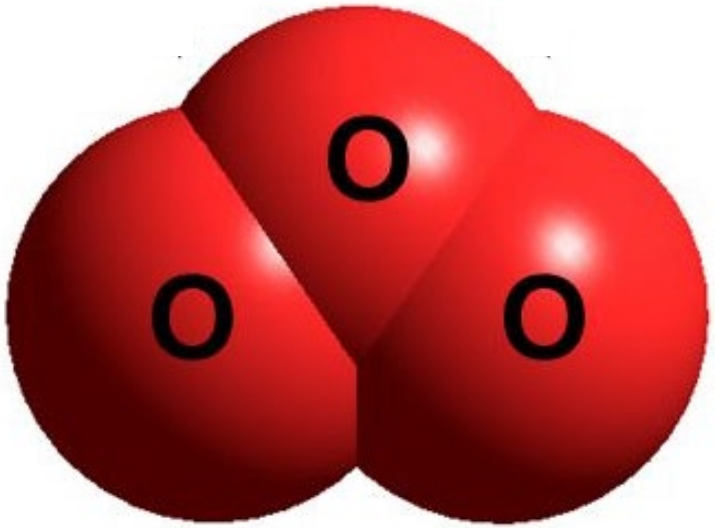
Overview

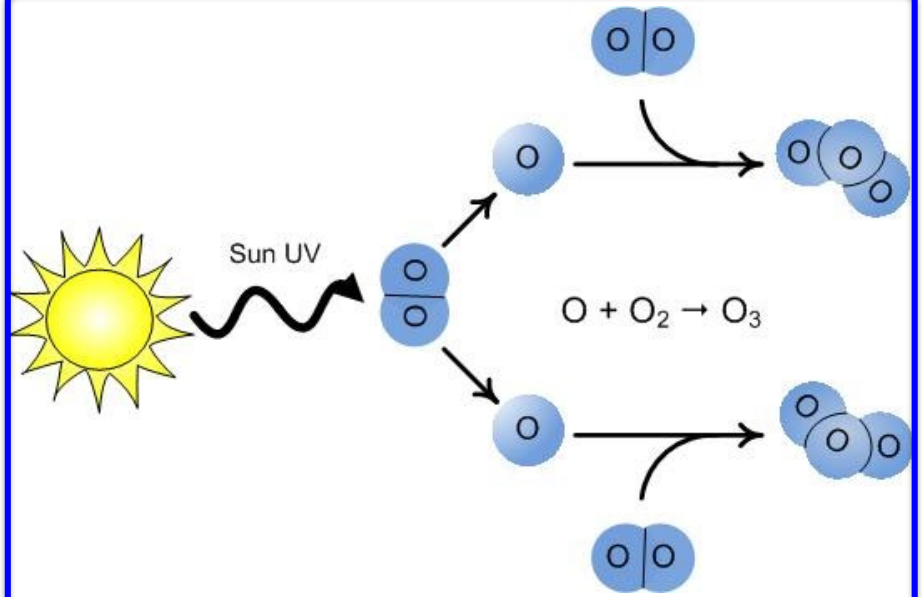
Superoxide anion metabolism

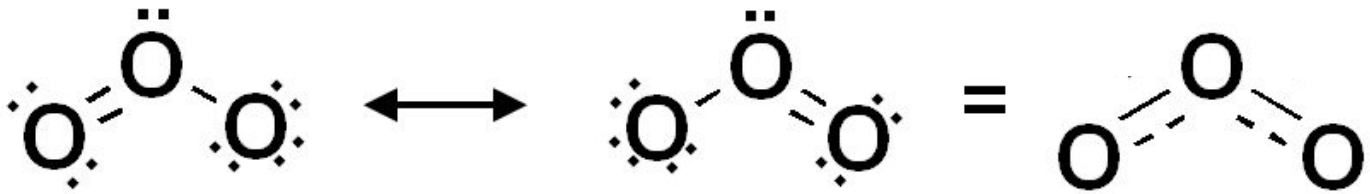


Overview

The ozone (O_3)

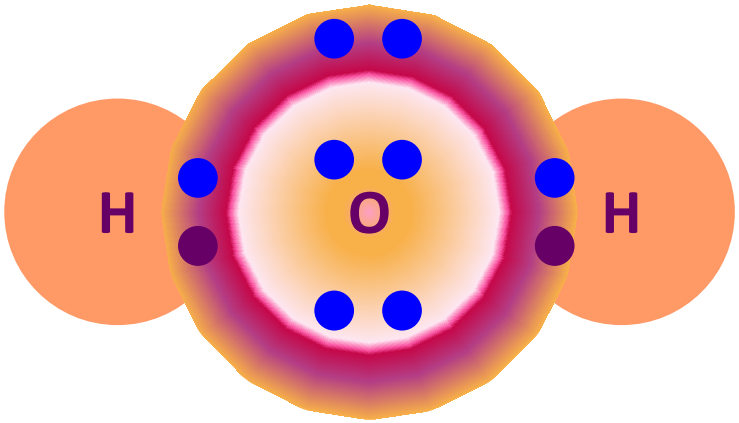




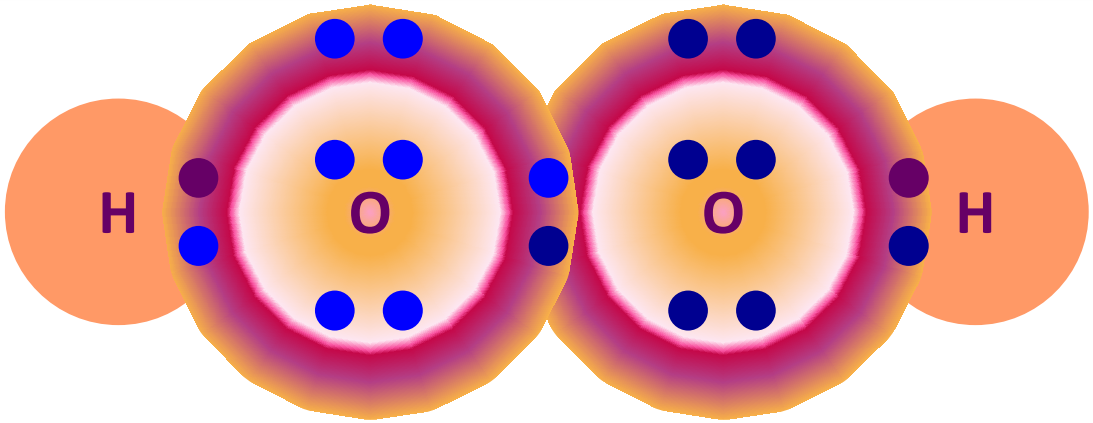


A powerful reactive oxygen species with therapeutic potential

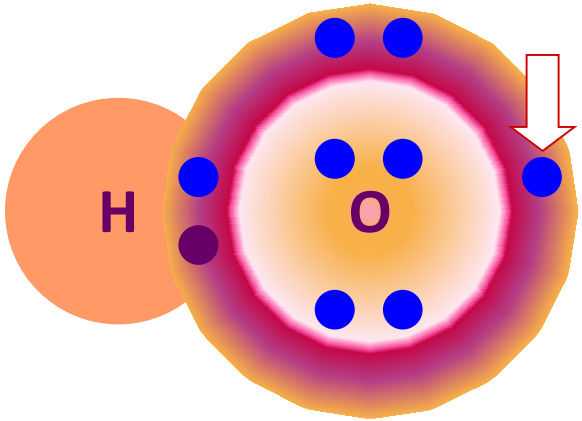
The main species of oxygen



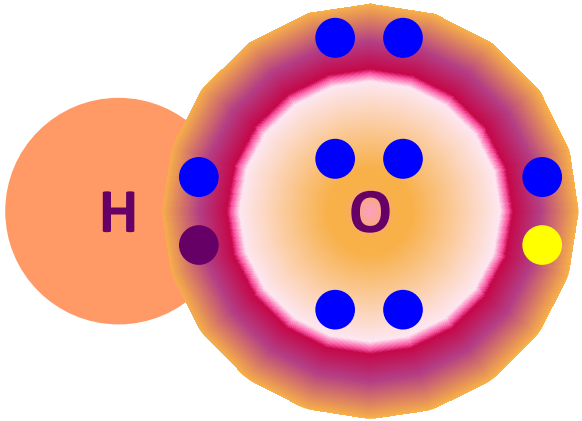
Water molecule all paired electrons, stable)



Hydrogen peroxide molecule (all paired electrons, unstable/reactive)



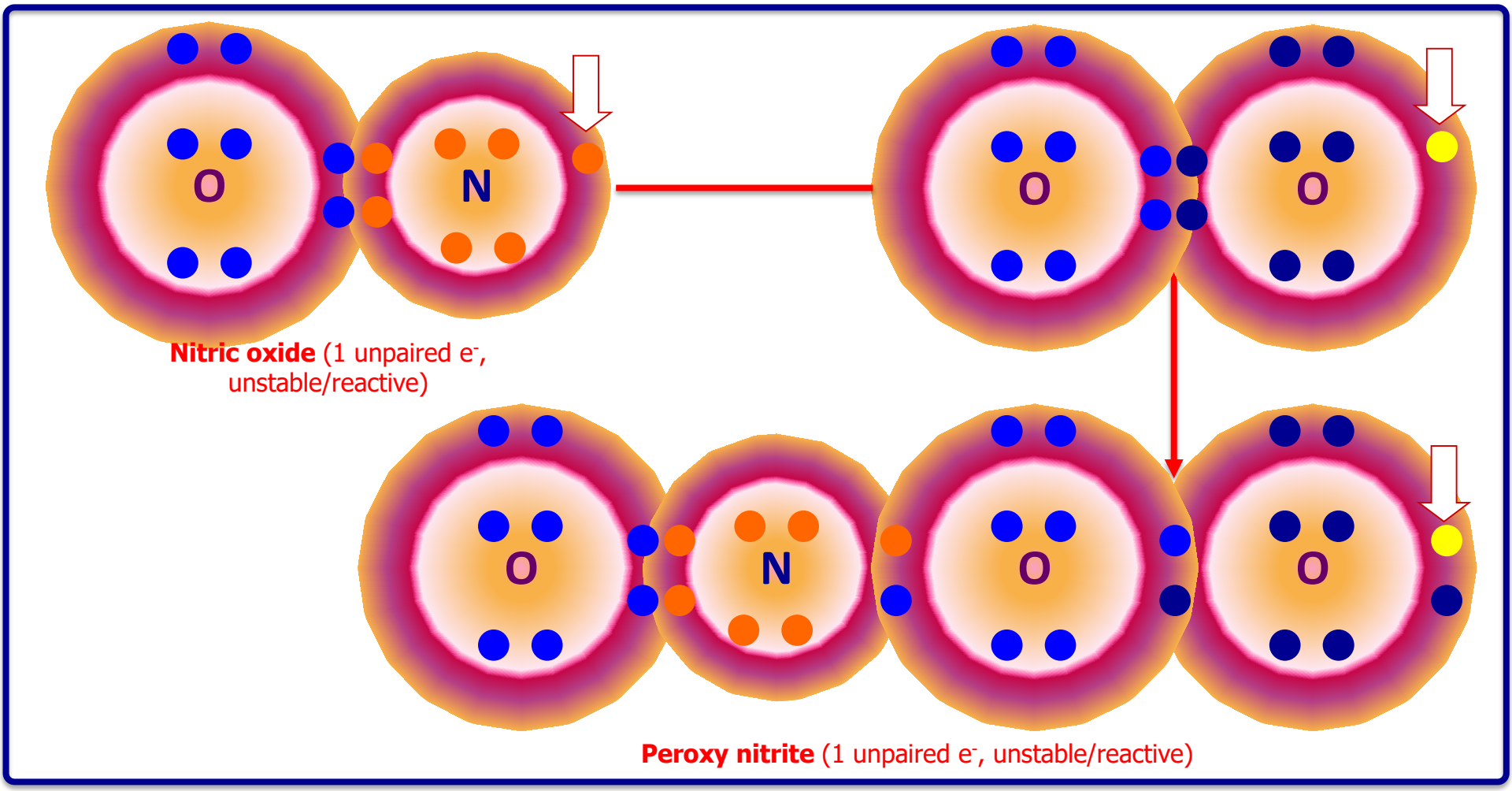
Hydroxyl radical (unstable, highly reactive)



Hydroxide anion (all paired e^-, stable)

Oxygen atom and superoxide anion are classical radicals with different reactivity and differs form singlet oxygen.

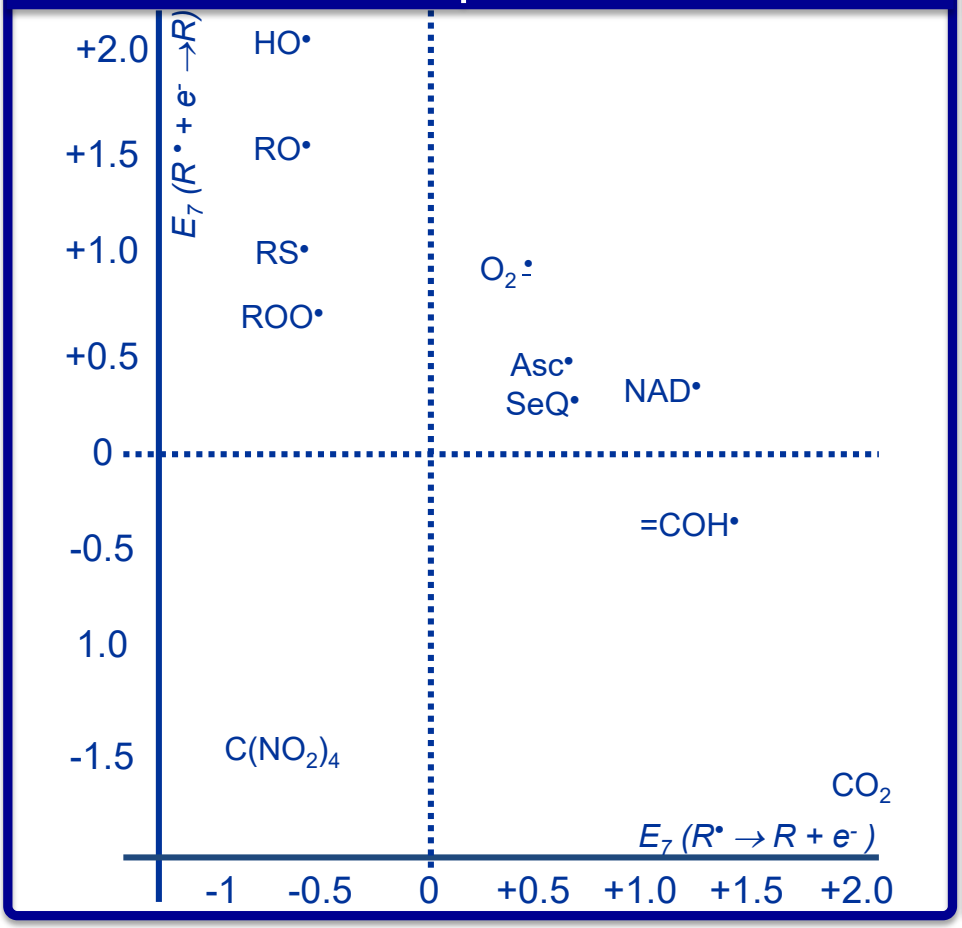
The two main reactive species of nitrogen



Nitric oxide can loose its positive effects due to the ability of superoxide anion to oxidise it to peroxynitrite

Reactivity is not an intrinsic feature of radicals i. e. some radicals are stable and unreactive!

Redox potential

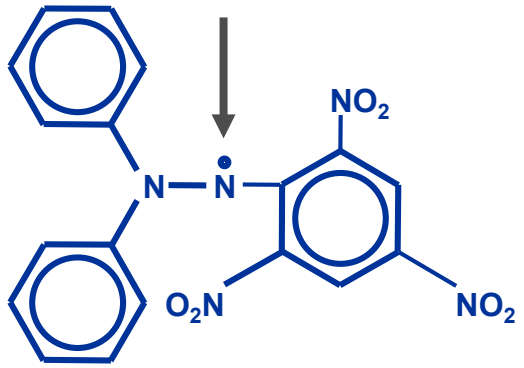


Charge/surface ratio

HO• hydroxyl radical

Cl• chlorine atom

highly reactive radicals (high charge/surface ratio)



Diphenylpicrylhydrazide (DPPH)

A scarcely reactive radical (low charge/surface ratio)

Redox potential and chemical structure predict radical reactivity

Reactive oxidant species are continuously and physiologically generated in all living organisms

Generation of reactive oxidant species ways

Without catalysts

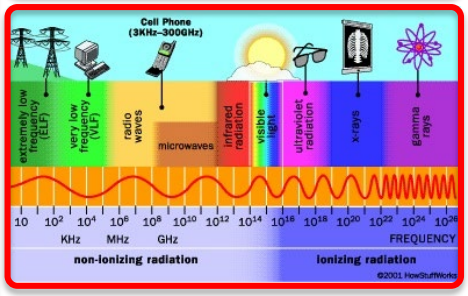
With catalysts

Physical agents

Chemical agents

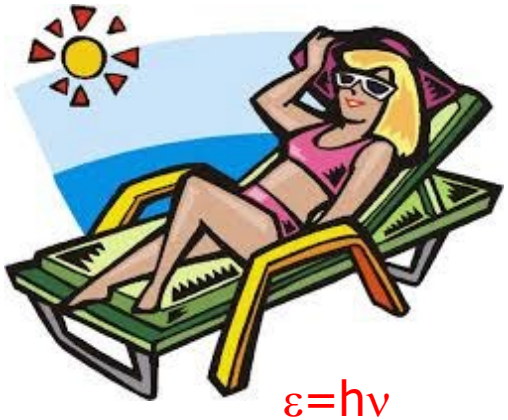
Inorganic (Fe/Cu)

Enzymes

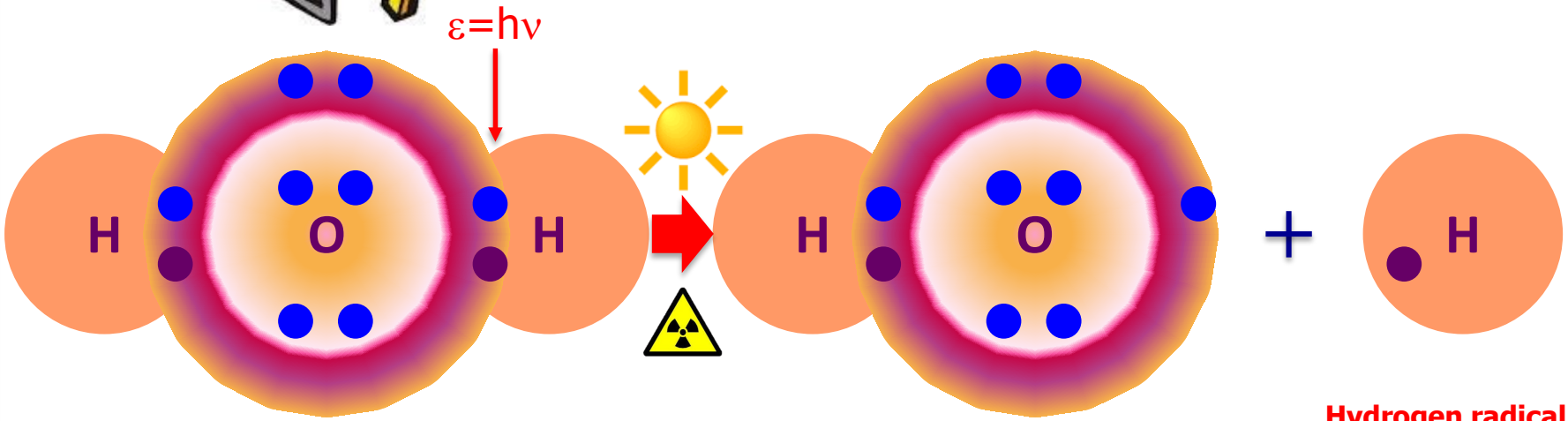


Reactive oxidant species are irreplaceable good/bad "journey companion" of cells life

The generation of free radicals due to the radiations



Estimated total daily amount of ROS generated from 18 grams of water: 2 to 4 x 6.02 x 10^{23}.



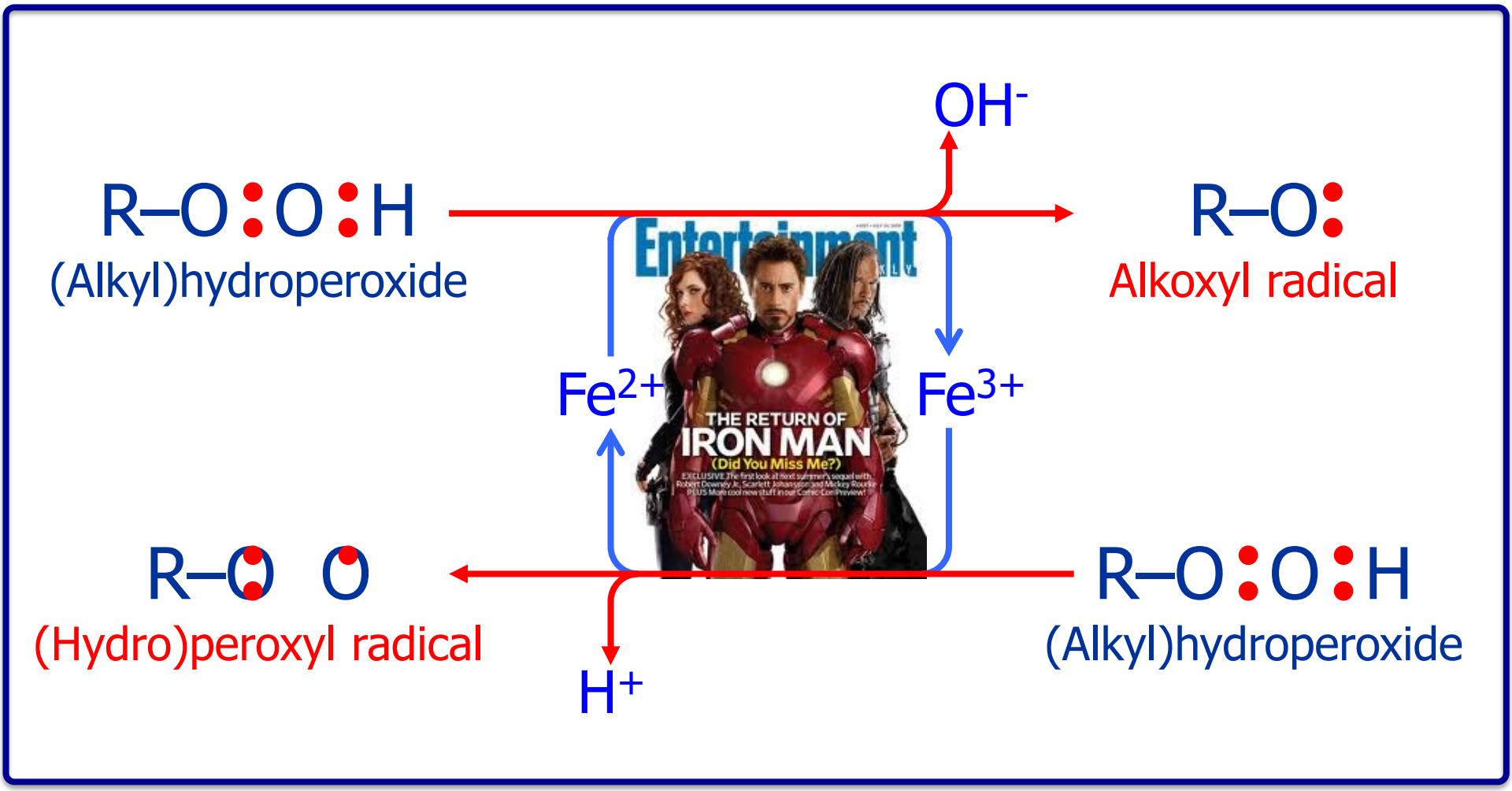
Water molecule
(all paired electrons, stable)

Hydroxyl radical (one unpaired electron, highly unstable/reactive)

Hydrogen radical (1 unpaired electron, unstable/reactive)

The water photolysis, a relevant phenomenon in the skin!

The generation of free radicals due to the free iron/copper catalytic action on peroxides

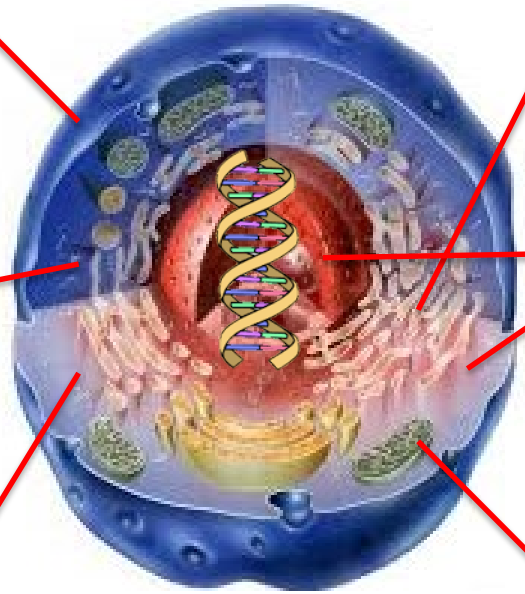


The so-called Fenton's reaction

The generation of reactive oxidant species associated to cell enzymatic activities

Membrane NADPH oxidase, NOS, lipooxygenase (?).

Endoplasmic reticulum Cyt P_{450} and $Cytb_5$



Peroxisome long chain fatty acid oxidative pathway

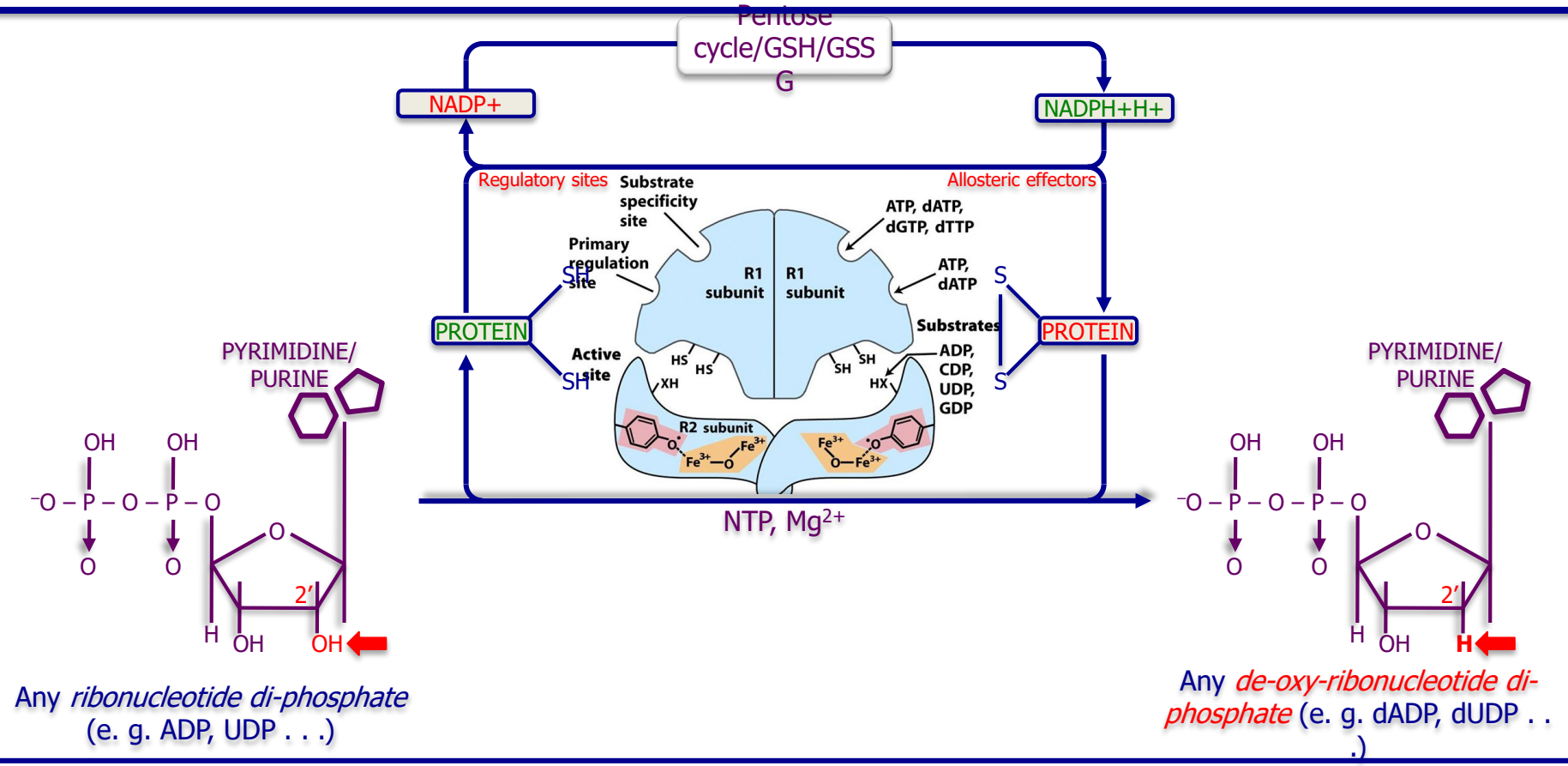
Other pathways (NDPr, FAD, MAO, DAAO, NOS, MPx,)

Cytosolic xanthine oxidase and aldehyde oxidase

Mitochondrial NADH dehydrogenase, Cyt oxidase

Many enzymes are physiologically involved in the production of reactive oxidant species

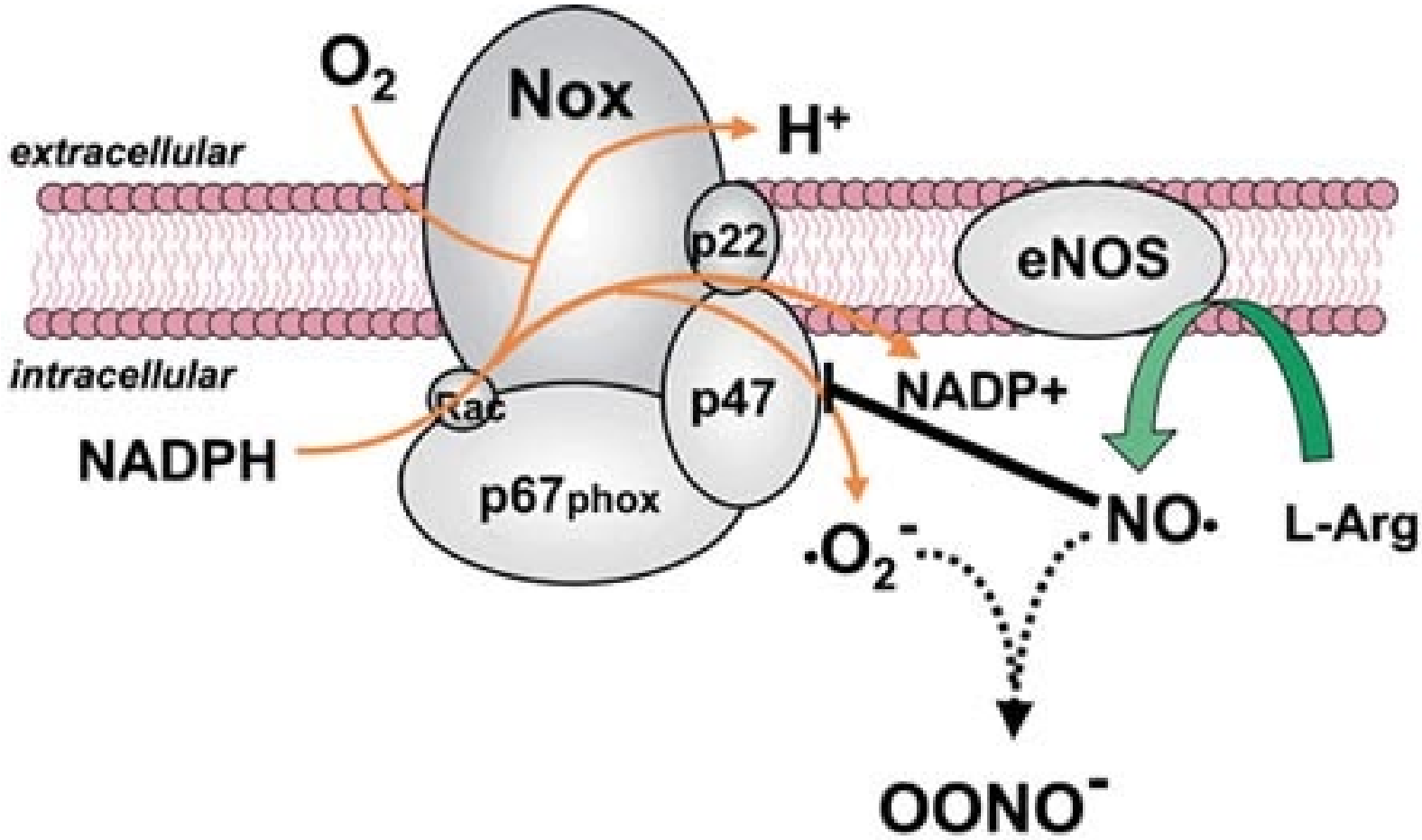
The nucleoside 5-diphosphate reductase converts ribonucleotides to 2′-de-oxy-ribonucleotides



Each site active of nucleoside 5-diphosphate reductase contains two thiols and a (-XH) that can be converted to an active-site radical; this group is probably the –SH of ^{439}Cys which functions as thiyl radical (-S·).

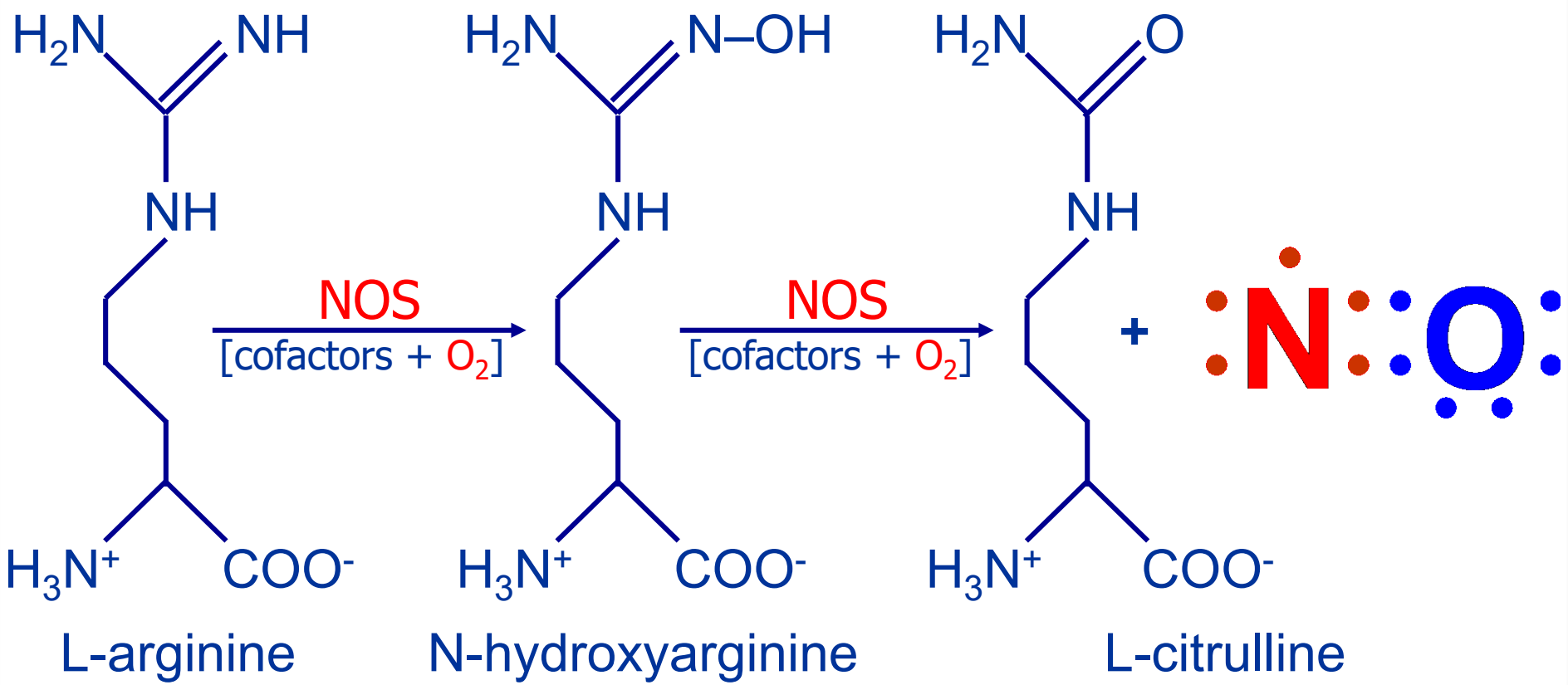
The nucleoside 5-diphosphate reductase, a redox enzyme, is the key enzyme of DNA biosynthesis!

The production of reactive oxidant species by cell membrane



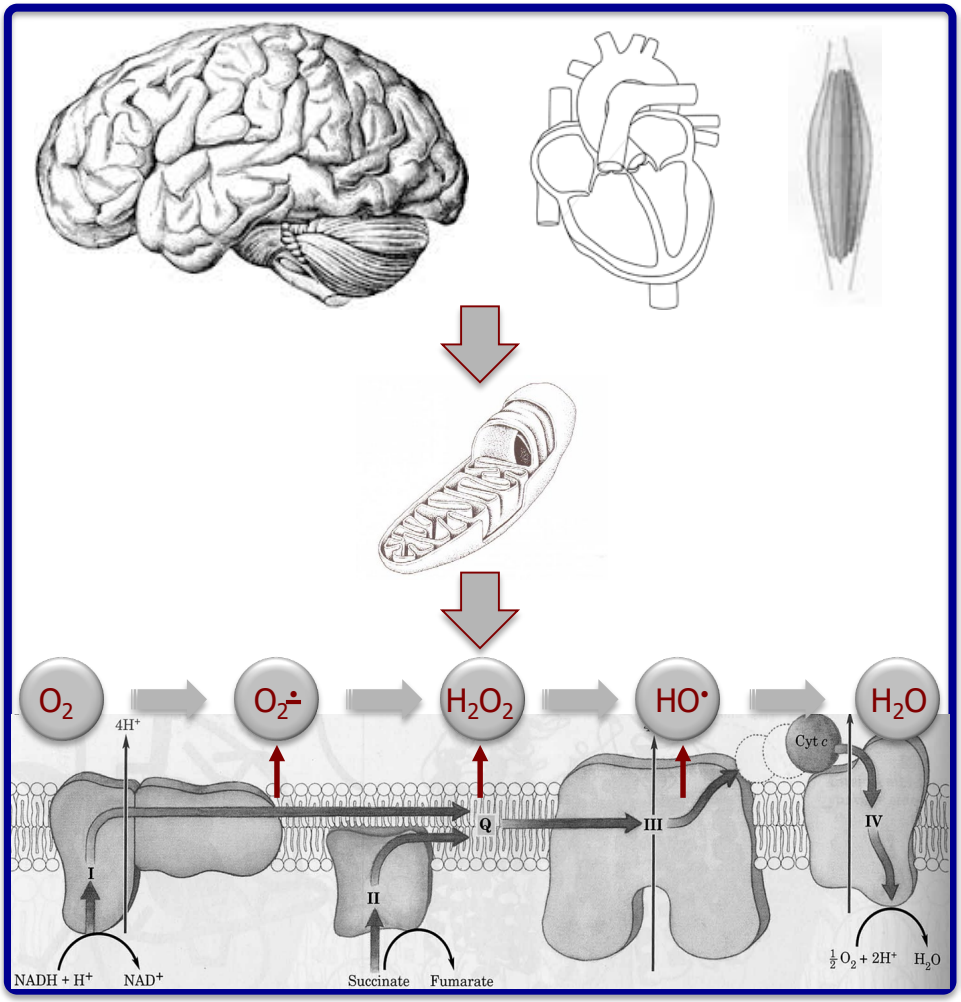
The interaction between NADPH oxidase (NOX) and endothelial nitric oxide synthase (eNOS)

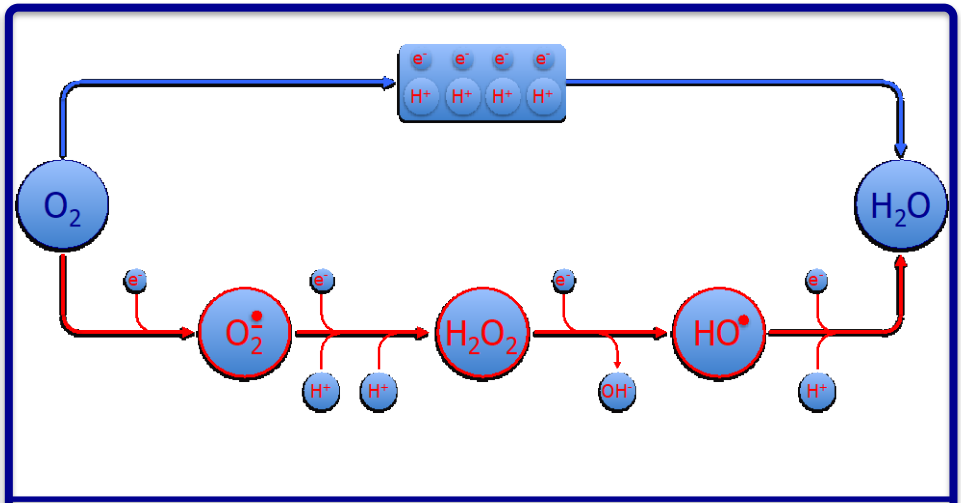
The biosynthesis of nitric oxide (NO) by nitric oxide synthase (NOS)



A particular redox reaction able to generate a radical gas

The production of reactive oxygen species in mitochondria





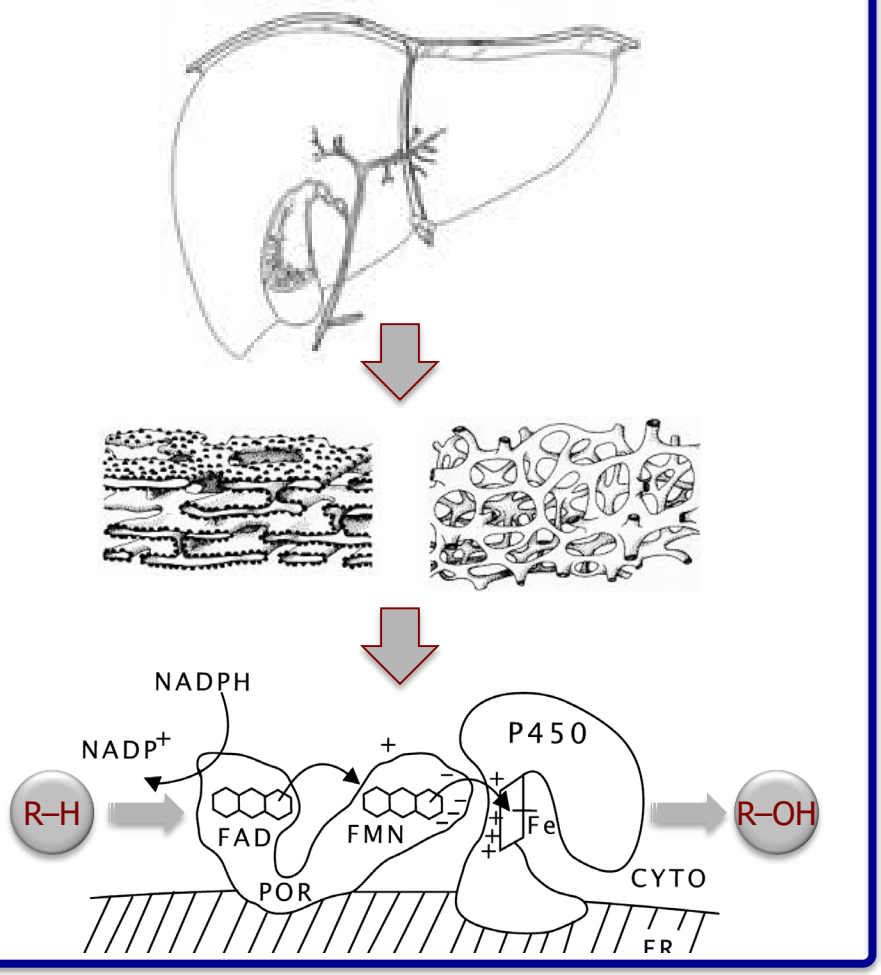
$$O_2 + e^- \rightarrow O_2^{\bullet-}$$

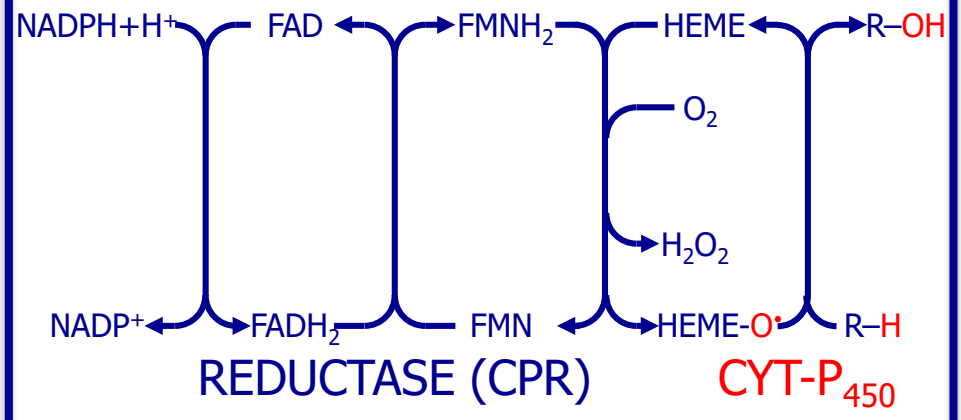
$$2\ O_2^{\bullet-} + e^- + 2\ H^+ \rightarrow H_2O_2$$

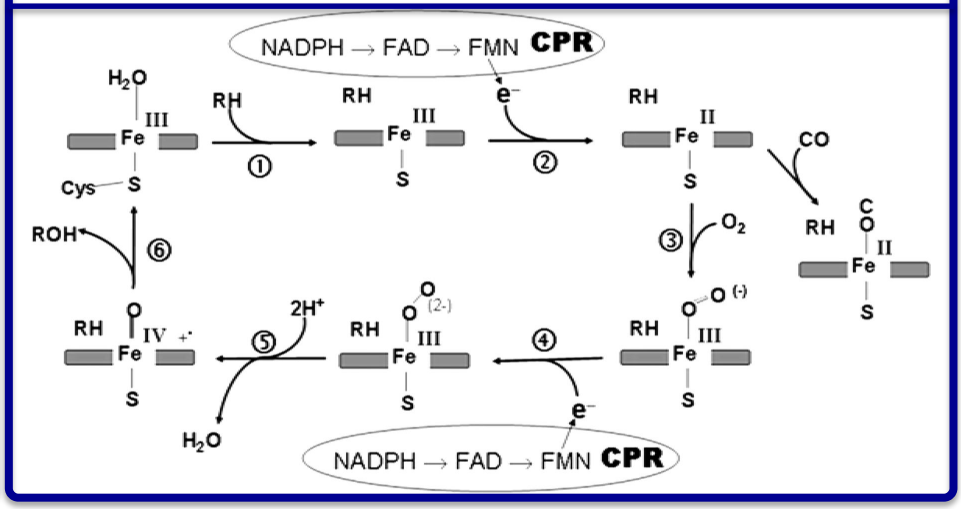
$$H_2O_2 + e^- \rightarrow OH^{\bullet} + OH^-$$

The so-called mono-valent reduction of molecular oxygen

The production of reactive oxidant species by endoplasmic reticulum/microsomes

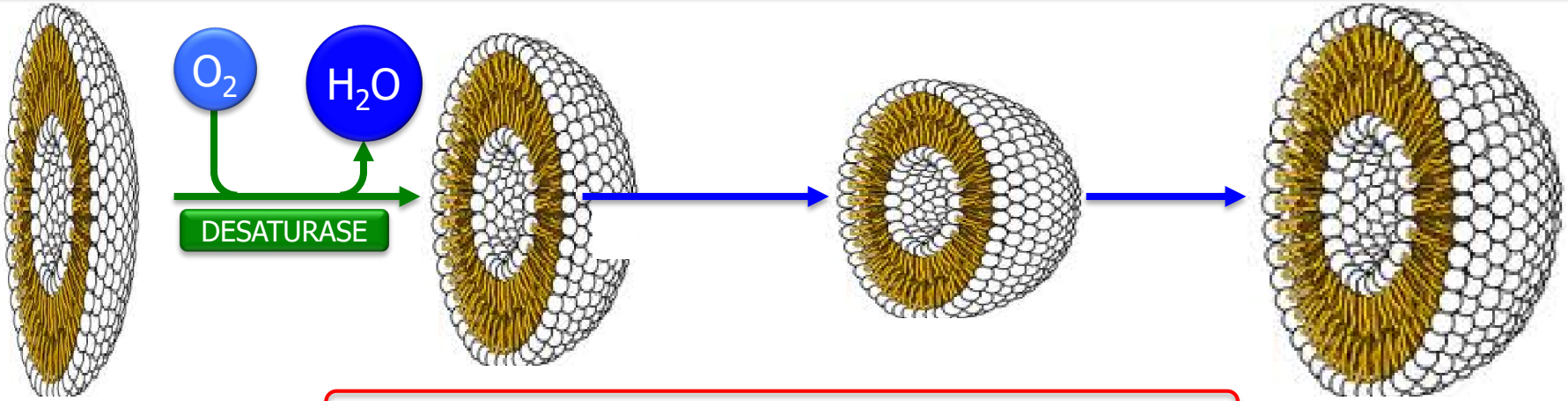


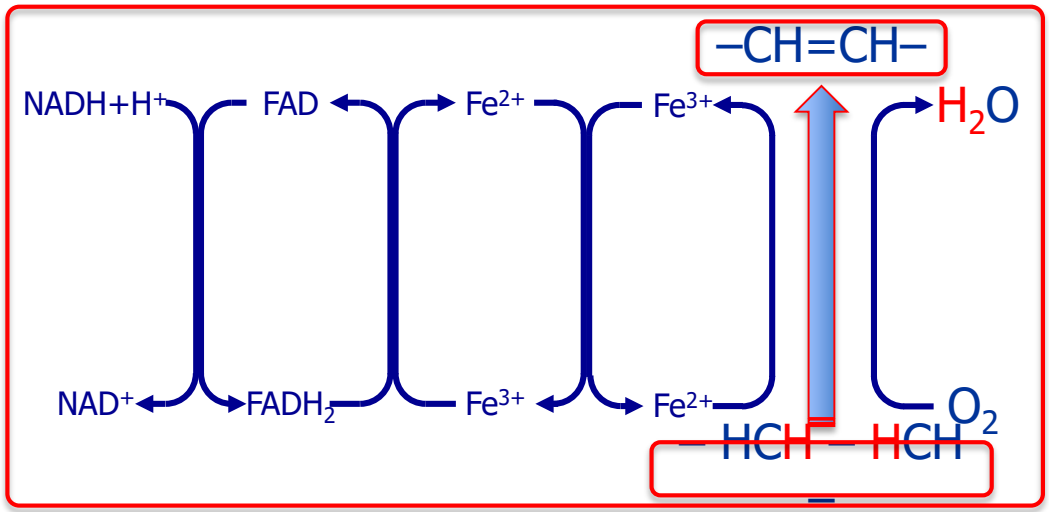




The detoxifying role of Cytochrome P450 liver enzymes

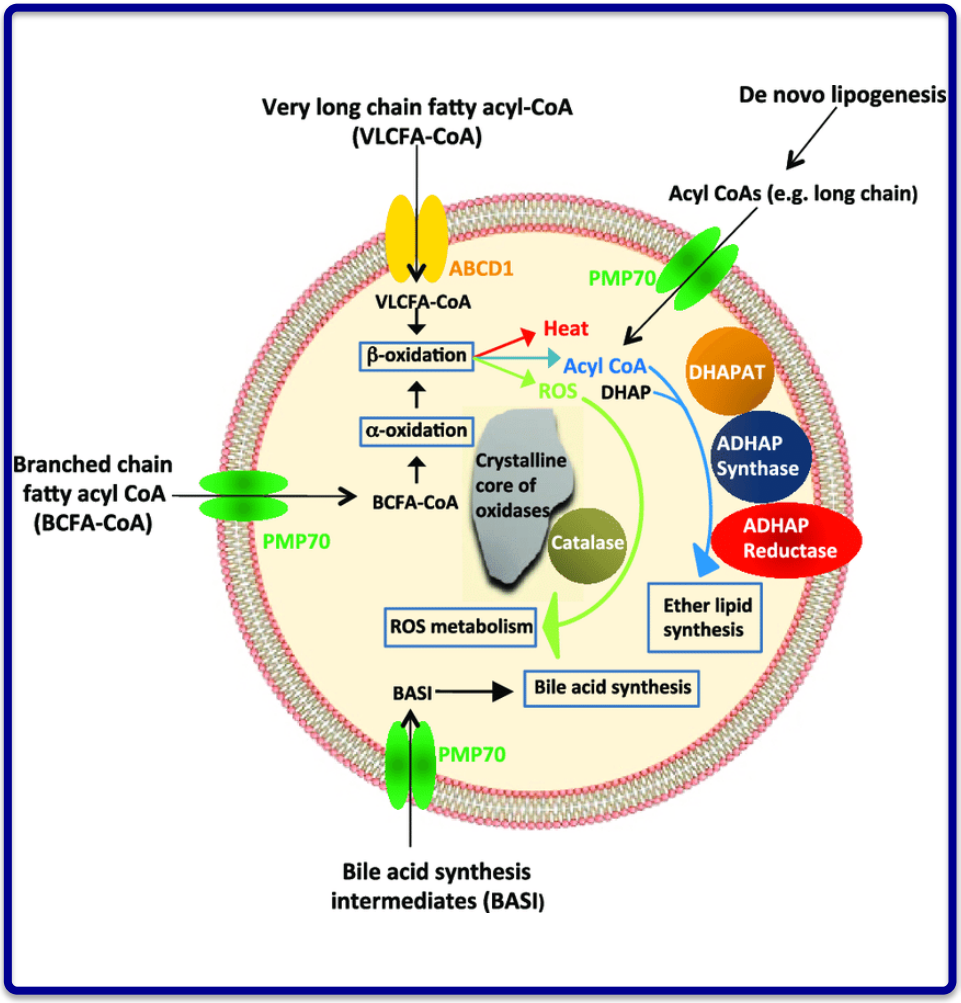
The ER-fatty acid desaturases (FADs) generates double carbon bonds from single carbon bonds

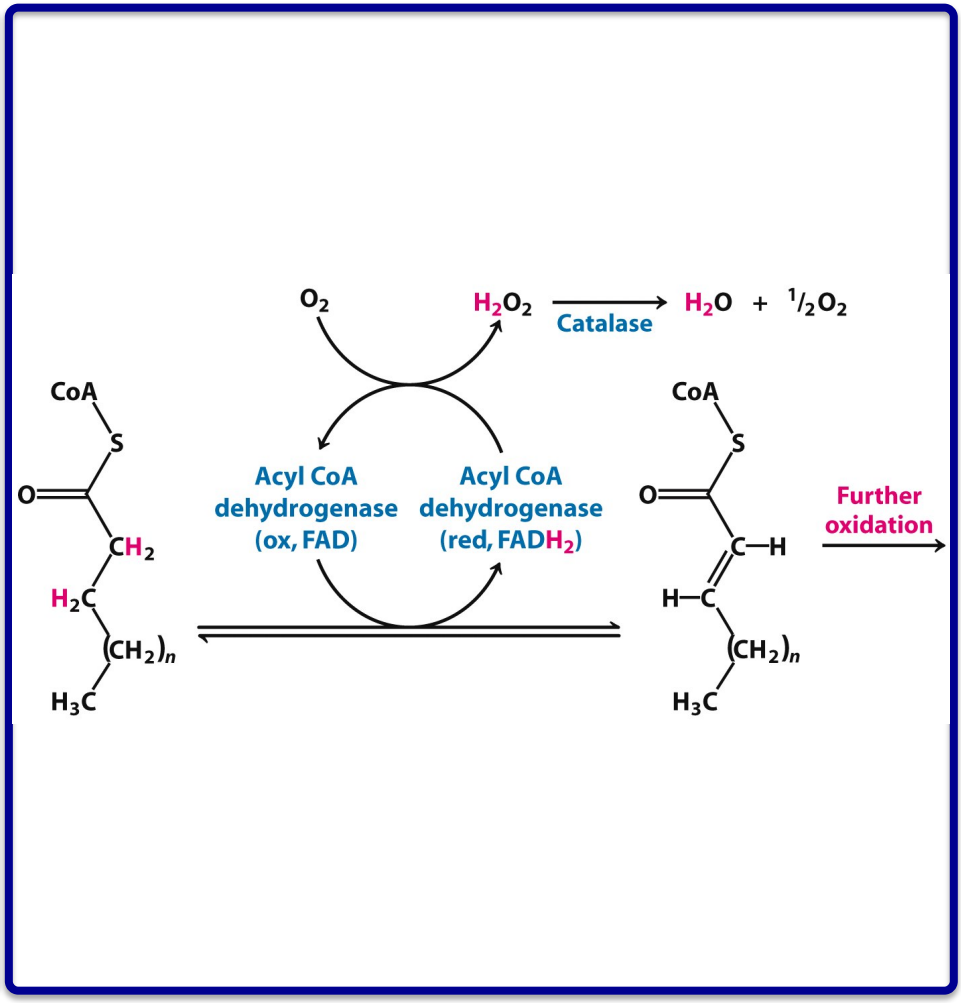




The FAD-dependent desaturation plays a key role in the biosynthesis, structure and function of fatty acids.

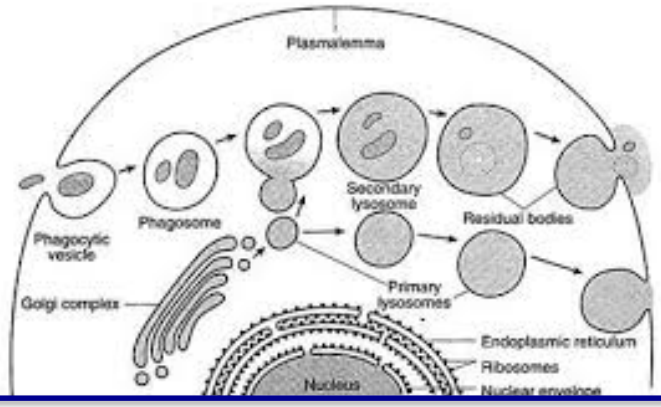
The production of reactive oxidant species by peroxisomes

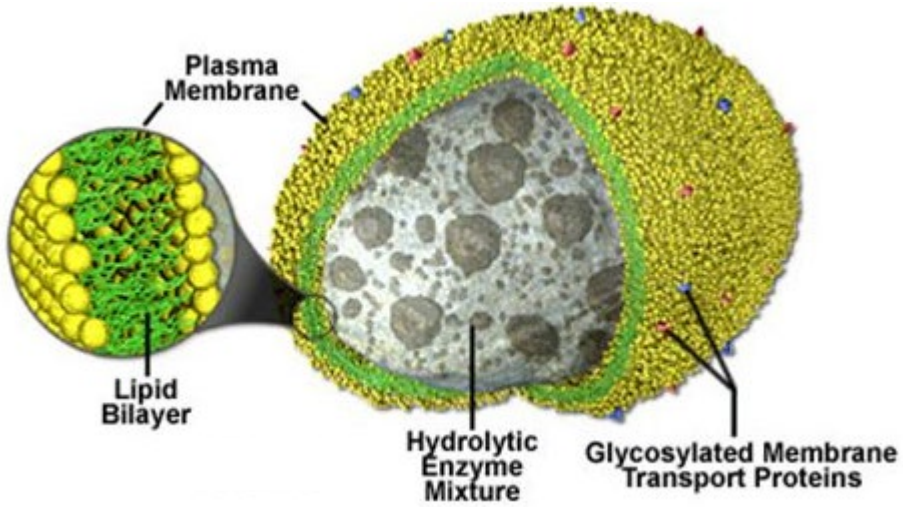




Peroxisomes generate hydrogen peroxide from very long chain fatty acid oxidation

The production of reactive oxidant species by lysosomes



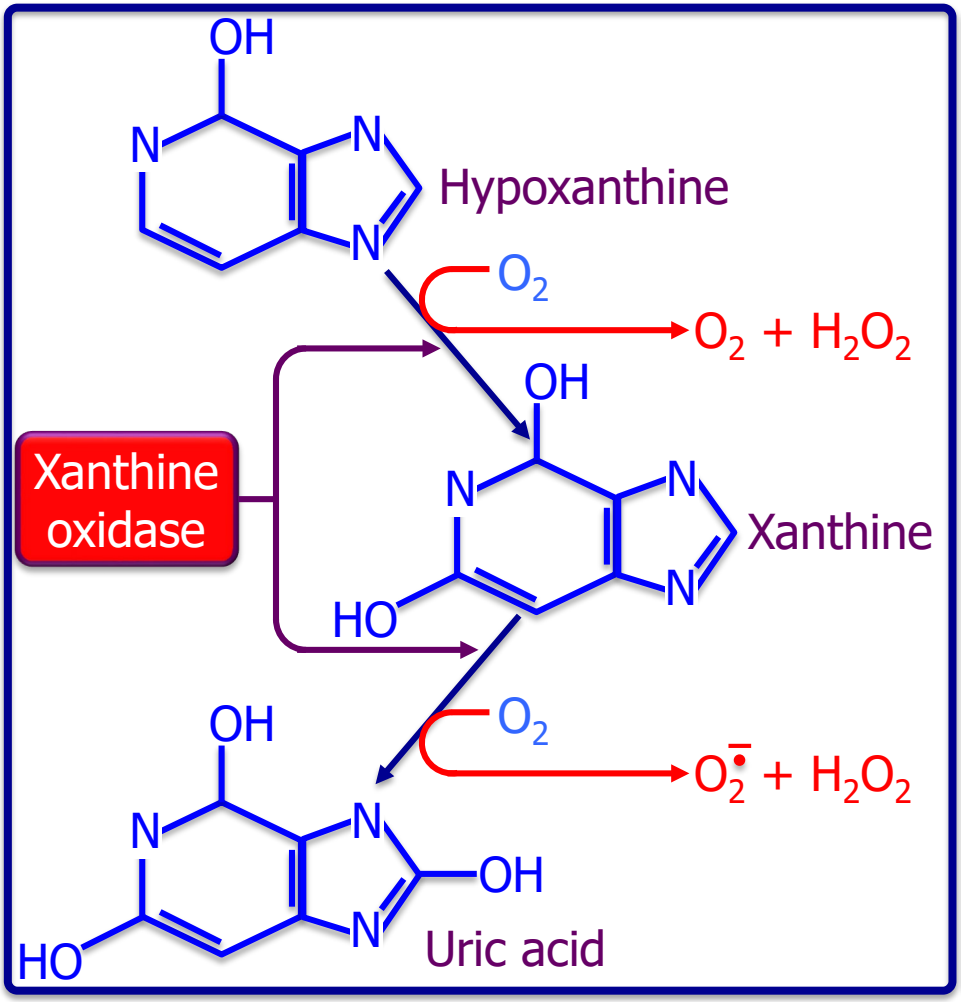


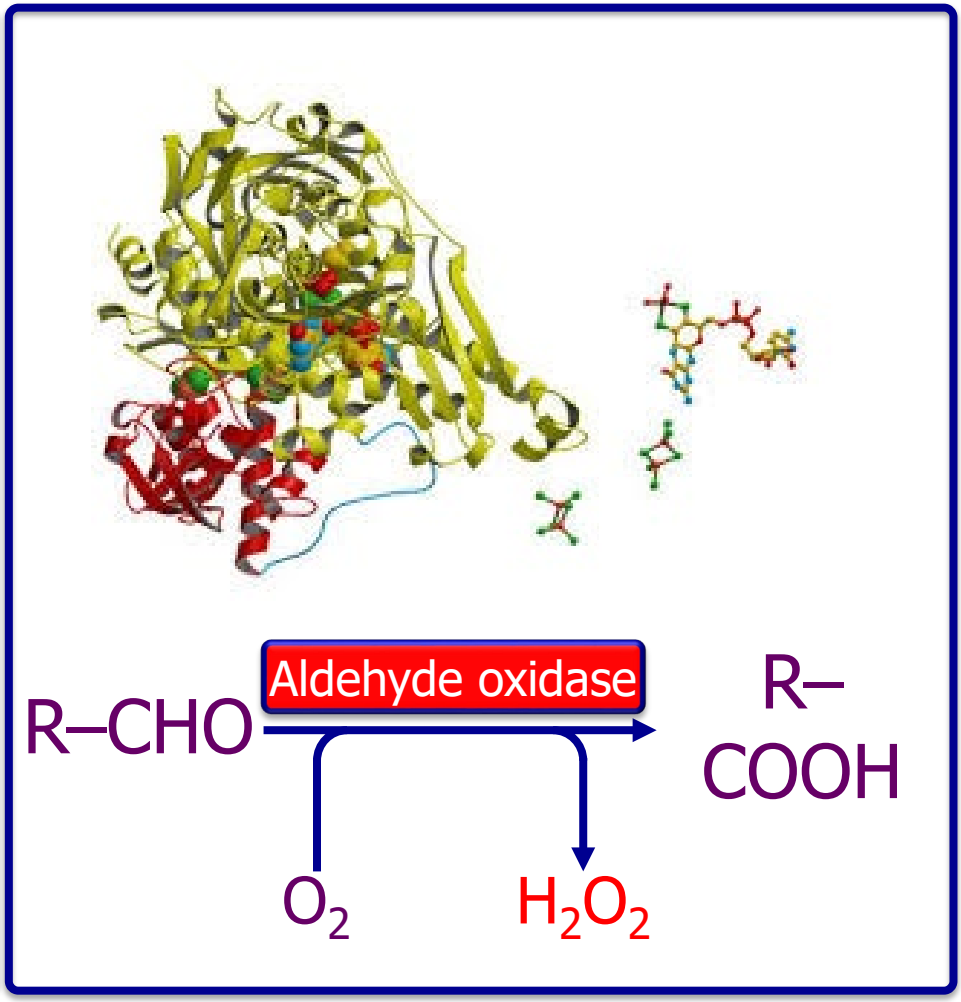
$$HCl + H_2O_2 \xrightarrow{\text{Myeloperoxidase}} HClO + H_2O$$

Hydrogen chloride — Hydrogen peroxide — Hypochlorous acid — Water

Myeloperoxidase is fundamental in the phagocytic process

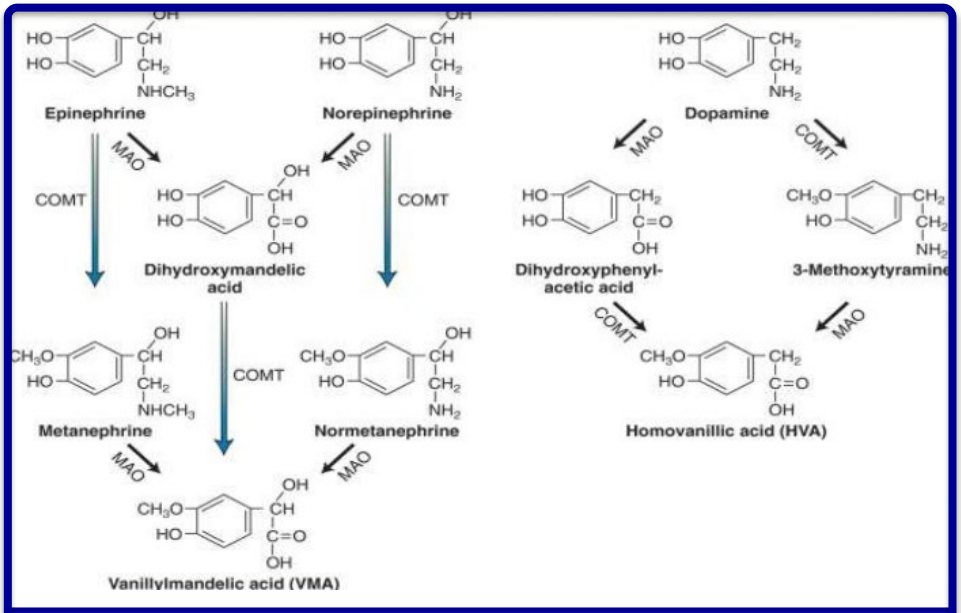
The production of reactive oxidant species by cytosol enzymes

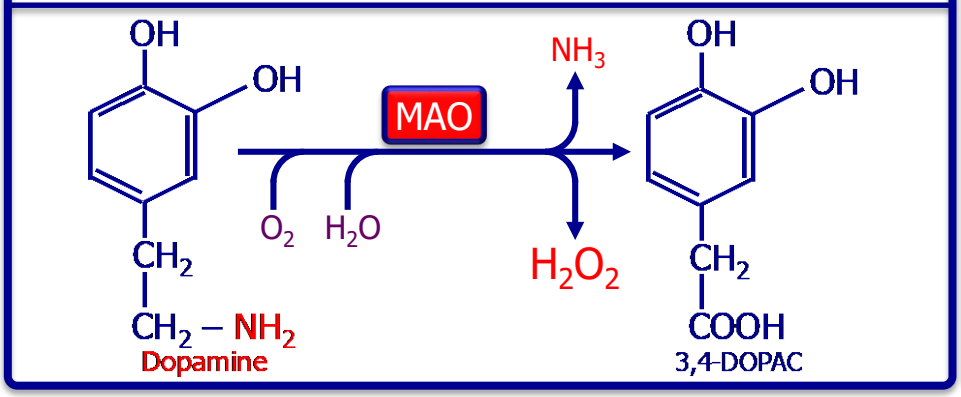


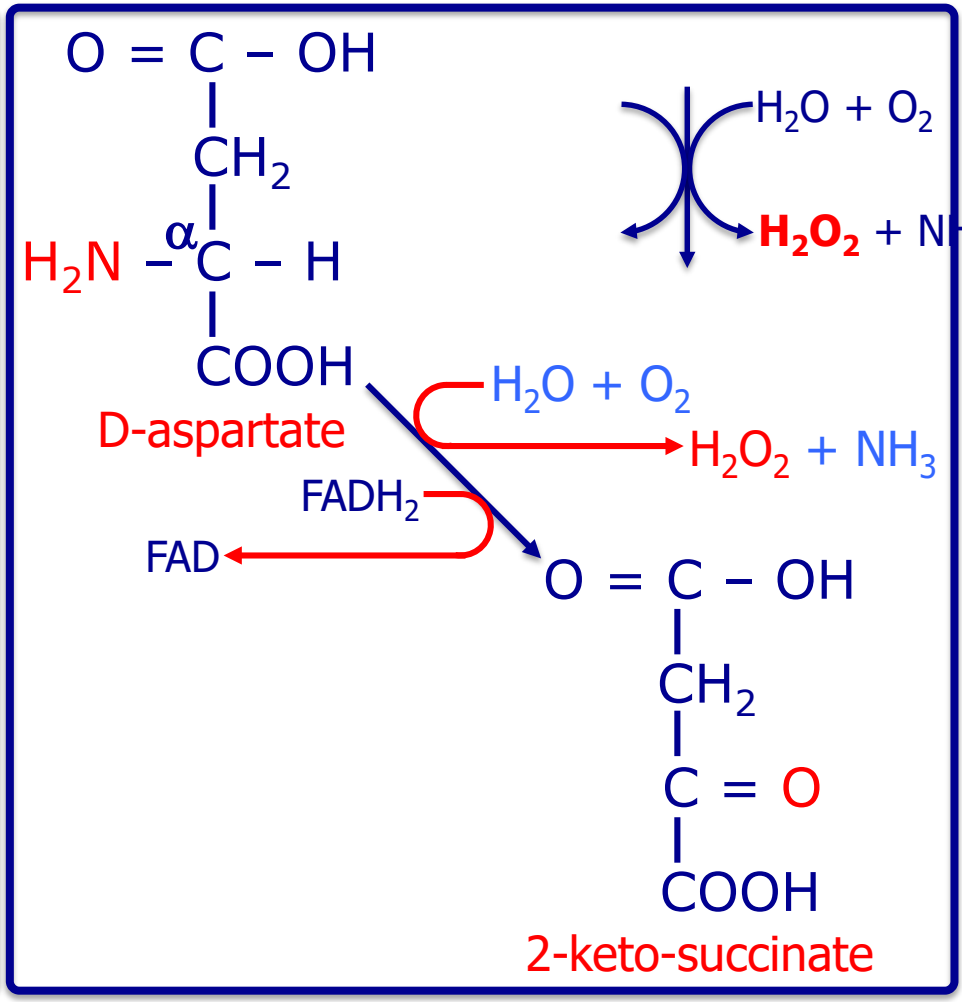


Xanthine oxidase and aldehyde oxidase

Other relevant or mysterious redox enzymes

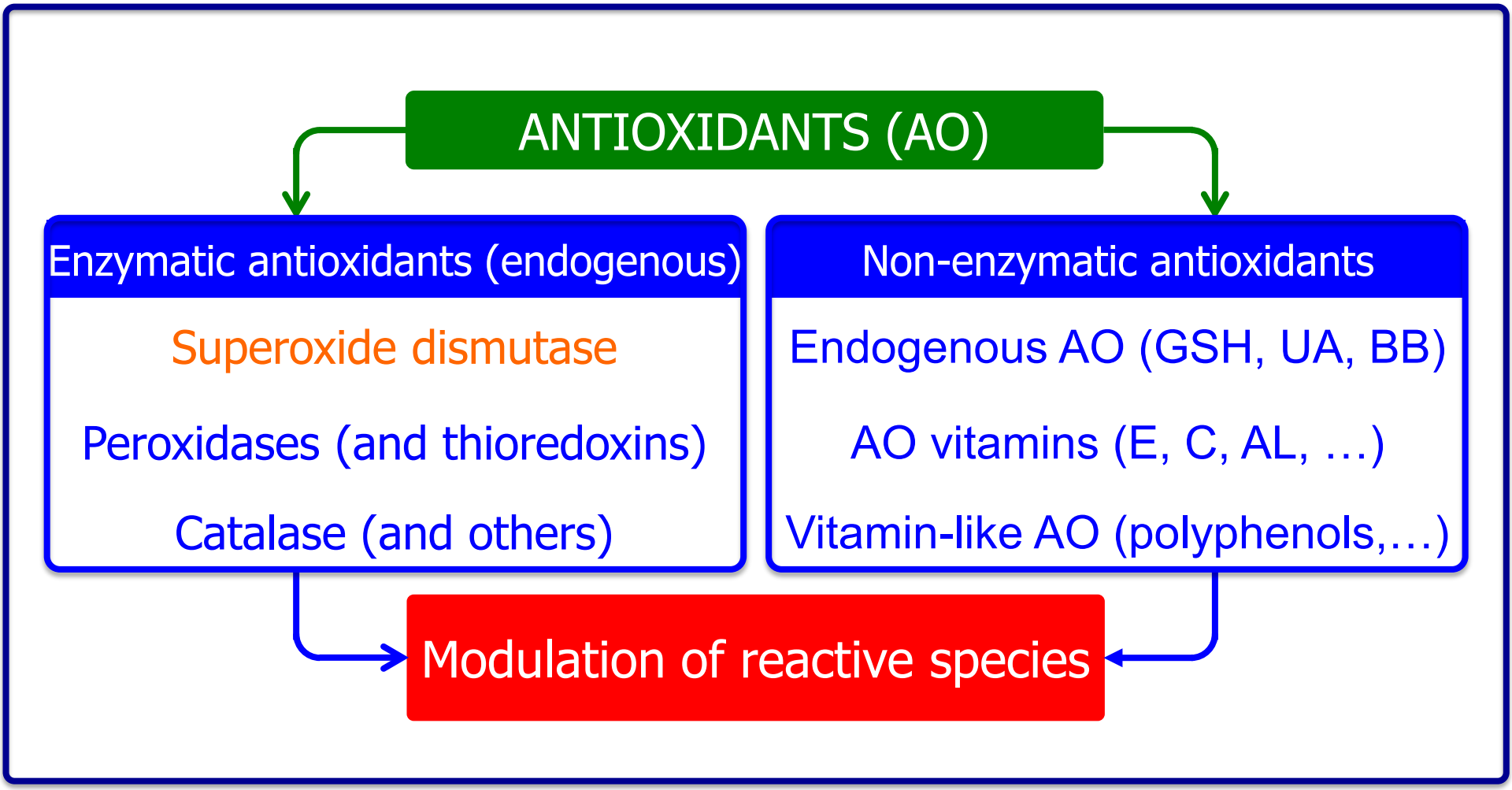






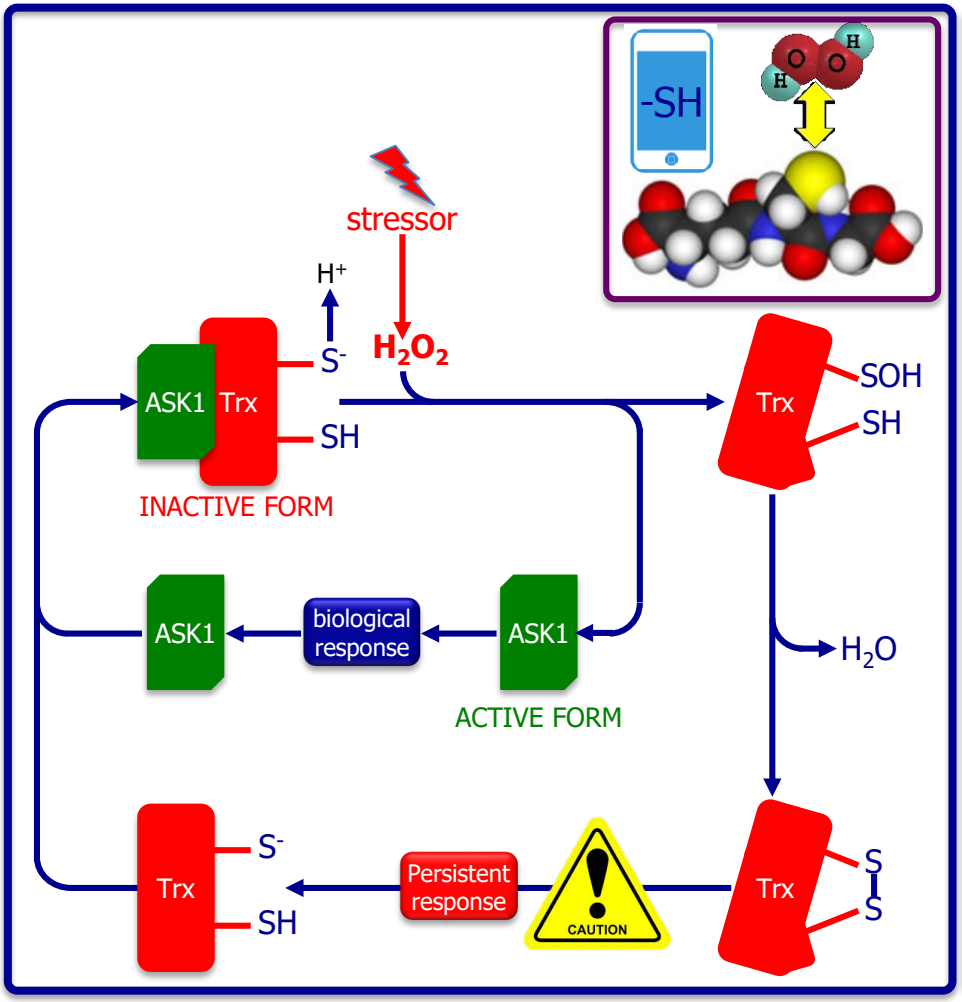
The unique role of monoamine oxidase (MAO) and the controversial role of D-amino acid oxidase (DAAO)

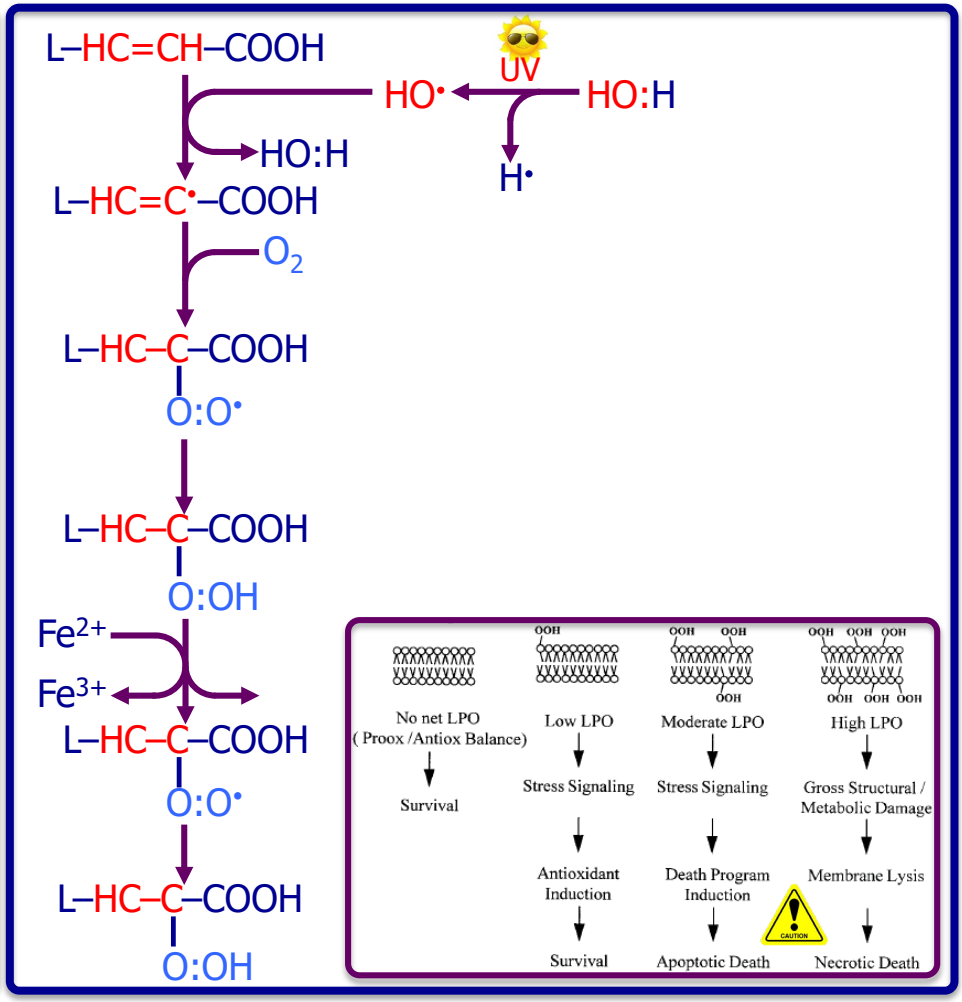
The antioxidants network acts physiologically by giving electrons to exceeding reactive species



By this way reactive species excess cannot disturb signalling or reach/damage unwanted biological targets

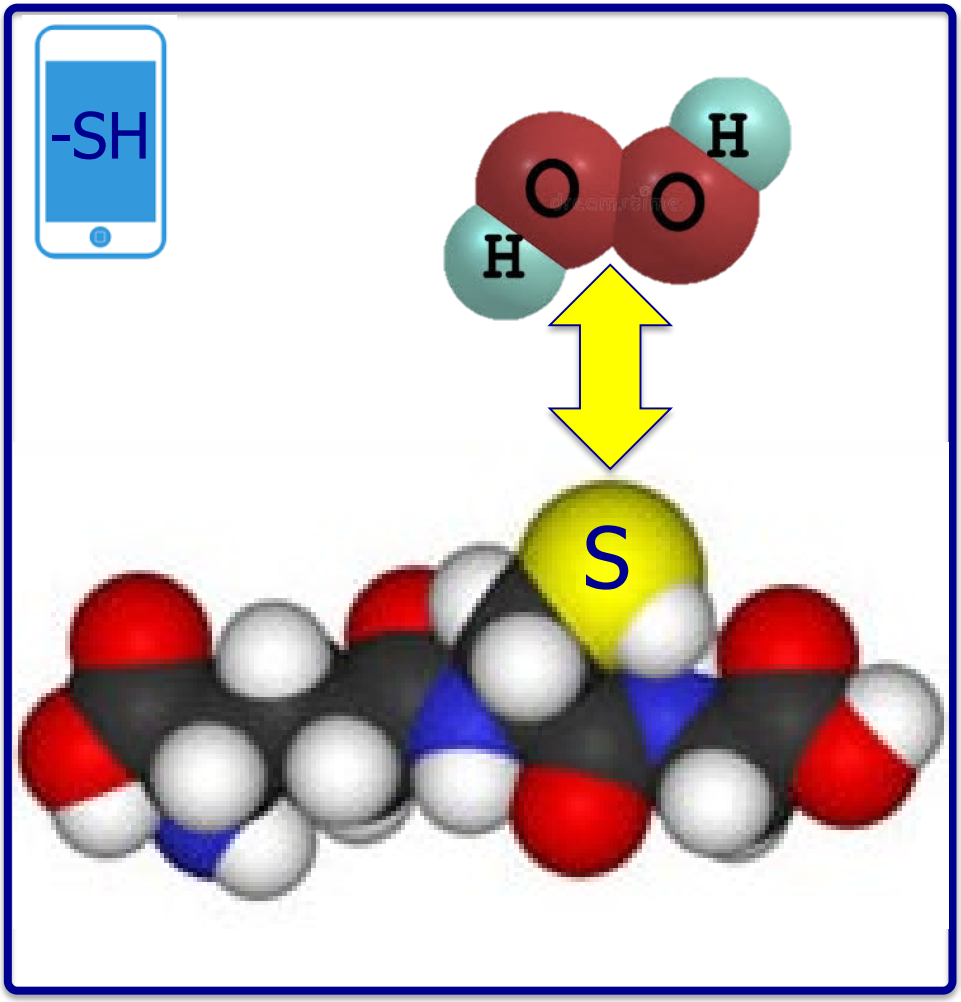
The interaction between reactive oxidant species and their biological substrates

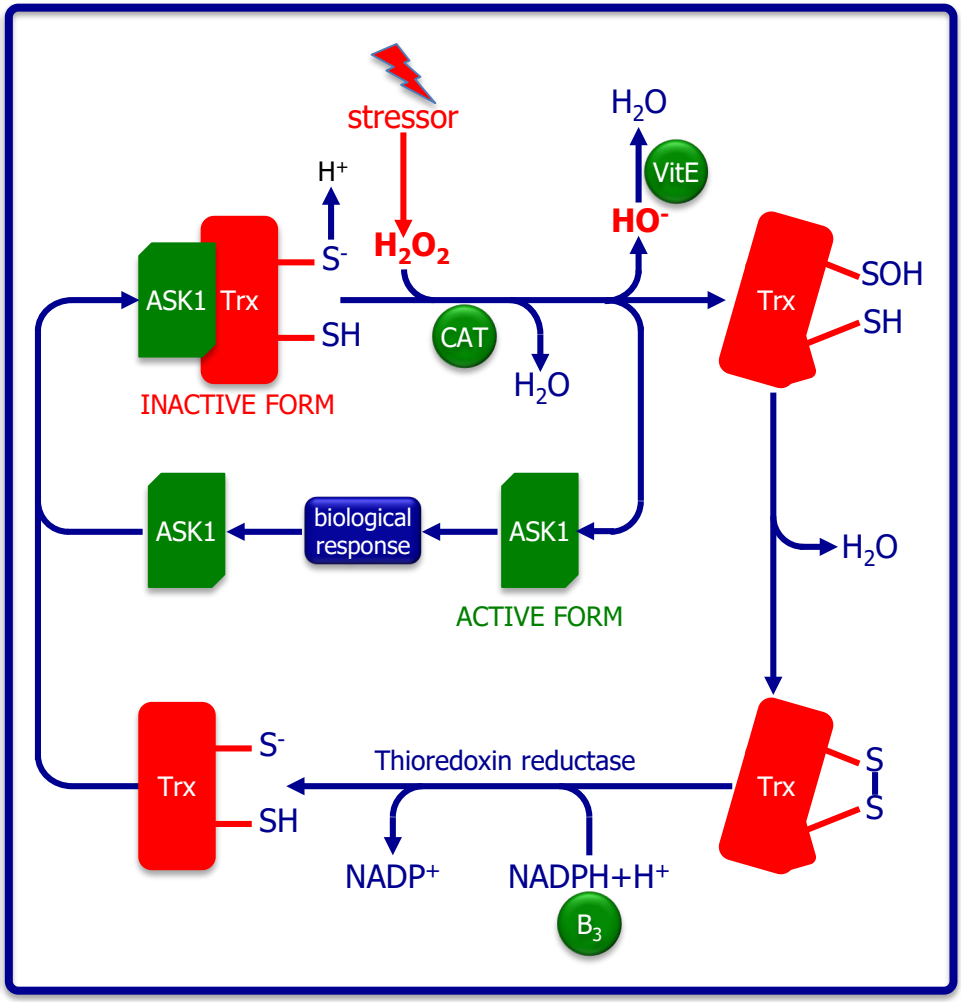




Different pathways/degree of oxidation cause different effects

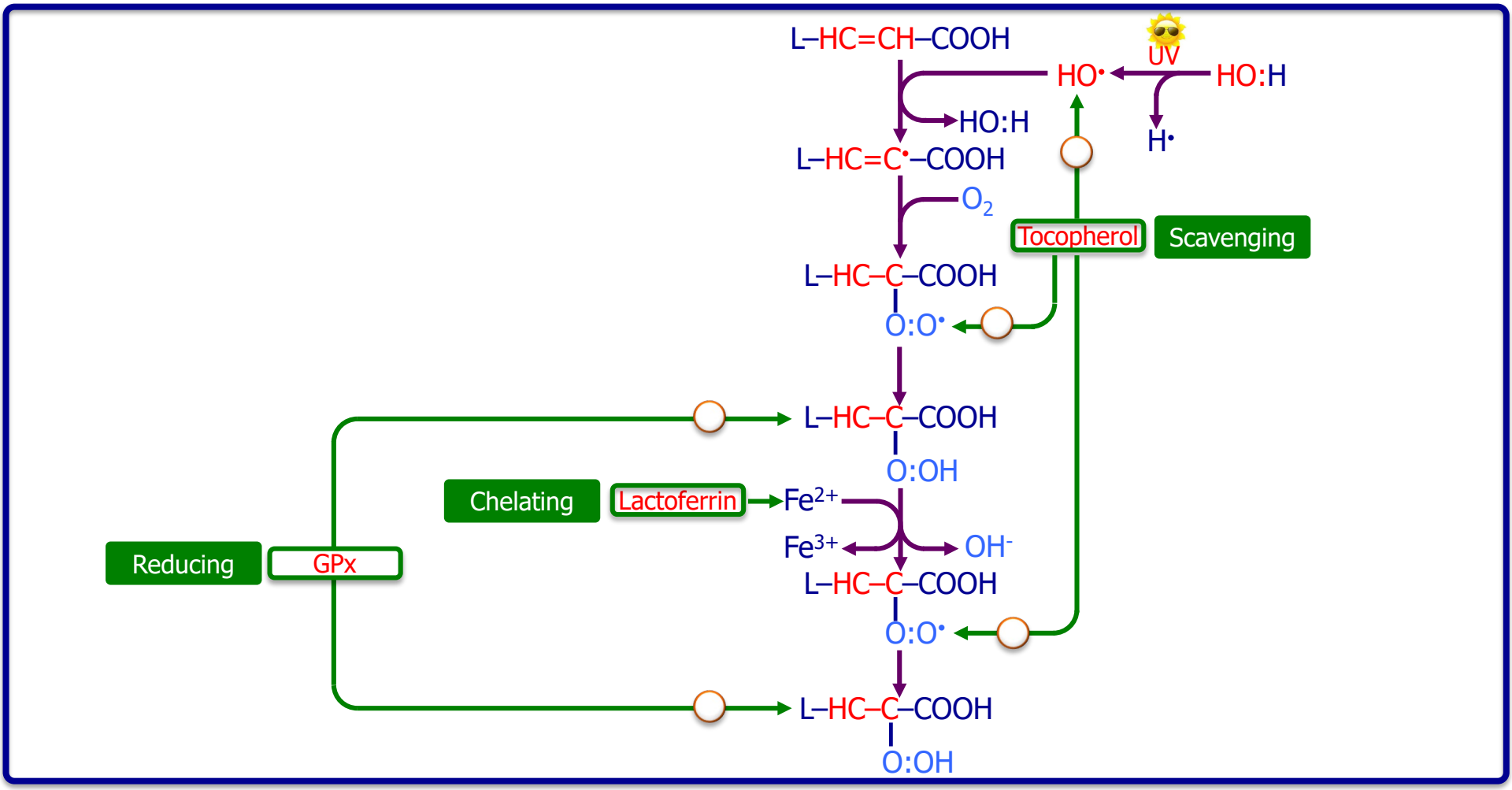
The REDOX network in action: the oxidant -antioxidant interplay in cell signalling.





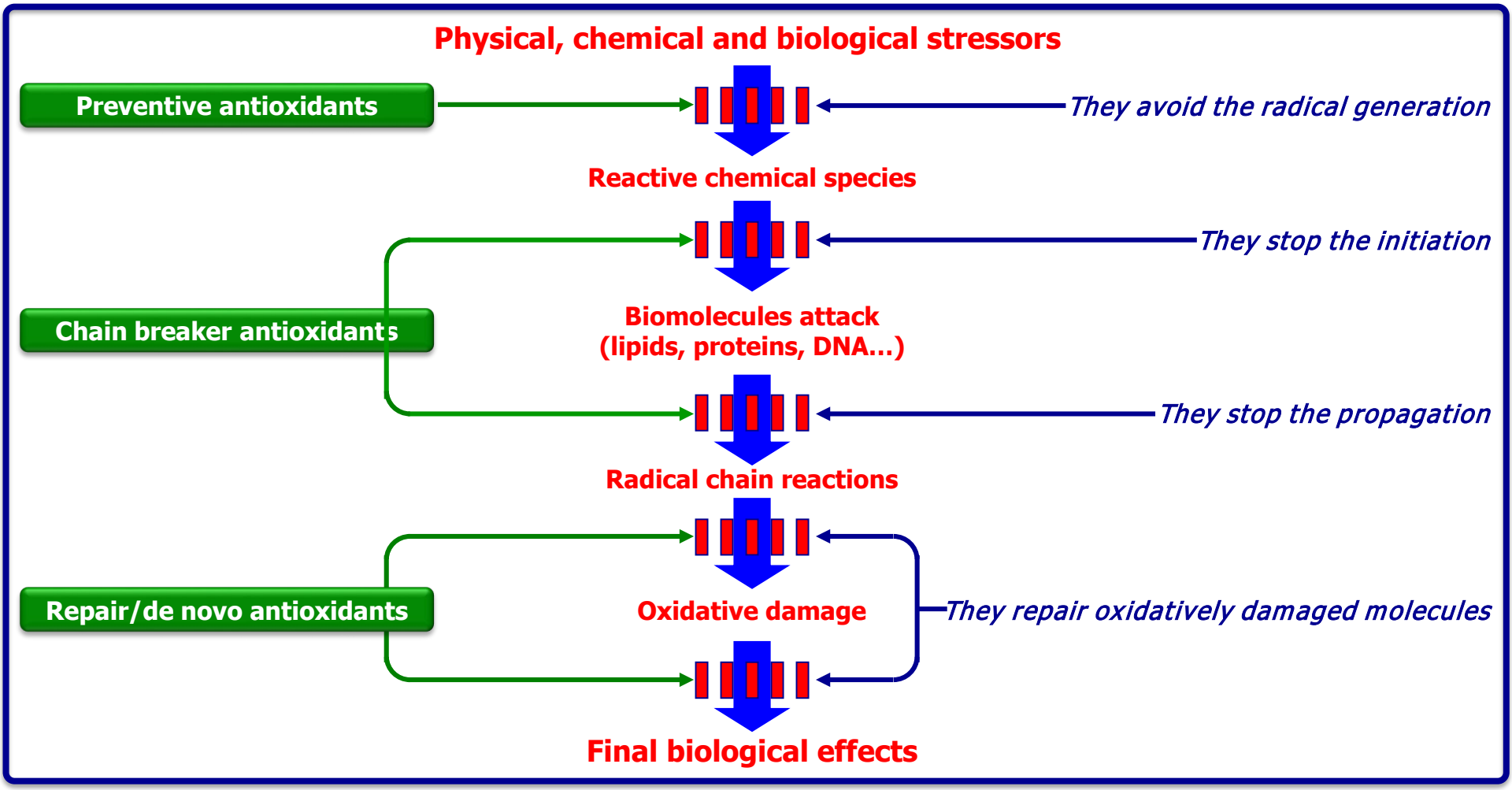
Hydrogen peroxide activates thiol-mediated ASK1/Trx signalling that is modulated by catalase/vitamin E/vit B_3

The "YANG" face of REDOX system: antioxidants modulate physiologically the ROS metabolism/actions.



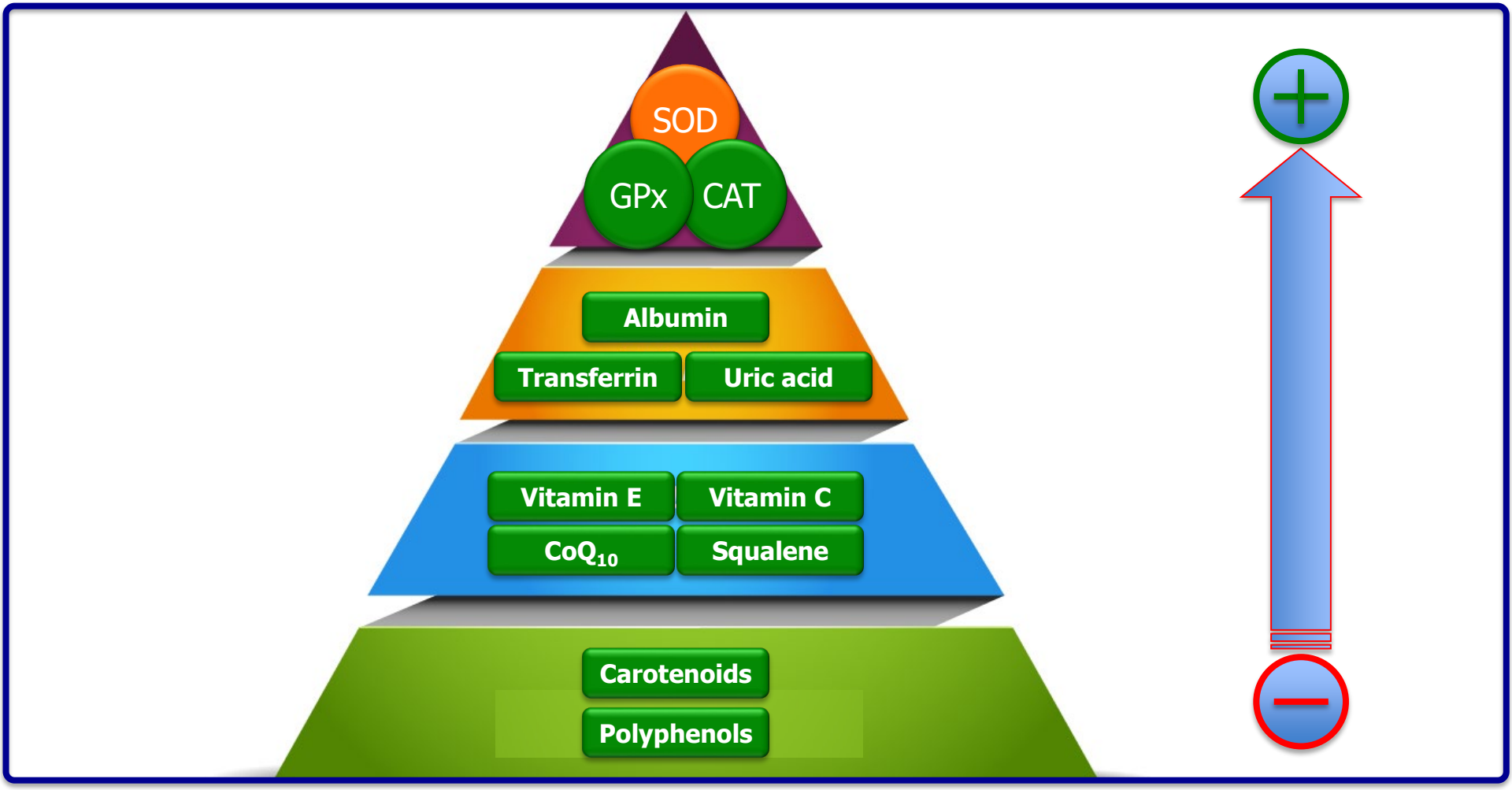
Reducing does not mean antioxidant
Antioxidant does not mean reducing

Antioxidants: how they work.



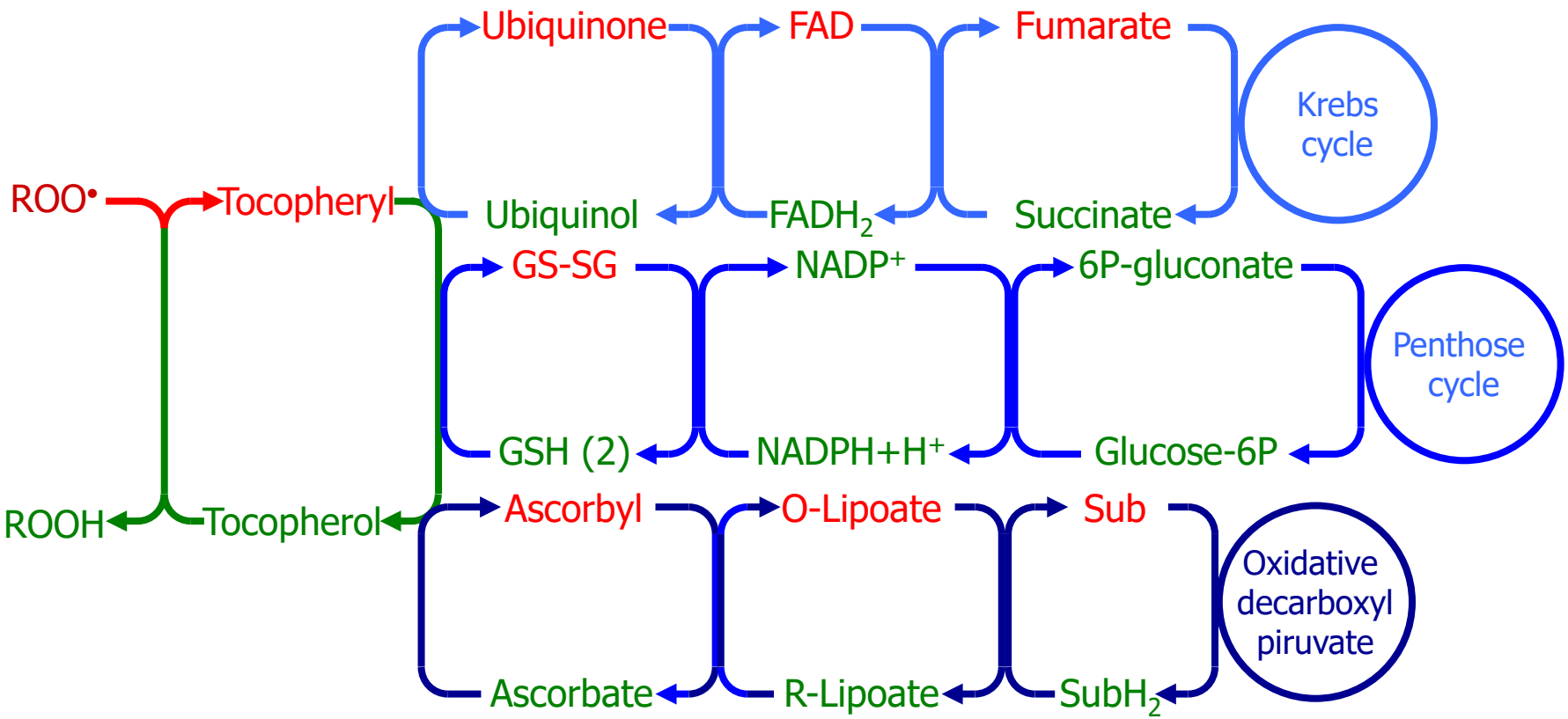
From the production of reactive oxidant species to the final biological effects: any step can be modulated.

The oxidant power hierarchy (vertical stratification)



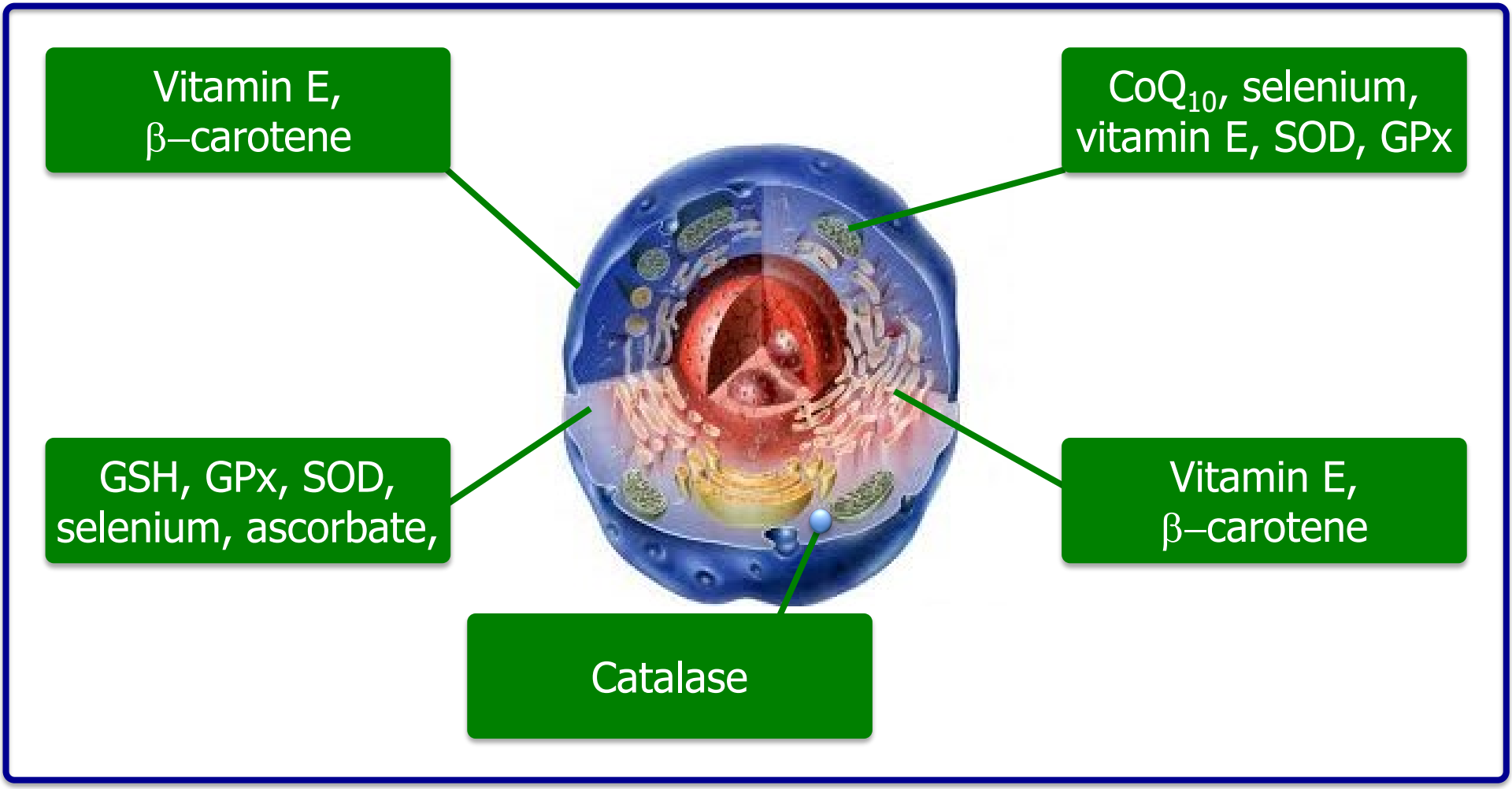
Endogenous antioxidants are potentially more effective than exogenous antioxidants

The orizontal stratification of antioxidants: a redox-dependent network.



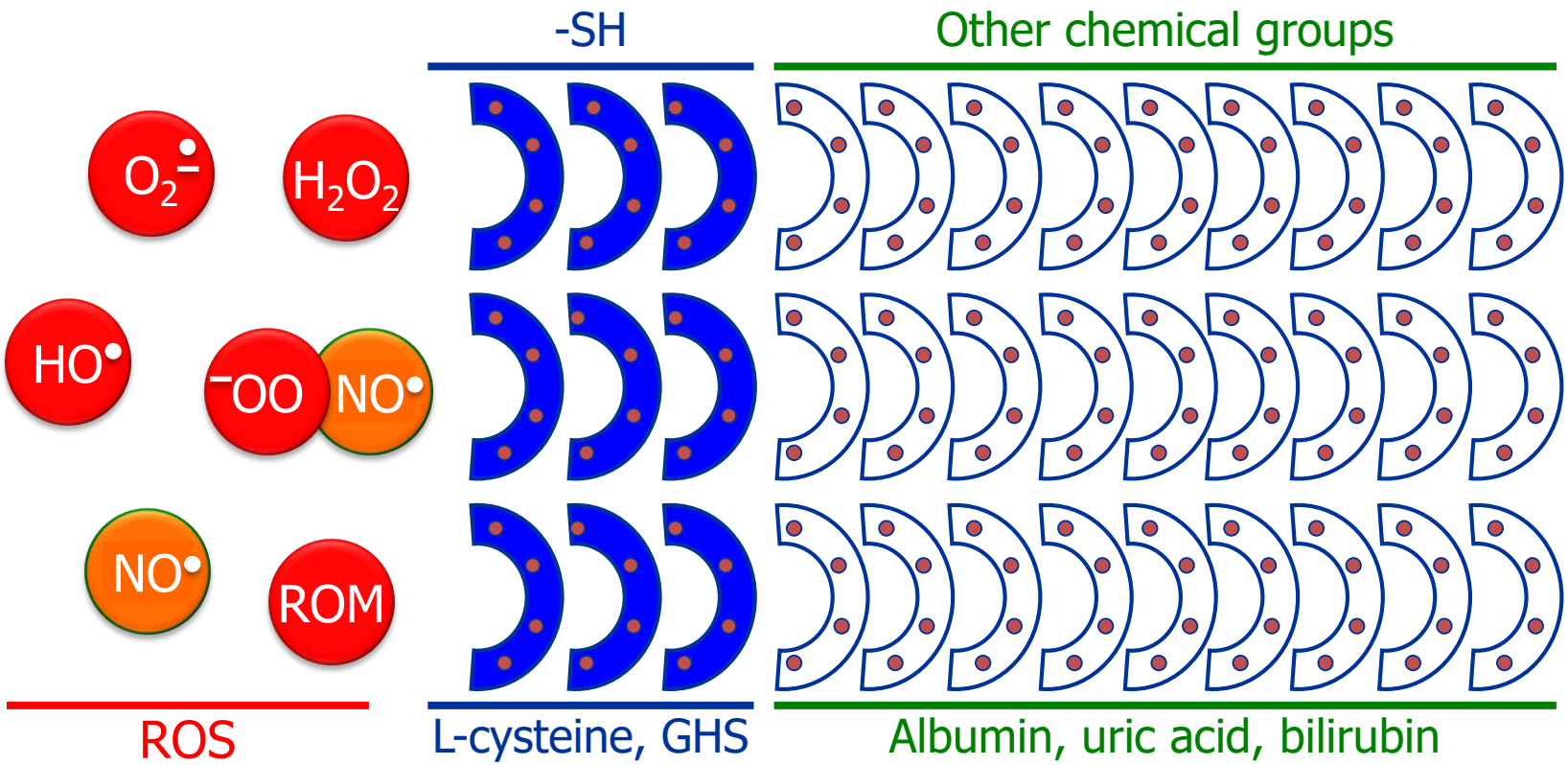
The relevance of recycling (to reduce the doses) and stoichiometry (to improve efficacy)

The antioxidant network is widely and regularly distributed inside the cell



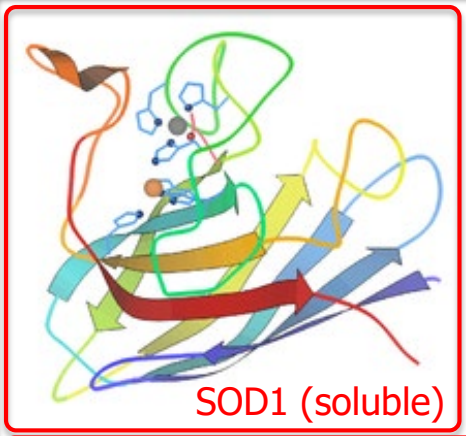
Lipophylic antioxidants control reactive oxygen species in the membranes

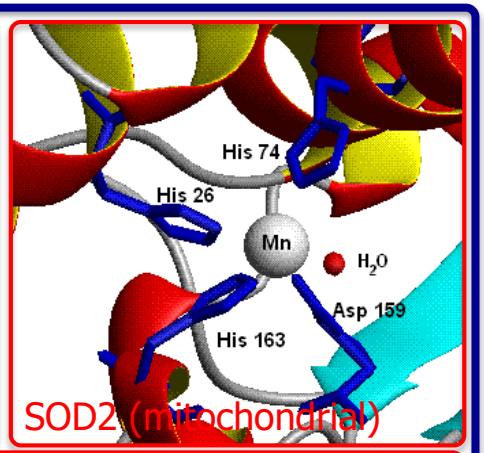
The antioxidant network is widely and regularly distributed in the extracellular fluids



Reduced thiols are a relevant component of the so-called plasma antioxidant barrier

Superoxide dismutase (SOD)


SOD1 (soluble)



SOD2 (mitochondrial)


SOD3 (extracellular)

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that alternately catalyses the dismutation (or partitioning) of the superoxide anion ($O_2^{\cdot-}$) into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Thus, SOD is an important but ambiguous enzyme in nearly all living cells exposed to oxygen, because it eliminates a potentially harmful reactive specie like superoxide anion but generate another potentially damaging reactive specie i. e. hydrogen peroxide. In Humans 3 forms of SOD are present.

☞SOD1 is located in the cytoplasm, is a dimer, and contains copper and zinc in its reactive centre. Its gene is located on chromosome 21 (21q22.1).

☞SOD2 is located in the mitochondria, is a tetramer, and contains manganese in its reactive centre. Its gene is located on chromosomes 6 (6q25.3).

☞SOD3 is the extracellular form, is a tetramer, and contains copper and zinc in its reactive centre. Its gene is located on chromosomes 4 (4p15.3-p15.1).

An ambiguous enzyme

Catalase (CAT)

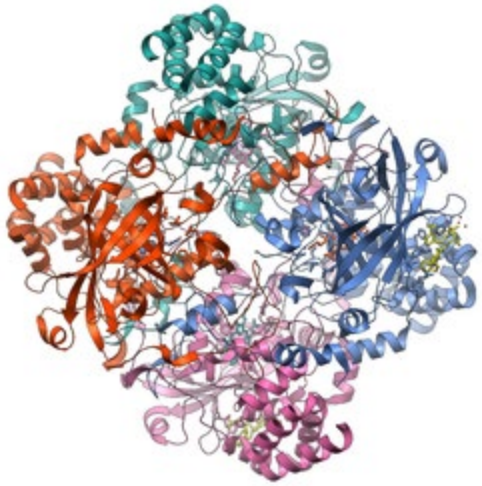
Catalase is a common enzyme found in nearly all living organisms exposed to oxygen, including bacteria. Located mainly in the peroxisomes (but also in mitochondria), it catalyses the decomposition of hydrogen peroxide – a multifunctional reactive oxidant species that can be produced by a lot of reactions (e. g. through the superoxide dismutase, the monoamine oxidase, te xanthine oxidase and so on) – to water and oxygen.

$$2H_2O_2 + O_2 \xrightarrow{CAT} 2H_2O + O_2$$

By this point of view its main function is to control the action of hydrogen peroxide thus avoiding its unwanted sife.

Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second.

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four iron-containing heme groups that allow the enzyme to react with the hydrogen peroxide.



☞While the complete mechanism of catalase is not currently known, the reaction is believed to occur in two stages:

H_2O_2 + Fe(III)-E → H_2O + O=Fe(IV)-E(.+)

H_2O_2 + O=Fe(IV)-E(.+) → H_2O + Fe(III)-E + O_2

Here Fe()-E represents the iron center of the heme group attached to the enzyme. Fe(IV)-E(.+) is a mesomeric form of Fe(V)-E, meaning the iron is not completely oxidized to +V, but receives some stabilising electron density from the heme ligand, which is then shown as a radical cation (.+).

The modulator of the multifunctional hydrogen peroxide

Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) is a ubiquitous enzyme (E) that catalyses the reduction of peroxides (ROOH) to alcohol (water for hydrogen peroxide), according to the following general reaction:

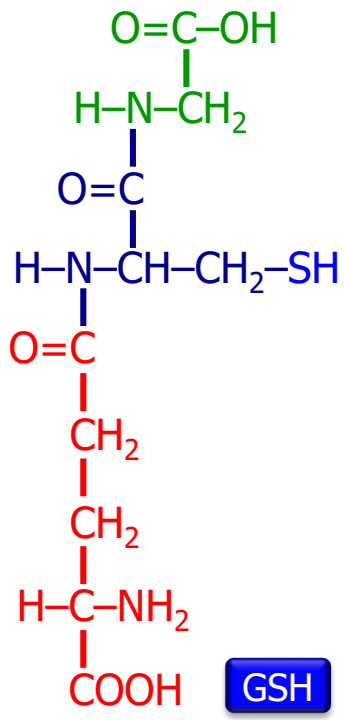
$$R{-}OOH + EH_2 \xrightarrow{GPx} R{-}OH + E + H_2O$$

It is expressed in 8 different isoforms, which vary in cellular location and substrate specificity.

- ☞GPx1 is the most abundant version, found in the cytoplasm of nearly all tissues, whose preferred substrate is hydrogen peroxide.
- ☞GPx2 is an intestinal and extracellular enzyme.
- ☞GPx3 is extracellular, especially abundant in plasma.
- ☞GPx4 has a high preference for lipid hydroperoxides (phospholipid hydroperoxidase); it is expressed in nearly every cell, though at much lower levels.
- ☞GPx5 is also know as epididymal androgen-related protein, while GPx6 is the so-called olfactory GPx

GPx requires glutathione (GSH) as coenzyme and selenium as cofactor. When the substrate is hydrogen peroxide, in the first step such ROS oxidise a seleno-cysteine residue of the enzyme to a selenol derivative. This latter react with the first molecule of GSH thus generating an GS-SeR intermediate, that finally reacts with the second molecule of GSH regenerating the original seleno-cysteine residue and releasing the oxidised form of glutathione (GSSG): the glutathione reductase then reduce GSSG to (2)GSH, according to the following sequence:

1) $RSeH + H_2O_2 \rightarrow RSeOH + H_2O$
2) $RSeOH + GSH \rightarrow GS\text{-}SeR + H_2O$
3) $GS\text{-}SeR + GSH \rightarrow GS\text{-}SG + RSeH$



$$R{-}OOH + 2GSH \longrightarrow L{-}OH + GSSG + H_2O$$

$$HO\text{-}OH + 2GSH \longrightarrow 2H_2O + GSSG$$

The powerful modulator of peroxides

Glutathione peroxidase 1 (GPx-1) activity is independently associated with an increased risk of CAD

The NEW ENGLAND JOURNAL of MEDICINE

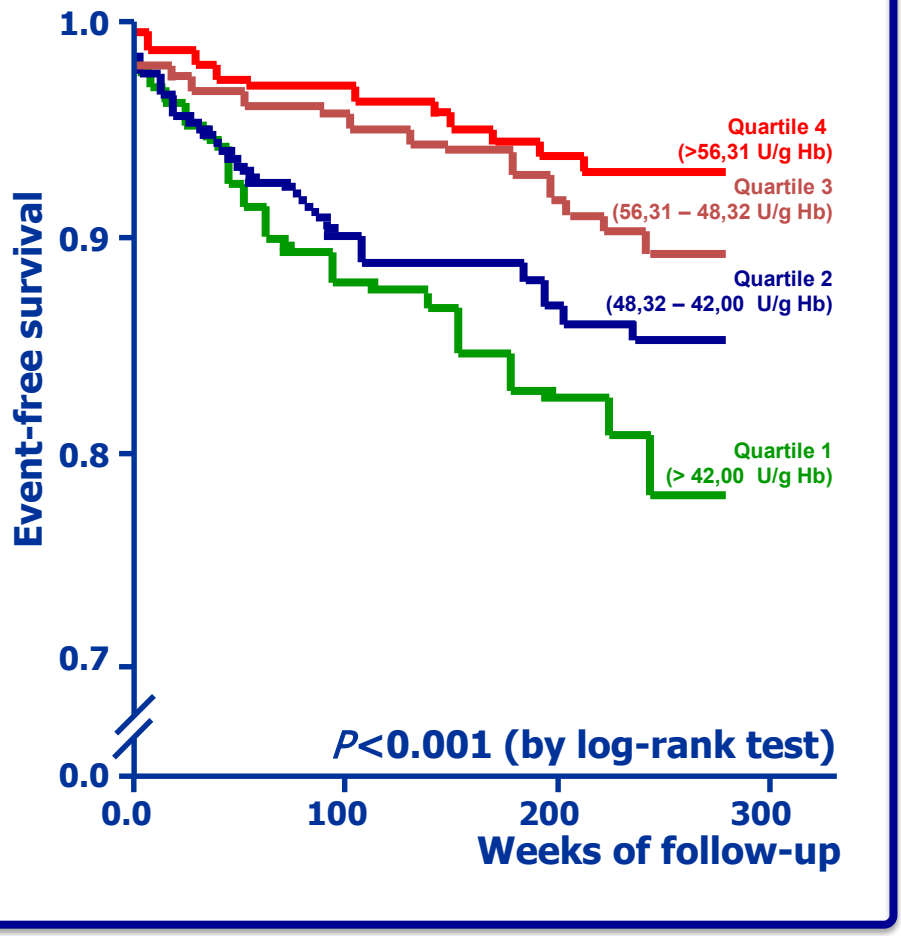
ORIGINAL ARTICLE

Glutathione Peroxidase 1 Activity and Cardiovascular Events in Patients with Coronary Artery Disease

Stefan Blankenberg, M.D., Hans J. Rupprecht, M.D., Christoph Bickel, M.D., Michael Torzewski, M.D., Gerd Hafner, M.D., Laurence Tiret, Ph.D., Marek Smieja, M.D., Ph.D., François Cambien, M.D., Jürgen Meyer, M.D., and Karl J. Lackner, M.D., for the AtheroGene Investigators*

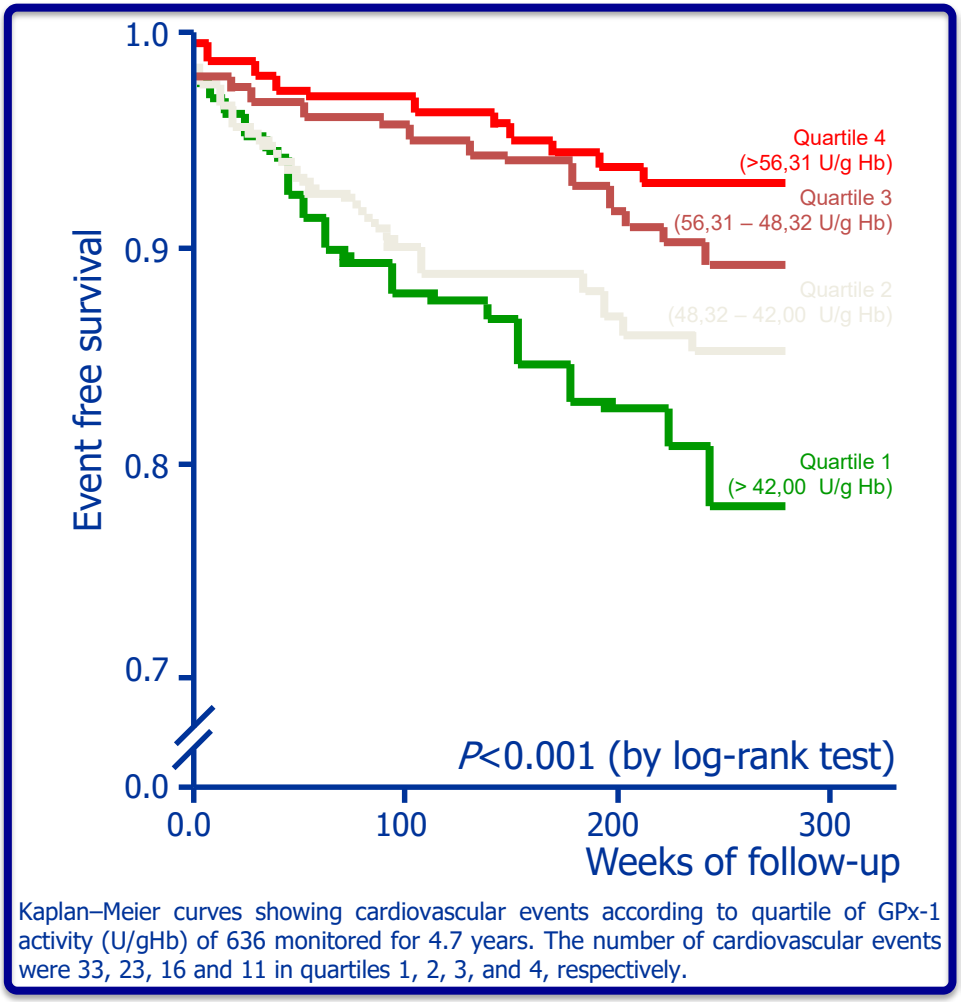
Kaplan–Meier curves showing cardiovascular events according to quartile of glutathione peroxidase 1 activity (units per gram of hemoglobin) (N=636).

The numbers of cardiovascular events were 33, 23, 16, and 11 in quartiles1, 2, 3, and 4, respectively.



Blankenberg et Al. NEJM. 2003. 349: 1605–1613.

Glutathione peroxidase activity (GPX1) in red blood cells is predictive of future cardiovascular events



Kaplan–Meier curves showing cardiovascular events according to quartile of GPx-1 activity (U/gHb) of 636 monitored for 4.7 years. The number of cardiovascular events were 33, 23, 16 and 11 in quartiles 1, 2, 3, and 4, respectively.

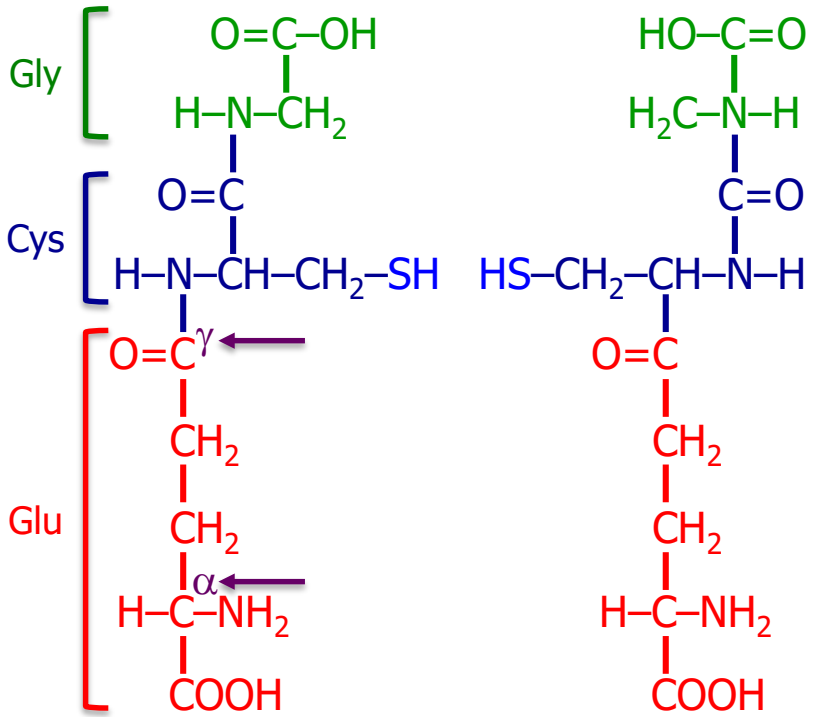
The iGENESIS REDOX SNPs panel

GENE	GENOTYPE	
SOD2_ rs48**	C	T
CAT_rs10*****	C	C
GPX1_rs10*****	C	T
EPHX_rs10*****	T	T
PON1_rs6**	A	A
NQO1_18*****	C	C
KEAP1_rs11******	C	C
TXN_rs41*****	A	G
TXNRD2_rs15*****	T	T
CYP1A2_rs76*****	A	A
NAT2 Haplotype	SLOW	
GSTT1_rs22*****	G	G
GSTM1_rs36*****	T	T
GSTP1_rs16**	A	A

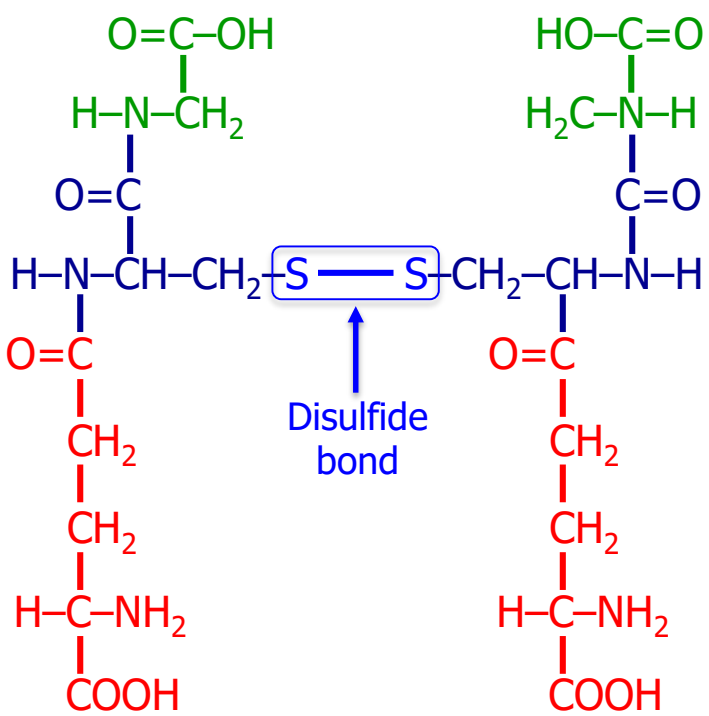
Blankenberg et Al. N Engl J Med. 2003. 349(17): 1605–1613.

The glutathione

Reduced glutathione (GSH, monomer)

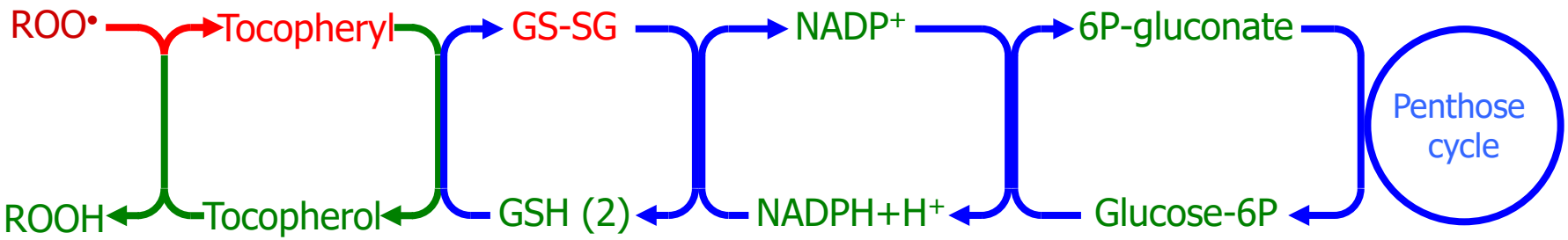


Oxidised glutathione (GS–SG, dimer)



L-γ-glutamyl-L-cysteinyl-glycine

The biological cycle of glutathione



Between tocopherol and pentose cycle

Main biological functions of glutathione

1. Specifically redox-related functions

- co-enzyme of glutathione peroxidases (GPxs)
- direct scavenger against free radicals
- it maintains vitamin E and C in their reduced, biologically active forms
- it can reduce transition metals
- it is involved in the detoxification phase I reactions
- it can modulate the functions of some thiol-proteins (e.g., enzymes)
- it prevents meta-haemoglobin formation.

2. Specifically related to its "conjugating" ability:

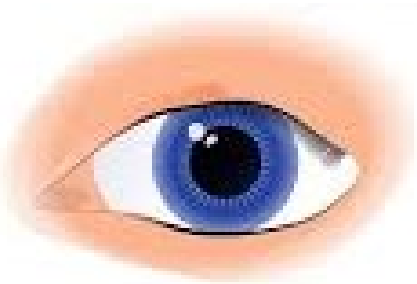
- it is involved in the detoxification phase II reactions
- can further positively or negatively modulate other proteins (glutathionylation)

3. Others:

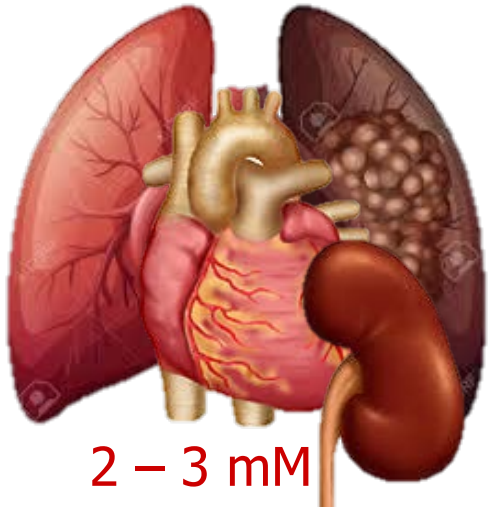
- it transports and maintains the L-cysteine in its non toxic form
- it is indirectly involved in the metabolism of phospholipids and S-adenosylmethionine/homocysteine
- as cofactor of metabolism of arachidonic acid it allows the biosynthesis of leukotrienes (LT)
- Ut can transport/modulate some functions of nitric oxide (NO)

Overview

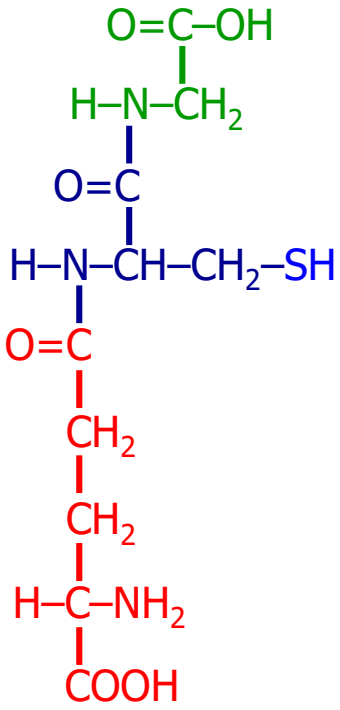
The tissue distribution of glutathione

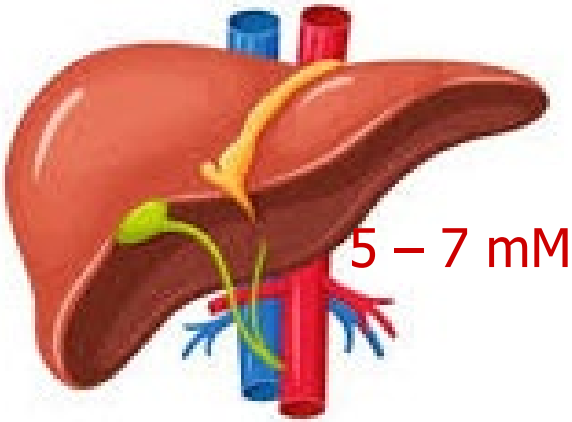


~ 10 mM



2 – 3 mM





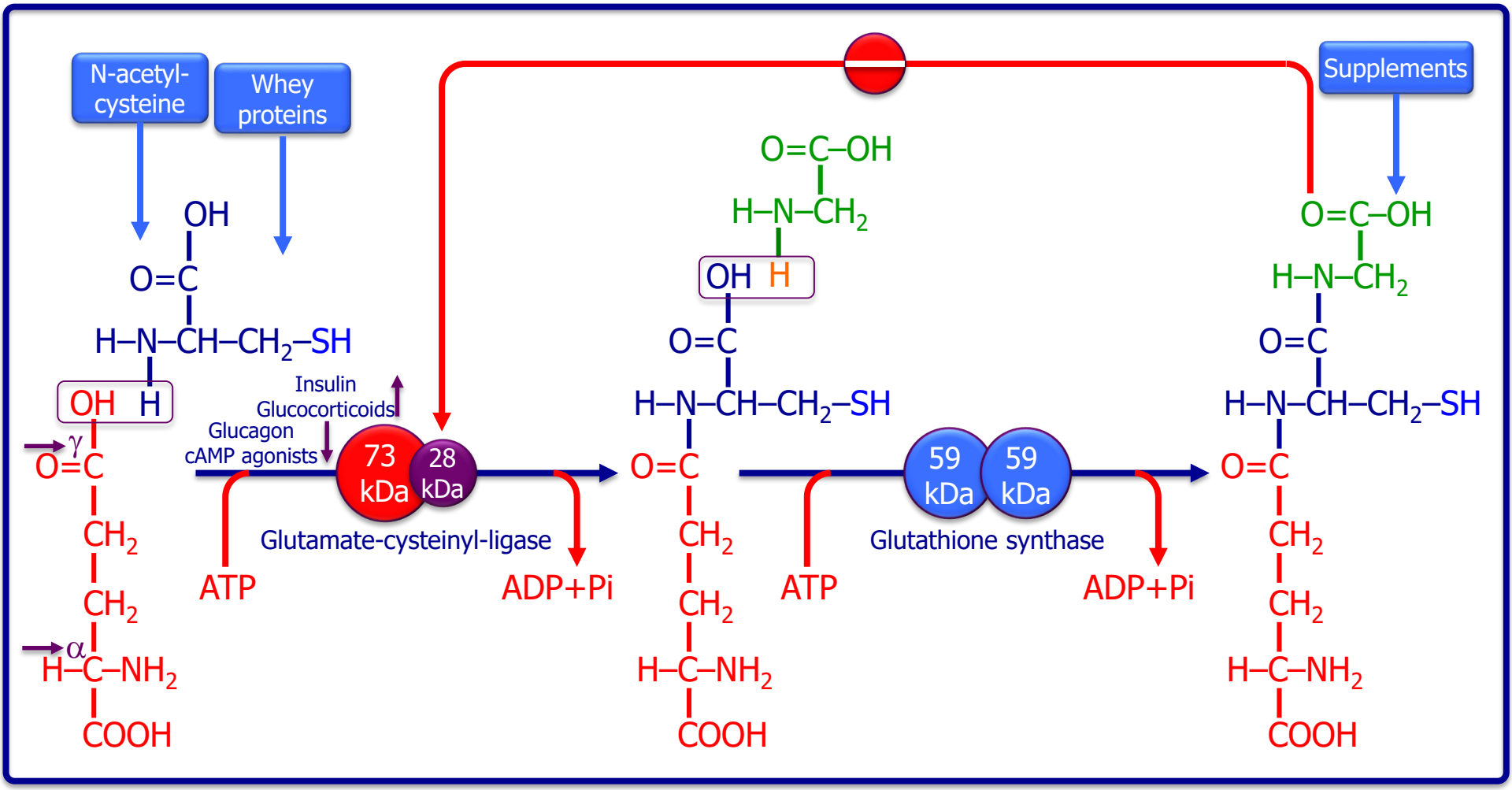
5 – 7 mM

~ 2 mM

<0.05 mM

The highest level in the eyes to effectively modulate reactive oxidant species

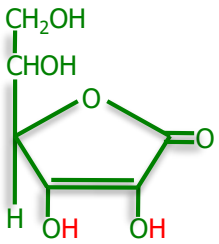
The *de novo* biosynthesis of glutathione



Alternative sources and metabolic control

Transport across the plasmamembrane

Ascorbic acid passes membranes by a Na^+ dpendent active transport (SVCT)

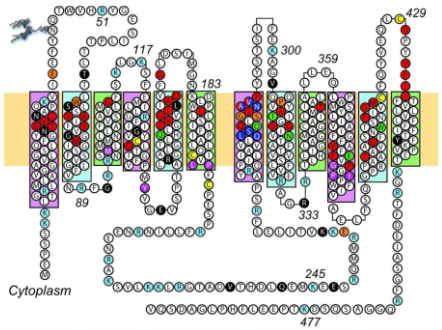


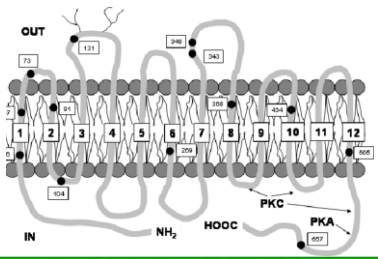
SVCT1

- Mostly expressed in epithelial cells
- Low affinity but high carrier capacity

SVCT2

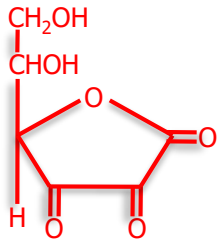
- Mostly expressed in brain neurons
- High affinity but low carrier capacity

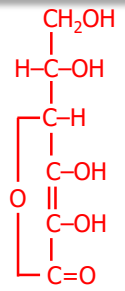


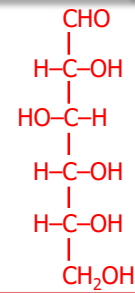


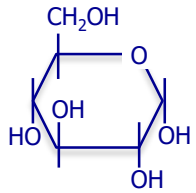
Documented polymorphisms

Ascorbic acid passes membranes by facilitated diffusion (GLUT)









GLUT1

- almost all cells

GLUT2

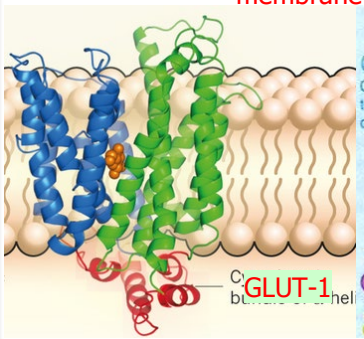
- liver, spleen, gut/kidney basolateral membrane

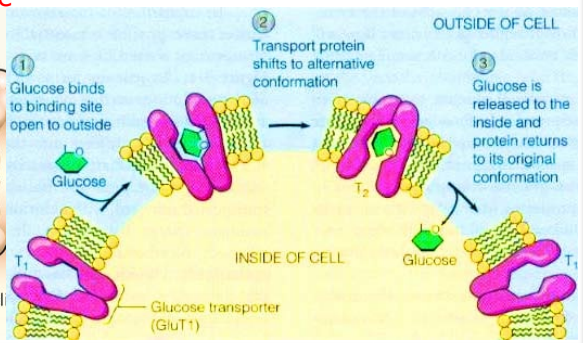
GLUT3

- brain neurons

GLUT4

- muscle and adipose cells

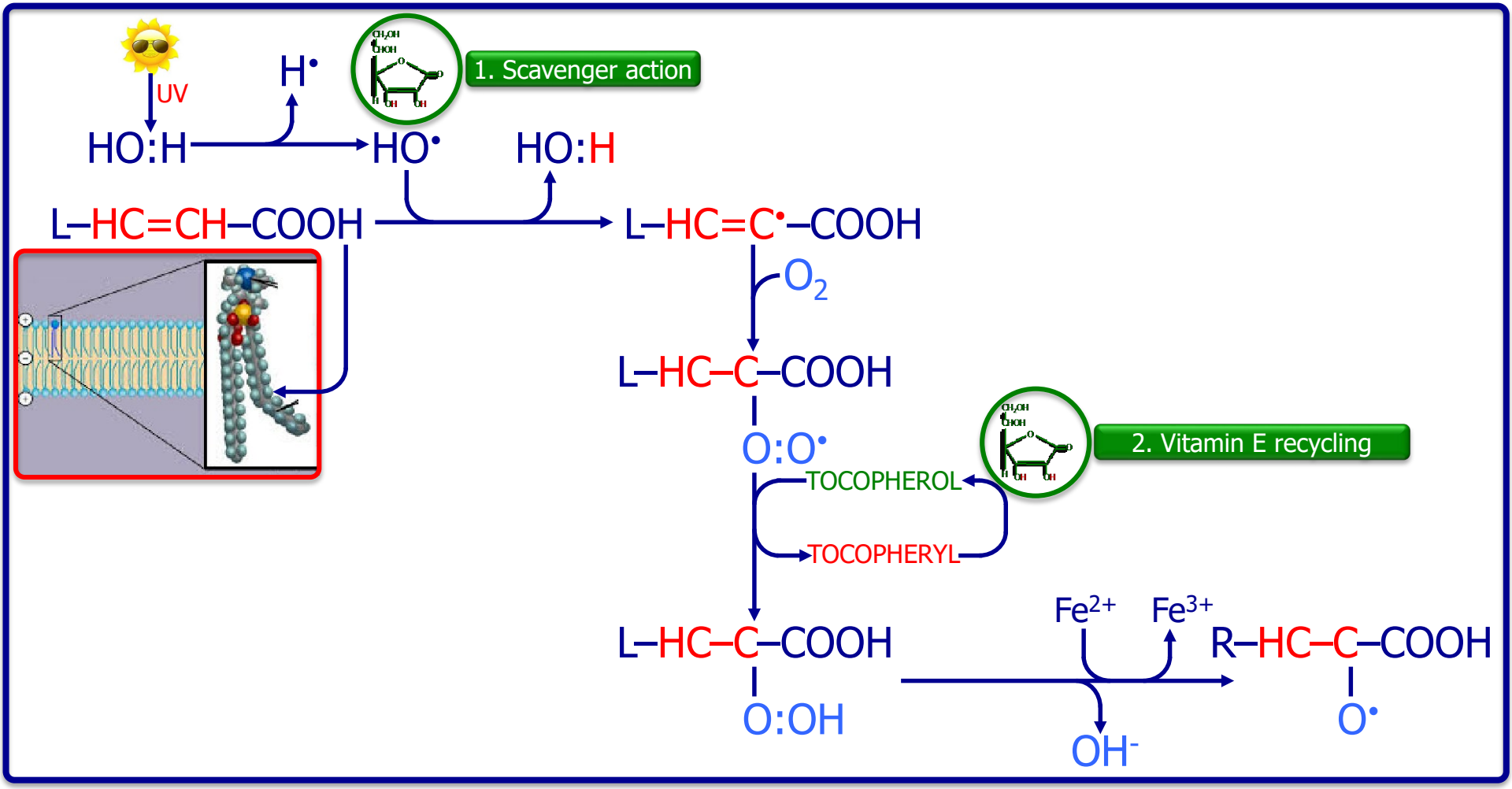




Dehydroascorbate and glucose as competitors for the same transporter

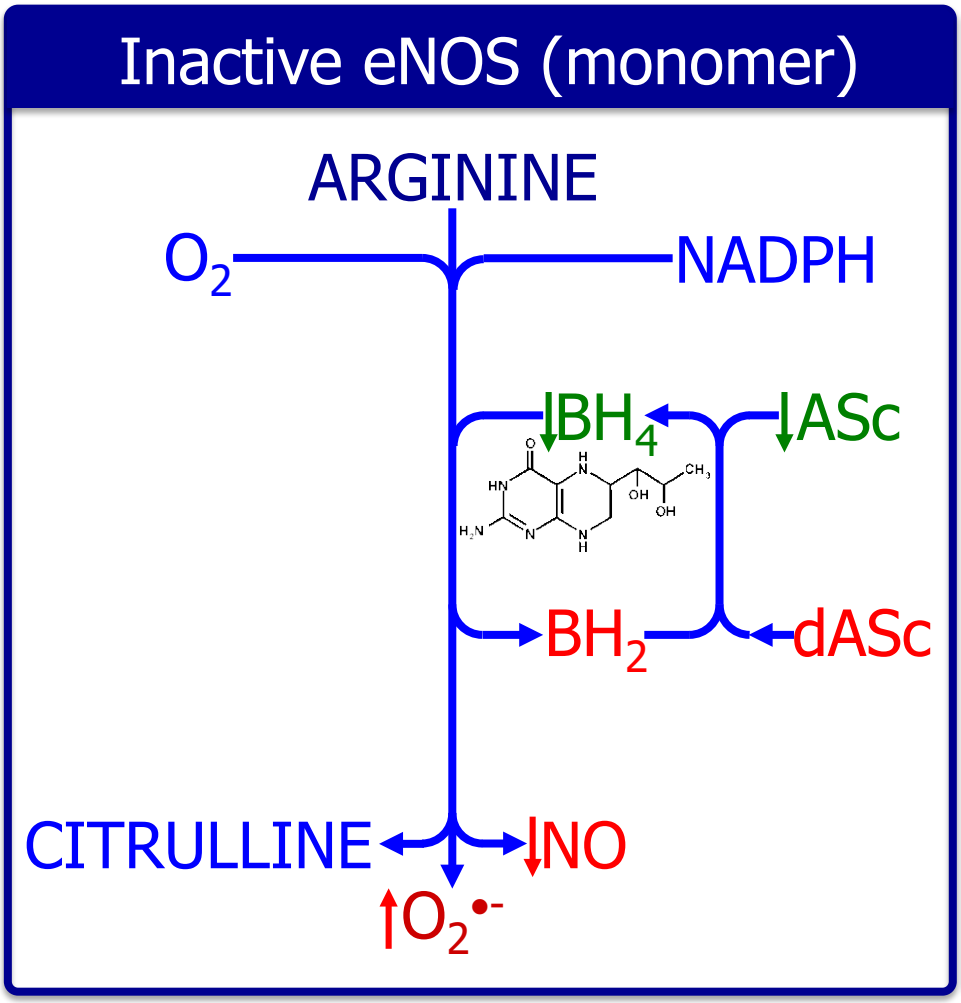
Two different transport systems: SVCT (for ascorbic acid) and GLUT (for dehydroascorbic acid)

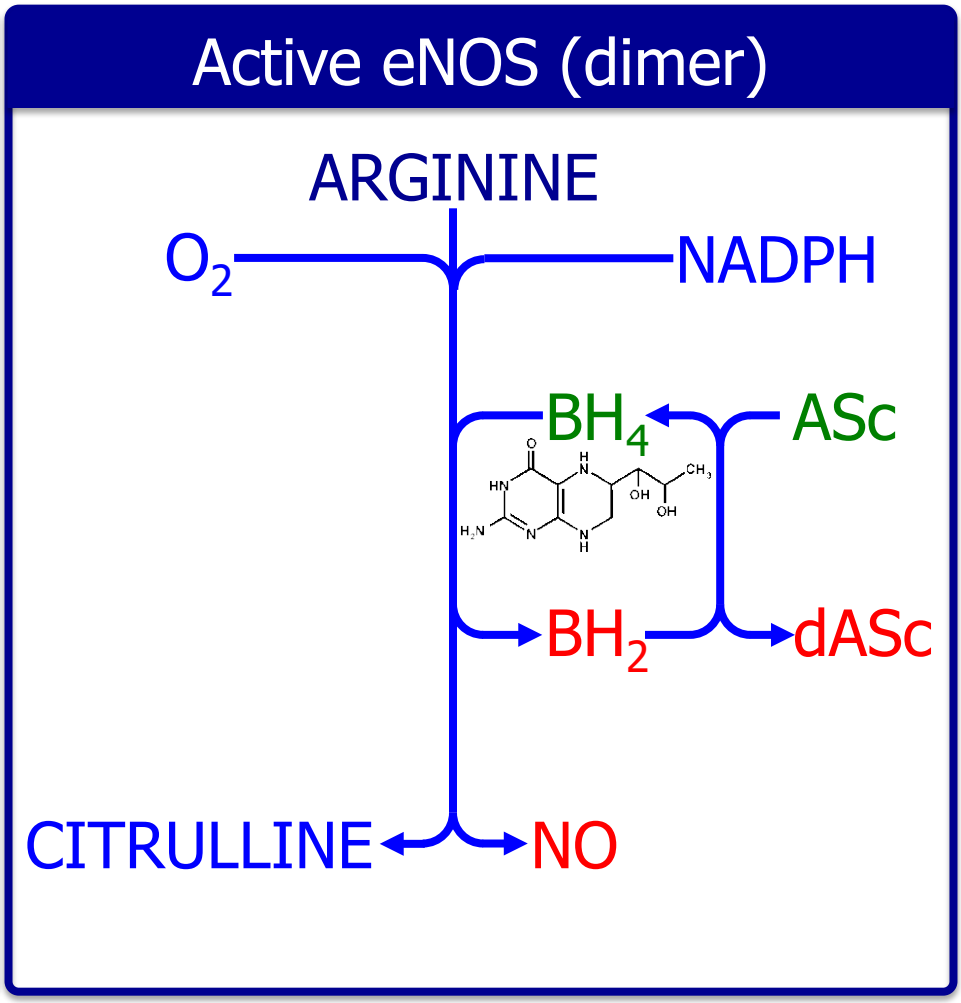
Vitamin C as antioxidant



Vitamin C scavenges free radicals and recyclates tocopheryl radicals

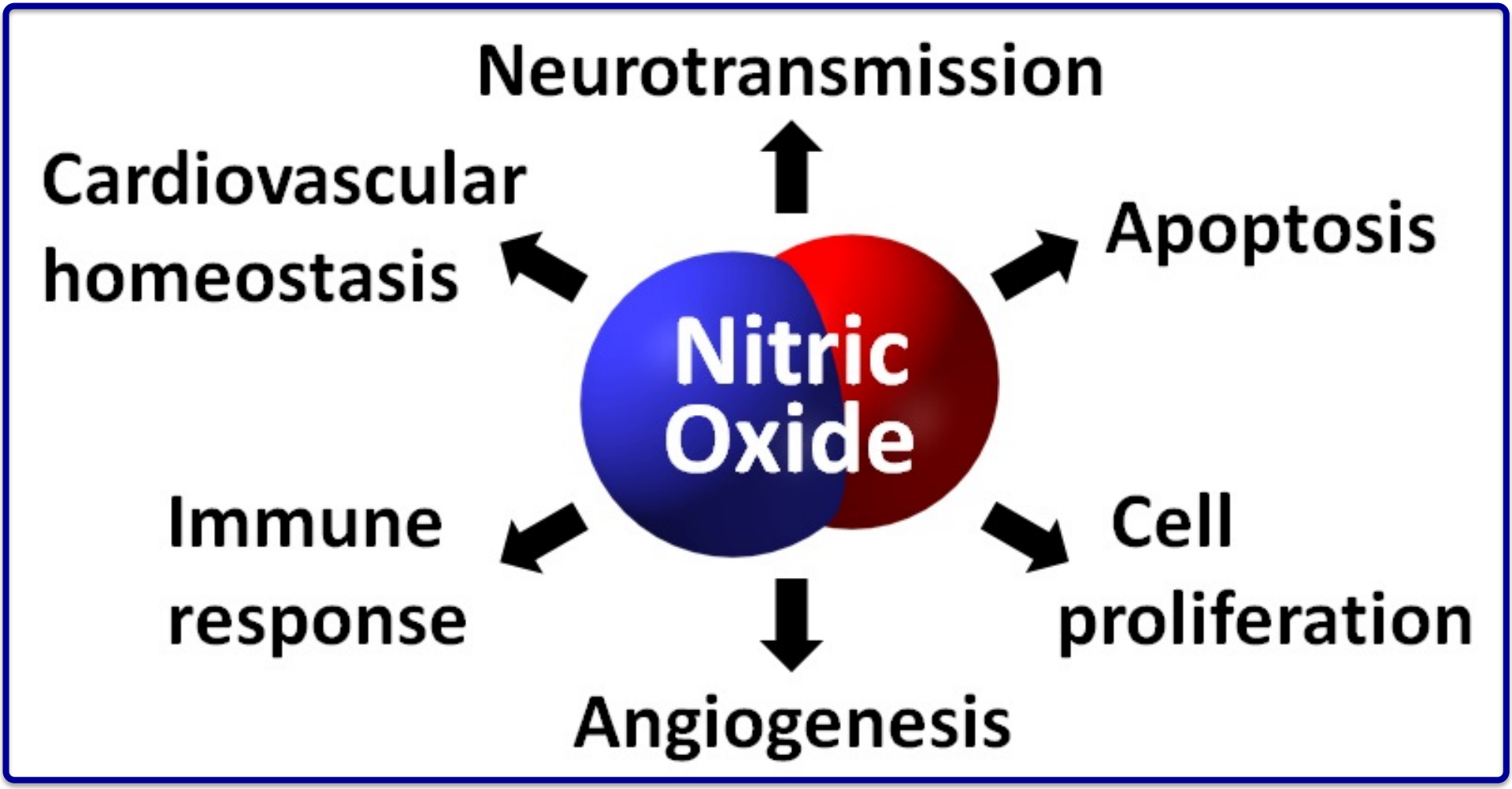
Vitamic C allows the reduction of di-hydrobiopterin (BH_2) to tetrahydrobiopterin (BH_4)





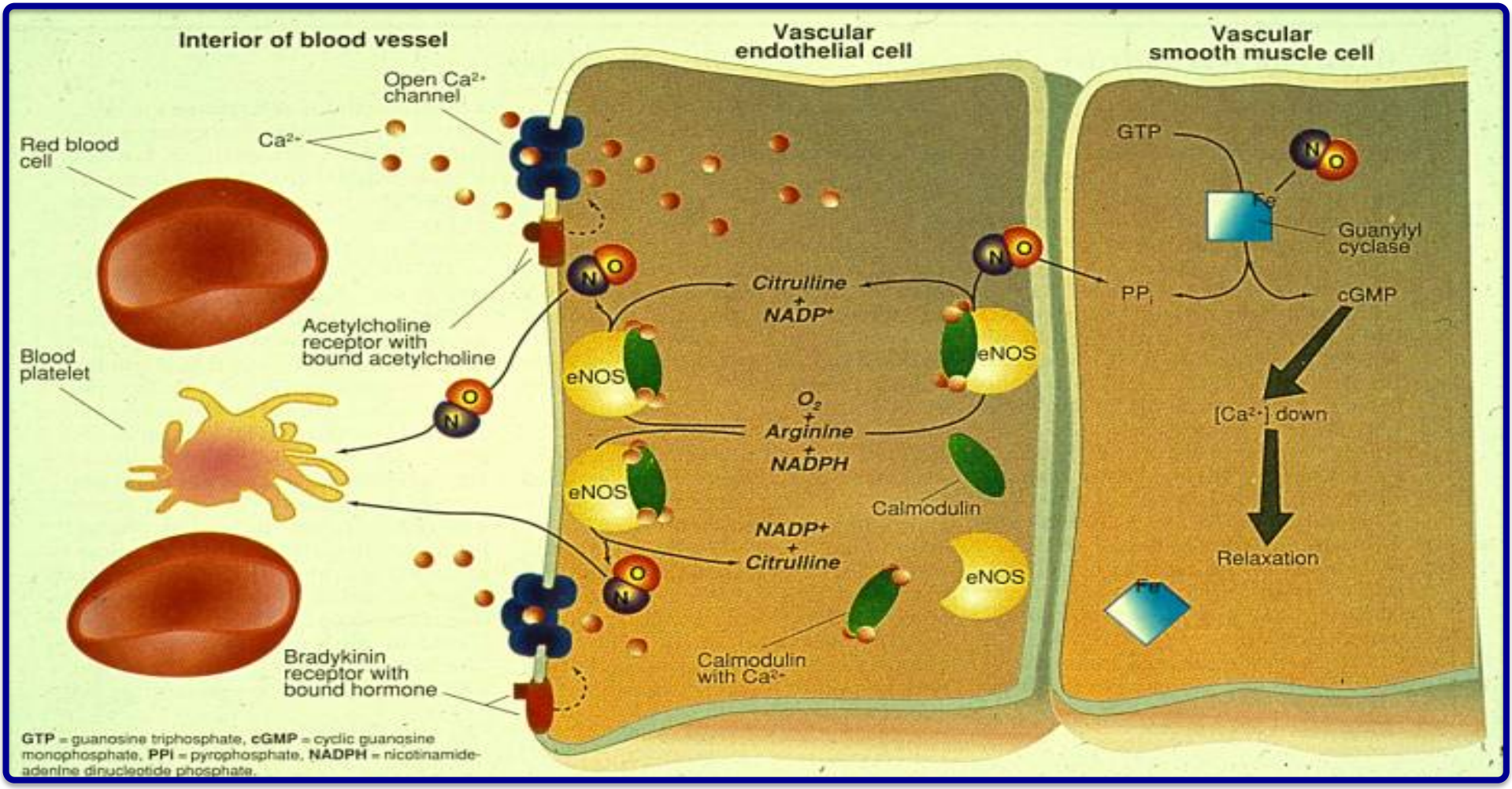
A crucial step in the nitric oxide (NO) generation

General biological role of nitric oxide



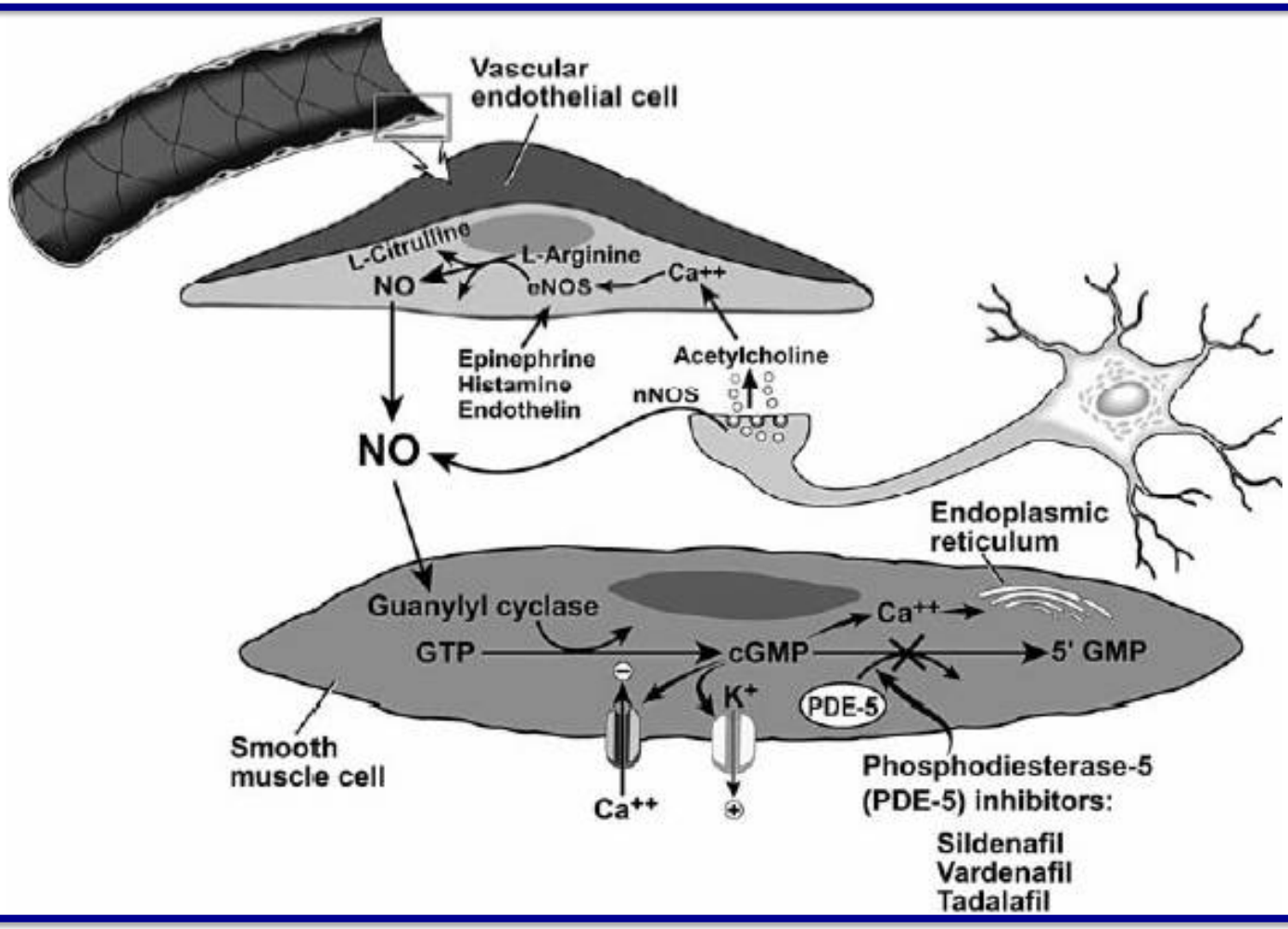
More that a radical gas

The biological role of nitric oxide (NO) in the microcirculation



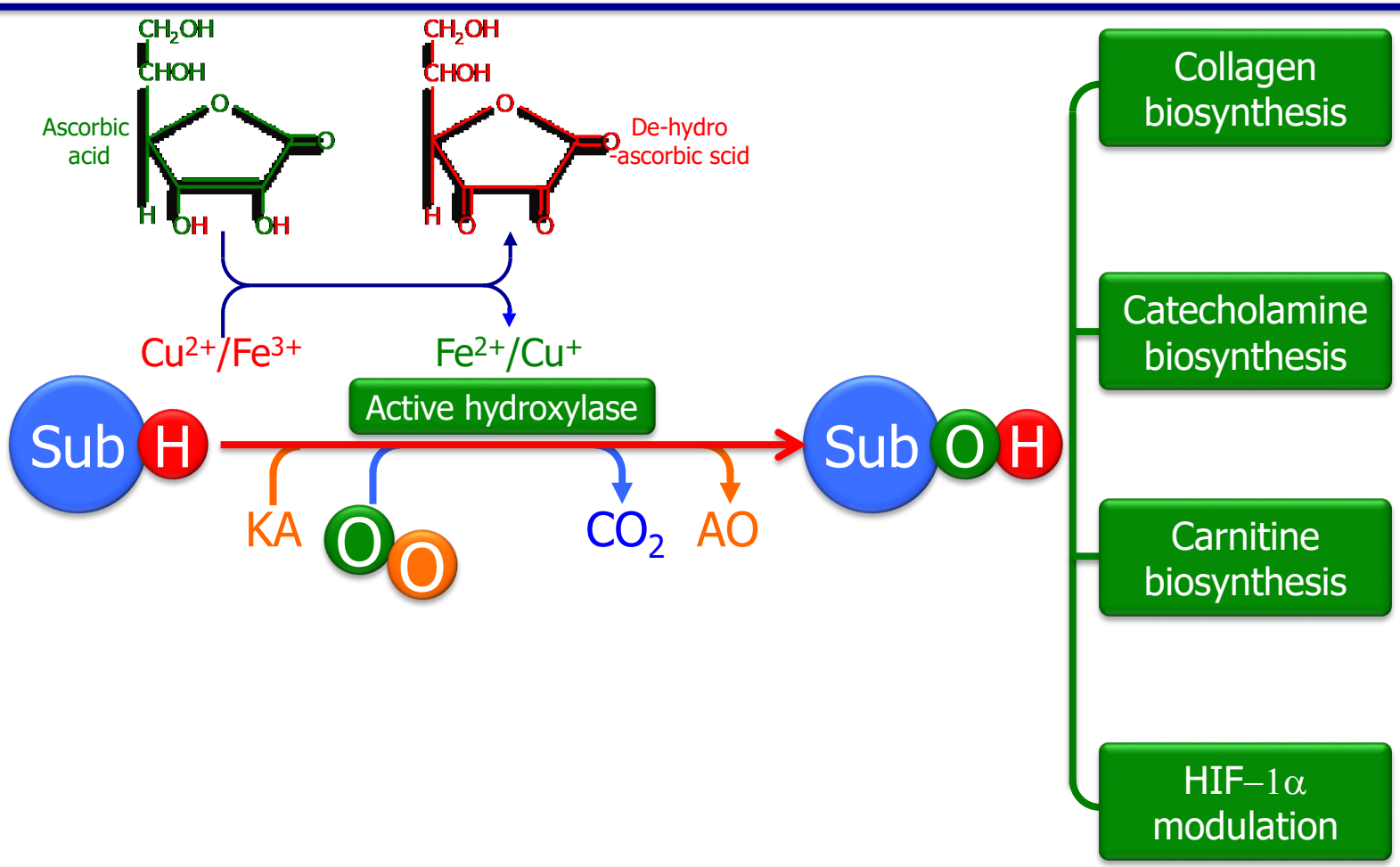
A Nobel price slide!

The role of nitric oxide in the erection



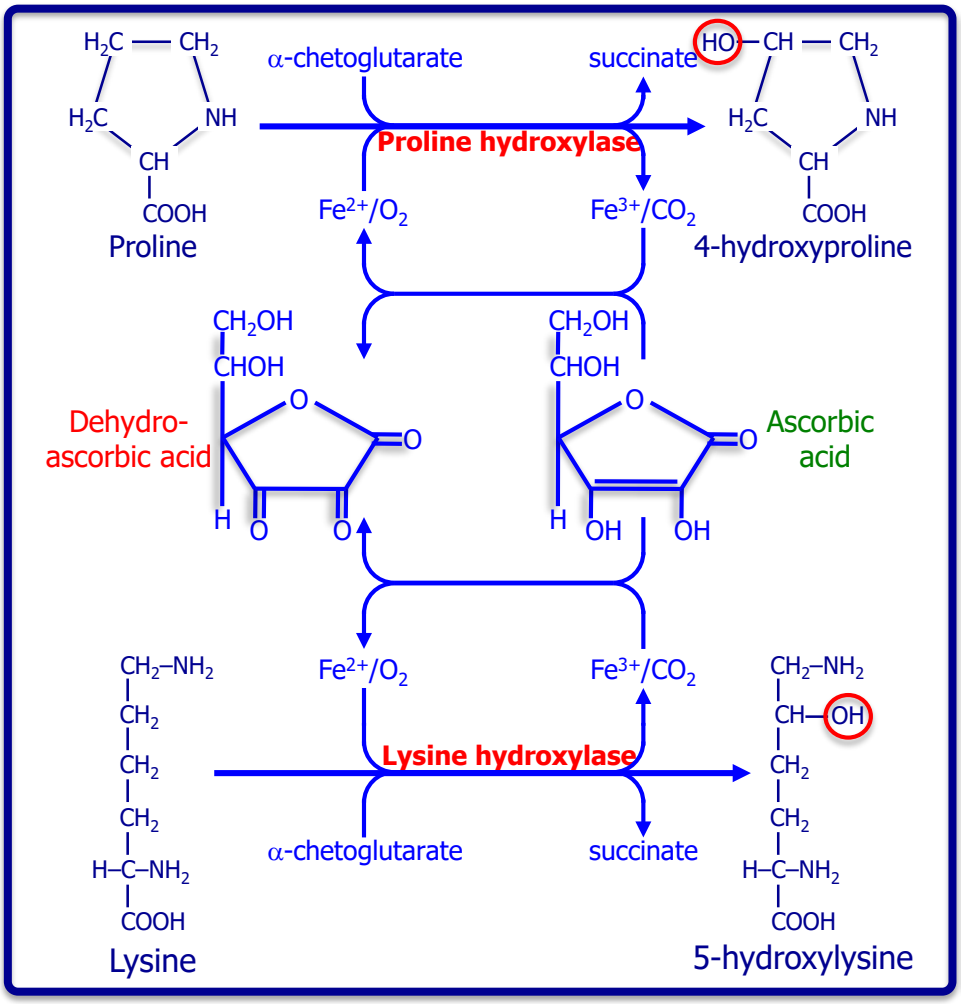
Due to its radical features, NO allows the biosynthesis of cGMP thus providind the biochemical basis for VIAGRA!

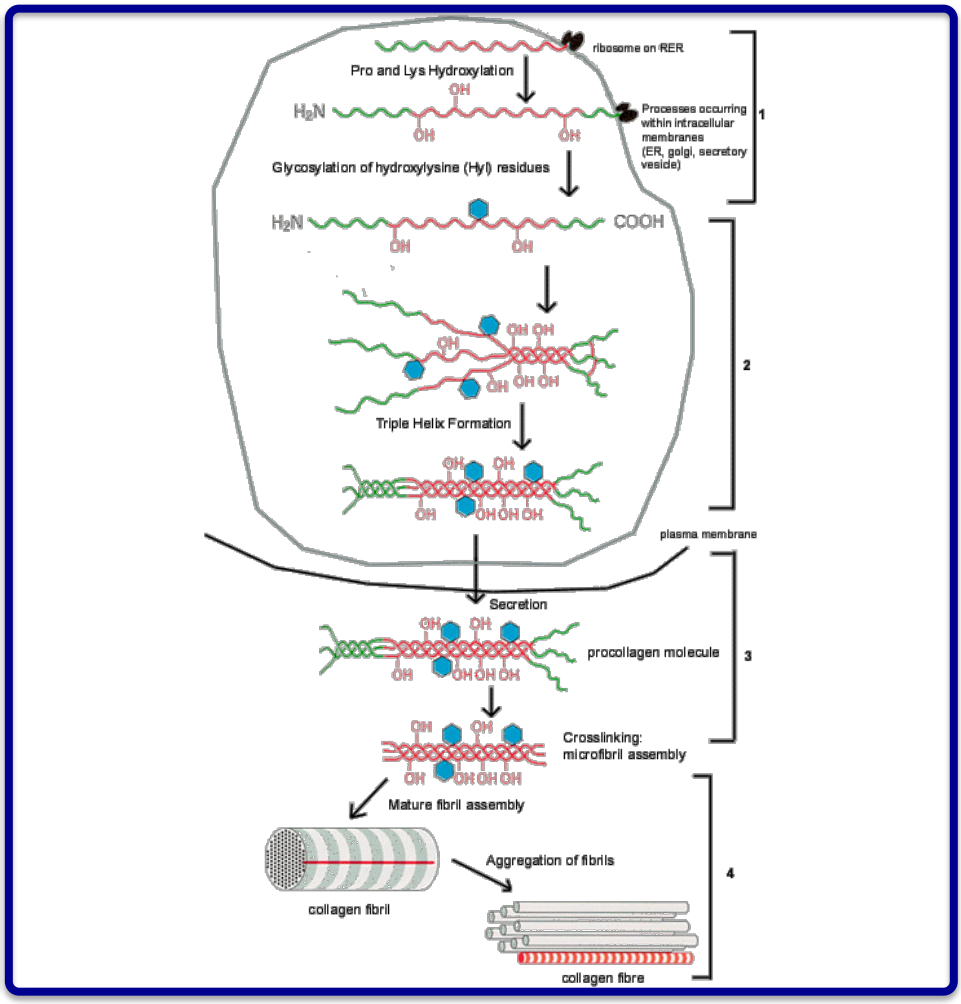
Vitamin C maintains in a reduced status metal cations that are essential for some hydrolylase activities



A complex pleiotropic mechanism

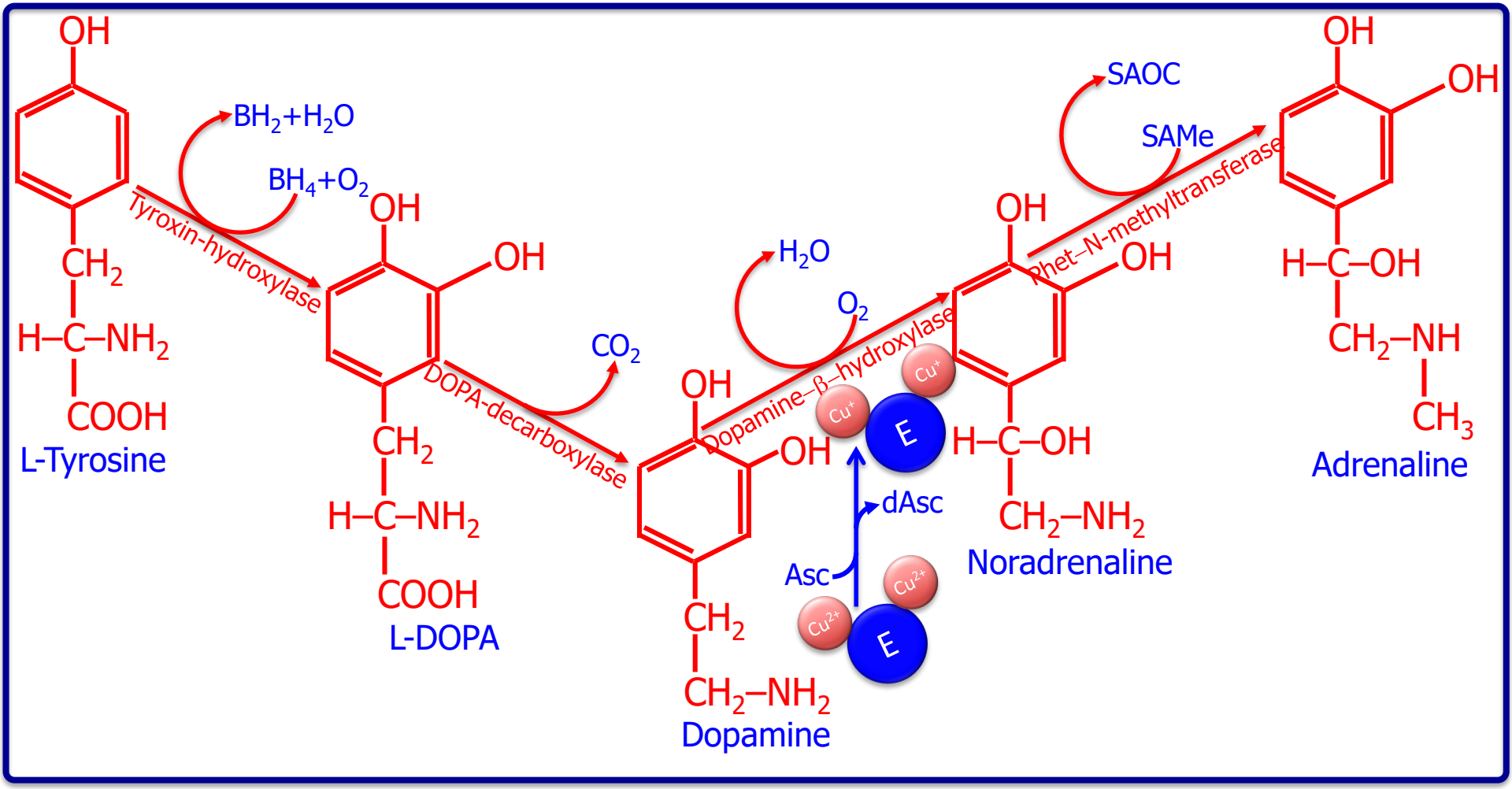
1. Vitamin C allows the hydroxylation of proline and lysine





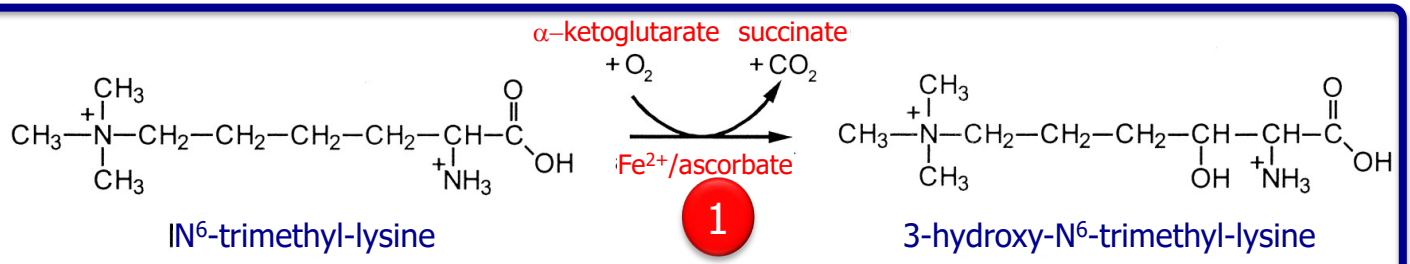
A fundamental step for the crosi-linking and stabilisation of collagene as well as epigenetic modulation

2. Vitamin C allows the hydroxylation of dopamine to noradrenaline

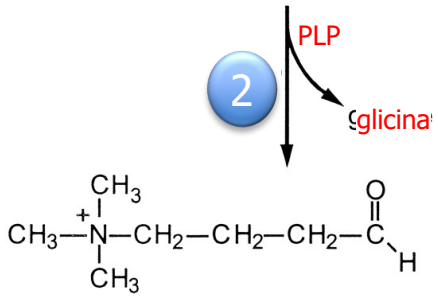


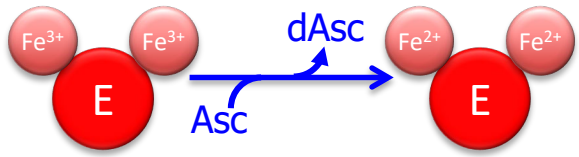
A fundamental step for many neuroendocrine functions

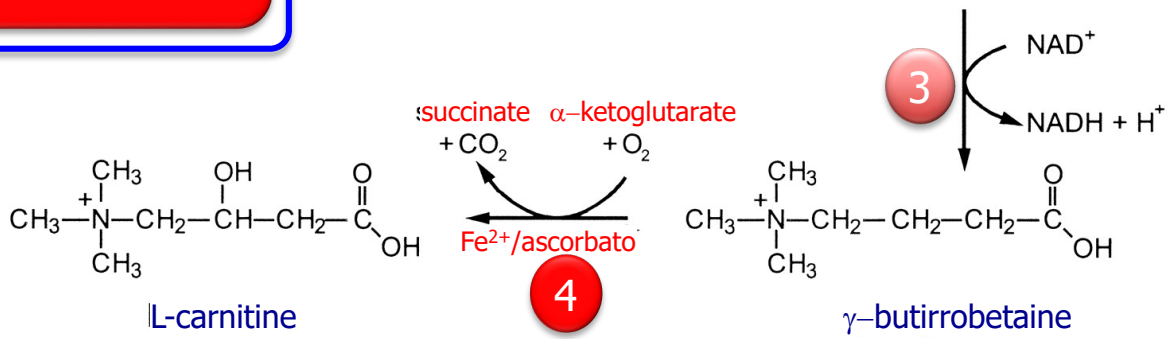
3. Vitamin C allows the biosynthesis of carnitine



1. N^6–trimethyllysine hydroxylase
2. 3–hydroxy–N^6–trimethyllysine aldolase
3. γ–butiric-betaine hydroxylase
4. 4–N^6trimethylammoniumbutiraldehyde dehydrogenase

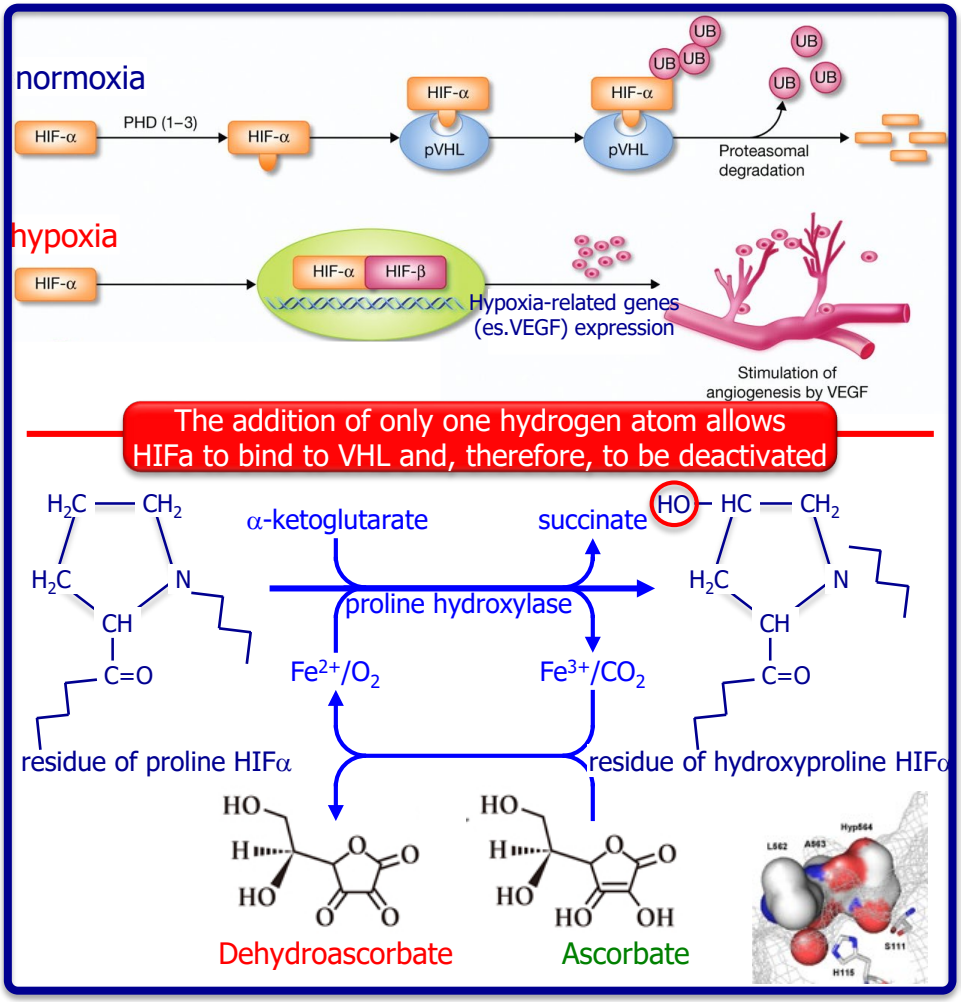


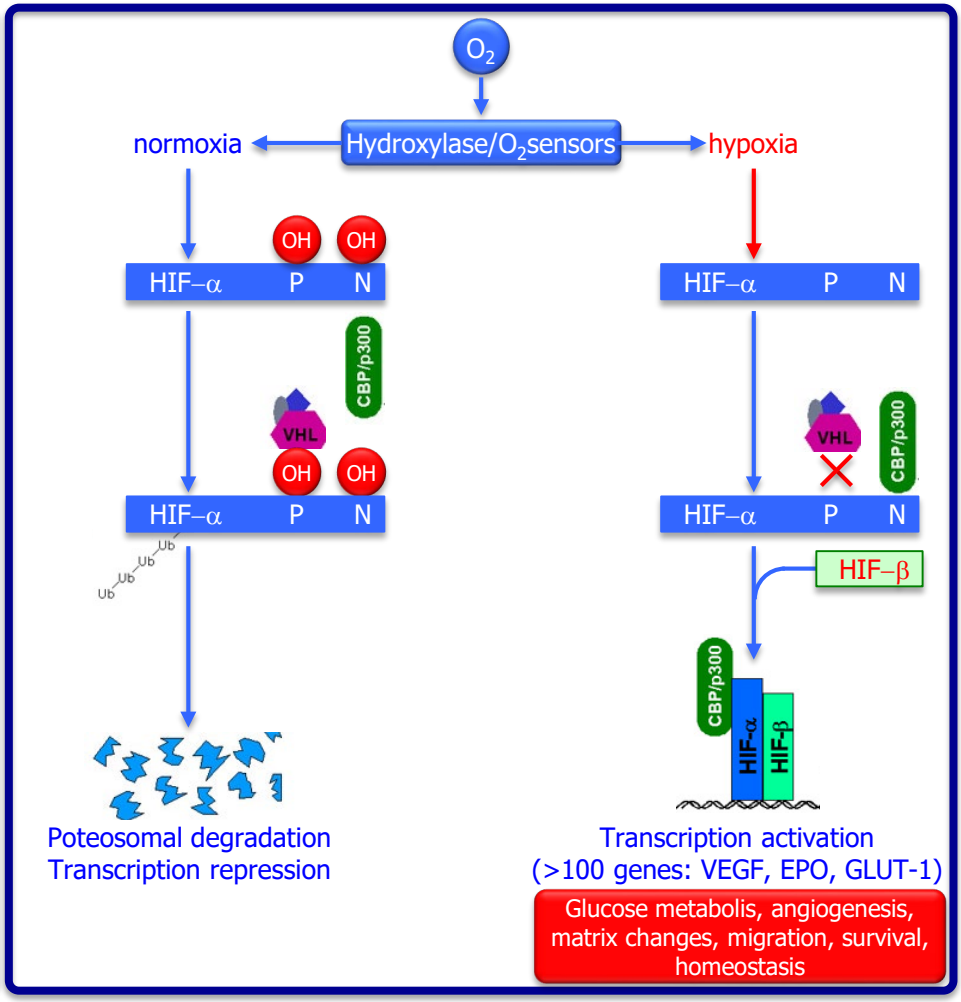




The production of an important pleiotropic molecule

4. Vitamin C allows to deactivate the hypoxia-inducible factor 1a (HIF1α)

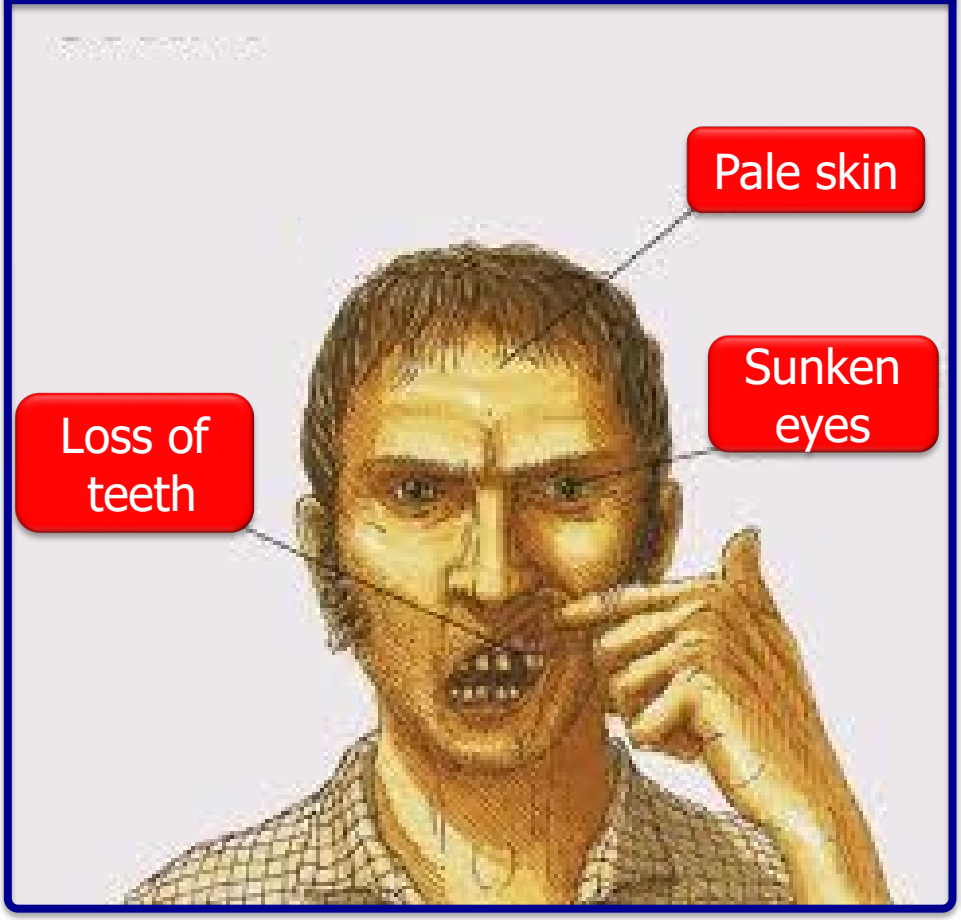




A modulation mechanism that is directly related to cell survival

The "scurvy" yesterday and today

SCURVY IN THE PAST



PRESUMABLE INCIDENCE 30%

C HYPOVITAMINOSIS Plasma level <11 μM

INTRACELLULAR FORMS PREVALENT

Un underestimated problem (compared to the vitamin D deficiency)

Presumable or documented causes of hypovitaminosis

1. Poor nutritional quality of ingested foods, unsuitable transformation processes (cooking, storage)
2. Inadequate lifestyles (unbalanced/monotonous diets, poor exercise, smoking, alcohol abuse)
3. Gastrointestinal diseases
4. Increased need linked to physiological conditions (pregnancy, lactation, growth)
5. Increased need for diseases (e.g., infections) and/or iatrogenic factors (e.g., antibiotics, contraceptive pill, etc.)

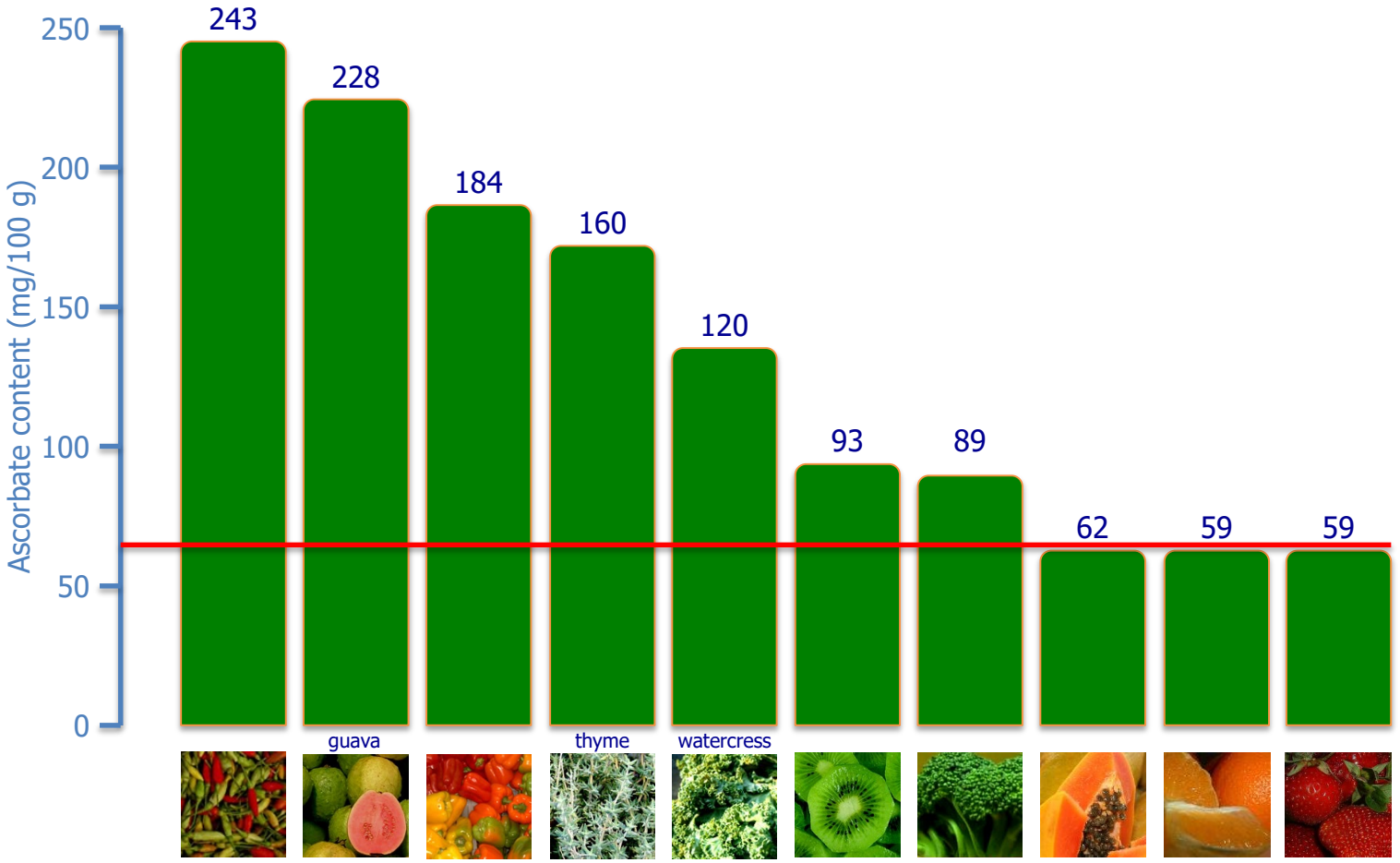
An emerging problem even in developed Countries

Mean loss (%) of vitamin C after food cooking

Treatment	Spinach	Asparagus	Carrots	Cabbage	Turnips	Potatoes	Peas	Tomatoes
Boiling	20-85	26-75	12-80	30-90	25-75	10-70	12-56	-
Pressure cooker	22	18-20	22-25	22-26	24-37	10-15	12-36	-
Steaming	24-70	22	14-25	33-70	39	15-40	24-29	
Boxing	60-65	-	-	-	-	-	-	25
Frying	-	-	-	-	-	30-60	--	-

Fried potatoes orphan of vitamin C!

Vitamin C content in some vegetables. THE TOP TEN.



Recommended daily levels for the Italian population: 60 mg per day.

The pro-oxidant effect of vitamin C. The lesson from Biochemistry.

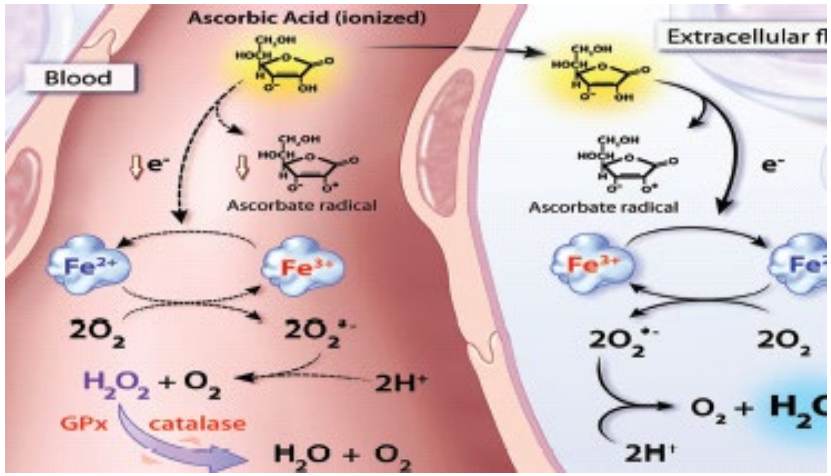
PNAS

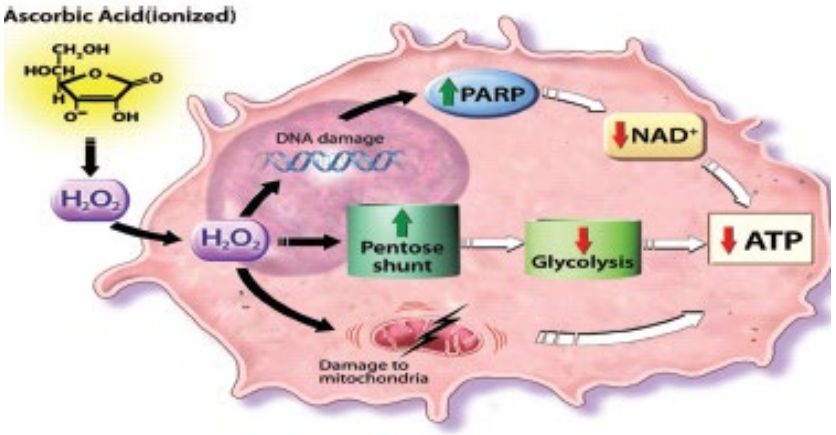
Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid *in vivo*

Qi Chen*, Michael Graham Espey†, Andrew Y. Sun*, Je-Hyuk Lee*, Murali C. Krishna†, Emily Shacter‡, Peter L. Choyke§, Chaya Pooput¶, Kenneth L. Kirk¶, Garry R. Buettner‖, and Mark Levine*,**

*Molecular and Clinical Nutrition Section and ¶Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; †Radiation Biology Branch and the §Molecular Imaging Program, National Cancer Institute, Bethesda, MD 20892; ‡Laboratory of Biochemistry, Center for Drug Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892; and ‖Free Radical and Radiation Biology Program, University of Iowa, Iowa City, IA 52242

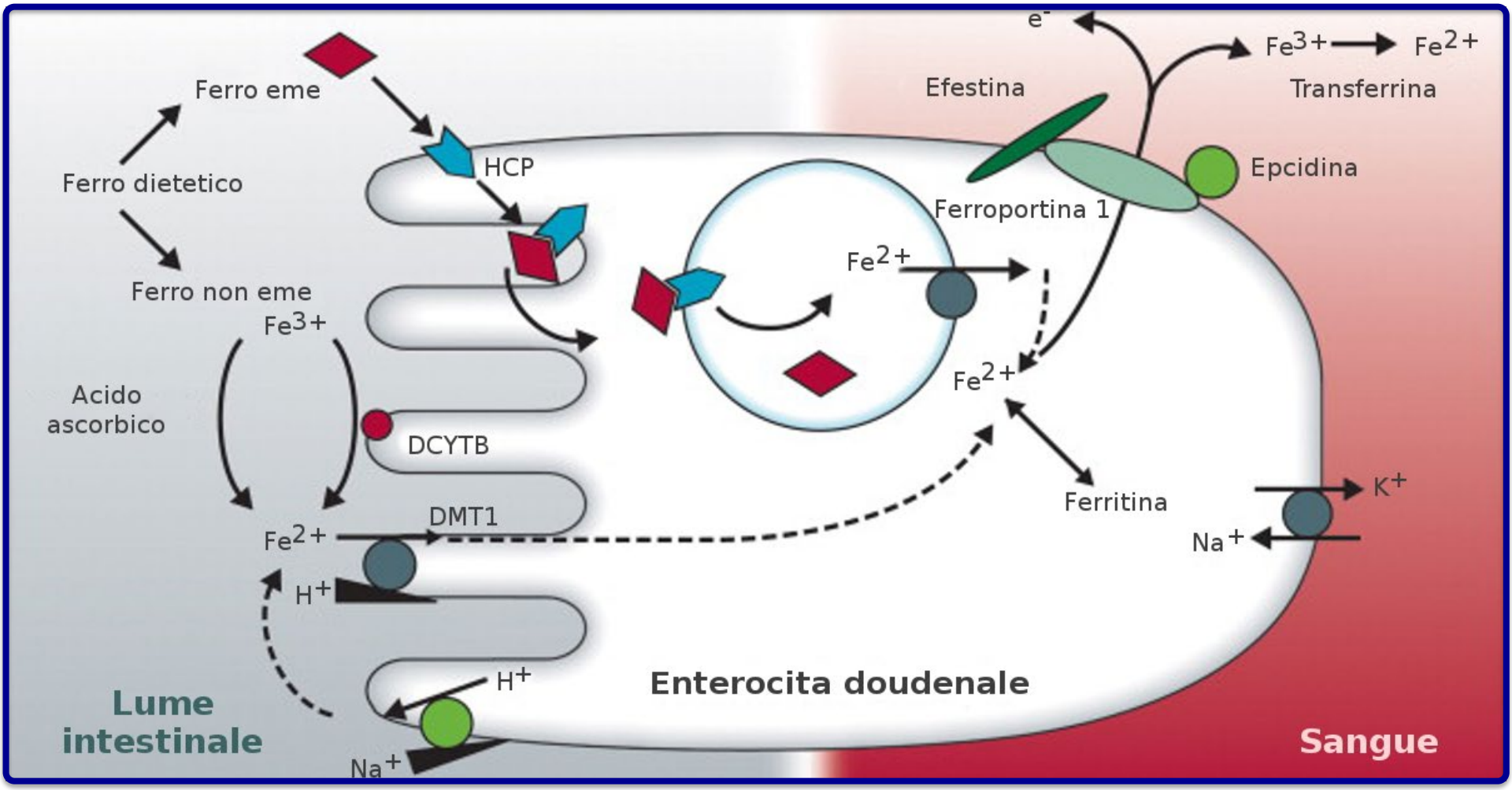
Communicated by E. R. Stadtman, National Institutes of Health, Bethesda, MD, March 27, 2007 (received for review January 31, 2007)





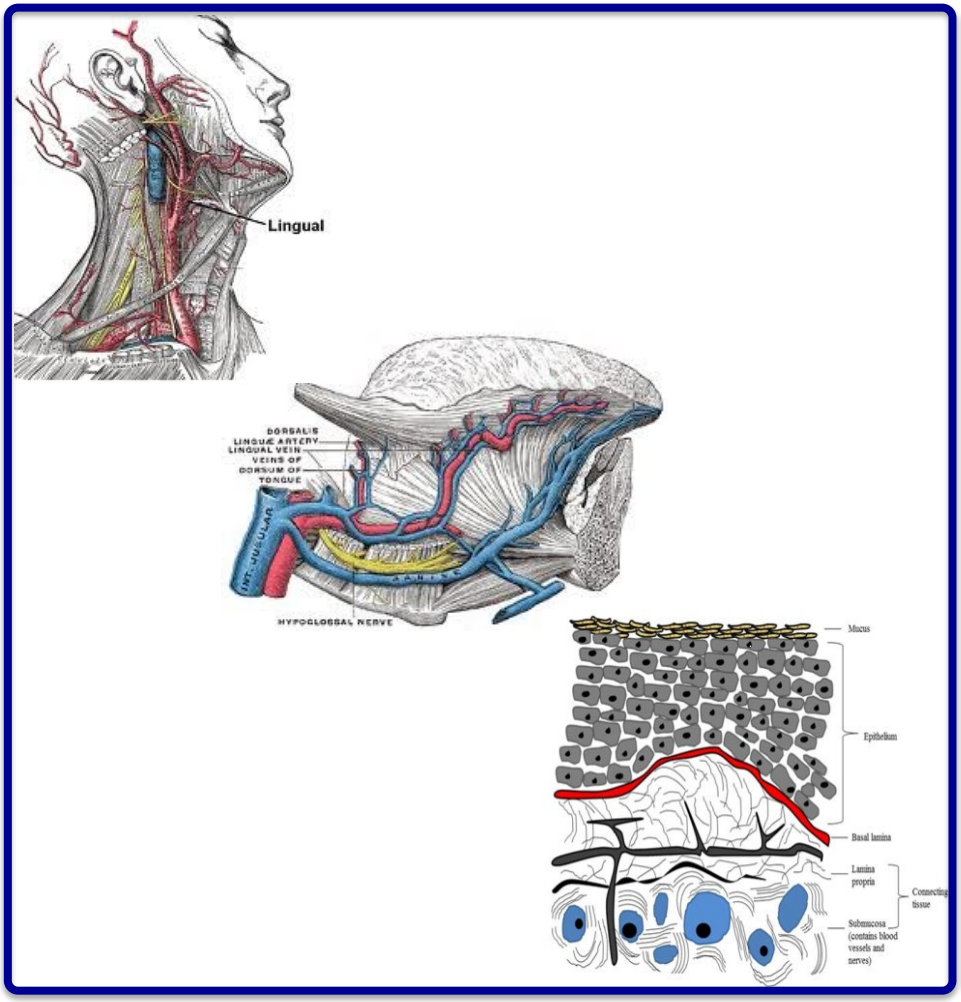
The unwanted side effects of high vitamin C: more hydrogen water inside the cells, possible apoptosis.

About vitamin C and iron. Another ”odd couple”?



Co-administration, a common practice, but . . .

News from the research



Advantages and challenges of sublingual way

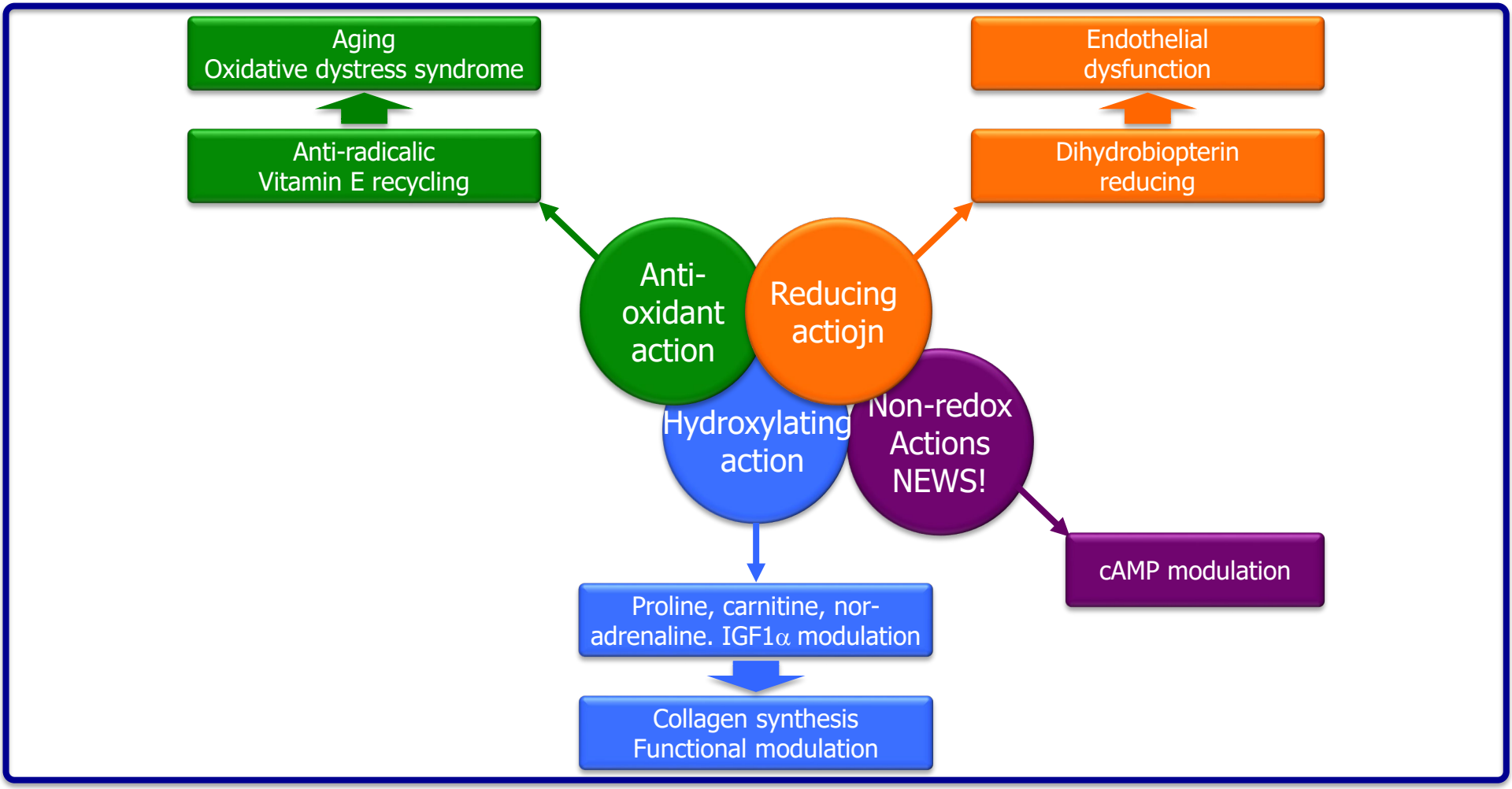
1. The buccal mucosa is relatively permeable with a rich blood supply, robust in comparison to the other mucosal tissues.
2. Bypass the first-pass effect and non-exposure of the drugs to the gastrointestinal fluids.
3. Easy access to the membrane sites so that the delivery system can be applied, localized and removed easily.
4. Improve the performance of many drugs, as they are having prolonged contact time with the mucosa.
5. High patient acceptance compared to other non-oral routes of drug administration.
6. Tolerance (in comparison with the nasal mucosa and skin) to potential sensitizers.
7. Increased residence time combined with controlled API release may lead to lower administration frequency.
8. Additionally significant cost reductions may be achieved and dose-related side effects may be reduced due to API localization at the disease site.
9. As a result of adhesion and intimate contact, the formulation stays longer at the delivery site improving API bioavailability using lower API concentrations for disease treatment.
10. Harsh environmental factors that exist in oral delivery of a drug are circumvented by buccal drug delivery.
11. The presence of saliva ensures relatively large amount of water for drug dissolution unlike in case of rectal or transdermal routes.
12. Provides an alternative route for the administration of various hormones, narcotic analgesics, steroids, enzymes, cardiovascular agents etc.
13. It allows the local modification of tissue permeability, inhibition of protease activity and reduction in immunogenic response. Thus, delivery of therapeutic agents like peptides, proteins and ionized species can be done easily.

Challenges for Buccal Drug Delivery System[19,20]: The main challenges of buccal administration are:

1. Limited absorption area- the total surface area of the membranes of the oral cavity available for drug absorption is 170 cm2 of which ~50 cm2 represents non-keratinized tissues, including buccal membrane.
2. Barrier properties of the mucosa.
3. The continuous secretion of the saliva (0.5-2 l/day) leads to subsequent dilution of the drug.
4. The hazard of choking by involuntarily swallowing the delivery system is a concern.
5. SalivaSwallowing can also potentially lead to the loss of dissolved or suspended drug and ultimately the involuntary removal of the dosage form.

Sublingual vitamin C

The biological actions of ascorbic acid. Not simply a vitamin!



Overview

Vitamin C

1. Vitamin C is a polyfunctional organic compound derived directly from glucose biosynthesis, of which it maintains a remarkable structural similarity, characteristic of great physiopathological impact (see diabetes mellitus, Alzheimer's disease).

2. The loss of the gene that encodes the gulonolactone oxidase obliges the Man and the Primates, together with a few other animals, to take the vitamin C from the outside through the feeding.

3. Once absorbed, vitamin C is distributed, oscillating between its oxidized and the reduced form, in the various compartments of the organism where it is diffrently concentrated, thus reaching maximum levels in the brain and in the adrenal medulla.

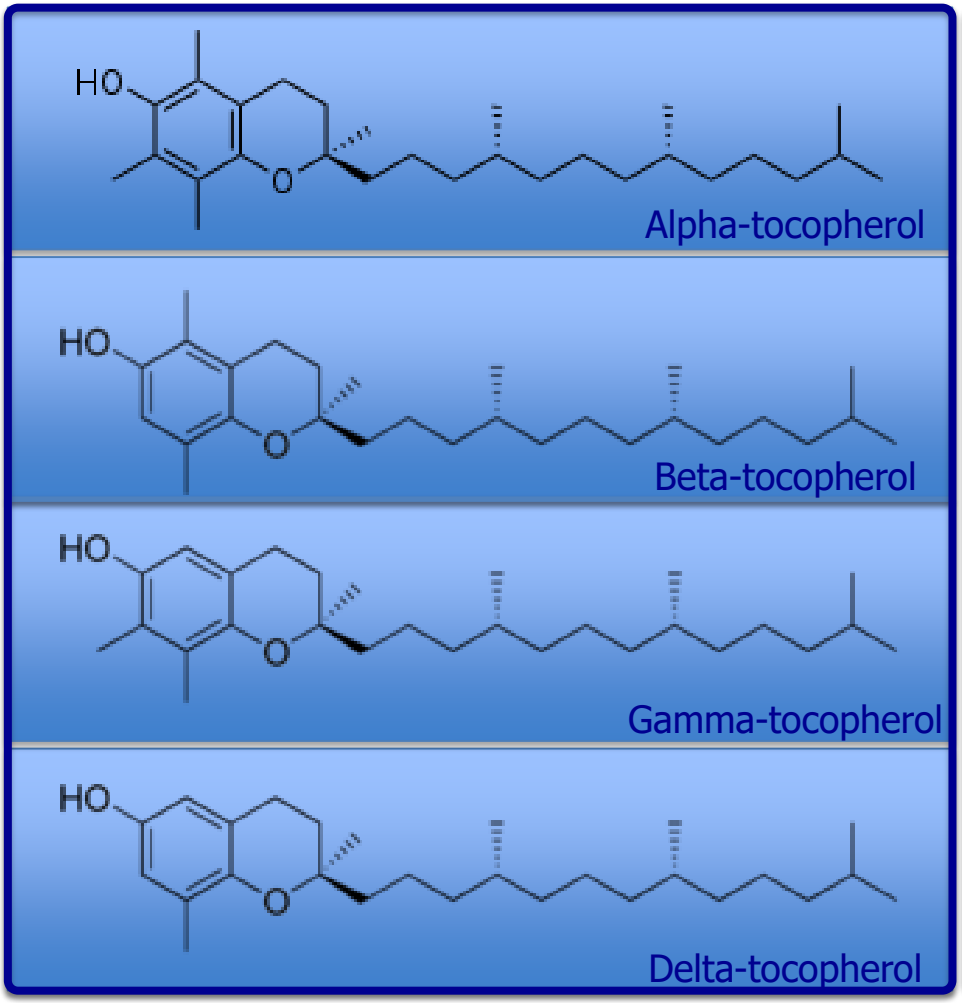
4. Vitamin C, through its triple antioxidant, reducing and hydroxylating action, is prefigured as a pleiotropic hormone-like substance indispensable in the modulation of the general homeostasis of the organism.

5. Scurvy has not disappeared from the nosography but is present today in a more subtle form, with a prevalent intracellular localization.

6. Vitamin C intake should possibly be prevented from taking into account the LARNs (60 mg/day). Any supplementation, always in documented relative or absolute deficient situations, can not be separated from the pro-oxidant effect of high dosages. In this context, the sublingual formulations are promising.

Concluding remarks

Tocopherols (vitamin E)



Tocopherols are a class of poly-isoprenoid lipid soluble compounds that exists in 4 main forms, e.g., alpha-, beta-, gamma-, and delta-tocopherol, with further subfamilies due to the phenomenon of stereoisomery. Alpha-tocopherol is the main food source of vitamin E that is abundant in olive oil while gamma-tocopherol is dominant in American diet. To tocopherols are related the so-called tocotrienol.

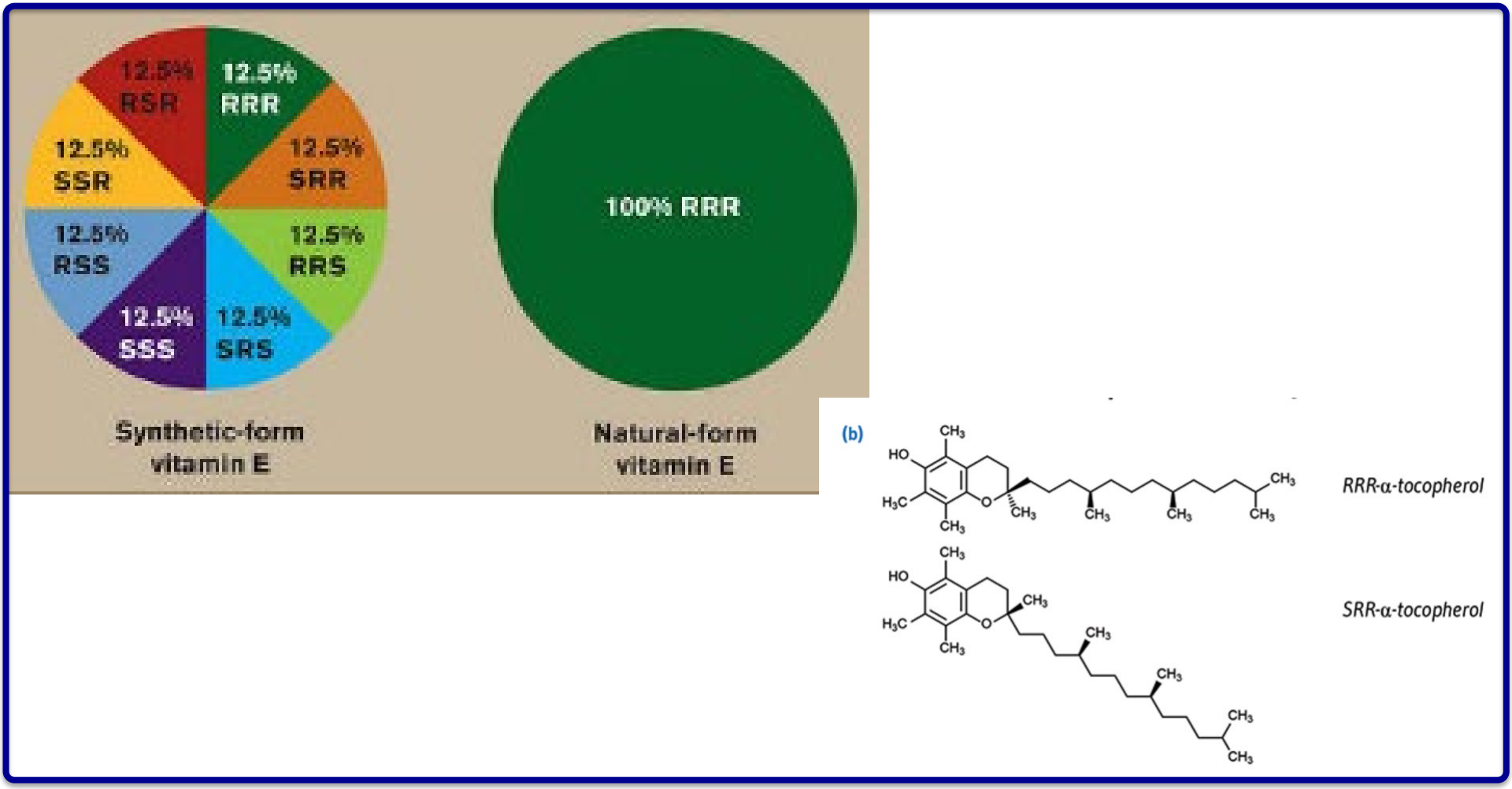
☞Tocopherols are essential compounds for Humans. Therefore tocopherols behave as vitamins (group E).

☞Tocopherols, well-known as powerful antioxidants, share many activities, including scavenging and chain breaking against free radicals, quenching against singlet oxygen and the control of some membrane protein (e.g., phospholipase A_2).

☞Tocopherol are recycled by vitamin C and ubiquinol, this latter being closely associated to it in circulating lipoproteins.

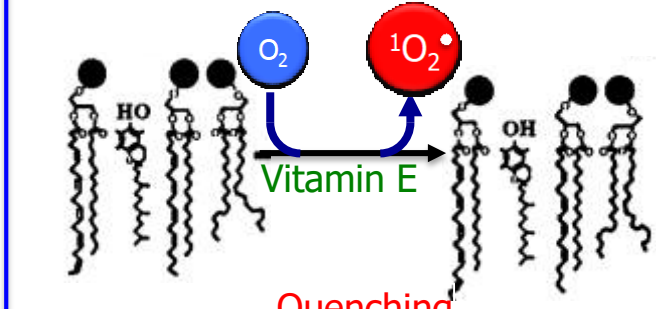
Main features

Natural versus synthetic tocopherol

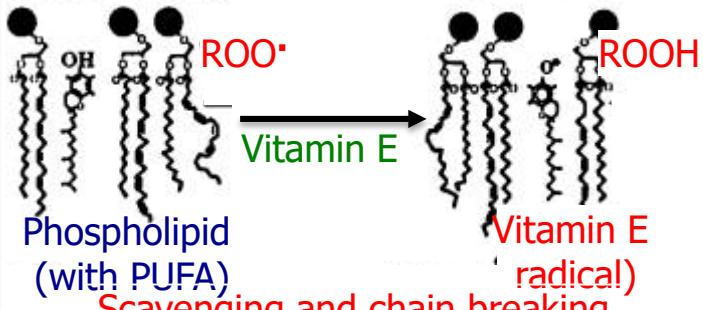


Ceasarone et Al. Panminerva Med. 2010. 52 (1): 15–19.

Tocopherols (vitamin E)



Quenching

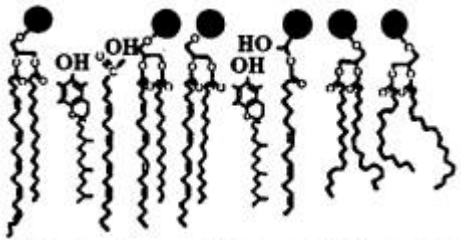


Phospholipid (with PUFA)

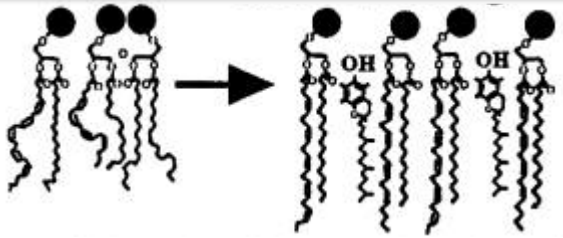
Vitamin E radical)

Scavenging and chain breaking

Effects on intracellular signalling, nuclear transcription factors, and gene expression.



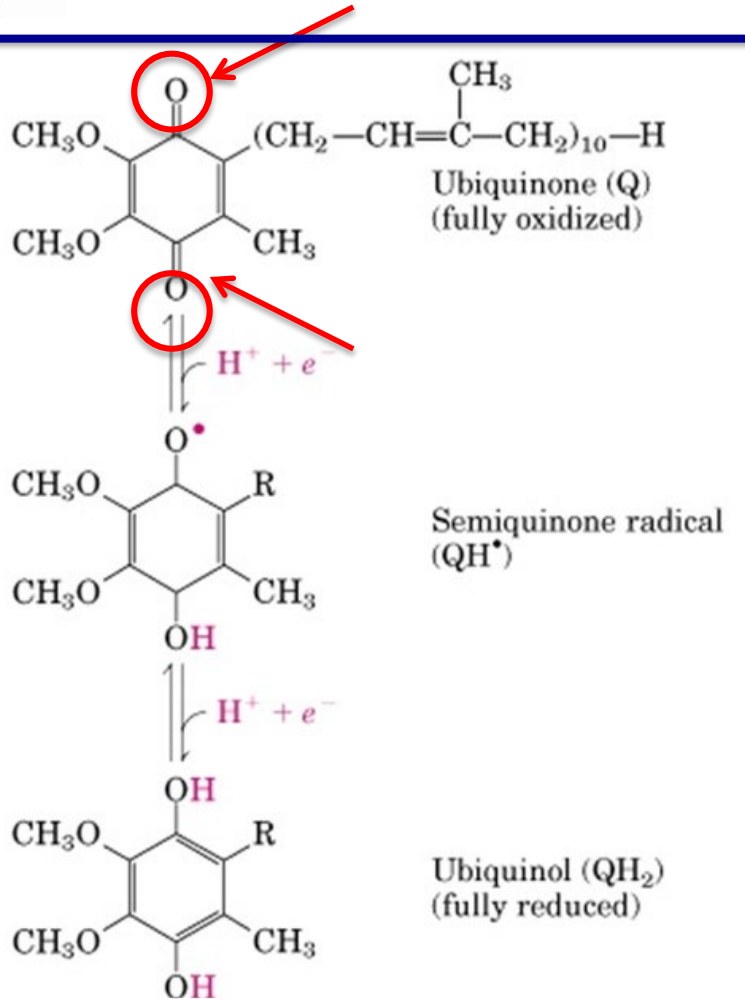
Phospholipase A_2 modulation (control of inflammation, vascular tone, etc.)



Intracellular signalling, modulation of protein kinase C activity

Main biological effects

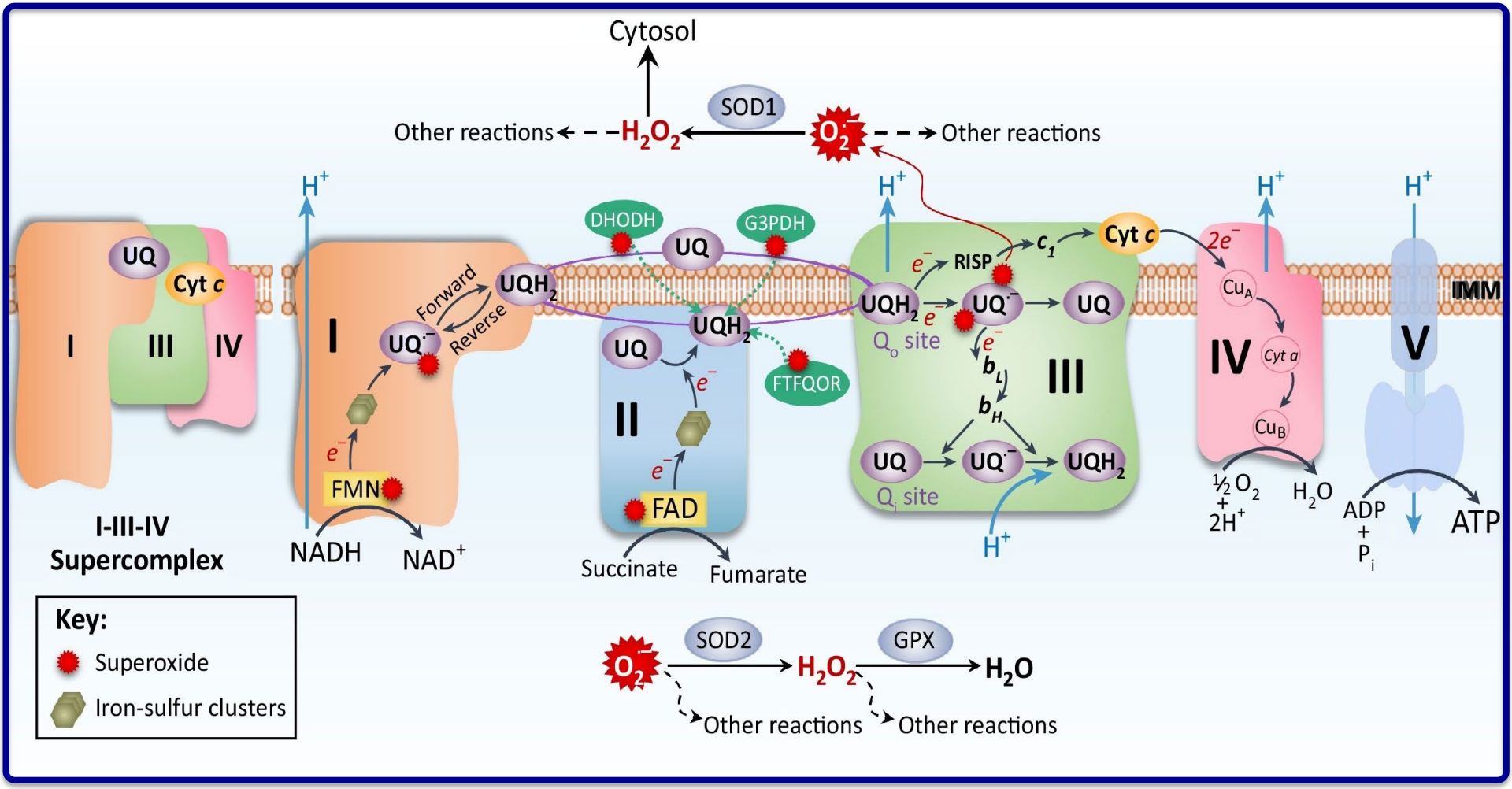
Coenzyme Q_{10}



- Sostanza lipofila a basso peso molecolare
- Scavenger nei confronti dei radicali ROO•
- Consente la rigenerazione dei tocoferoli

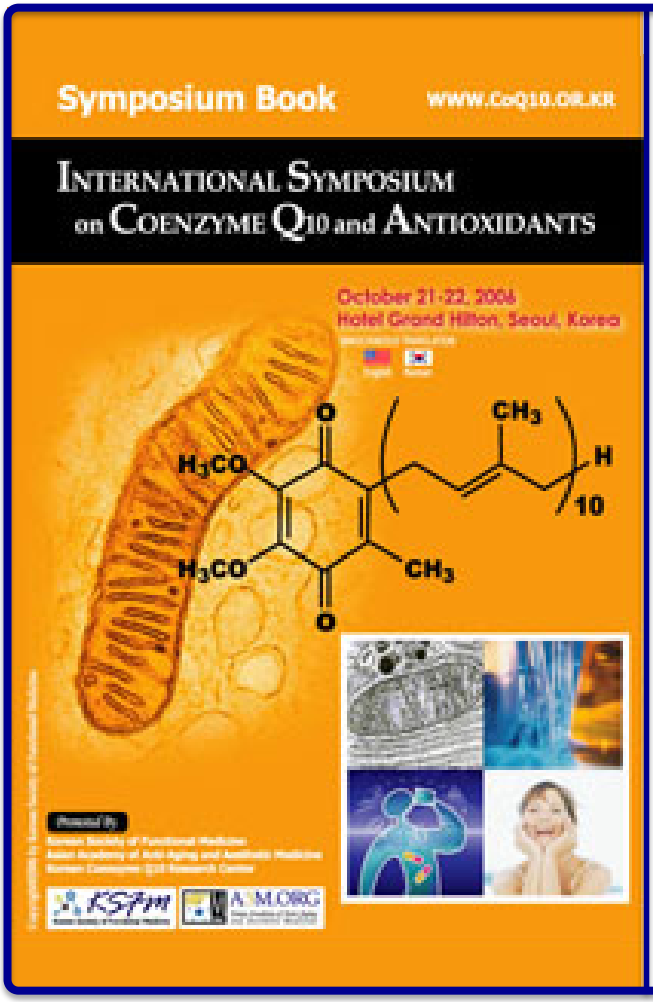
Biologically active form: ubiquinol.

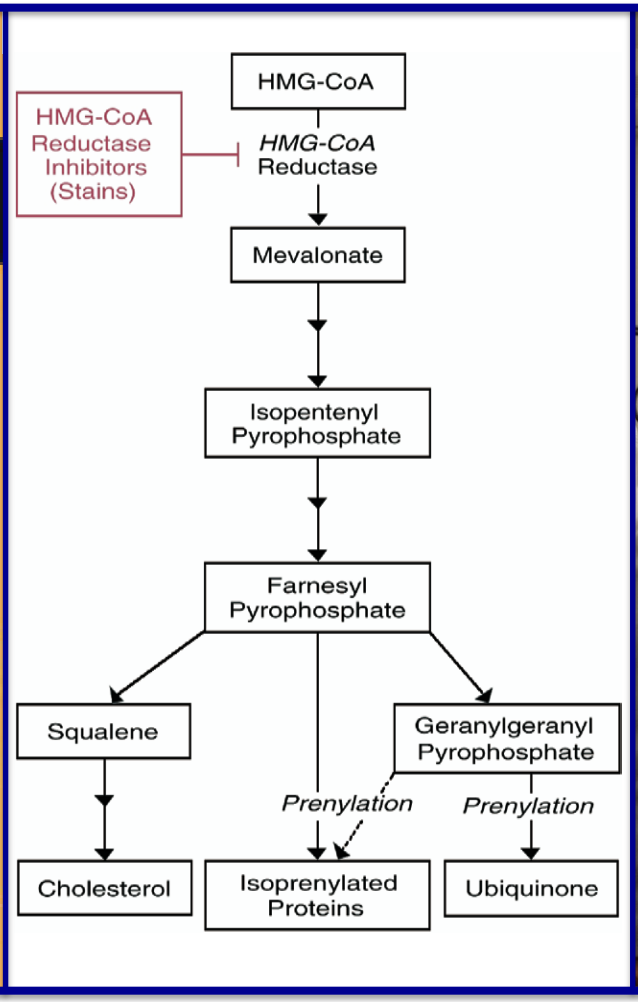
The biological role of coenzyme Q_{10} in the mitochondrial respiratory chain

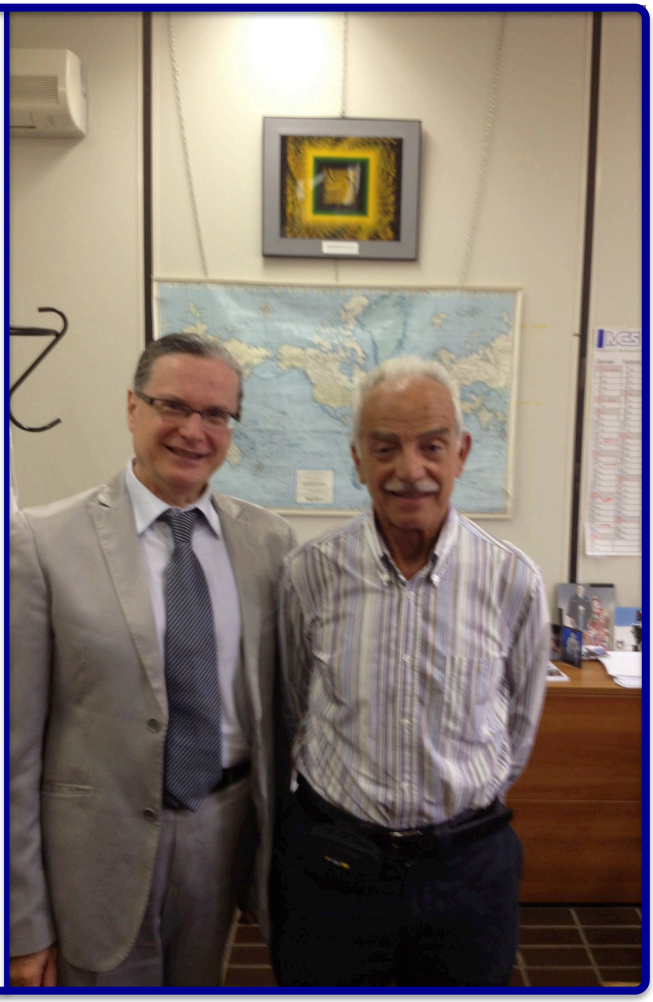


A two-faceted molecule

The "statin paradox"







Together with prof Littarru, a worldwide recognised expertise of uniquinol!

THE STATIN PARADOX. 1.

Potgieter et Al. *Primary and secondary coenzyme Q10 deficiency: the role of therapeutic supplementation*. Nutr Rev 2013. 71(3): 180–188.

☞ Coenzyme Q_{10} (CoQ10) is the only lipid-soluble antioxidant that animal cells synthesize de novo. It is found in cell membranes and is particularly well known for its role in the electron transport chain in mitochondrial membranes during aerobic cellular respiration. A deficiency in either its bioavailability or its biosynthesis can lead to one of several disease states.

☞ Primary deficiency has been well described and results from mutations in genes involved in CoQ10 biosynthesis. Secondary deficiency may be linked to hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), which are used for the treatment of hypercholesterolemia. Dietary contributions of CoQ10 are very small, but supplementation is effective in increasing plasma CoQ10 levels.

☞It has been clearly demonstrated that treatment with CoQ10 is effective in numerous disorders and deficiency states and that supplementation has a favorable outcome. However, CoQ10 is not routinely prescribed in clinical practice. This review explores primary as well as statin-induced secondary deficiency and provides an overview of the benefits of CoQ10 supplementation.

AN OLD STORY!

THE STATIN PARADOX. 2.

Needham et Al. *Statin myotoxicity*. Neuromuscul Disord. 2014. 24(1): 4–15.

☞The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors (statins) are among the most common medications prescribed worldwide, but their efficacy and toxicity vary between individuals. One of the major factors contributing to intolerance and non-compliance are the muscle side-effects, which range from mild myalgia through to severe life-threatening rhabdomyolysis.

☞One way to address this is pharmacogenomic screening, which aims to individualize therapy to maximize efficacy whilst avoiding toxicity. Genes encoding proteins involved in the metabolism of statins as well as genes known to cause inherited muscle disorders have been investigated. To-date only polymorphisms in the SLCO1B1 gene, which encodes the protein responsible for hepatic uptake of statins, and the COQ2 gene, important in the synthesis of coenzyme Q10, have been validated as being strongly associated with statin-induced myopathy.

☞The aim of this review is to summarize studies investigating genetic factors predisposing to statin myopathy and myalgia, as the first step towards pharmacogenomic screening to identify at risk individuals

AN OLD STORY!

THE STATIN PARADOX. 3.

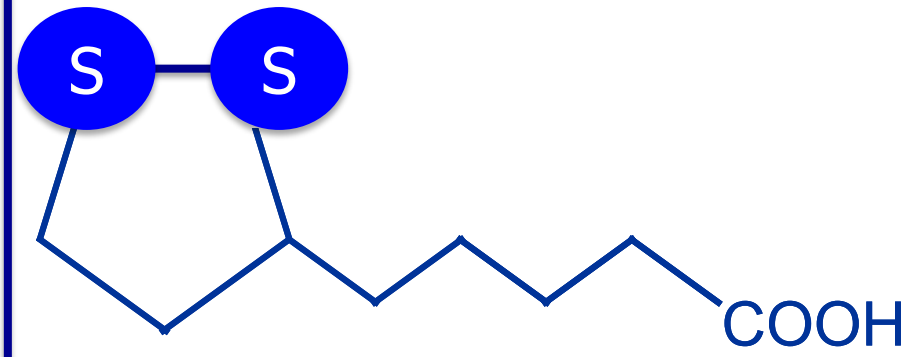
Littlefield et Al. *Statin's effect on plasma levels of Coenzyme Q10 and improvement in myopathy with supplementation*. J Am Assoc Nurse Pract.2014. 26 (2): 58–90.

PURPOSE: Heart disease is the leading cause of death in the United States. HMG-CoA reductase inhibitors, or statins, are medications at the forefront of the battle against cardiovascular disease. Despite their effectiveness, patient compliance with statins has lagged because of medication cost and adverse effects, namely myopathy. Myopathy is the most common side effect of statin use. The purpose of this review is to report plasma levels of CoQ10 in patients taking statins and then to determine the benefit of Coenzyme Q10 (CoQ10) supplementation on statin-related myopathy as evidenced by symptomatic improvement and increase in serum levels of CoQ10.
DATA SOURCES:CINAHL, Medline, Health Source: Nursing/Academic Edition, and Cochrane Library.
CONCLUSIONS: Evidence from this review suggests that studies showed a significant relationship between statin intake and decreased serum levels of CoQ10. A few studies showed a benefit in symptoms of myalgia or improvement of serum levels of CoQ10 with supplementation. One study showed no benefit of CoQ10 supplementation when taken with statins. There were no risks of supplementation reported in any of the studies.
IMPLICATIONS FOR PRACTICE: CoQ10 supplementation might benefit those patients suffering from statin-induced myopathy as evidenced by the results of these studies. Supplementation of CoQ10 at a dose of between 30 and 200 mg daily has shown to have beneficial effects on statin myopathy with no noted side effects. Further research is necessary.

Statins as antiinflammatory compounds?

Lipoic acid

Forma ridotta (attiva)

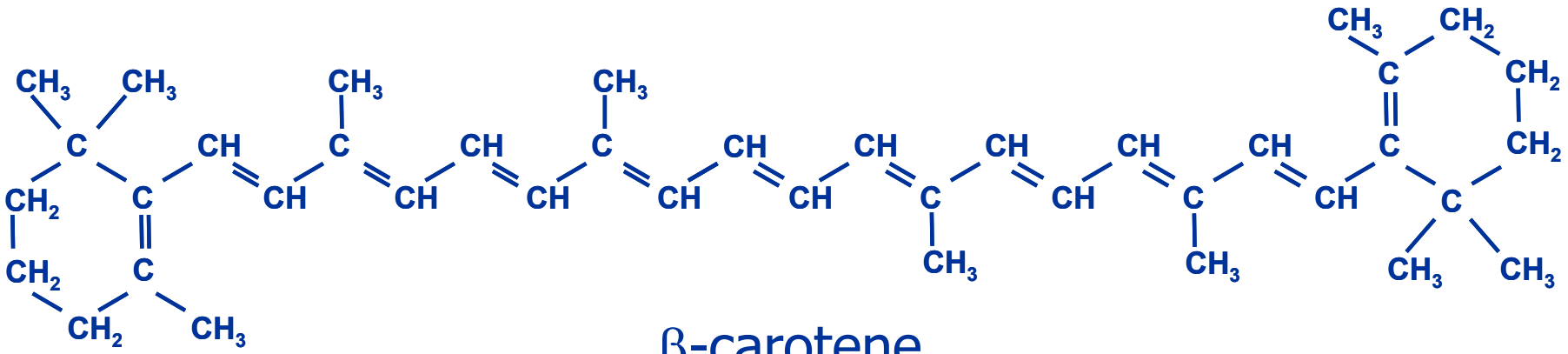


Forma ossidata

- ☞Sostanza a basso peso molecolare, relativamente idrofila.
- ☞Scavenger nei confronti di vari ossidanti ($HO^{\bullet}$, O_2*, HClO).
- ☞Chelante nei confronti dei metalli di transizione (Fe, Cu)
- ☞Consente la rigenerazione delle vitamine C ed E

Main features

Carotens and xantofils

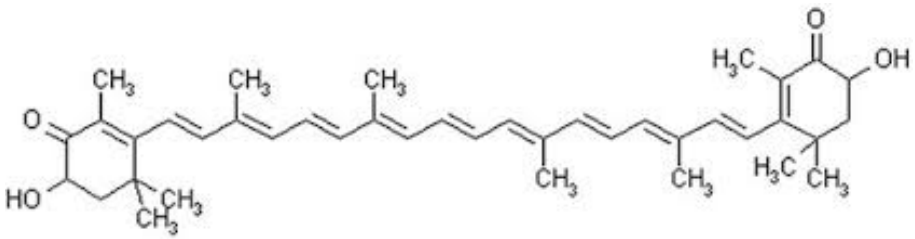


β-carotene

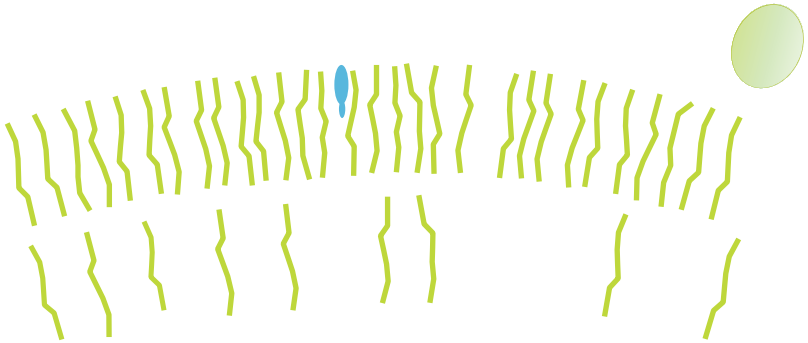
- Sostanze lipofile contenute in frutta e verdure.
- Quencher dell'ossigeno singoletto (O_2*).
- Interrompono le reazioni a catena dei perossili (R-OO•)

Main features

Astaxanthin

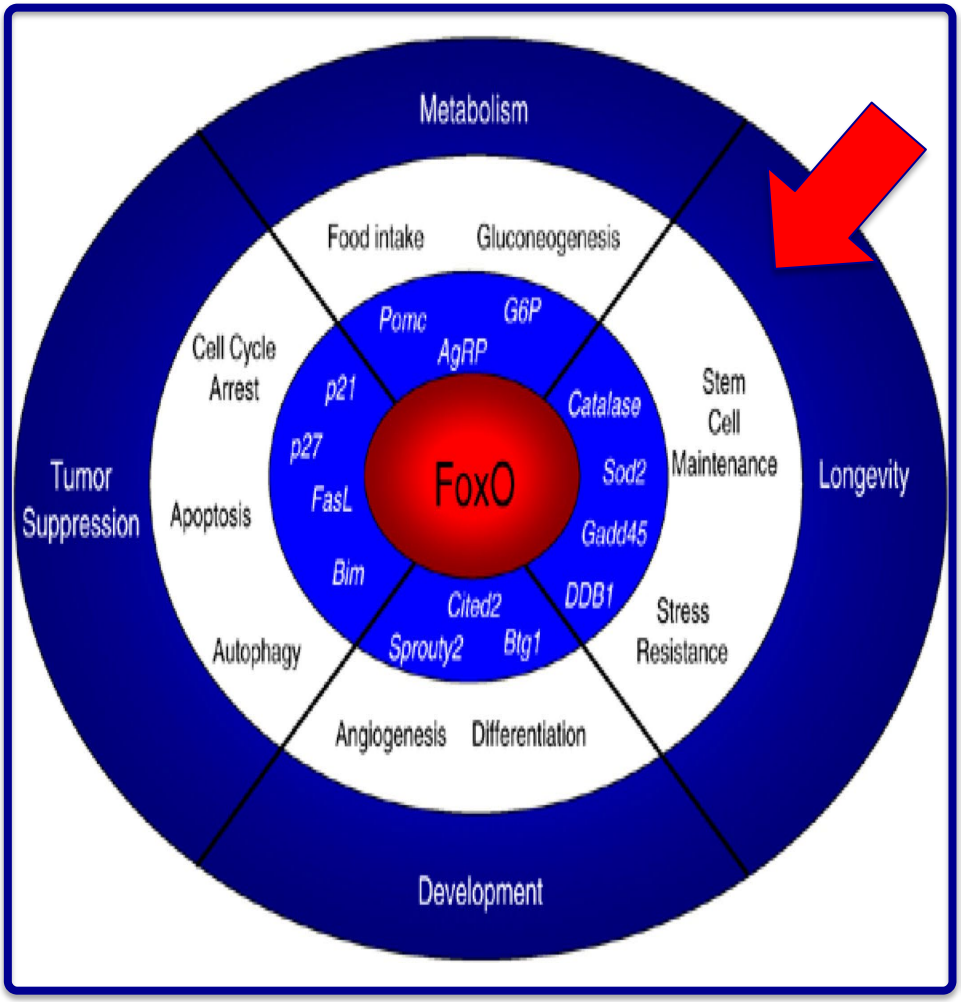


Astaxanthin



Not simply an antioxidant

The FOXO transcriptional factrs. One of the main targets of astaxanthin.



☞I fattori di trascrizione FOXO sono proteine caratterizzate dalla presenza di un dominio molecolare a forma di forcella (*forkhead*) che consente ad esse di adattarsi al DNA, regolandone in questo modo la trascrizione di specifici geni.

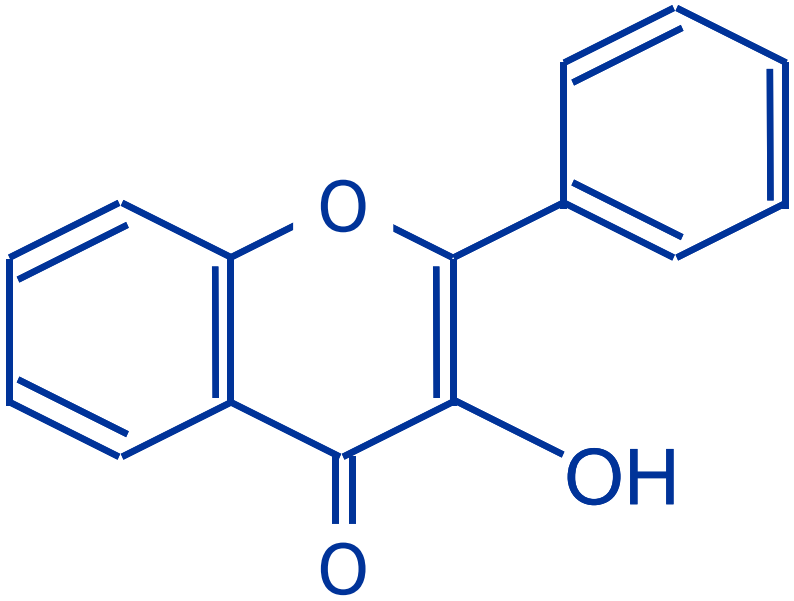
☞Fra le diverse varianti, l'attività di FOXO1, FOXO4 e FOXO6 viene inibita mediante la fosforilazione da parte di altre proteine quali le Akt/PKB attivate nella via di segnalazione PI3K (ad eccezione di FOXO6, che può essere costitutivamente nucleare). Invece, l'attività di FOXO3a può essere modulata anche mediante modificazioni post-traduzionali quali l'acetilazione e la metilazione.

☞FOXO3a interviene nel processo di apoptosi, attraverso la sovra-regolazione dei geni necessari per la morte delle cellule, come Bim e PUMA,[o nella sotto-regolazione dei geni di fattori anti-apoptotici come FLIP; in entrambi i casi viene promossa la morte delle cellule. Una variante di FOXO3 in particolare è risultata associata con la longevità negli esseri umani: la maggior parte dei centenari, infatti, risulta portatrice di tale mutazione.

Boyette et Al. J Clin Med. 2014. 3(1): 88-134.

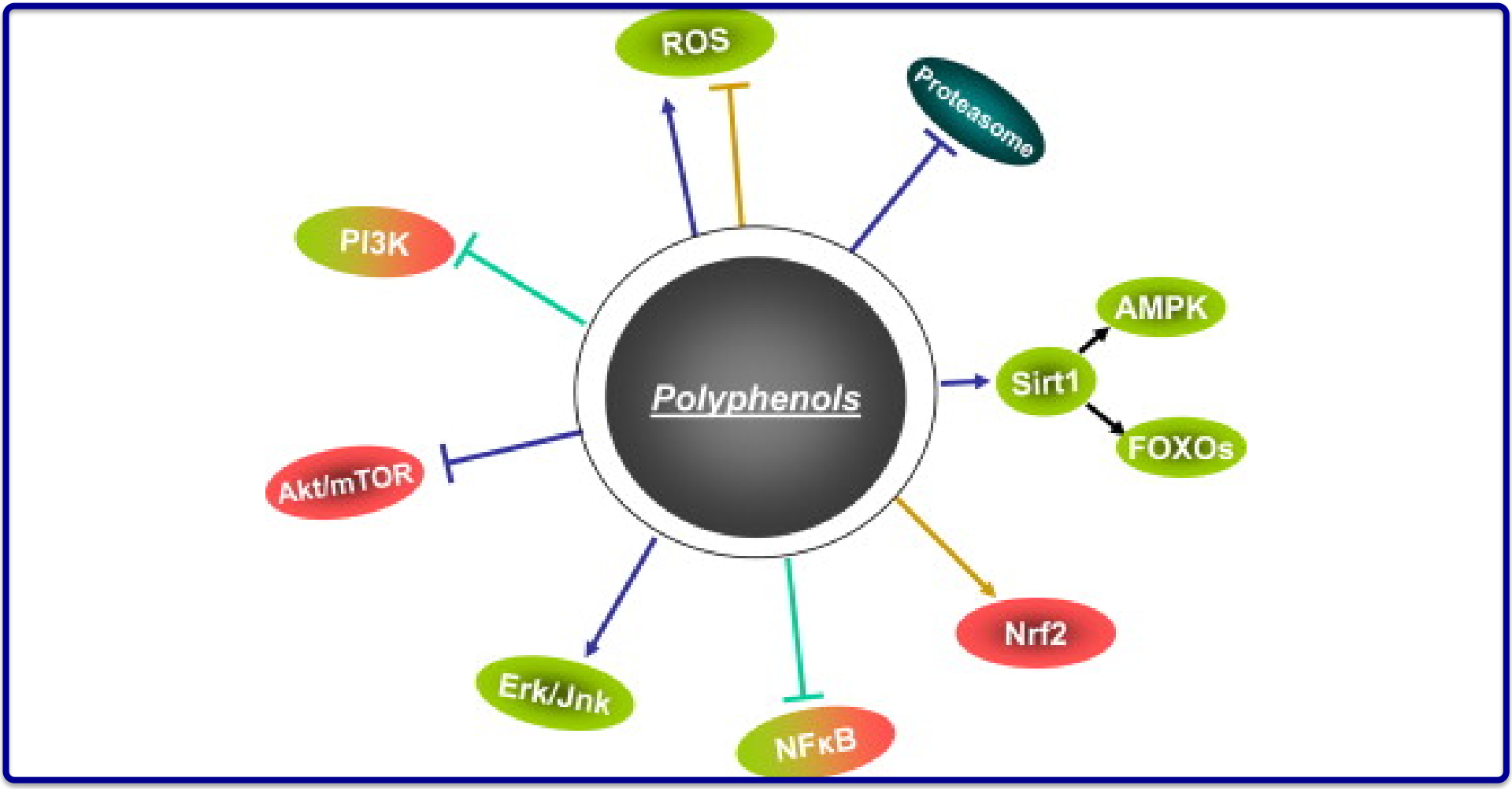
Polyphenols and flavonoids

- Ampia classe di sostanze naturalmente occorrenti in natura
- Comprendono antociani ed antoxantine (frutta e verdura)
- Azione scavenger nei confronti dei radicali $HO^{\bullet}$ e $O_2^{\bullet}$
- Possibile azione anti-aterogena



Main features

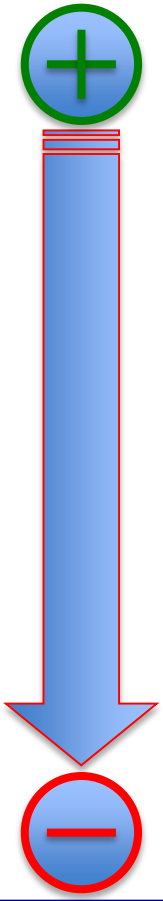
Polyphenols and flavonoids



Main activities

Bioavailability. The "polyphenols case".

Classes of compounds	Active principles
Hydroxybenzoic acids	Gallic acid
Hydroxycinamic acids	Ferulic acid
Isoflavones	Daidzeidin, glycetin
Catechines	Epicatechin, epigallocatechin
Flavanones	Naringin, esperidin
Flavanols	Quercetin, rutin
Proanthocyanidines	Dimeric, trimeric
Anthocyanidines	Cyanidin, malvidin



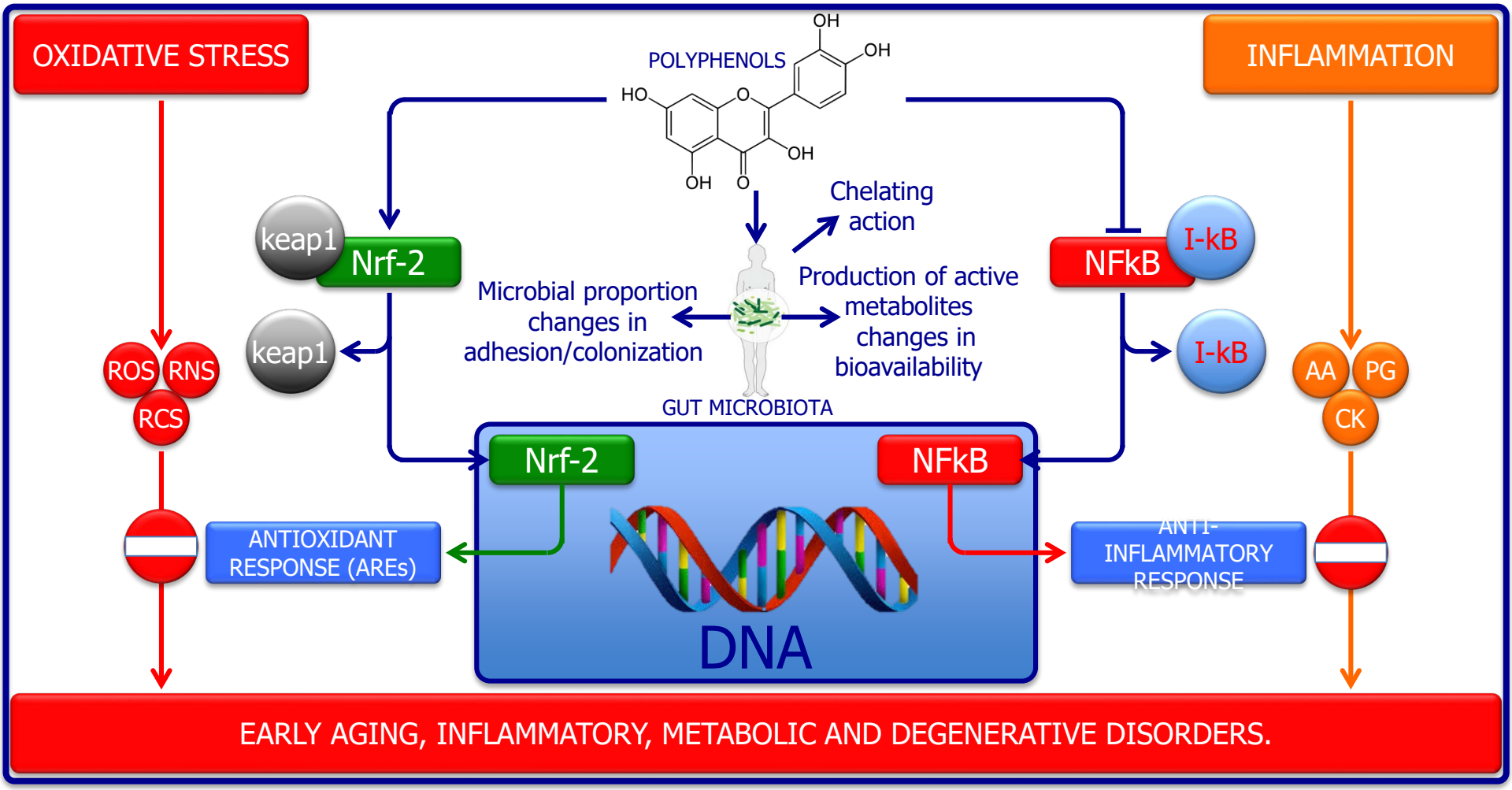
Only a few molecules can go into the cells!

Biological activity and targets

Inhibiting activity	Flavone	Quercetin
On lipooxygenase	50	4
On cyclooxygenase	8	16
On DNA oxidation (bi-phasic)	NO	YES
On LDL oxidation	100	2
On lipids peroxidation	2	675

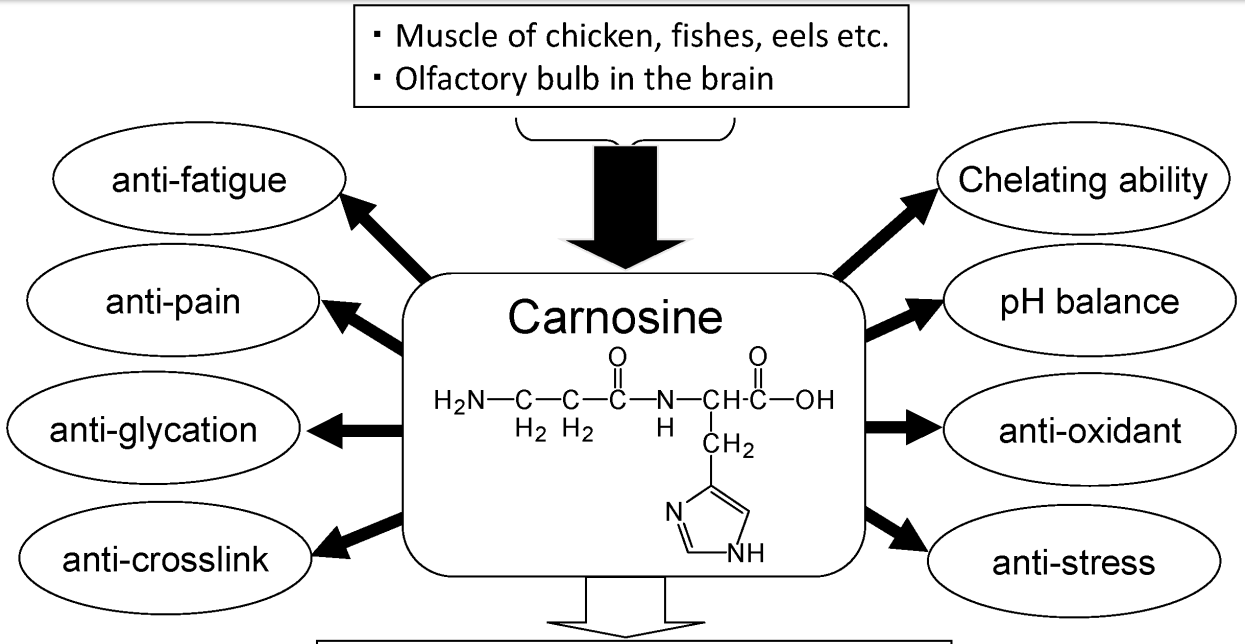
The right formula as a function of the expected effect

Polyphenols as possible functional modulators



Savikin et Al. J Med Food. 2014. 17 (5): 582–587.

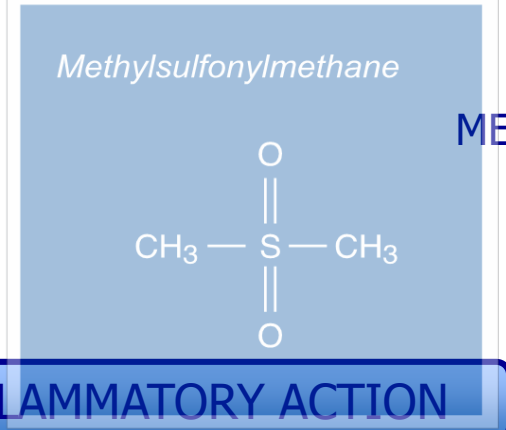
Carnosine

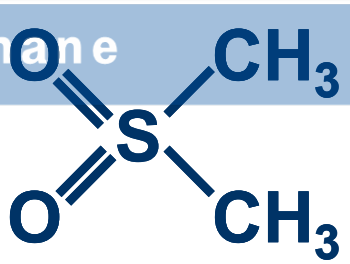


Main features

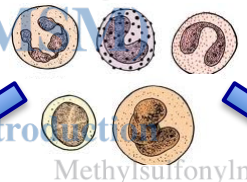
MSM

Methylsulfonylmethane Monograph





METILSULFONILMETANO
(MSM)



ANTIINFLAMMATORY ACTION

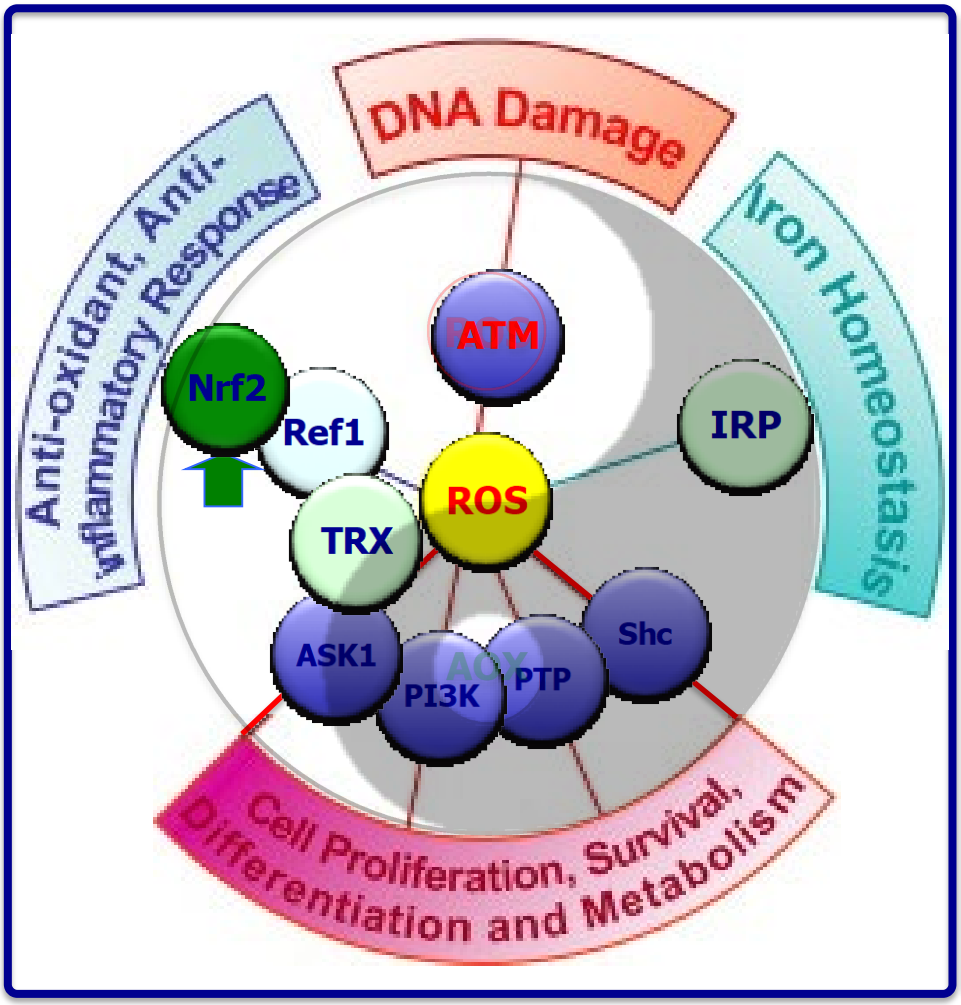
ANTI-ALLERGIC ACTION

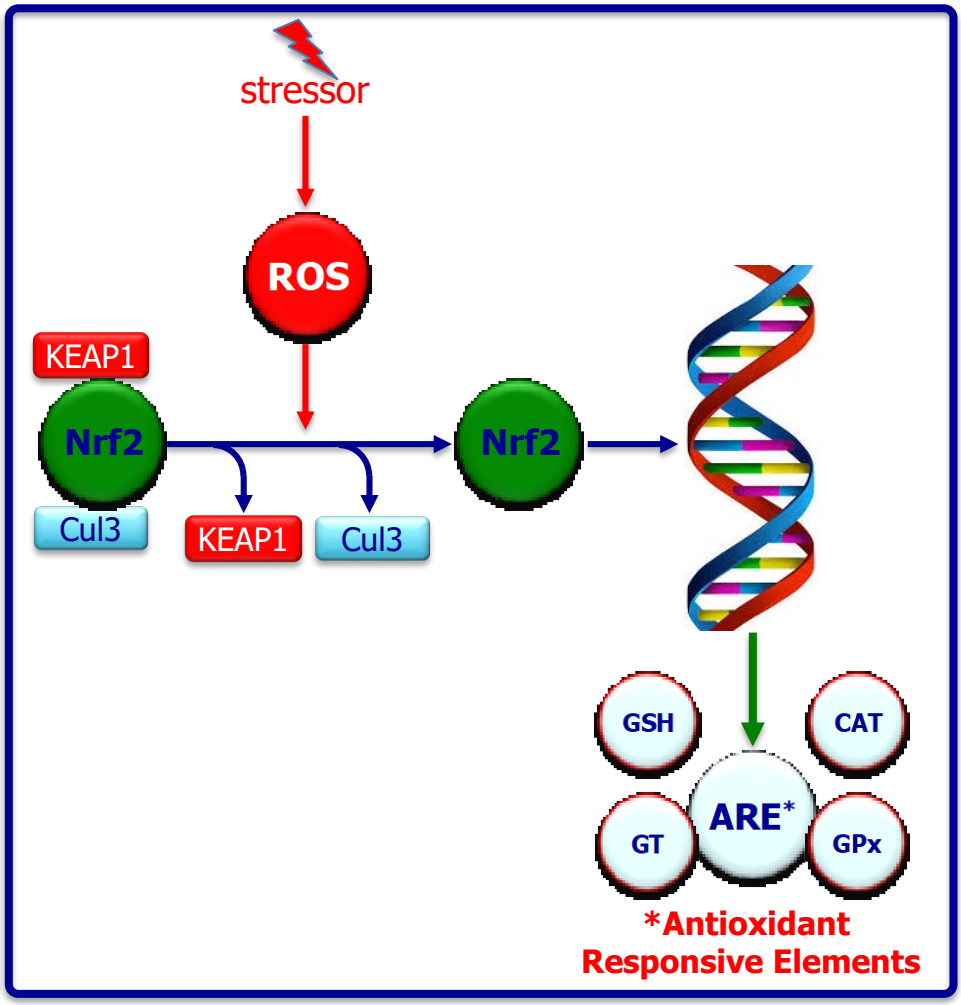
AGAINST ARTHRITIS, FIBROMYALGIA . . .

AGAINST DERMATITIS, RHINITIS . .

A powerful functional modulator

The REDOX system mediates adaptive response to oxidising stressors by modulating DNA expression





Reactive species modulate almost all the biological processes responsible of cell homeostasis and survival

The REDOX system modulates the telomeres length

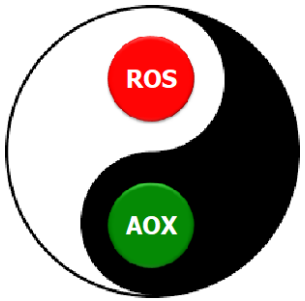
Mediterranean diet
Physical activity
Healthy Lifestyle
Meditation
Paternal age
Sleep duration

Exposures **positively** associated with telomere length

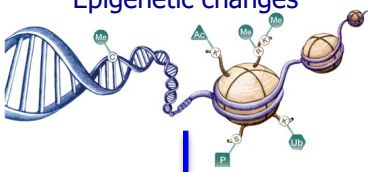
Others ?

Stress, phobic anxiety
Cigarette smoking
High BMI
Occupational exposures
Early social deprivation
Rotating night shifts

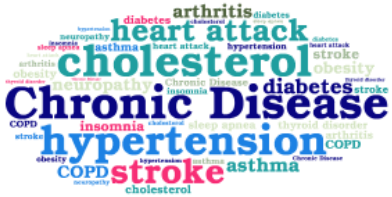
Exposures **negatively** associated with telomere length



Epigenetic changes



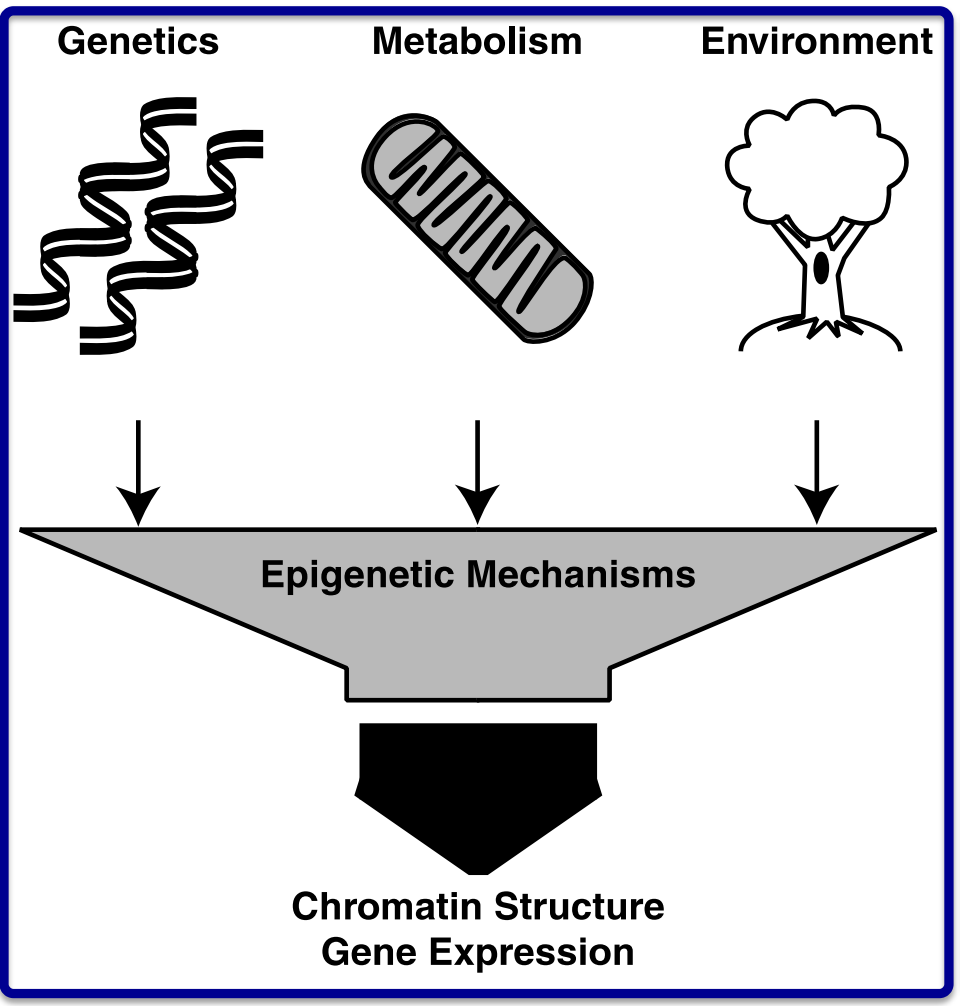
Genetic susceptibility

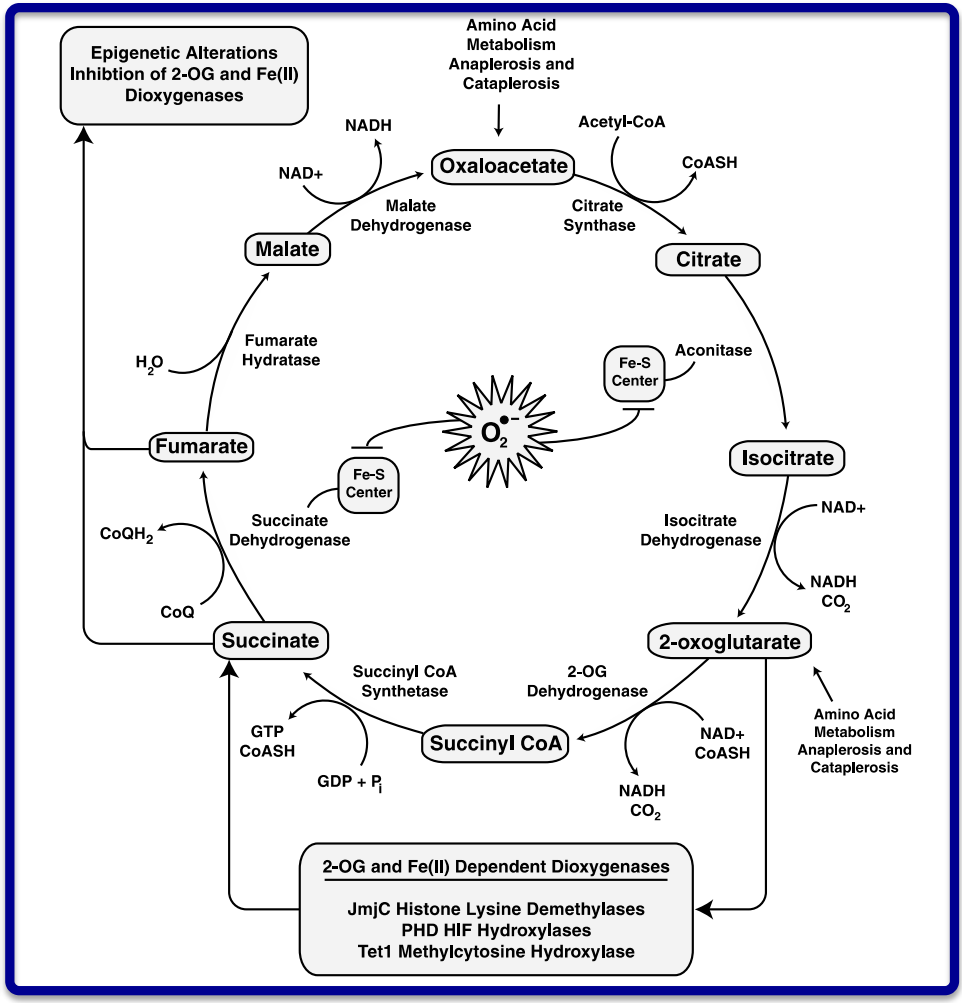


Basal conditions

De Vivo et Al. 2015 (*modified by Iorio*)

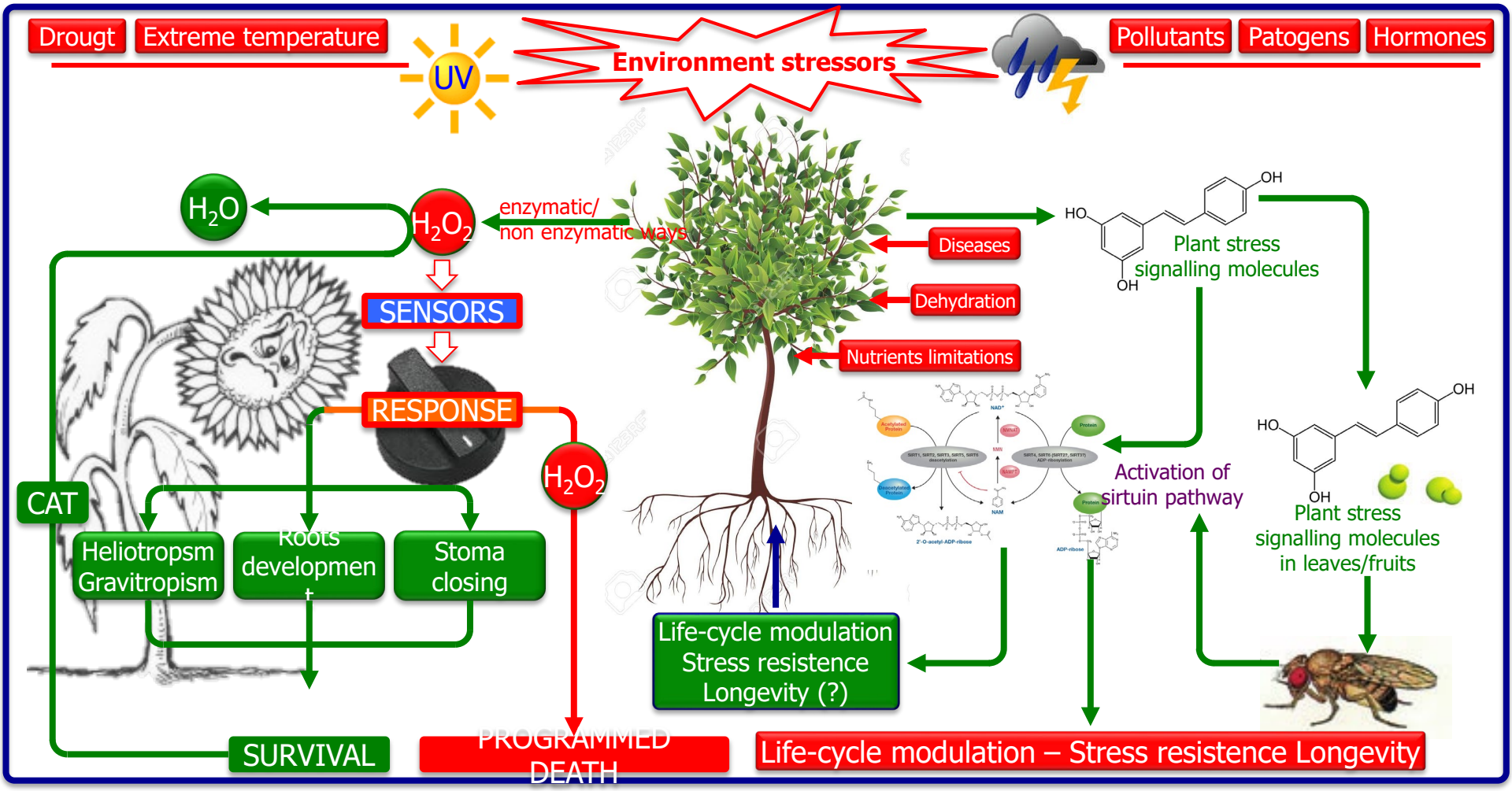
Redox changes play a relevant role in epigenetics





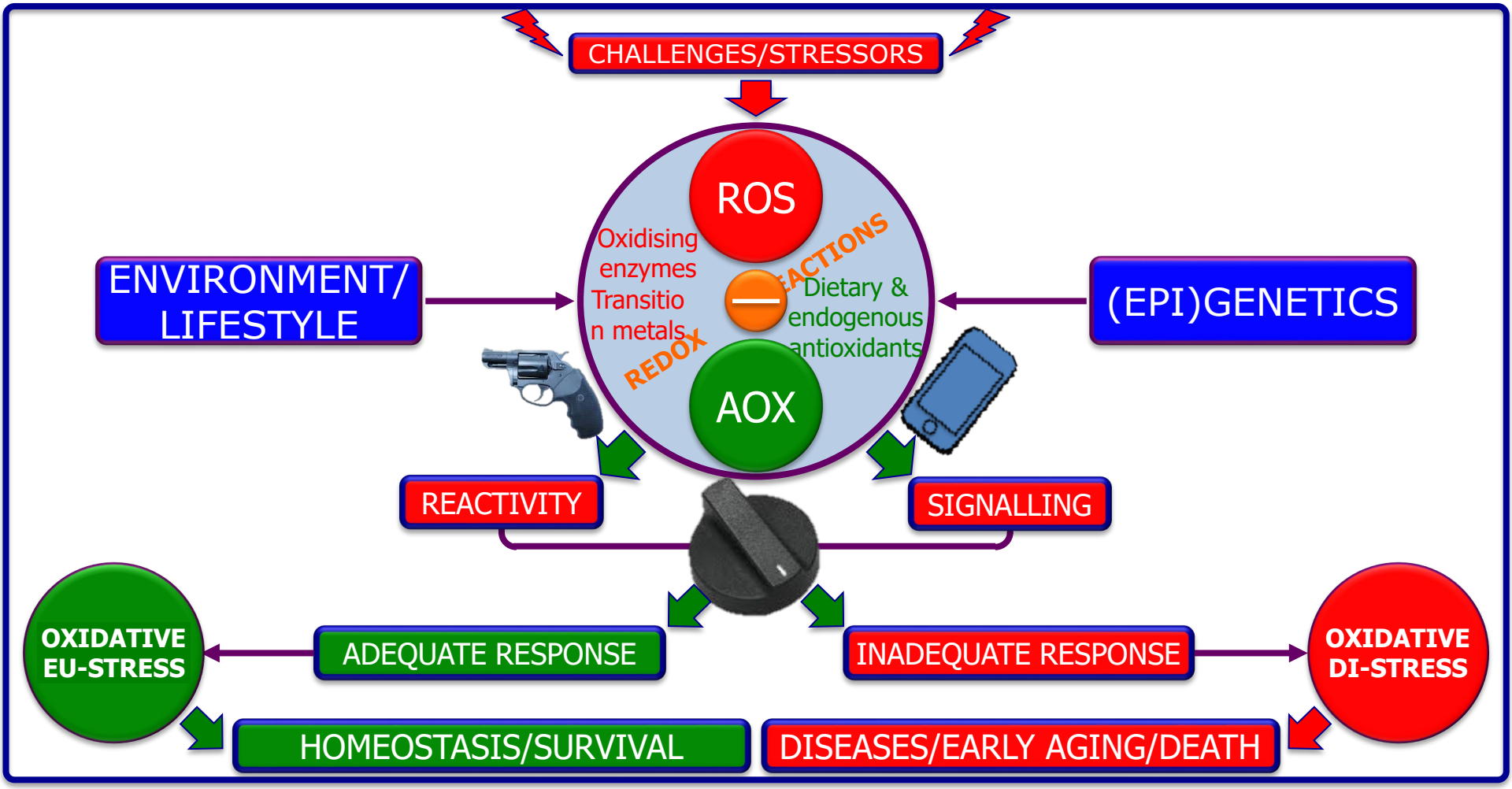
Cyr et Al. Antiox Redox Signal. 2011.

The redox system is one of the most ancient and efficient adaptive system, as results from studies in Plants.



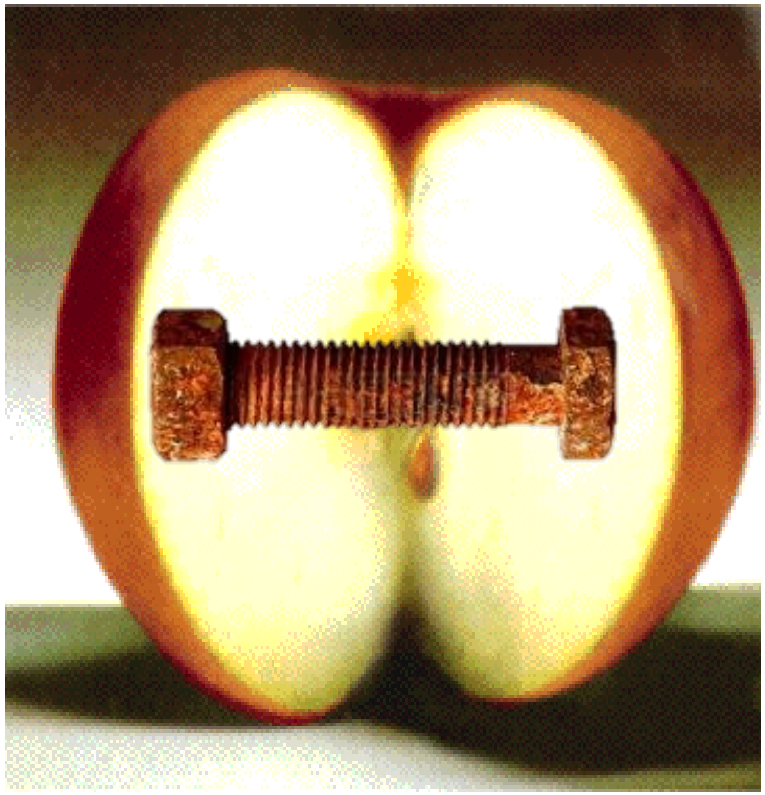
The redox system mediates stress response and allows Plants to dial indirectly with other living organisms

Even in Humans the redox system mediates stress response and allows to adapt and survive



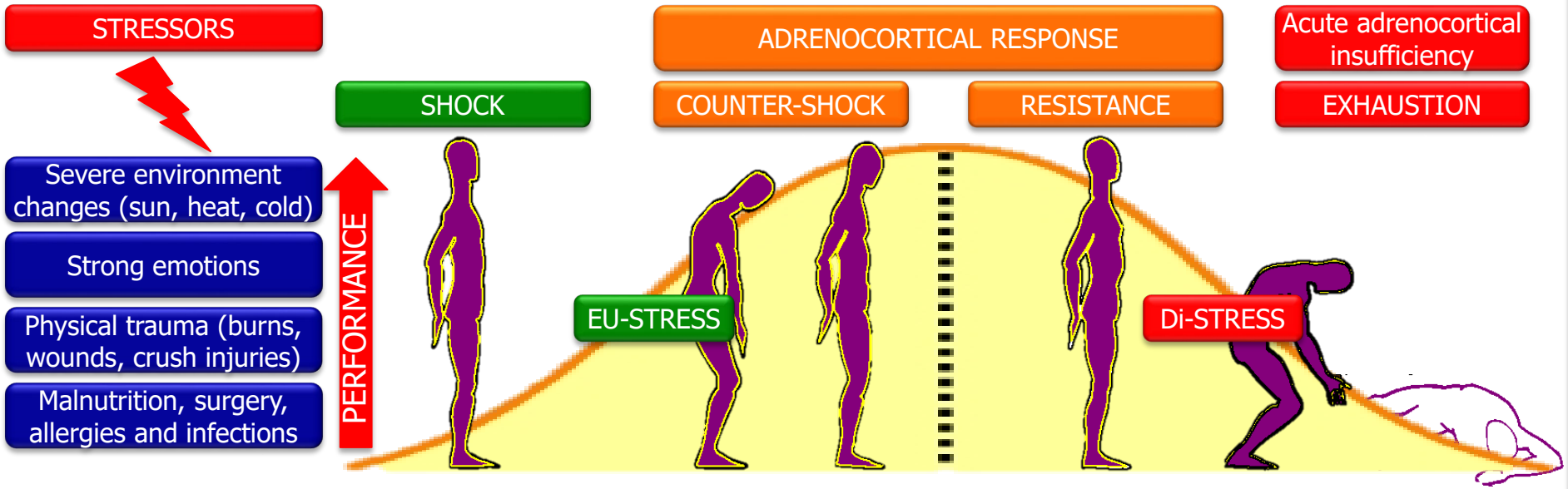
The REDOX system provides the basis for OXIDATIVE STRESS and functional REDOXOMICS

Oxidative stress



Two key words: oxidation and stress!

Going back to the original concept of "stress": fight or flight?



Stress triggers the so-called "fight or flight" response, a reaction that involve neurological, endocrine and immune systems, through the production of specific mediators like ACTH, cortisol and adrenaline.

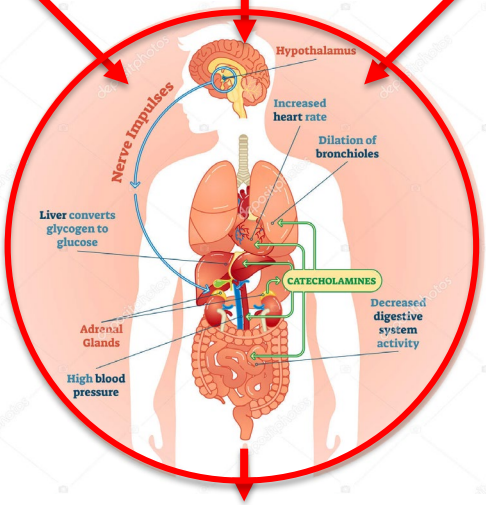
Hans Selye (1907–1982)

Biological stress is managed by nervous/endocrine chemical mediators (the hypothalamus-adrenal axis)

Physical stressors (e. g. UVr)

Biological stressors (e.g. bacteria)

Chemical stressors (e. g. pollutants)



ADAPTIVE RESPONSE

Adequate

Inadequate

EU-STRESS

DI-STRESS

Physical stressors (e. g. Uvr)

Biological stressors (e. g. bacteria)

Chemical stressors (e. g. pollutants)

ROS

AOX

SIGNALLING

DETOXIFICATION

DEFENCE

ADAPTIVE RESPONSE

Adequate

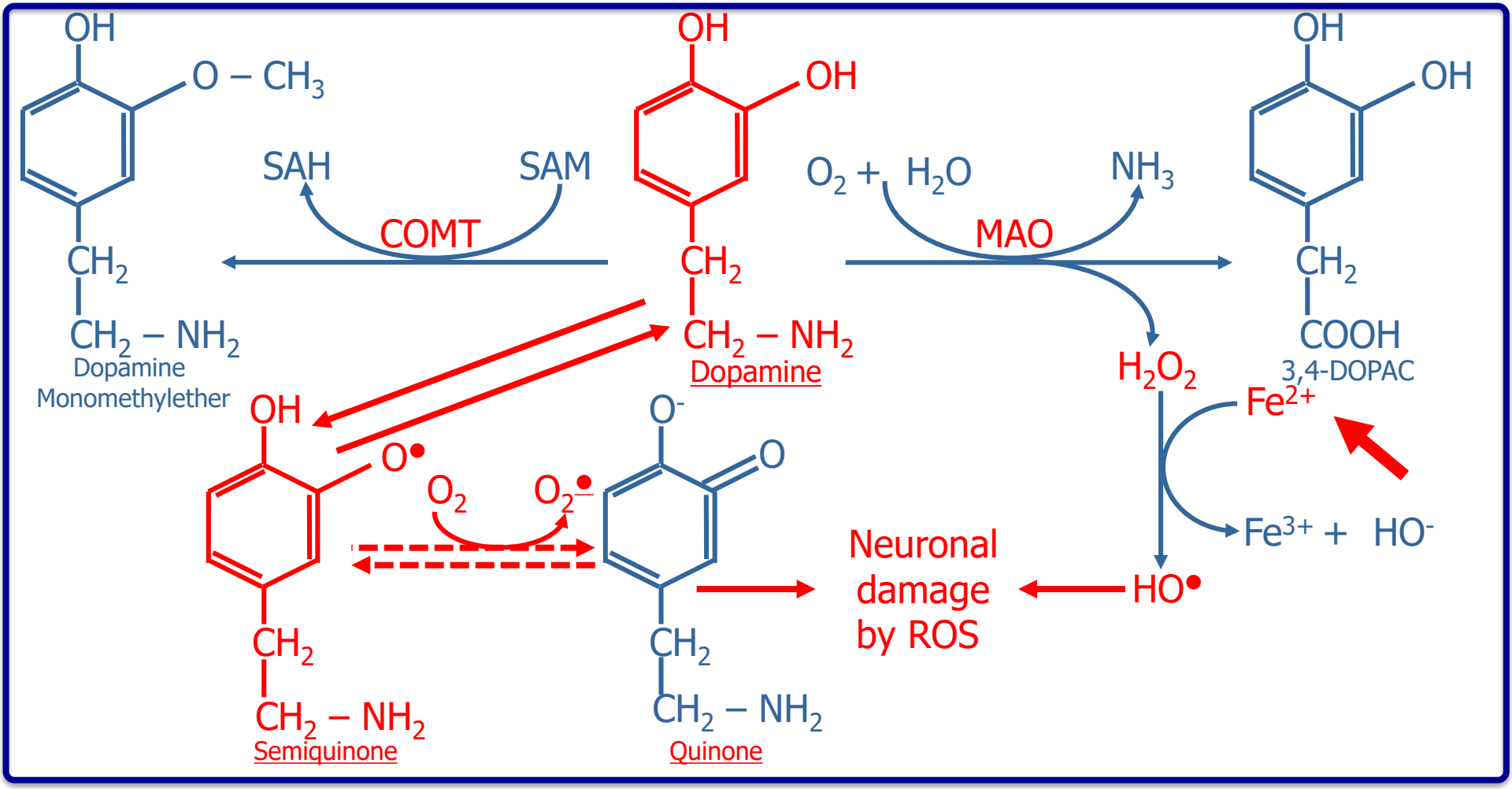
Inadequate

OXIDATIVE EU-STRESS

OXIDATIVE DI-STRESS

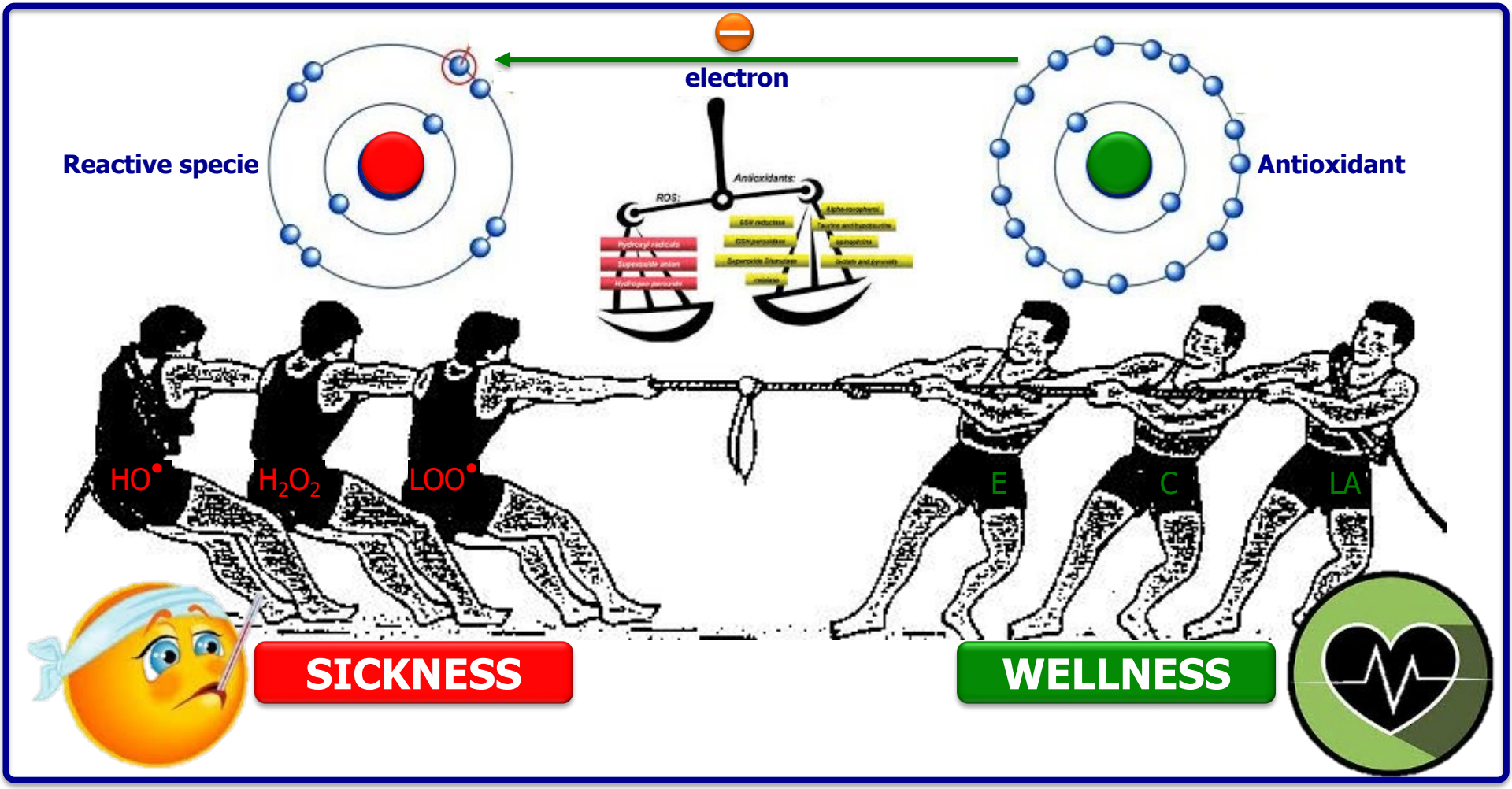
Oxidative stress is managed by reactive oxidant/ reducing chemical species (the REDOX system)

Oxidative stress provides a suitable chemical basis for emotional stress



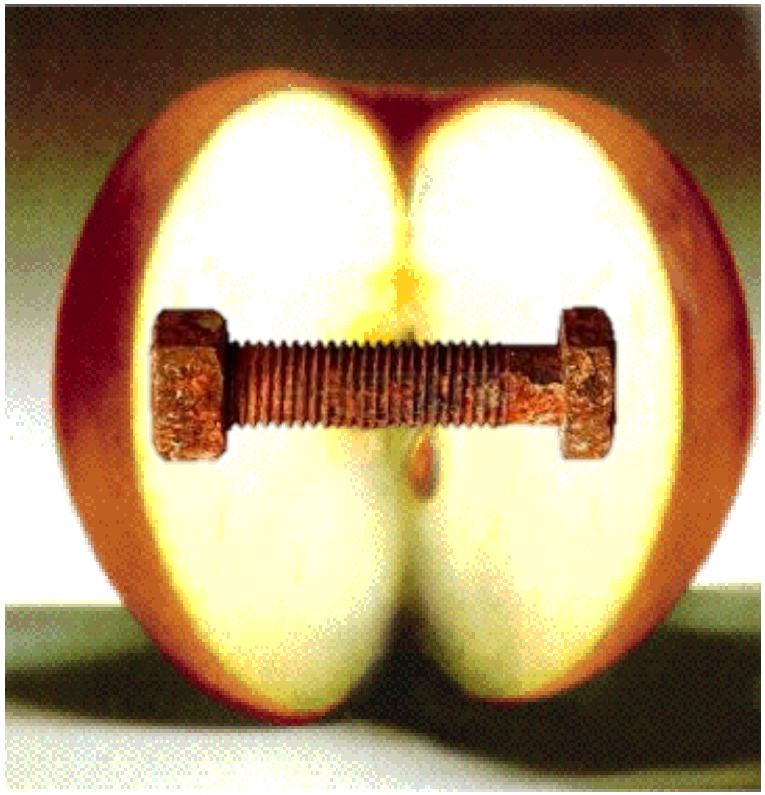
The catabolism of adrenaline generated hydrogen peroxide (H_2O_2), i. e. an oxidising chemical specie!

The traditional concept of oxidative stress, an emerging health risk factor.



The breakdown of balance between radical/ /non-radical reactive species and antioxidants

Un'equazione classica: ossidazione = danno biologico.



Un mito da sfatare!

The traditional concept of oxidative stress, an emerging health risk factor.

The breakdown of balance between radical/ /non-radical reactive species and antioxidants

The REDOX system works as an adaptive interface between exposome and (epi)genome

EXPOSOME

SENSORS

Oxidising enzymes
Transitio n metals

ROS

REACTIONS

REDOX

AOX

TRANSDUCERS

ADAPTIVE RESPONSE

Adequate

Inadequate

EU-STRESS

DI-STRESS

(EPI)GENOME

GENOME, EPIGENOME

EDITED TRANSCRIPTOME

PROTEOME, EPIPROTEOME

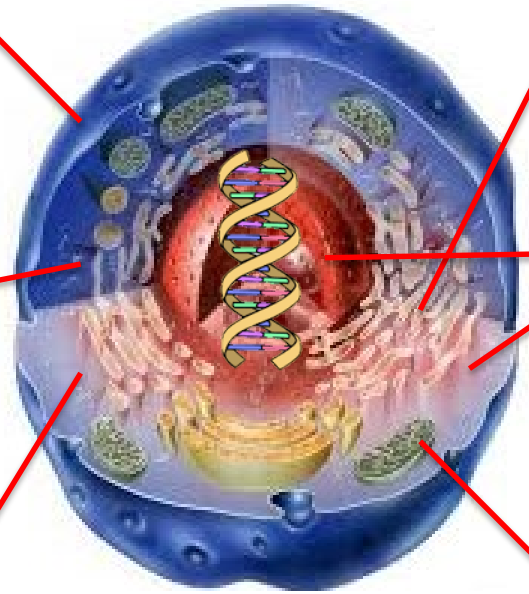
LIPIDOME, METABOLOME

The REDOX system modulates oxidative eu-stress, i.e. a physiological adaptive response for homeostasis/survival

The generation of reactive oxidant species associated to cell enzymatic activities

Membrane NADPH oxidase, NOS, lipooxygenase (?).

Endoplasmic reticulum Cyt P_{450} and $Cytb_5$



Peroxisome long chain fatty acid oxidative pathway

Other pathways (NDPr, FAD, MAO, DAAO, NOS, MPx,)

Cytosolic xanthine oxidase and aldehyde oxidase

Mitochondrial NADH dehydrogenase, Cyt oxidase

Four main source of reactive oxidant species becomes four models for 4 different kind of oxidative stress

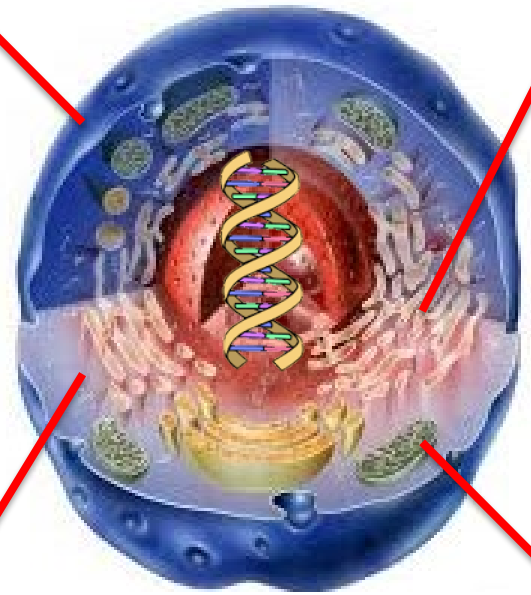
Most of reactive oxidant species are enzymatically generated in four main cell sites

Membrane NADPH oxidase, NOS, lipooxygenase (?).

Reactive oxidative stress (classical oxidative stress)

Endoplasmic reticulum Cyt P_{450} and $Cytb_5$

Toxic oxidative stress (xenobiotic/hyatrogenic)



Hypoxic oxidative stress (ischemic-riperfusive)

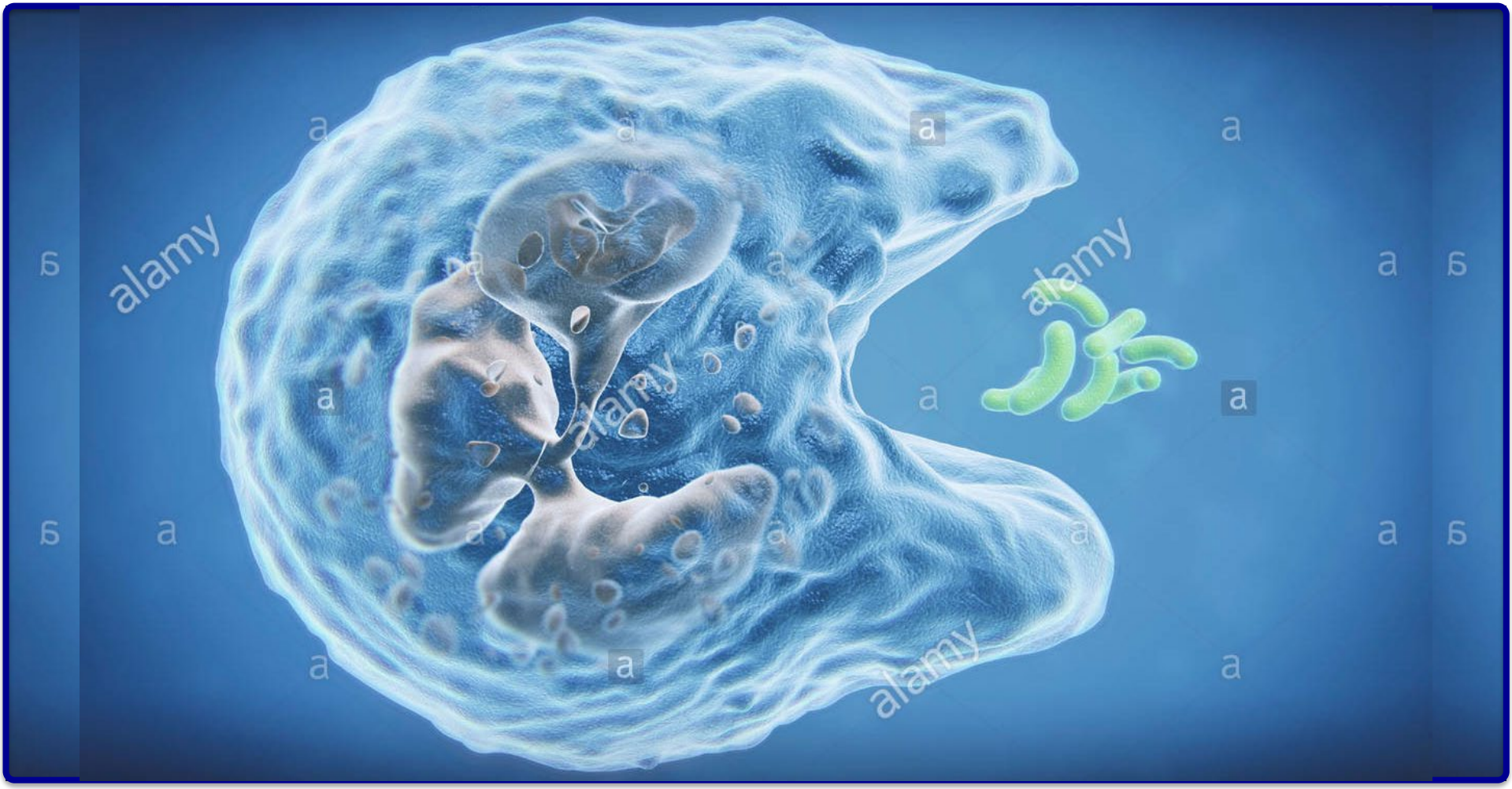
Respiratory oxidative stress (metabolic oxidative stress)

Cytosolic xanthine oxidase and aldehyde oxidase

Mitochondrial NADH dehydrogenase, Cyt oxidase

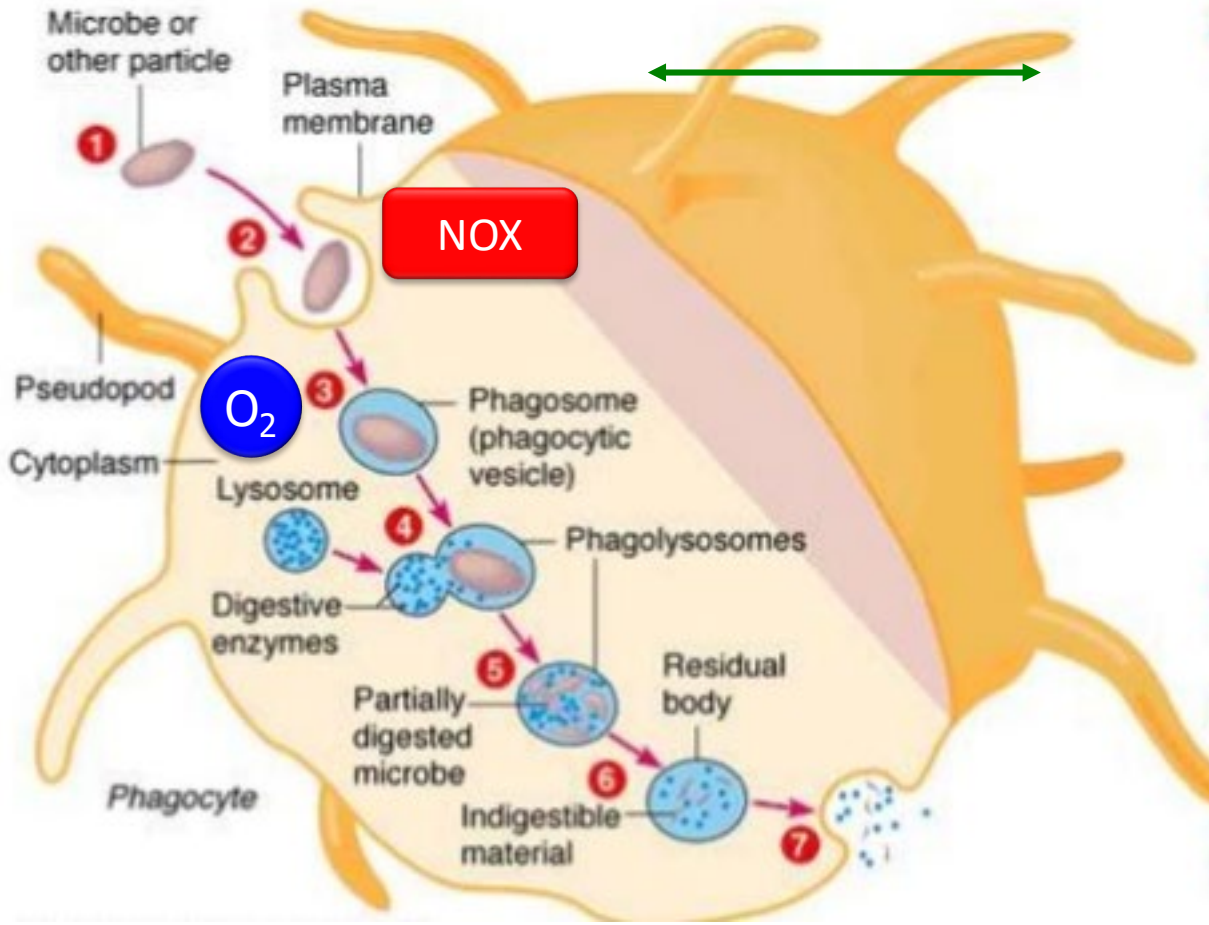
4 main source of reactive oxidant species becomes 4 models for 4 different kind of oxidative stress

The phagocytosis. A crucial function of aspecific immune response.



A relevant step of infection-triggered inflammation. The paradigma of reactive oxidative stress (oxinflammation).

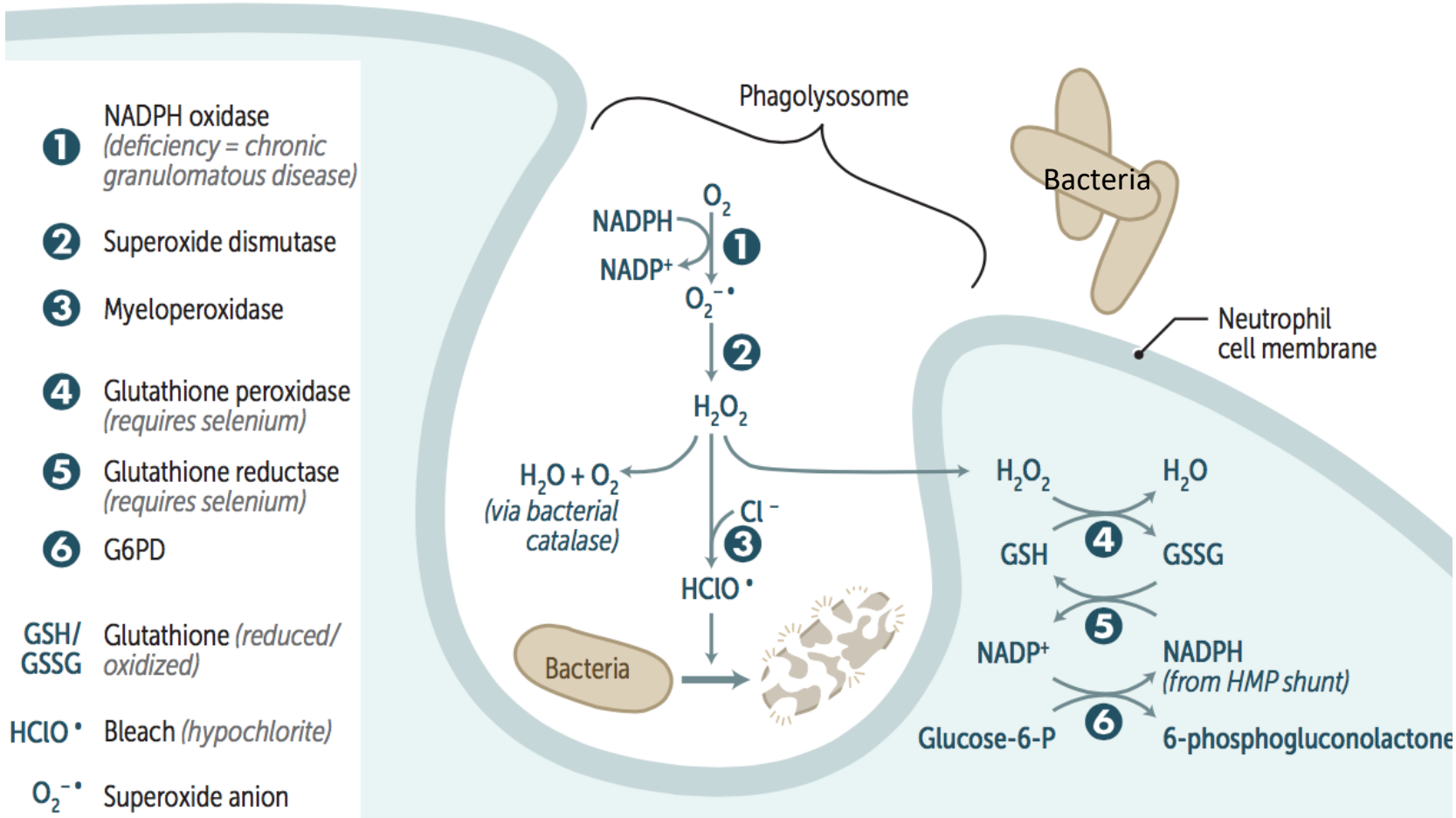
Phagocytosis requires two important conditions



1. Chemotaxis and adherence of microbe to phagocyte.
2. Ingestion of microbe by phagocyte.
3. Formation of a phagosome.
4. Fusion of the phagosome with a lysosome to form a phagolysosome.
5. Digestion of ingested microbe by enzymes.
6. Formation of residual body containing indigestible material.
7. Discharge of waste materials.

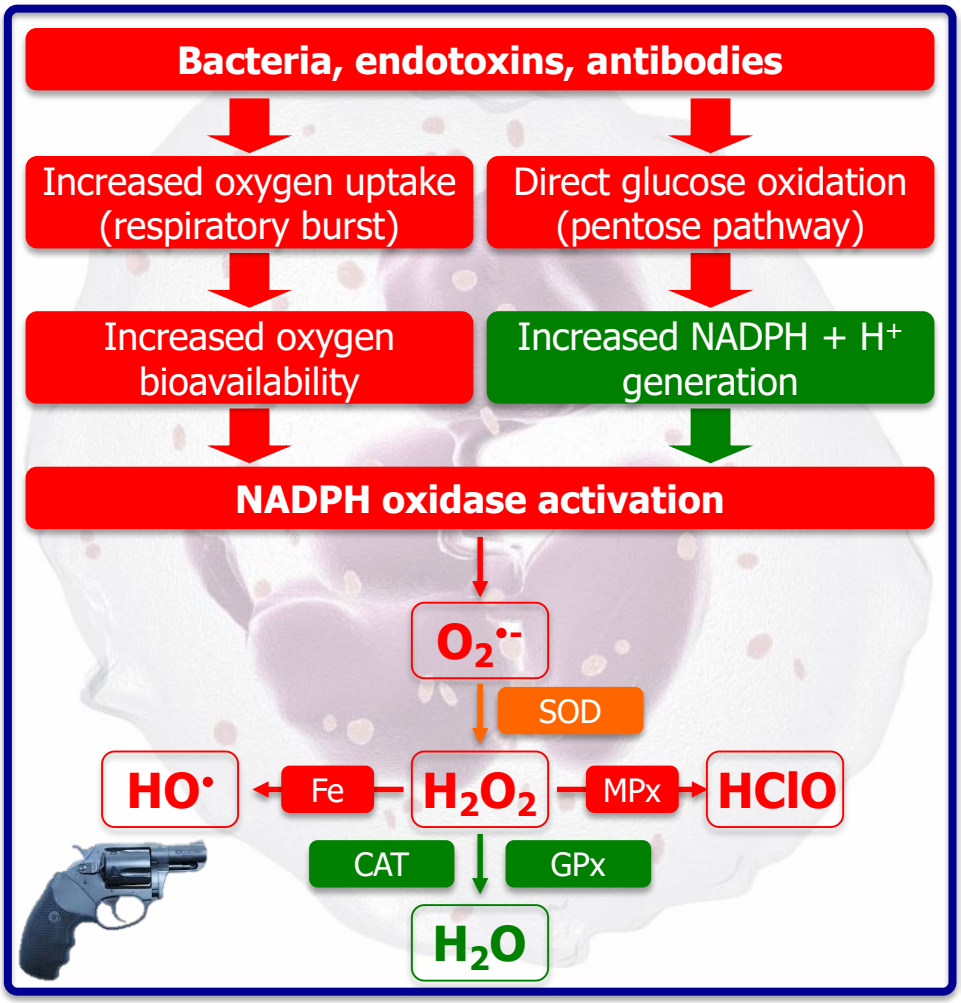
The activation of membrane NADPH-oxidase (NOX) and a increased uptake of oxygen from circulating blood

Biochemical changes in the early phagosome



From oxygen to hydrogen peroxide and hypochlorite to kill bacteria

The REDOX network in action: the oxidant-antioxidant interplay in the infectious/inflammatory response



NADPH oxidase (NOX)

$$NADPH + O_2 \rightarrow NADP^{\bullet} + H^+ + O_2^{\bullet-}$$
$$NADP^{\bullet} + O_2 \rightarrow NADP^+ + O_2^{\bullet-}$$

Superoxide dismutase (SOD)

$$2\ O_2^{\bullet-} + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Catalase (CAT)

$$2\ H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione peroxidase (GPx)

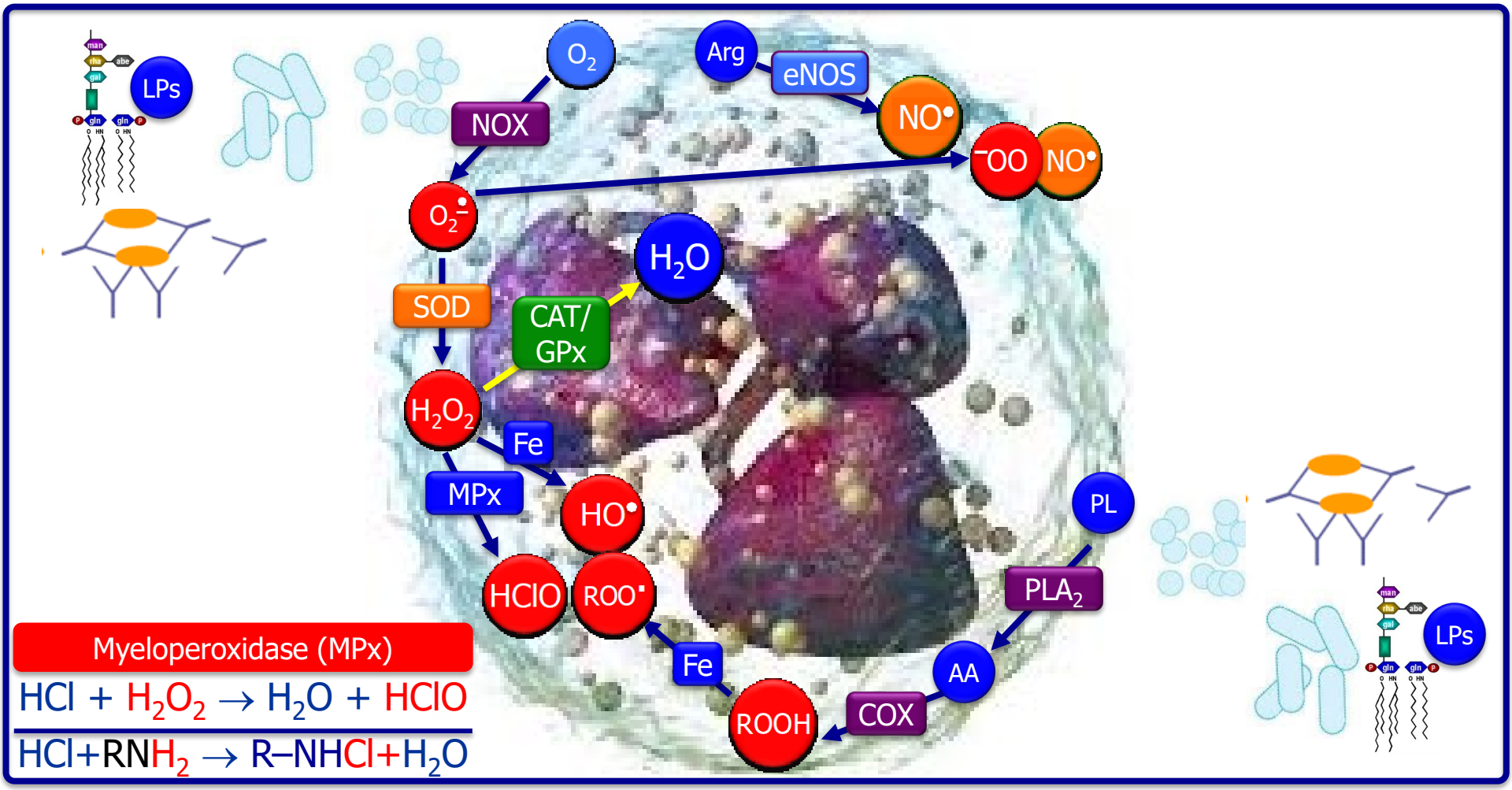
$$2\ GSH + H_2O_2 \rightarrow 2H_2O + GSSG$$

Myeloperoxidase (MPx)

$$HCl + H_2O_2 \rightarrow H_2O + HClO$$

Superoxide dismutase, catalase, and glutathione peroxidase modulate NOX-derived ROS effects.

The oxygen/nitrogen-centred species pathway in the polymorphonuclear leukocytes/monocytes/macrophages



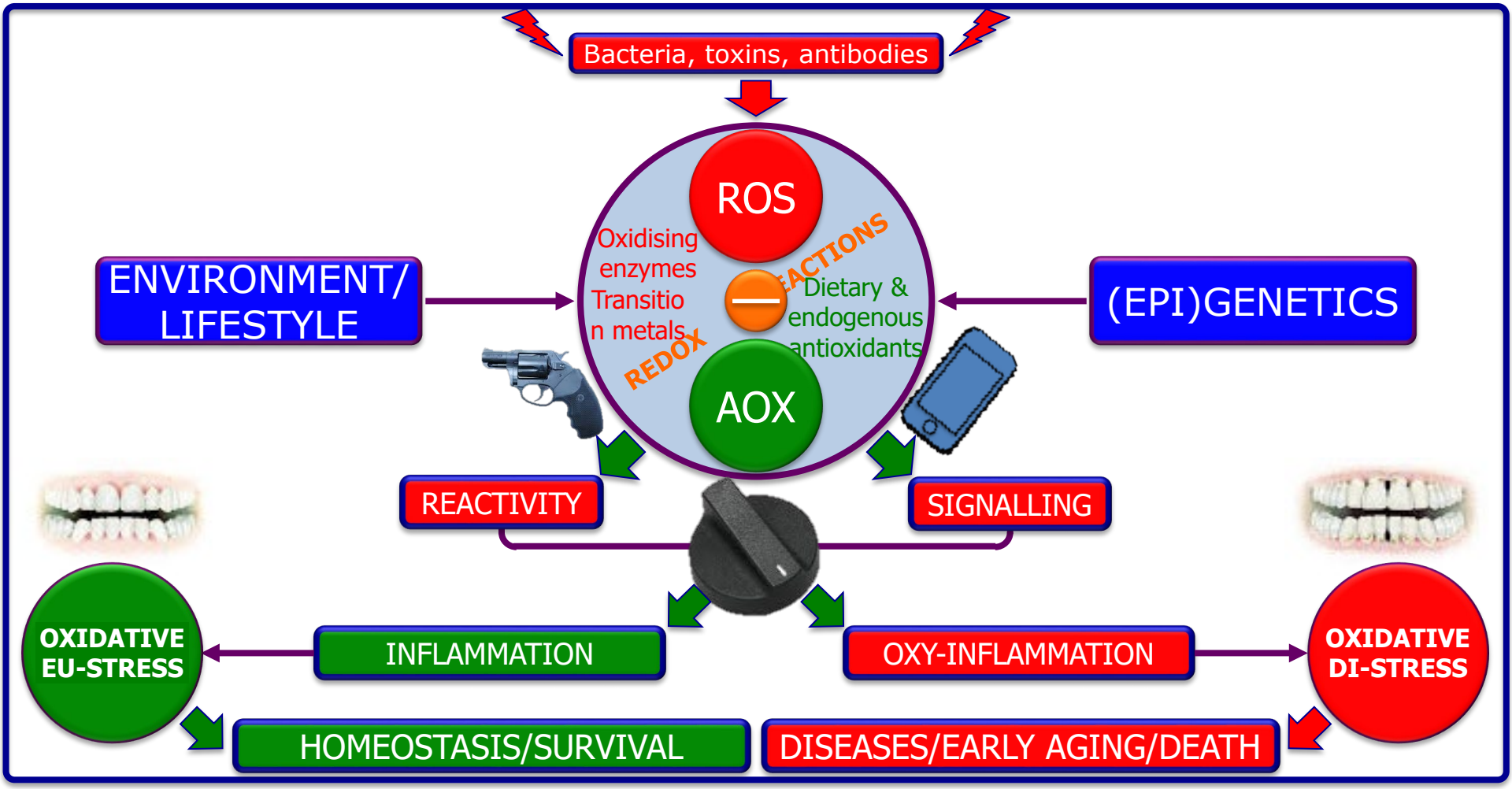
Myeloperoxidase (MPx)

$HCl + H_2O_2 \rightarrow H_2O + HClO$

$HCl + RNH_2 \rightarrow R\text{–}NHCl + H_2O$

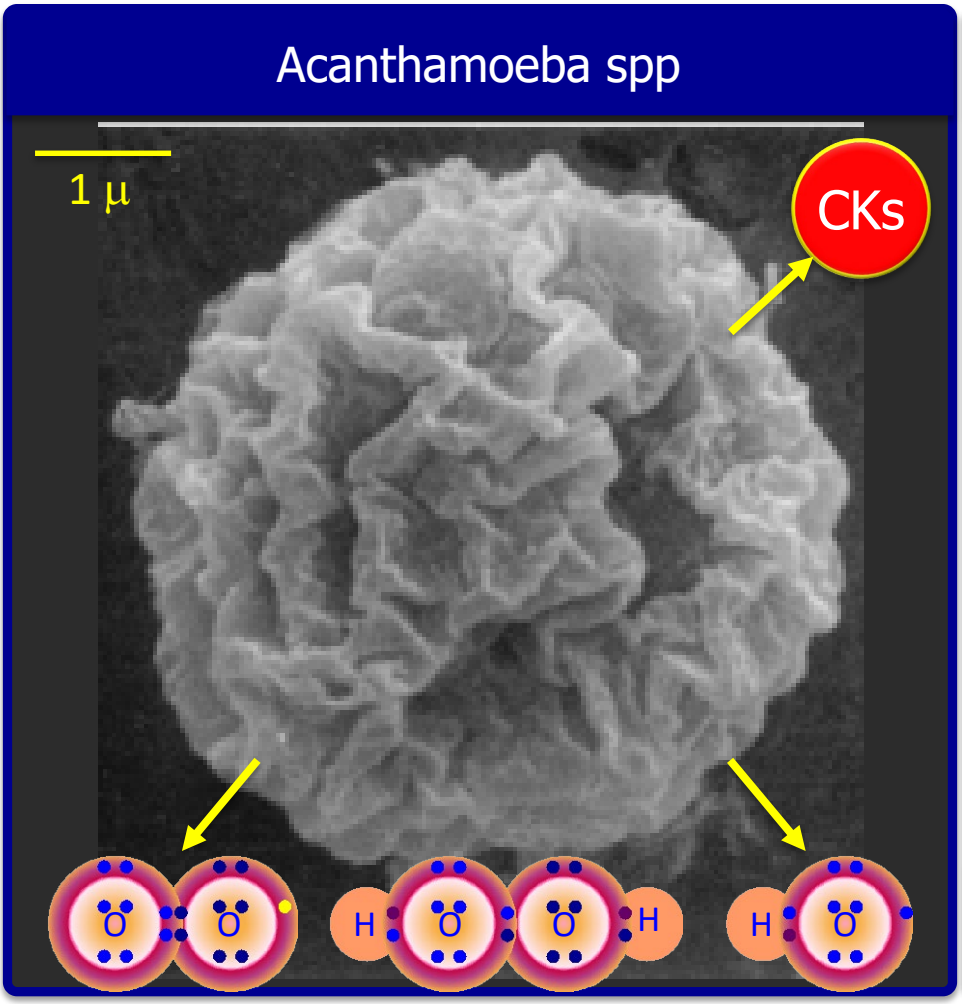
An ancient and well conserved defence mechanism

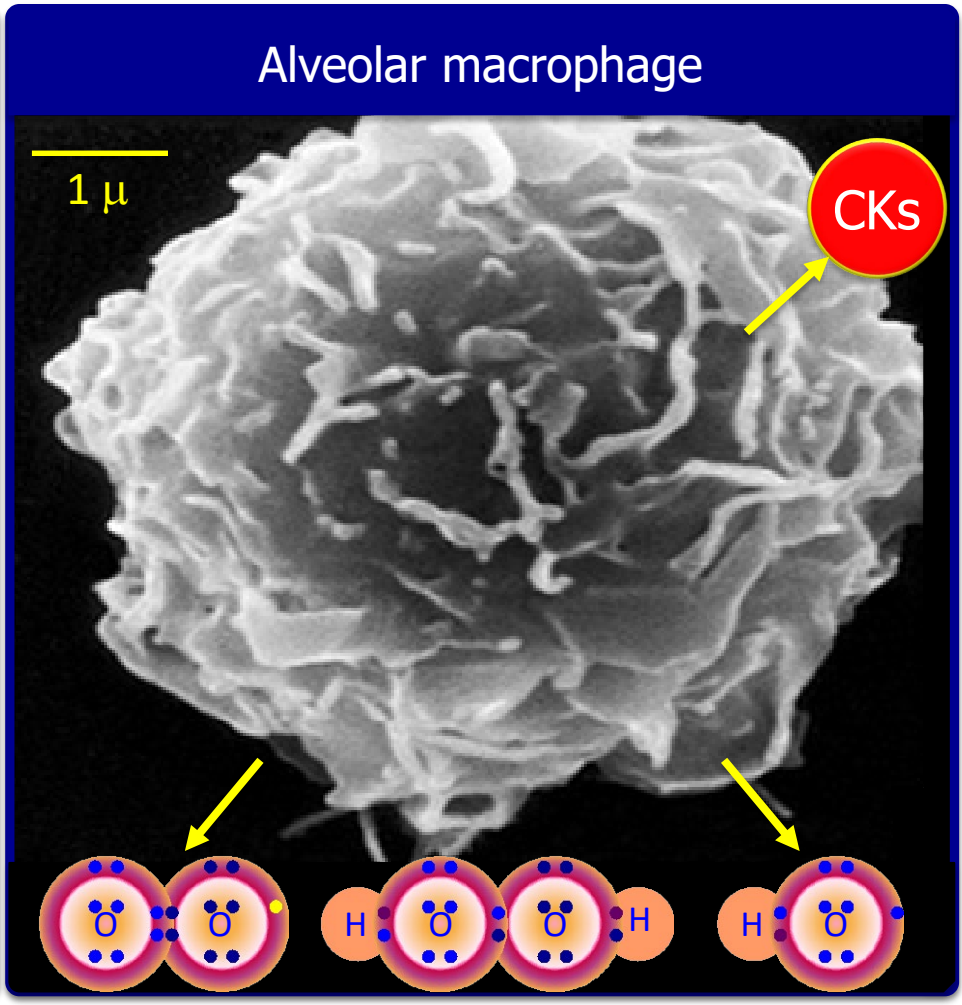
From reactive oxidative eu-stress (inflammation and healing) to reactive oxidative di-stress (oxinflammation)



Common dental infectious diseases can switch to periodontitis, a health risk factor for vascular diseases

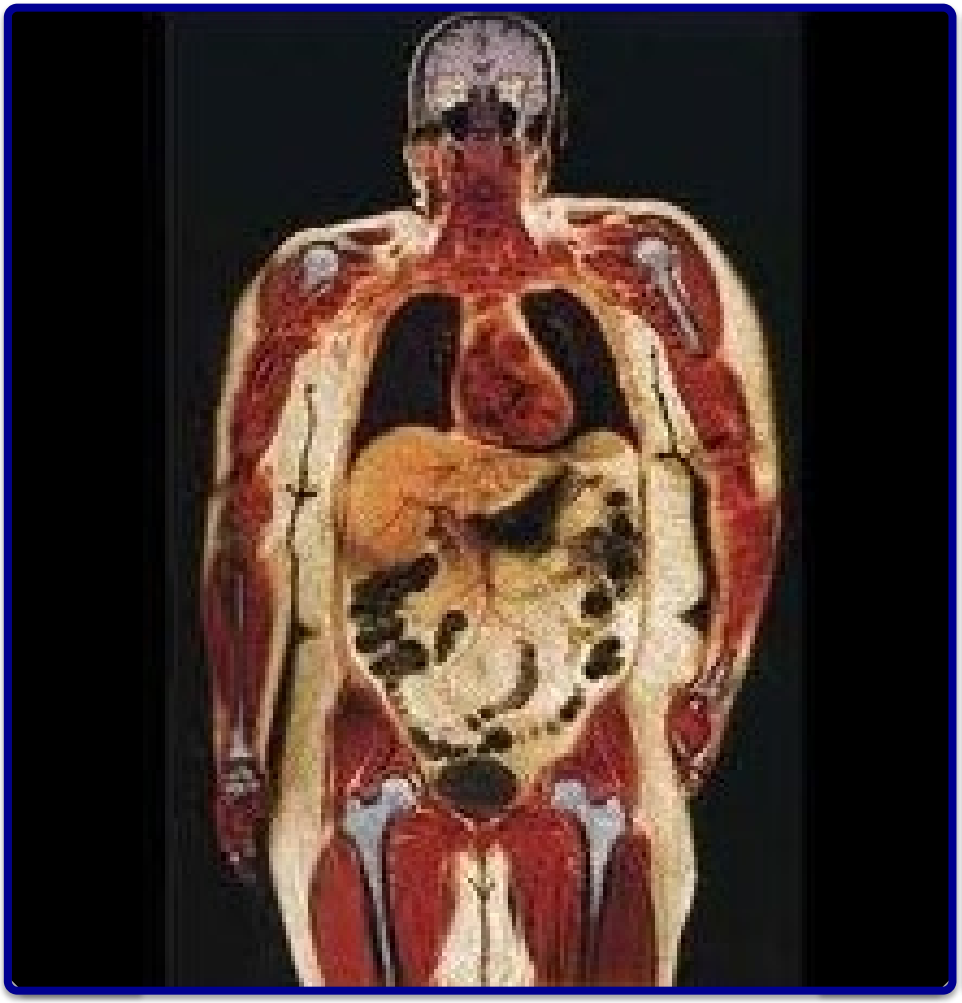
ROS production from leukocytes and macrophages may derive from an ancestral alliance





The inclusion of amoeba in our body as macrophage allowed our ancestors to exploit ROS as protectors

Macrophages are associated to the visceral fat (very wide) in order to take energy for their life



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Minireview

Acanthamoeba is an evolutionary ancestor of macrophages: A myth or reality?

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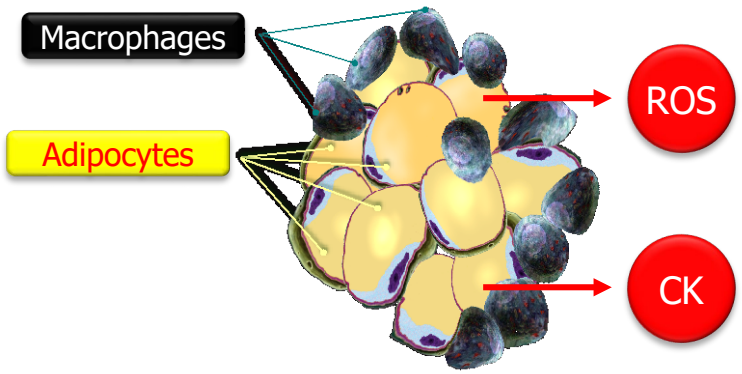
ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form 17 November 2011
Accepted 19 November 2011
Available online 28 November 2011

Keywords:
Acanthamoeba
Evolution
Macrophages

ABSTRACT

Given the remarkable similarities in cellular structure (morphological and ultra-structural features), molecular motility, biochemical physiology, ability to capture prey by phagocytosis and interactions with microbial pathogens, here we pose the question whether *Acanthamoeba* and macrophages are evolutionary related. This is discussed in the light of evolution and functional aspects such as the astonishing resemblance of many bacteria to infect and multiply inside human macrophages and amoebae in analogous ways. Further debate and studies will determine if *Acanthamoeba* is an evolutionary ancestor of macrophages. Is this a myth or reality?



From inflammation to oxidative stress and metabolic syndrome (and cellulite?)

The intriguing relationships between stress, inflammation and metabolism.

Upregulation of Phagocyte-Derived Catecholamines Augments the Acute Inflammatory Response

Michael A. Flierl[1], Daniel Rittirsch[1], Brian A. Nadeau[1], J. Vidya Sarma[1], Danielle E. Day[1], Alex B. Lentsch[2], Markus S. Huber-Lang[3], Peter A. Ward[1]*

1 Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, United States of America, 2 The Laboratory of Trauma, Sepsis & Inflammation Research, Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, United States of America, 3 Department of Trauma-, Hand- and Reconstructive Surgery, University of Ulm Medical School, Ulm, Germany

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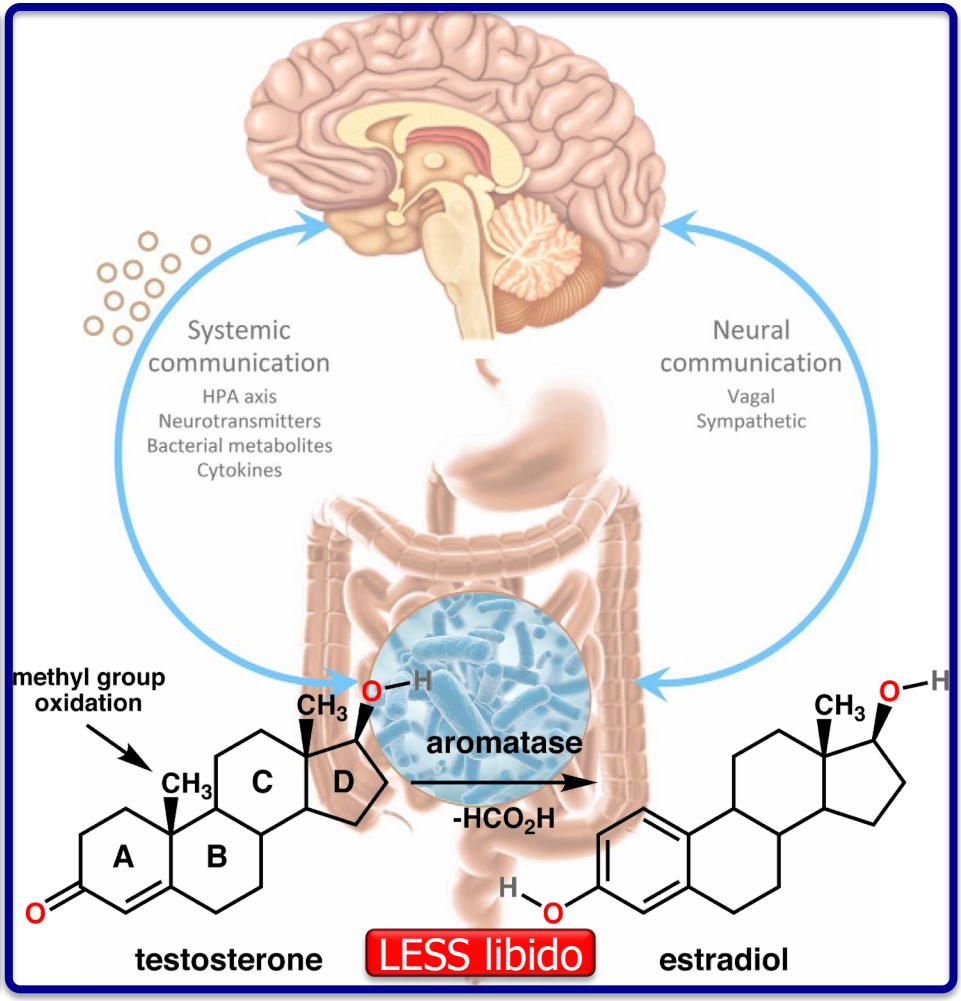
Mor G, Yue W, Santen RJ, et Al. *Macrophages, estrogen and the microenvironment of breast cancer*. J Steroid Biochem Mol Biol. **1998**. 67 (5-6): 403-411.

Xu F, Wu C, Lin J. *Microbial endocrinology: impact of interactions between microbes and neuroendocrine hormones on infection-a review*. **2013**. 53 (9): 901–907.

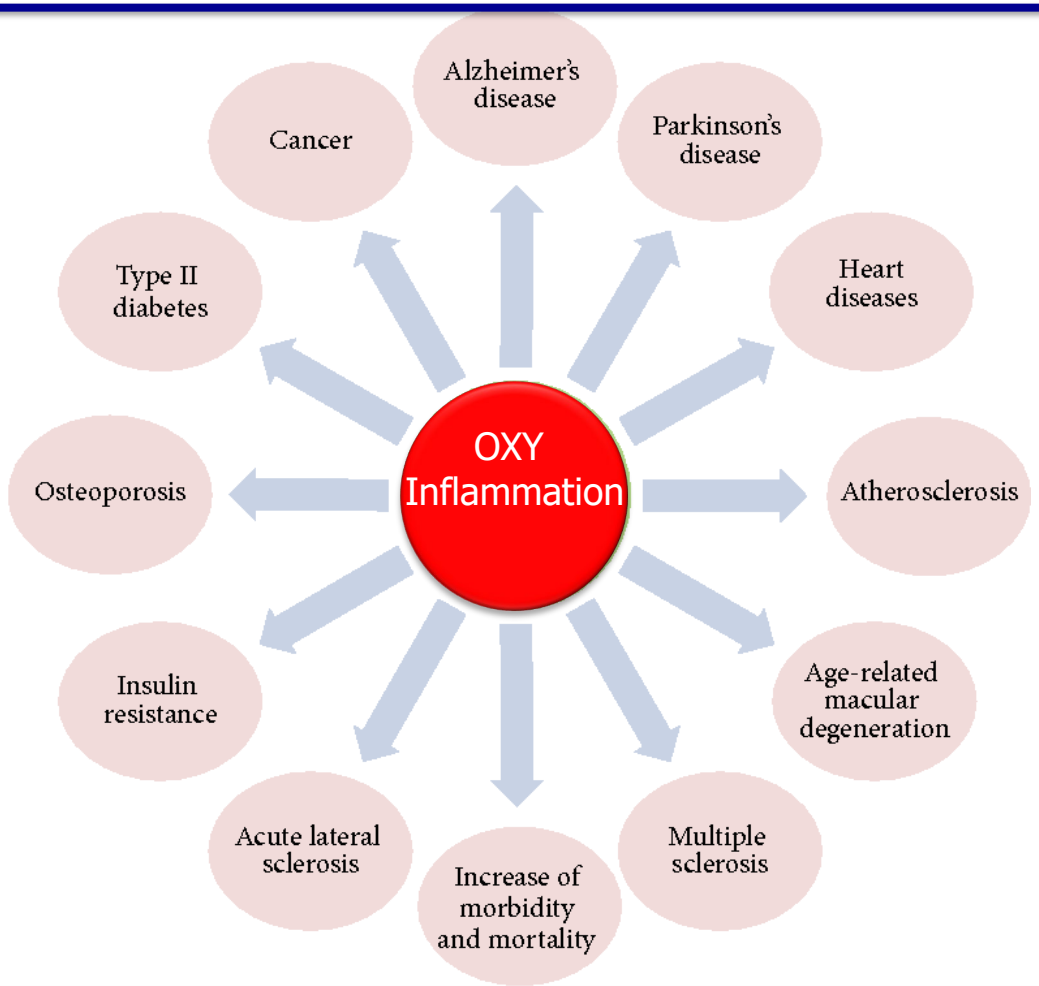


Monocytes-macrophages, as a "circulating sympathetic ganglion", produces catecholamines and also aromatase.

In the beginning it was the oxidative stress . . . . then came the inflammation!

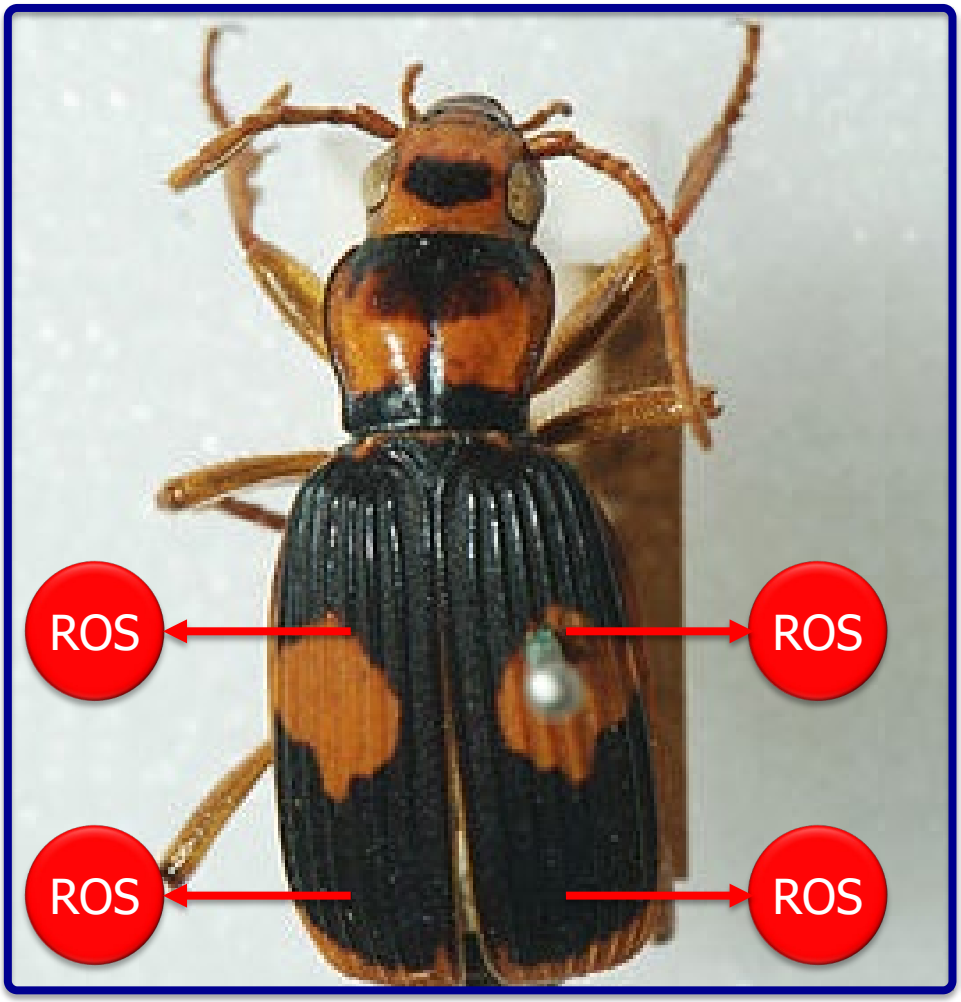
There can be oxidative stress without inflammation but not inflammation without oxidative stress!

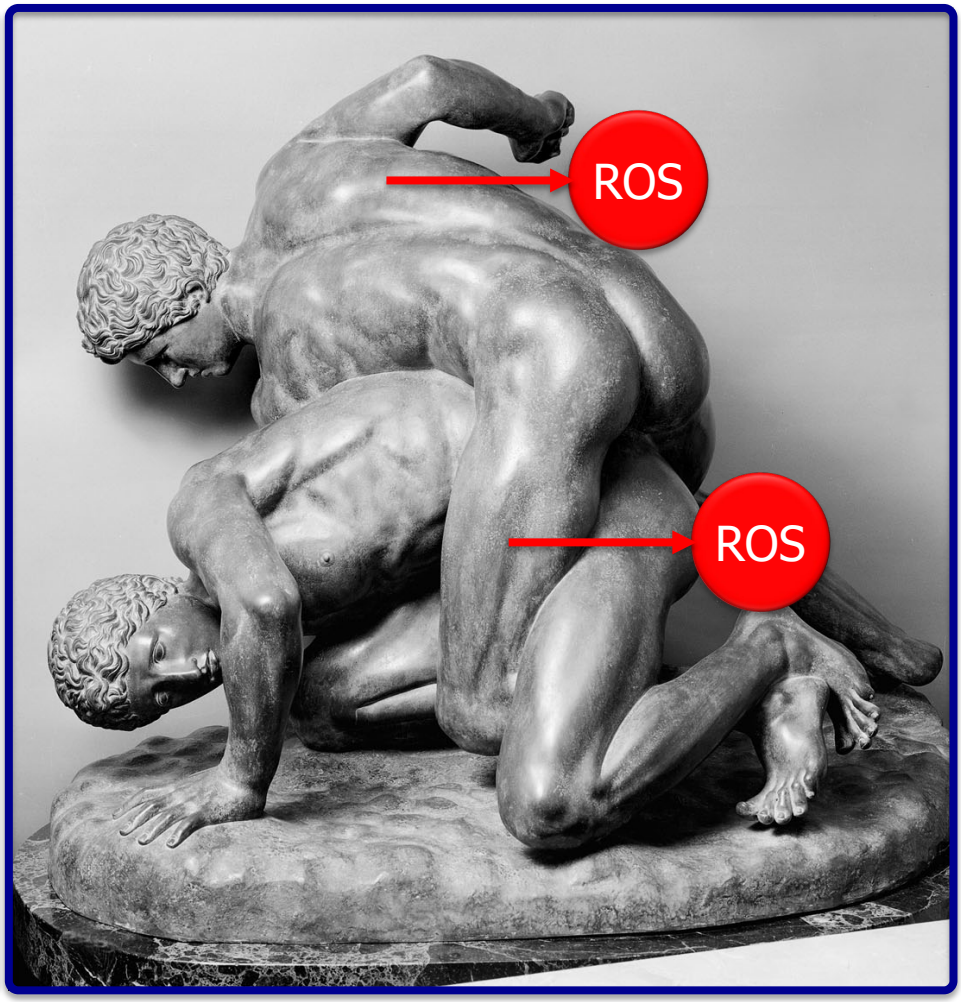
The impact of reactive oxidative di-stress on health



Closely relationships between oxidative di-stress and inflammation in the pathophysiology of most diseases

Fight or flight? The incredible bombardier beetle's story.





More sophisticated weapons to defend themselves from aggressors: going back to Greek-roman fight!

Exercise and redox system: a breath of life or a breath of death?

The paradigma of respiratory oxidative stress and the sport paradox

The sport paradox

OXYDATIVE STATUS AND THE PARADOX OF SPORT SHORTENING AND PROLONGING LIFE

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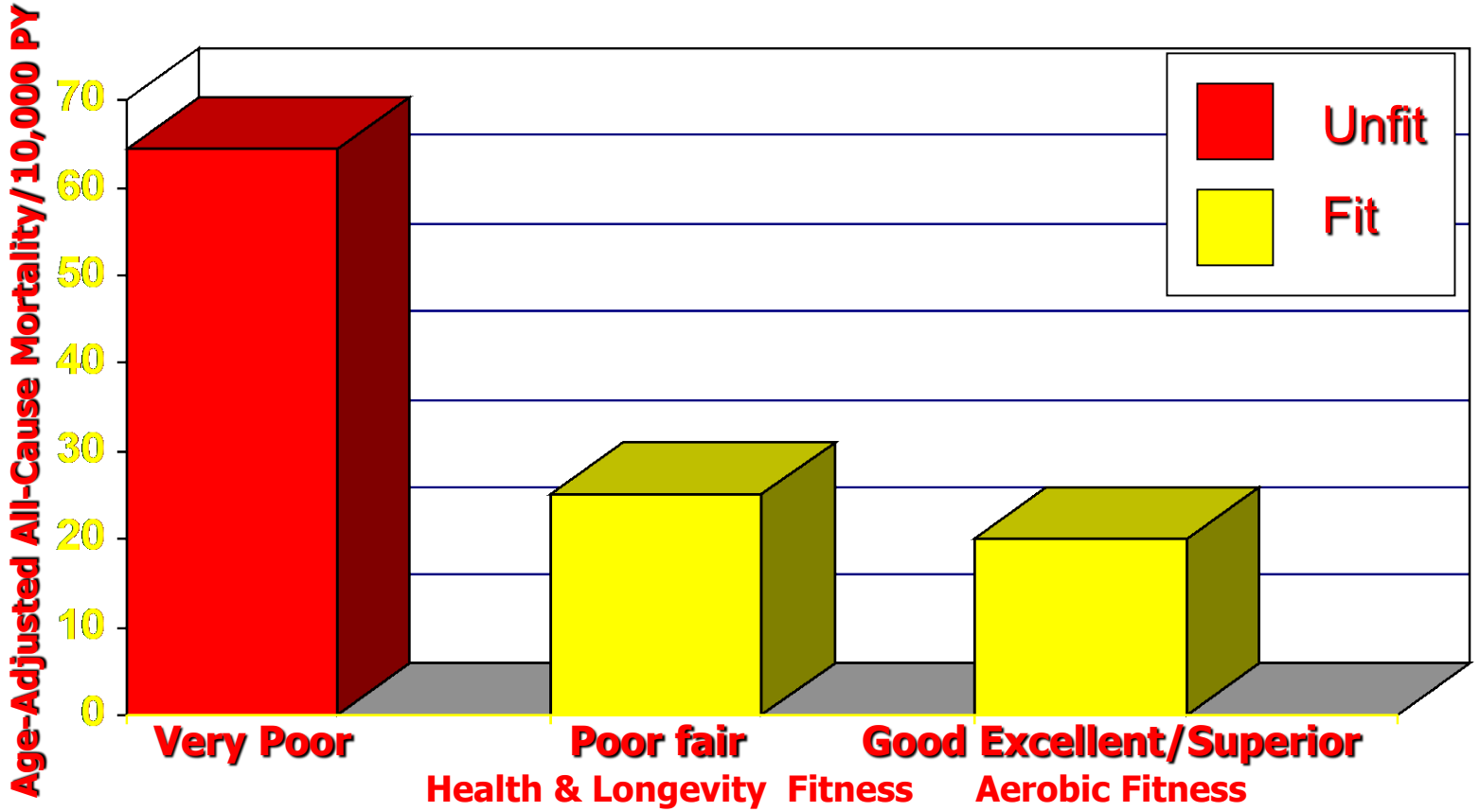
1 Laboratoire de Physiologie des Adaptations, Performance Motrice et Santé (EA), Univ. de Nice-Sophia-Antipolis, France
2 Myd Company sarl, France

The free radical theory of aging has received increased recognition during the past two decades (Sohal & Weindruch, 1996). Aging is characterized by the decrease in capacity to cope with stress and associated to the decrease in antioxidant defence and increase in oxidative stress. The reinforcement in the antioxidant response could in part ameliorate age induced adverse effects (Parkes, 1998). This effect could be expected as a result of the physical training-induced antioxidant system response. Acute physical exercise is associated with an increase in free radical production and consequently oxidative stress and damage. The repetition of such stress induces adaptive effects such as an increase in antioxidant defence. This adaptive response is however alleviate with aging (Ji et al., 2002). One could doubt the positive effects of physical exercise on aging as physical exercise induces oxidative stress. These effects are of interest if repeated physical exercise, so called training, is well conducted. In physiological situations, the magnitude of the stress determines the magnitude of adaptive effects. However, exercise has to be adapted to the current subjects conditioning. If current capacities of the subject are not adapted to cope with the free radical production, oxidative stress occurs, the antioxidant defence is overwhelmed, and adaptive effects are not related to exercise-induced stress. In an overuse or overtraining state the magnitude of the adaptive response is not related to the exercise-induced stimulation (Palazzetti et al., 2003). More is not certainly better. Sport can be expected to "prolong" life, if the training is regular, well conducted and adapted to the current physiological state. We have recently evidenced that nutrition and exercise-induced oxidative stress are interdependent factors whose combination determines the training-induced antioxidant adaptive response et al., 2003). It can be assumed that positive effects of physical activity closely depend on the training strategy and the dietary intake. A feedback of the strategy can be obtained by monitoring the oxidative stress by evaluating the total serum hydroperoxides. Using this tool could be useful to control exercise and training-induced oxidative stress. In this attempt, it could be of interest to monitor exercise bouts effects, and training effects more frequently than currently done, due to methodological and practical limiting factors. As the H_2O_2 production increases with both age and physical exercise, it can be monitored by analysing these reactive oxygen species with the d-ROMs test, which is a rapid, sensitive method (Verde et al., 2002) with good reproducibility

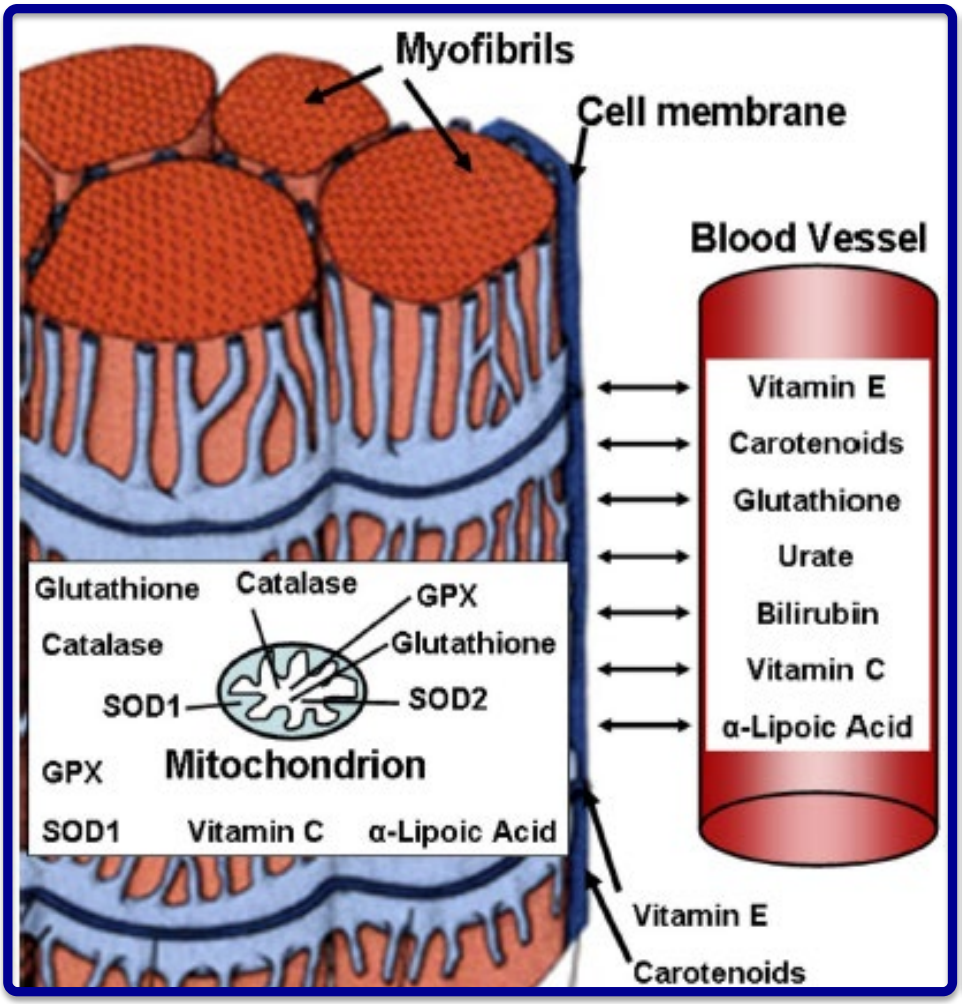
Margaritis. 2001.

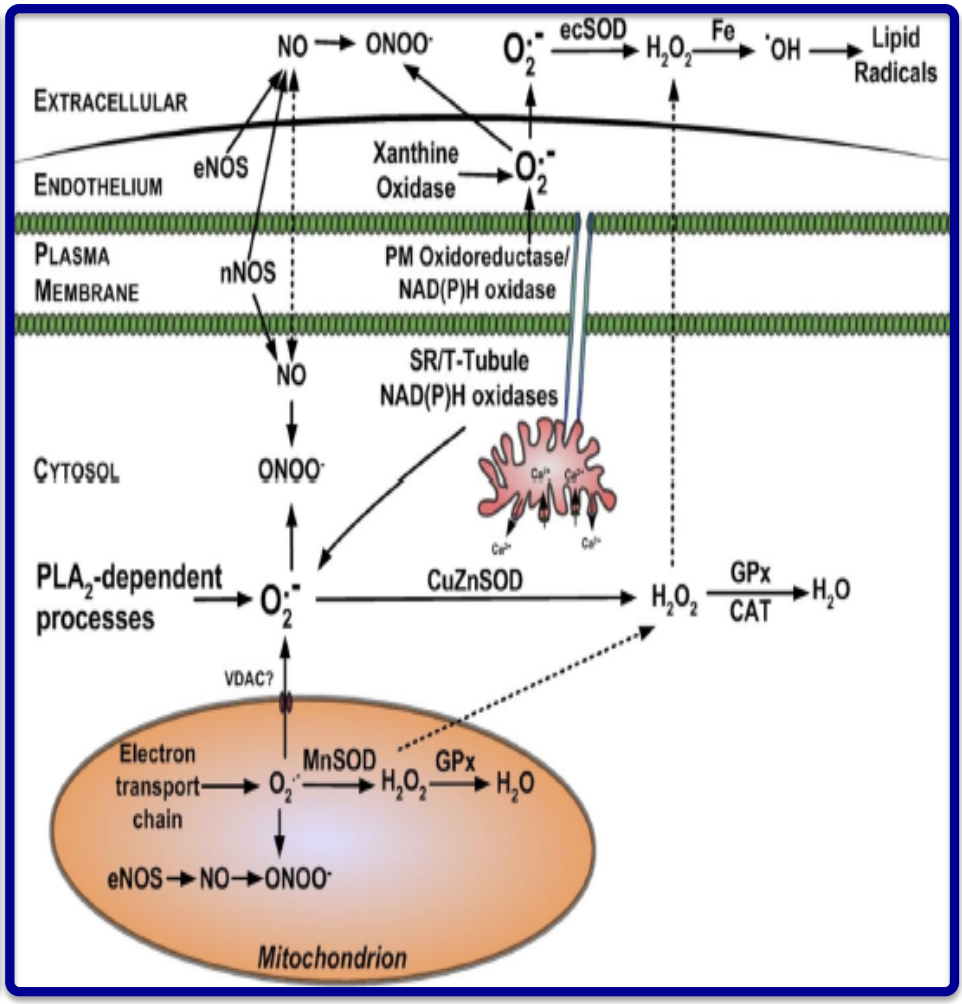
Poor to fair exercise reduce all-cause mortality. No further significant advantages for strong aerobics.



The fitness longevity trial of Cooper KH

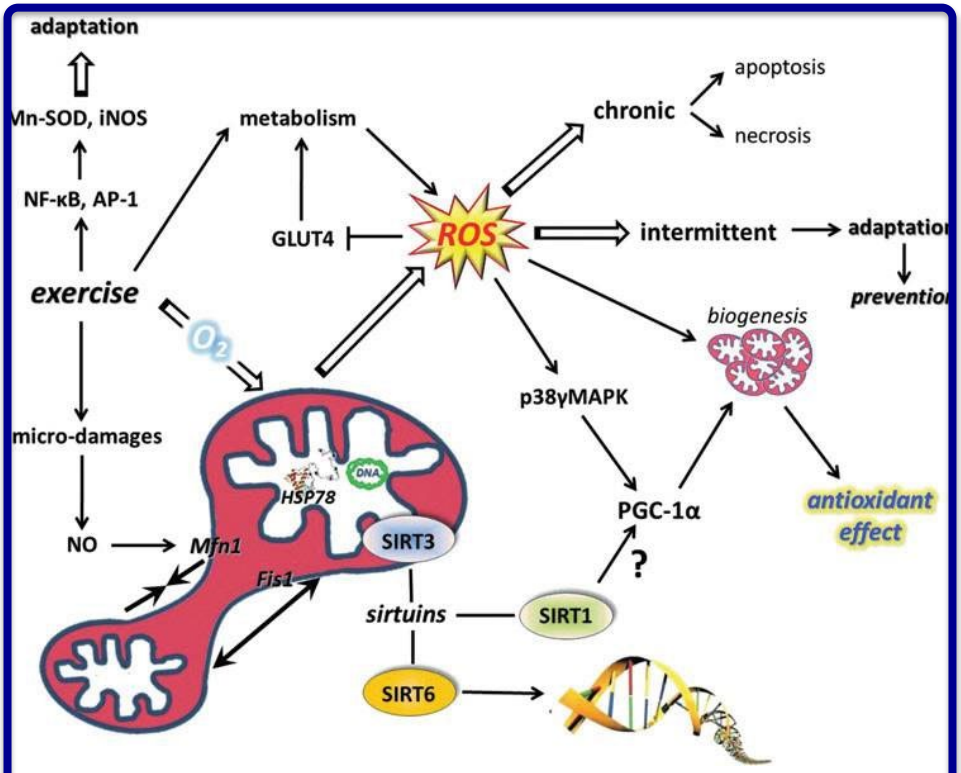
The redox system is widely distributed inside muscle tissues





The intracellular and extracellular oxidant/antioxidants network

Exercise results in large metabolic challenges to skeletal muscle that are related to ROS production/effects



AP-1, activator protein-1; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; Mn-SOD, manganese superoxide dismutase; NF-kB, nuclear factor-kappa B; PGC-1α, peroxisome proliferator-activated receptor-c coactivator 1α.

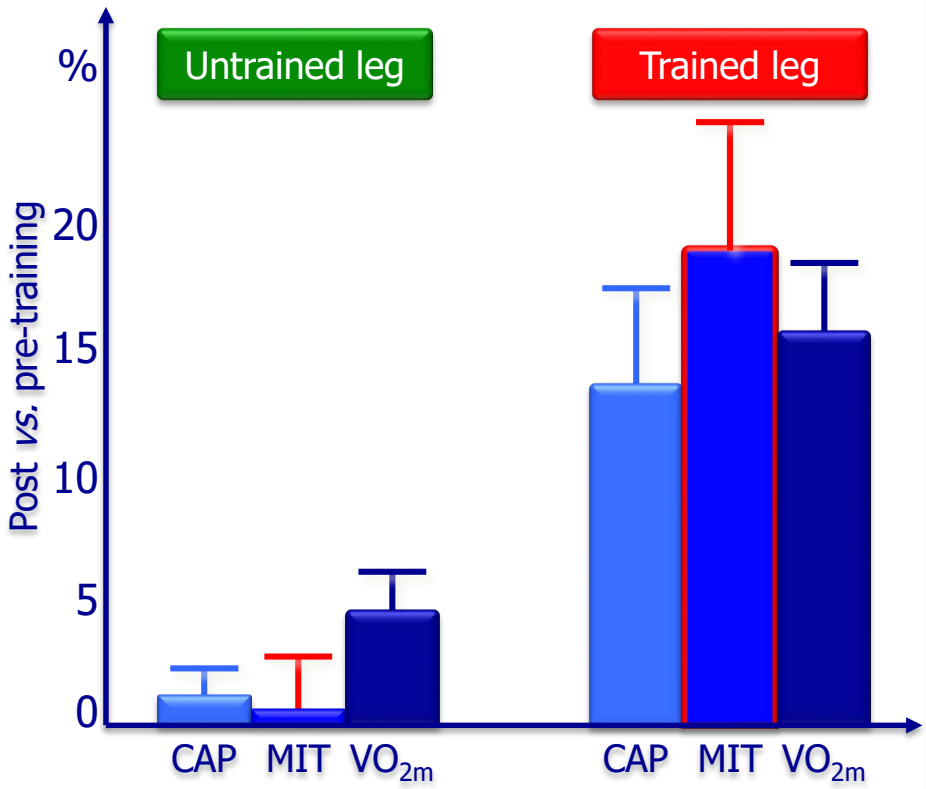
✓ROS are important signalling molecules for muscle contractions, PGC-1α, MAPK, as well as for transcription factor NF-jB.

✓NAD^+/NADH levels are readily modified by ROS and could affect the activity of sirtuins.

✓ROS are involved in mitochondrial biogenesis, fusion and fission.

Radak et Al. Antioxidants & Redox Signaling. 2013.

Endurance training increases the mitochondrial content (biogenesis of mithocondria)



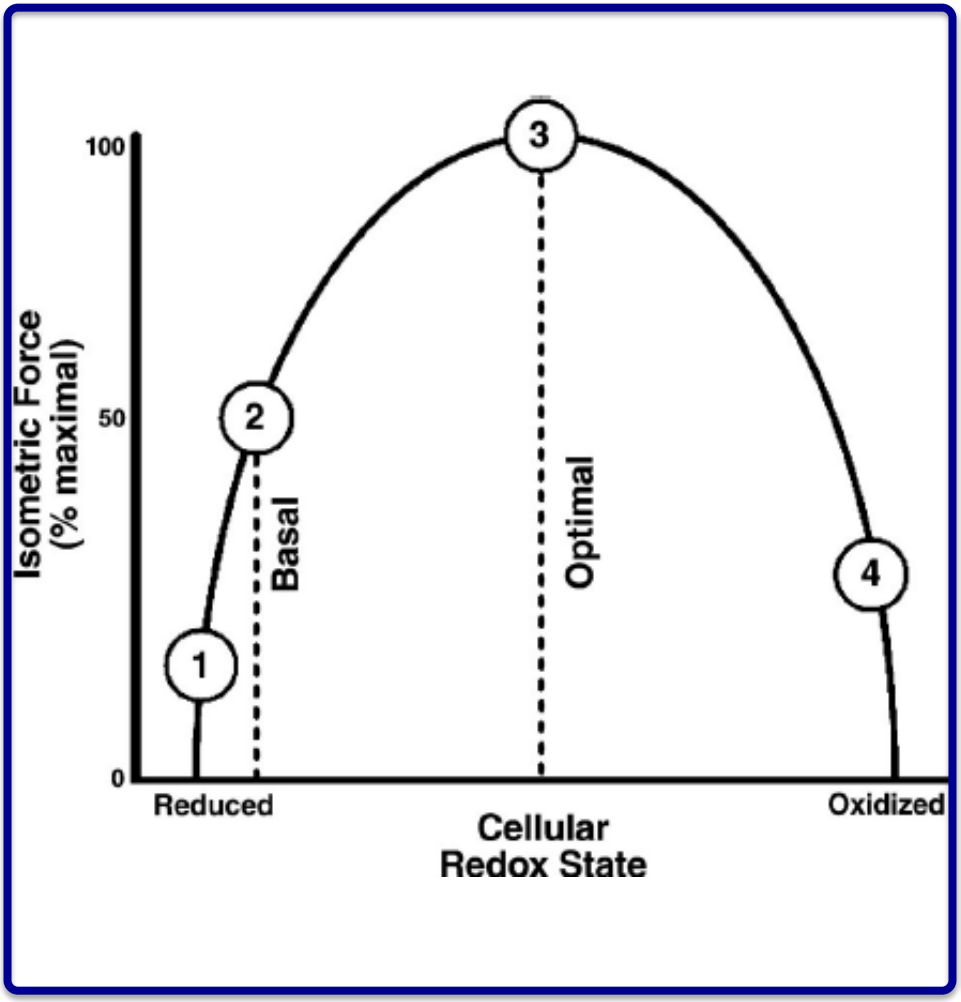
✓Subjects endurance trained with one leg and rested the other.

✓After the training period, the capillary density, mitochondrial content and peak oxygen uptake achieved when cycling with that leg were measured.

✓The results showed that endurance training increased the mitochondrial content of the trained leg by ≈ 20% and also the peak oxygen uptake that was achieved when working with the trained leg only.

Saltin et Al. Acta Physiol Scand. 1976. 96: 289–305.

A theoretical model that describes the biphasic effect of ROS on skeletal muscle force production.



Point 1 represents the force production by unfatigued muscle exposed to antioxidants or a reducing agent.

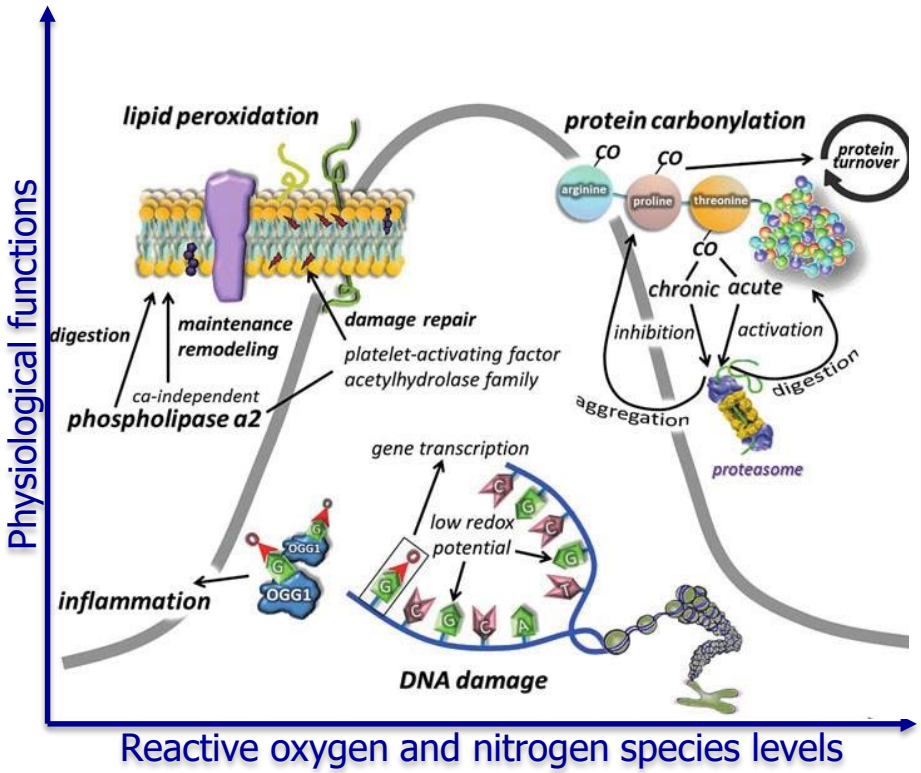
Point 2 illustrates the force generated by muscle in its basal state (i.e., no antioxidants or oxidants added).

Point 3 illustrates the force produced by unfatigued skeletal muscle exposed to low levels of oxidants; this represents the optimal redox state for force production.

Point 4 illustrates the deleterious effects of excessive ROS on skeletal muscle force.

Searching for optimal state (3)

Moderate levels of oxidative stress could be important to the induction of the oxidative damage repair system



8-oxoG: 8-Oxo-7,8 dihydroguanine; OGG1, 8-oxoguanine-DNA glycosylase.

✓Lipid peroxidation by-products, carbonyl groups, and the 8-oxoG levels are easily detectable, suggesting that these ROS-induced modifications could be necessary for cells.

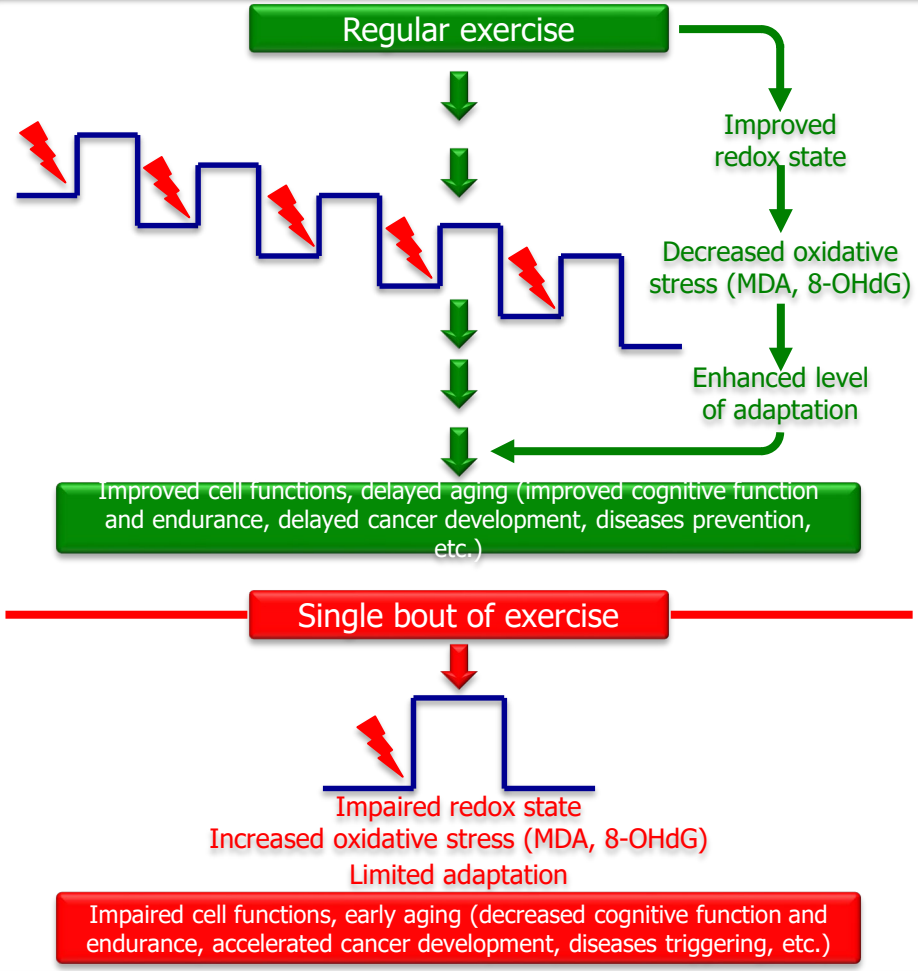
✓Lipid peroxidation can be induced by enzymatic processes and could be important to membrane remodelling.

✓Carbonylation of amino acid residues could be an important mediator of protein turnover, since carbonylation can serve as a tag for proteolytic degradation.

✓8-oxoG is necessary for transcription of specific genes and for the opening of chromatin.

Radak et Al. Antioxidants & Redox Signaling. 2013.

A regular exercise improves oxidative balance

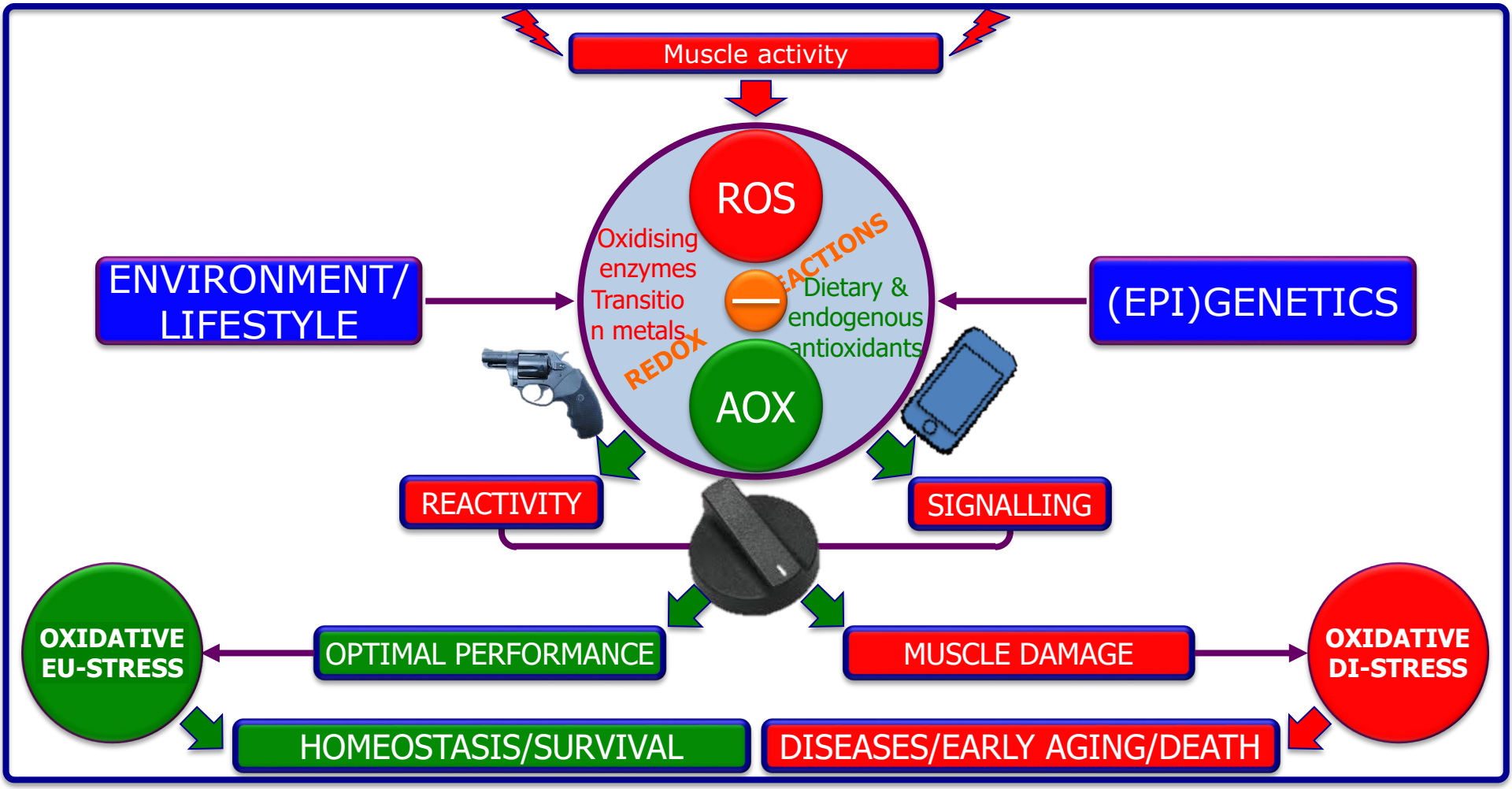


✓Moderate levels of oxidative damage could be important to the induction of the oxidative damage repair system.

✓The regular exercise induced adaptation, due to the intermittent feature of exercise and rest periods, allows induction of the antioxidant and damage repair systems, which results in enhanced protection against oxidative stress, attenuates the aging process, and promotes health with increased functional capacities.

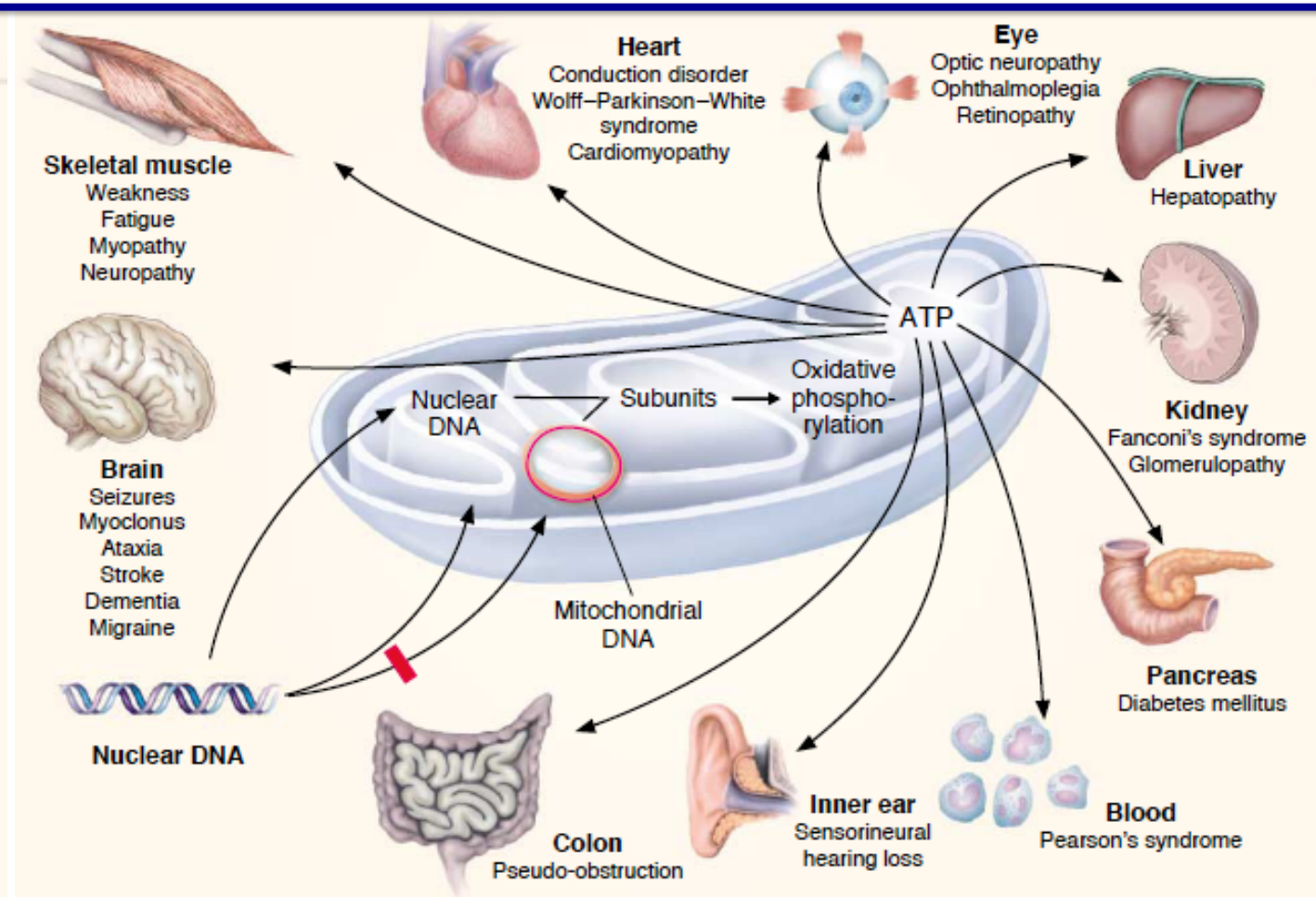
Radak et Al. Antioxidants & Redox Signaling. 2013.

From respiratory oxidative eu-stress (optimal performances) to its di-stress form (muscle damage)



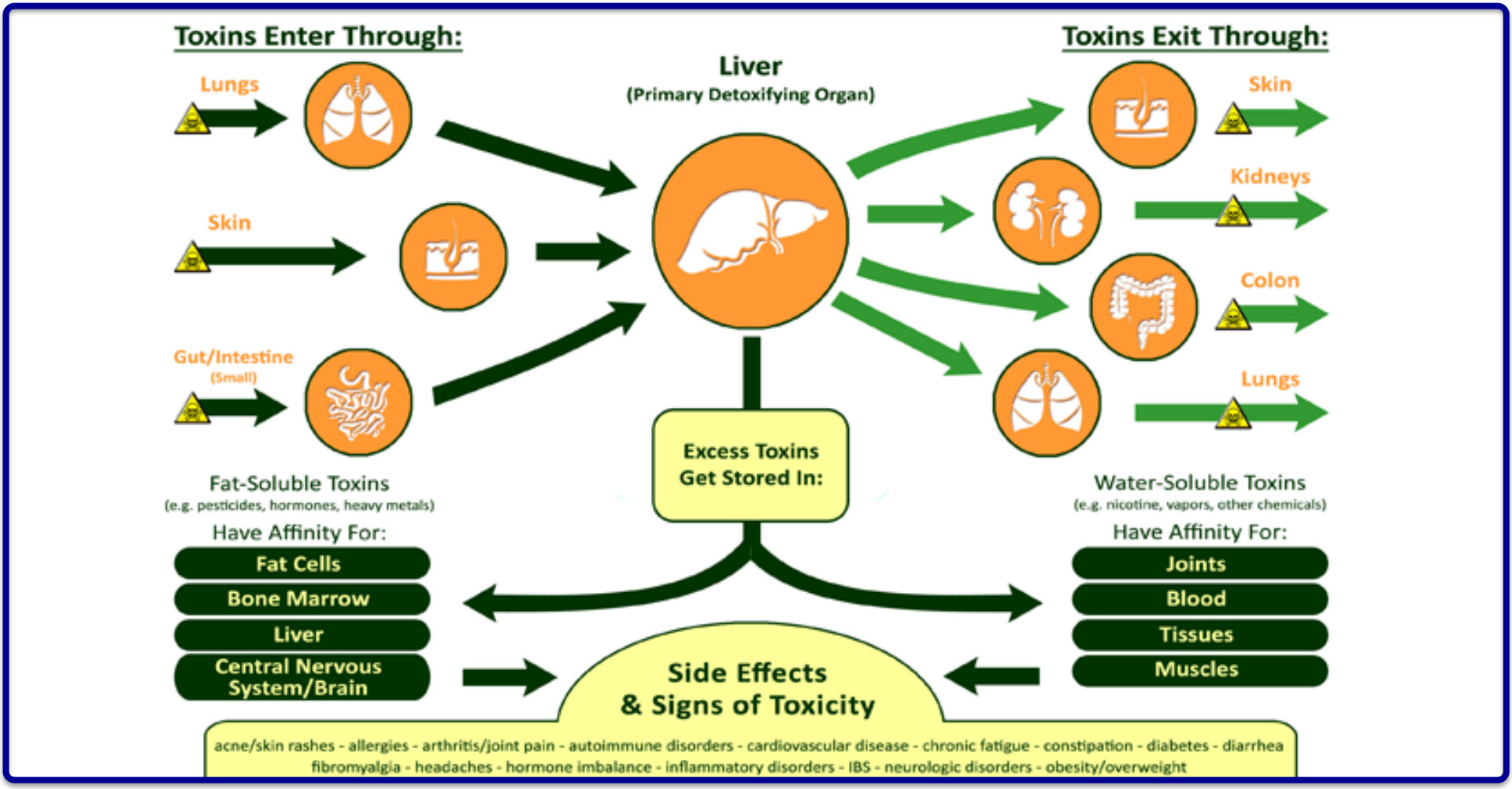
Strenuous uncontrolled aerobic exercise may shorten the life (the sport paradox)

Mitochondrial diseases



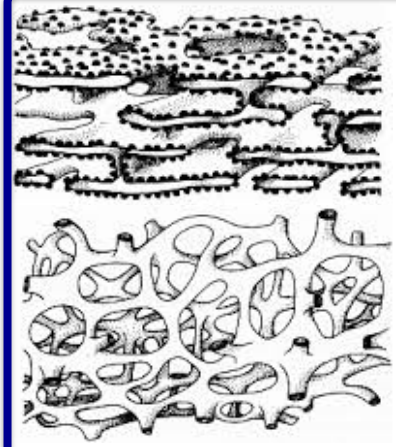
An emerging health problem

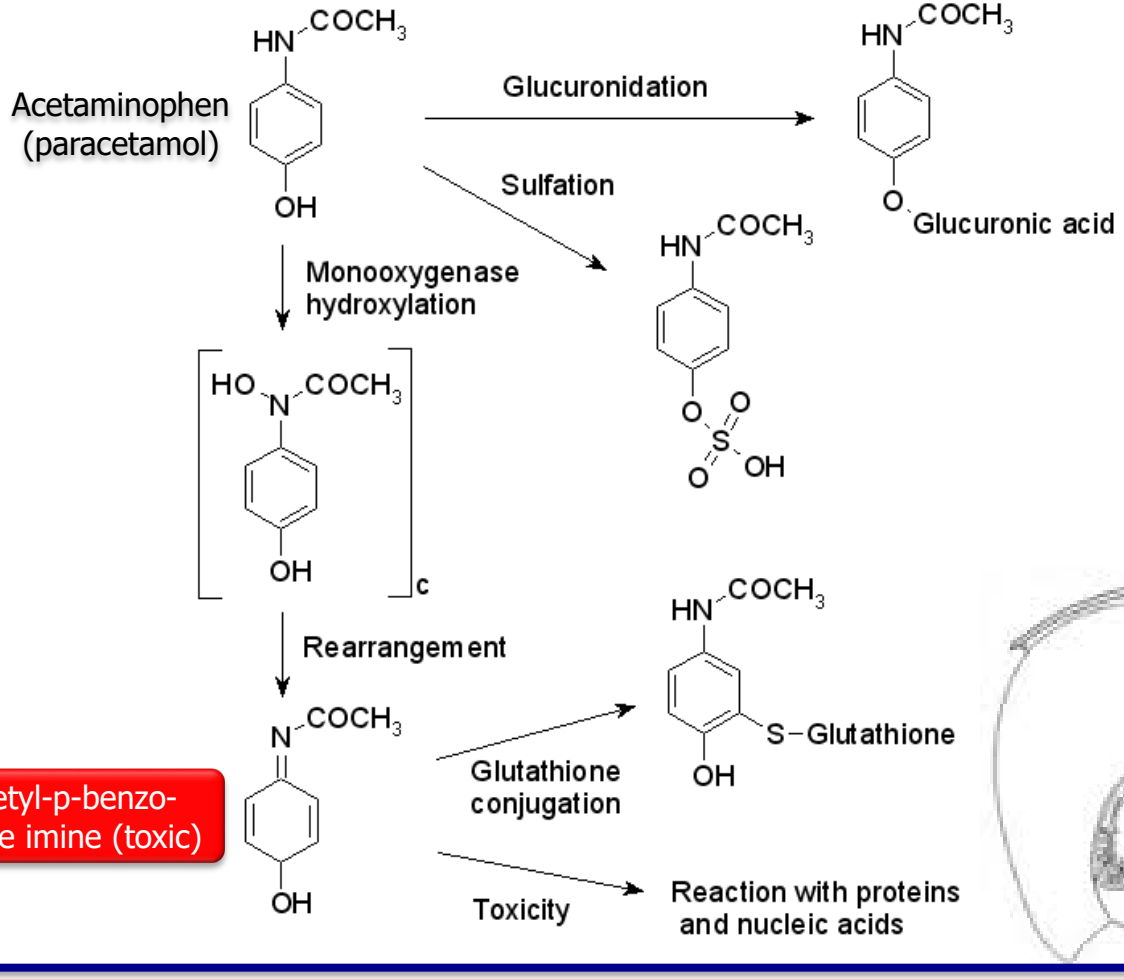
The detoxification. A crucial function for adaptation to the environmental challenges.

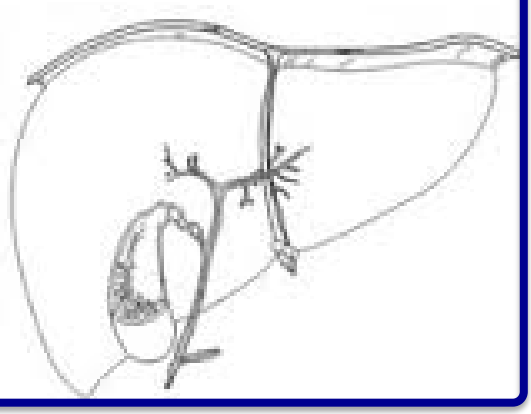


The paradigma of toxic oxidative stress (toxidative stress)

The role of microsomial system in drug detoxification: focus on acetaminophem and contrceptive pills!

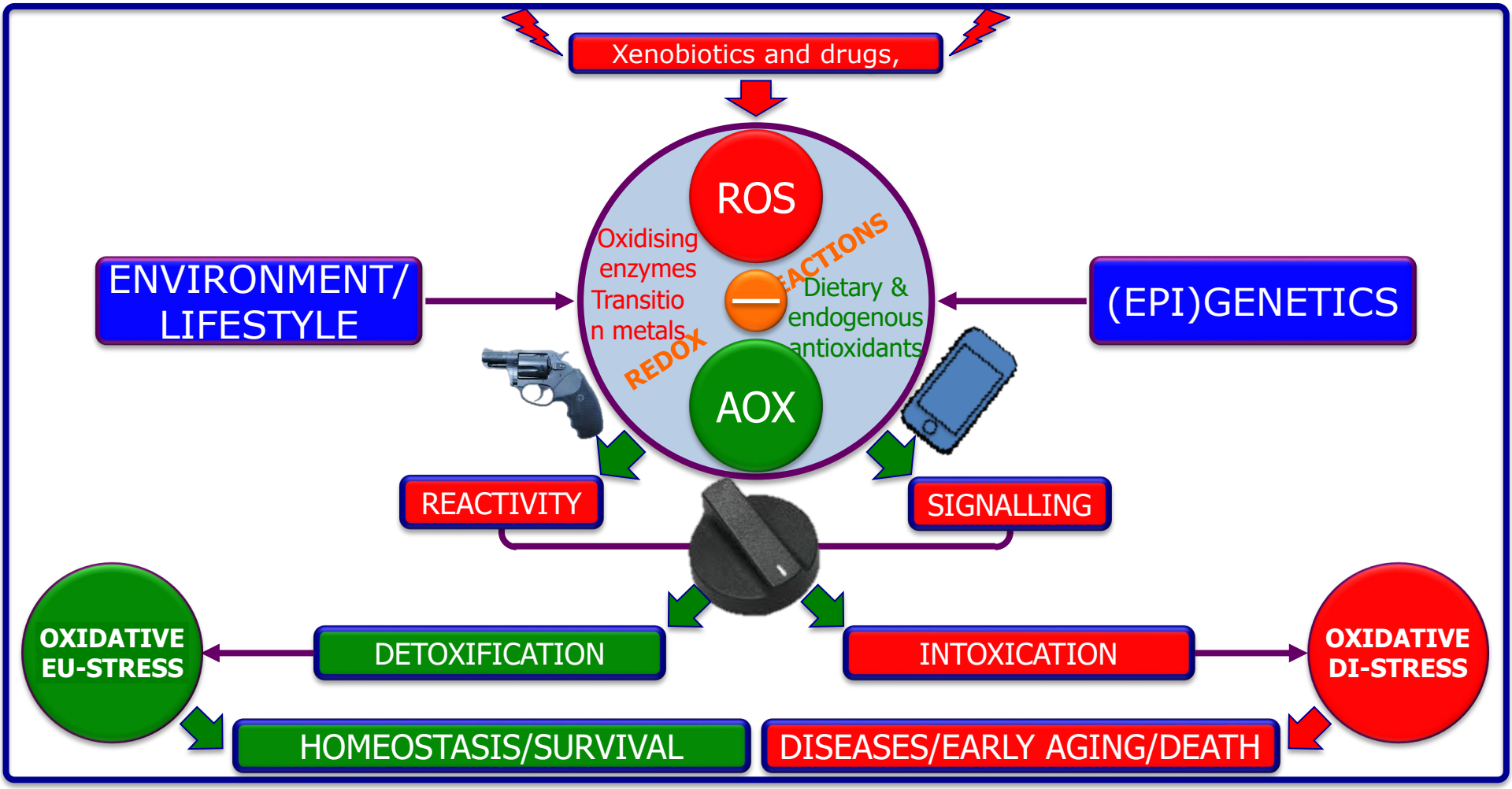






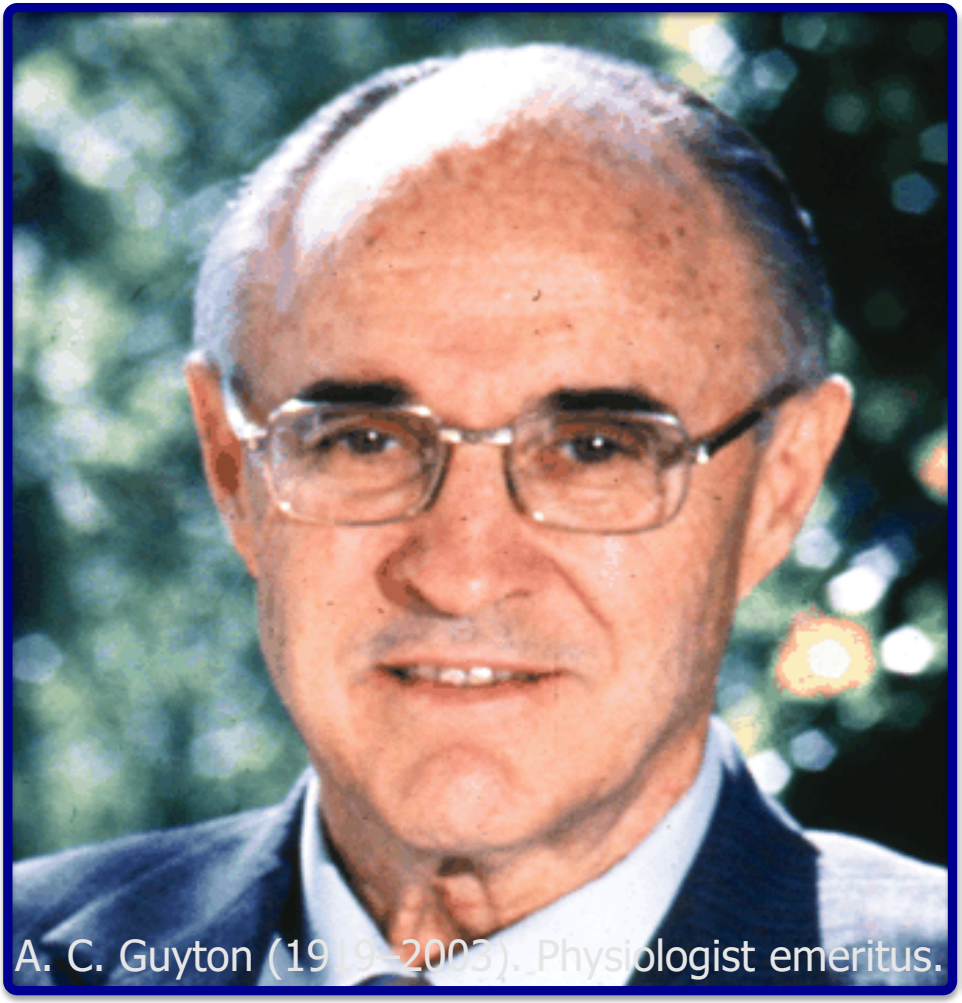
Detoxification from acetaminophen (paracetamol) into the microsomes may increase ROS liver production

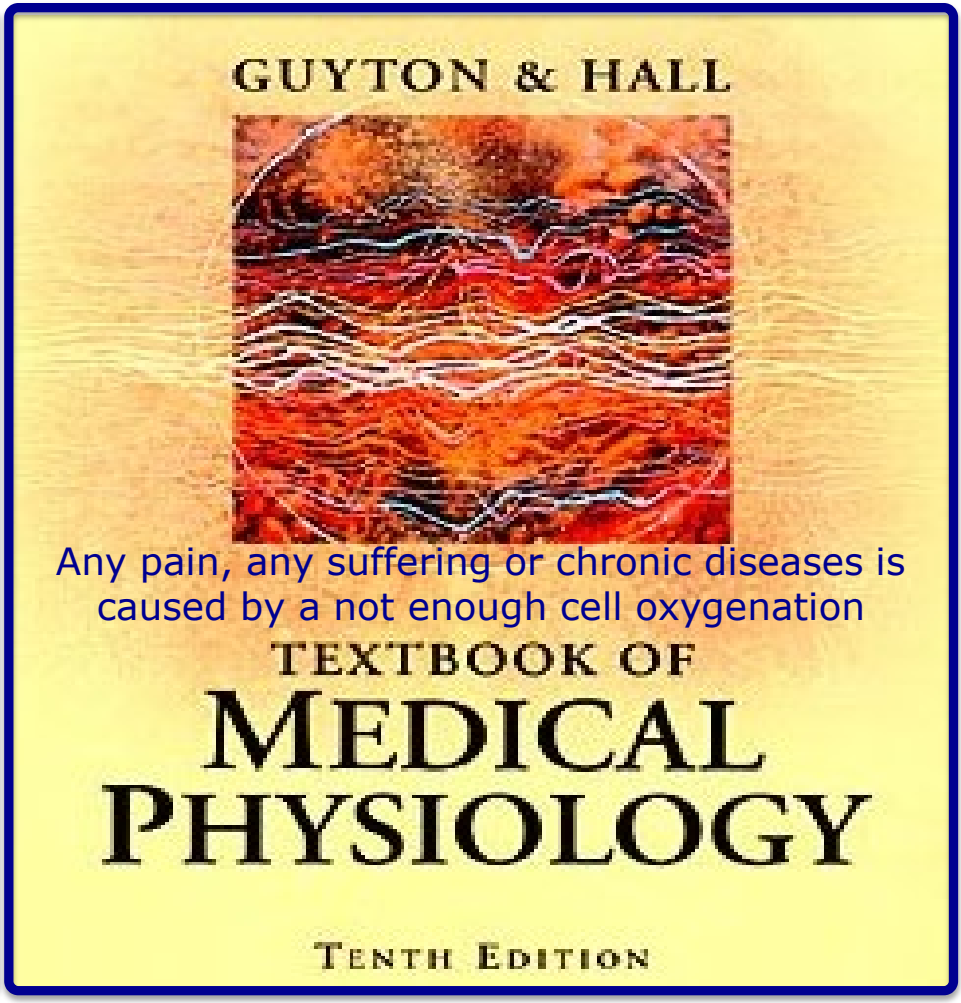
From toxic oxidative eu-stress (safe toxins elimination) to its di-stress form (chronic intoxication)



Improper redox-mediated detoxification function may lead to chronic diseases and death

The challenge of hypoxia


A. C. Guyton (1919–2003). Physiologist emeritus.



Any pain, any suffering or chronic diseases is caused by a not enough cell oxygenation

The paradigma of hypoxic oxidative stress

Uncontrolled/chronic hypoxia is the leading cause of tissues function/structure impairment

Decrease of inspired oxygen

Hemoglobin impaired function

Blood flow slowing

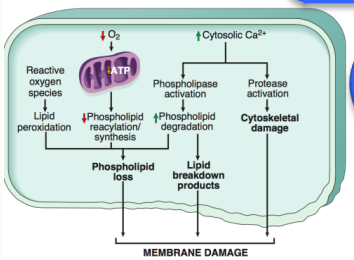
Mitochondrial dysfunction

HYPOXIC HYPOXIA

ANEMIC HYPOXIA

STAGNANT HYPOXIA

HISTOTOXIC HYPOXIA

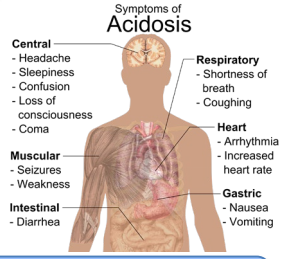


ATP↓

Weakness
Fatigue

Haemoglobin dissociation
Vasodilation

H^+↑

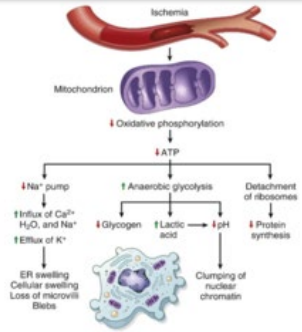


CELL FUNCTIONAL RESERVES OVERCOMING

Xanthine dehydrogenase to oxidase

Ischemia-reperfusion damage

INTRACELLULAR OXIDATIVE STRESS



Iron release/ROOH breakdown

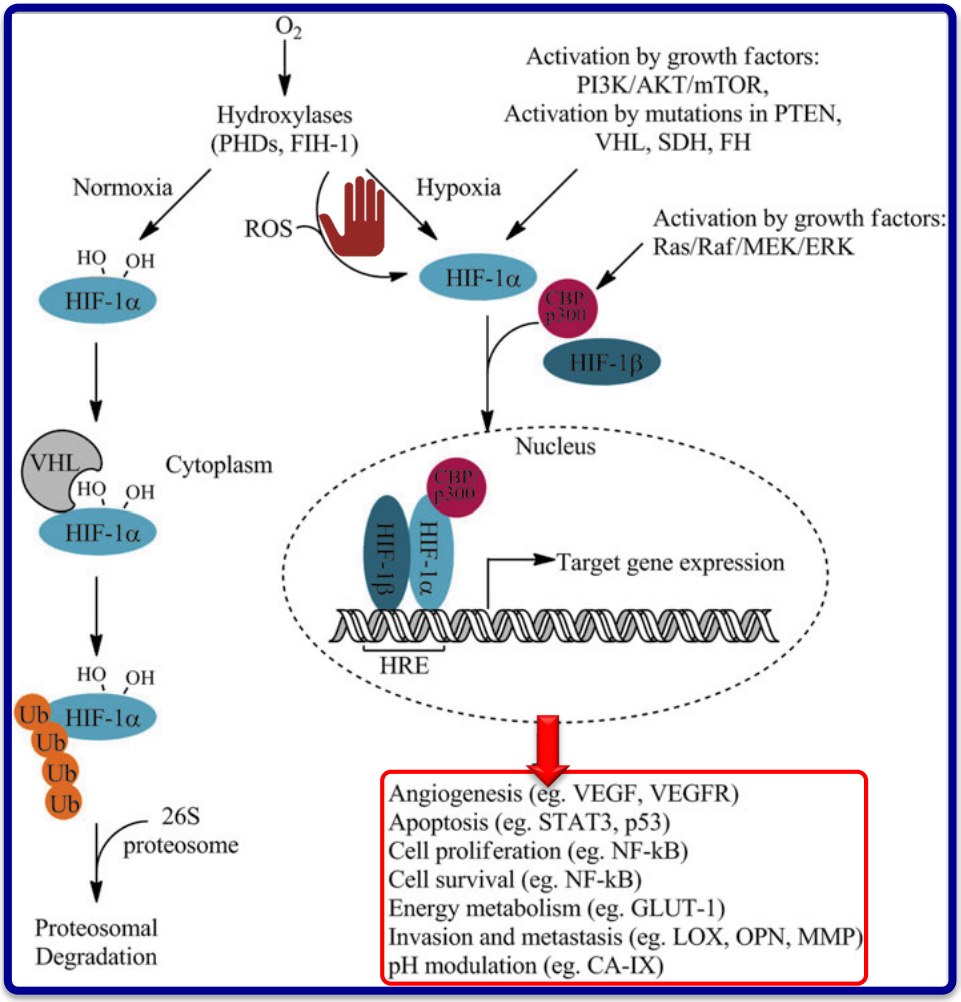
Endothelial dysfunction/ox-LDL/extracellular matrix injury

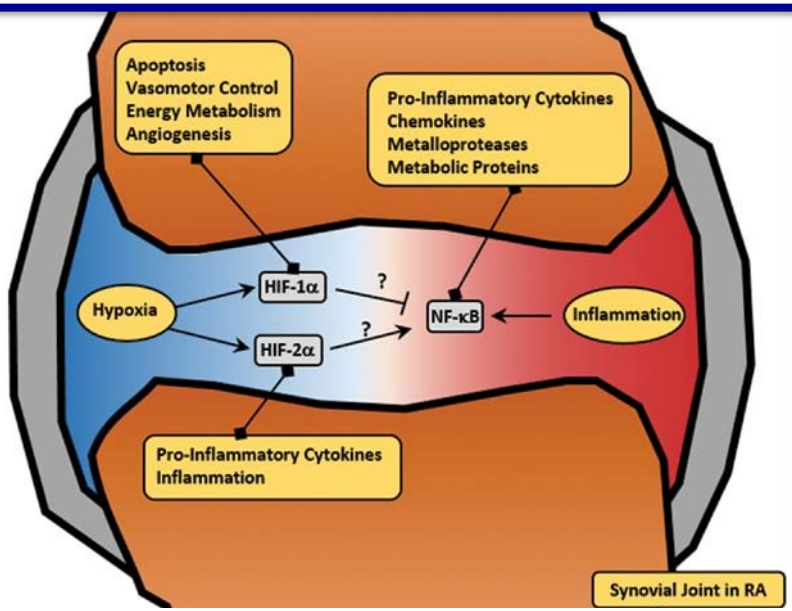
EXTRACELLULAR OXIDATIVE STRESS

REDUCED PERFORMANCES
DELAYED RECOVERY

Improving oxygen bioavailability is mandatory in order to prevent oxidative stress and its unwanted side effects!

Role of the hypoxia-inducible factor 1–α (HIF–α)





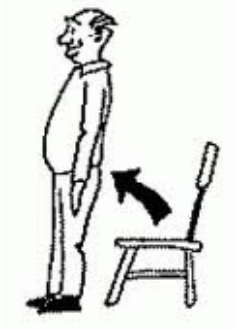
☞In arthritis, the synovial join is characterised by hypoxic and inflammatory regions (in blue and red, respectively).
☞Hypoxia leads to the activation of HIF-1α, which is involved in several cellular processes (such as apoptosis, vasomotor control, energy metabolism and angiogenesis).
☞Additionally, hypoxia leads to the activation of HIF-2α, which is involved in the activation of pro-inflammatory cytokines.
☞In RA, inflammation leads to the activation of NF-κB, which activates a pro-inflammatory programme, including pro-inflammatory cytokines, chemokines, metalloproteases and metabolic proteins.
☞While HIF-1α has been implicated in the repression of the NF-κB pathway, HIF-2α has been shown to increase inflammation.

Relationships between hypoxia, oxidative stress and inflammation.

The paradigma of hypoxic oxidative stress

ISCHEMIA

REPERFUSION



Ipoxanthine → Xanthine → Uric acid

XANTHINE DEHYDROGENASE

XANTHINE OXIDASE

$H_2O + O_2$ → H_2O_2

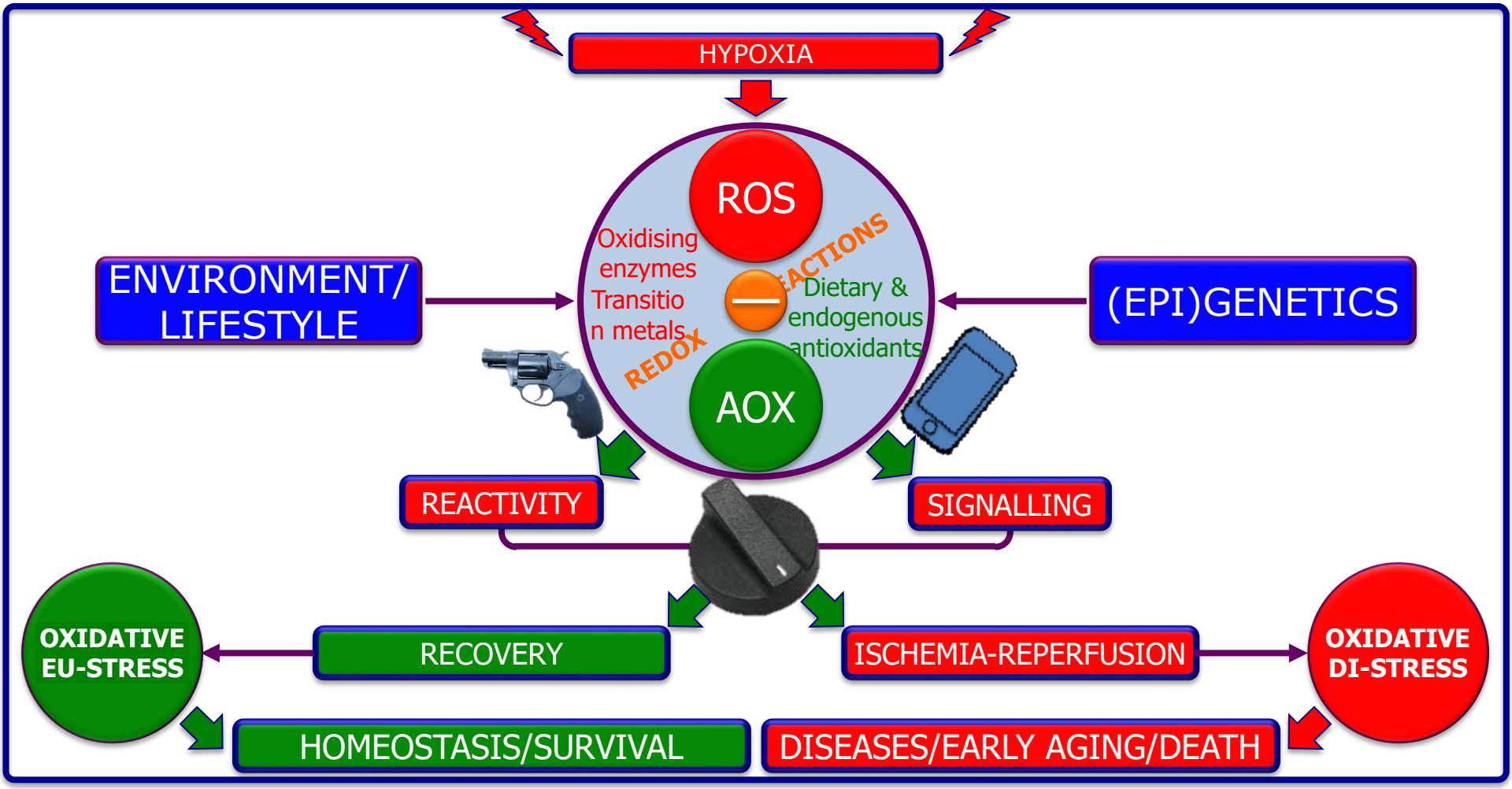
XANTHINE DEHYDROGENASE

XANTHINE OXIDASE

O_2 → $O_2^{\bullet -}$

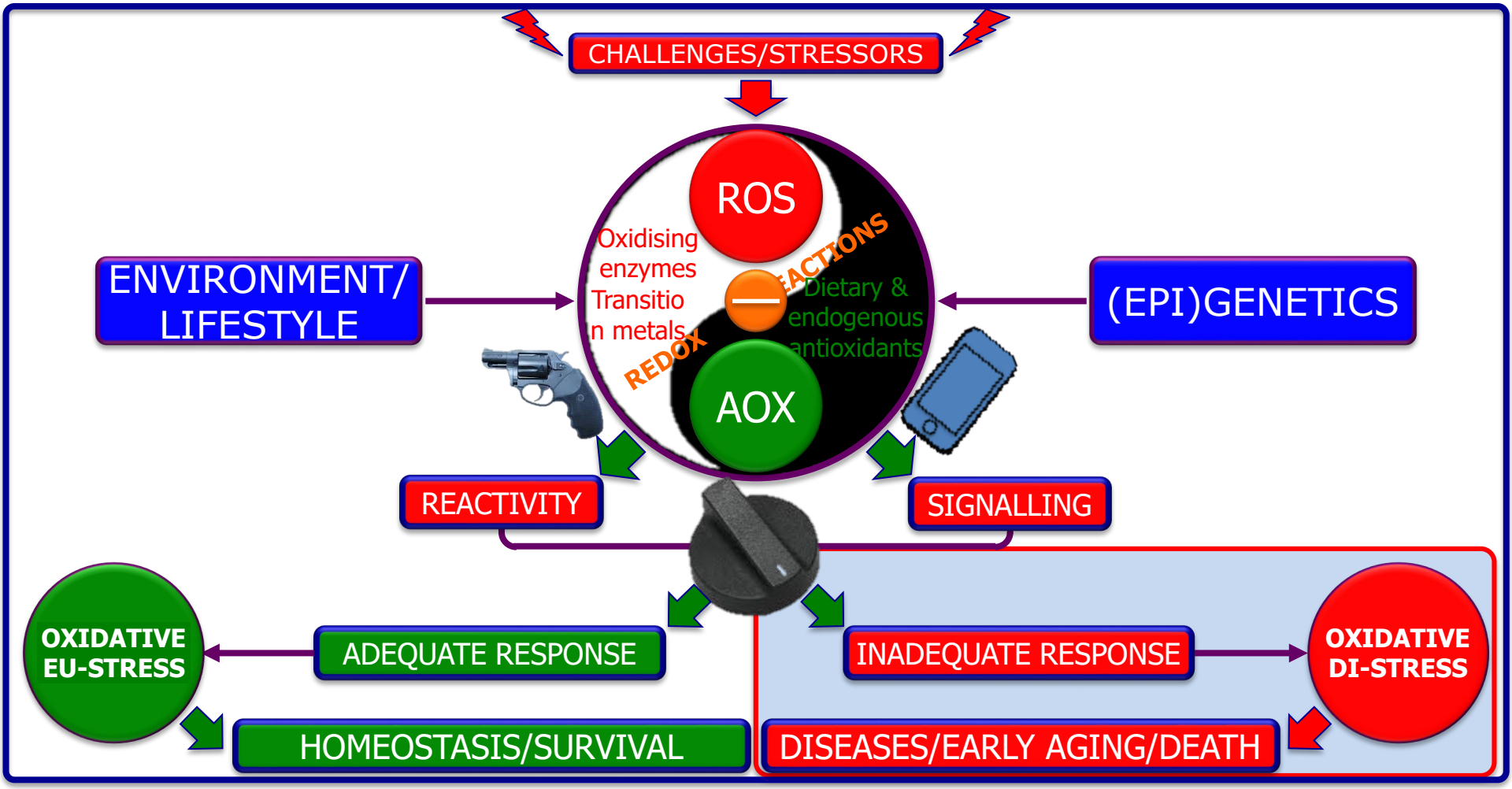
Reperfusion after ischemia may lead to reactive oxygen species production due to xanthine oxidase activation

From hypoxic oxidative eu-stress (safe recovery) to its di-stress form (ischemia-reperfusion damage)



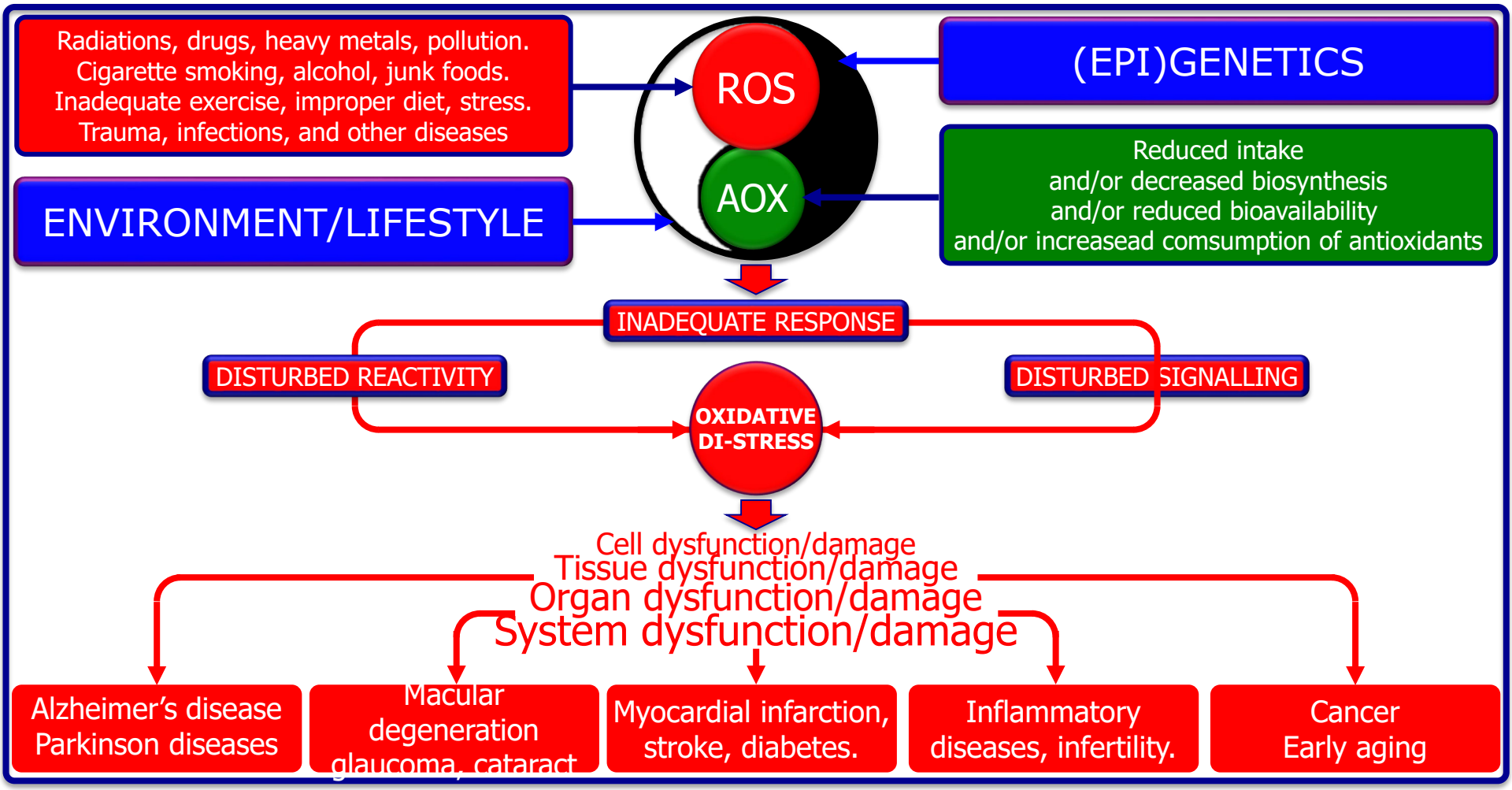
Improper redox-mediated detoxification function may lead to chronic diseases and death

From redox system and oxidative stress . . .



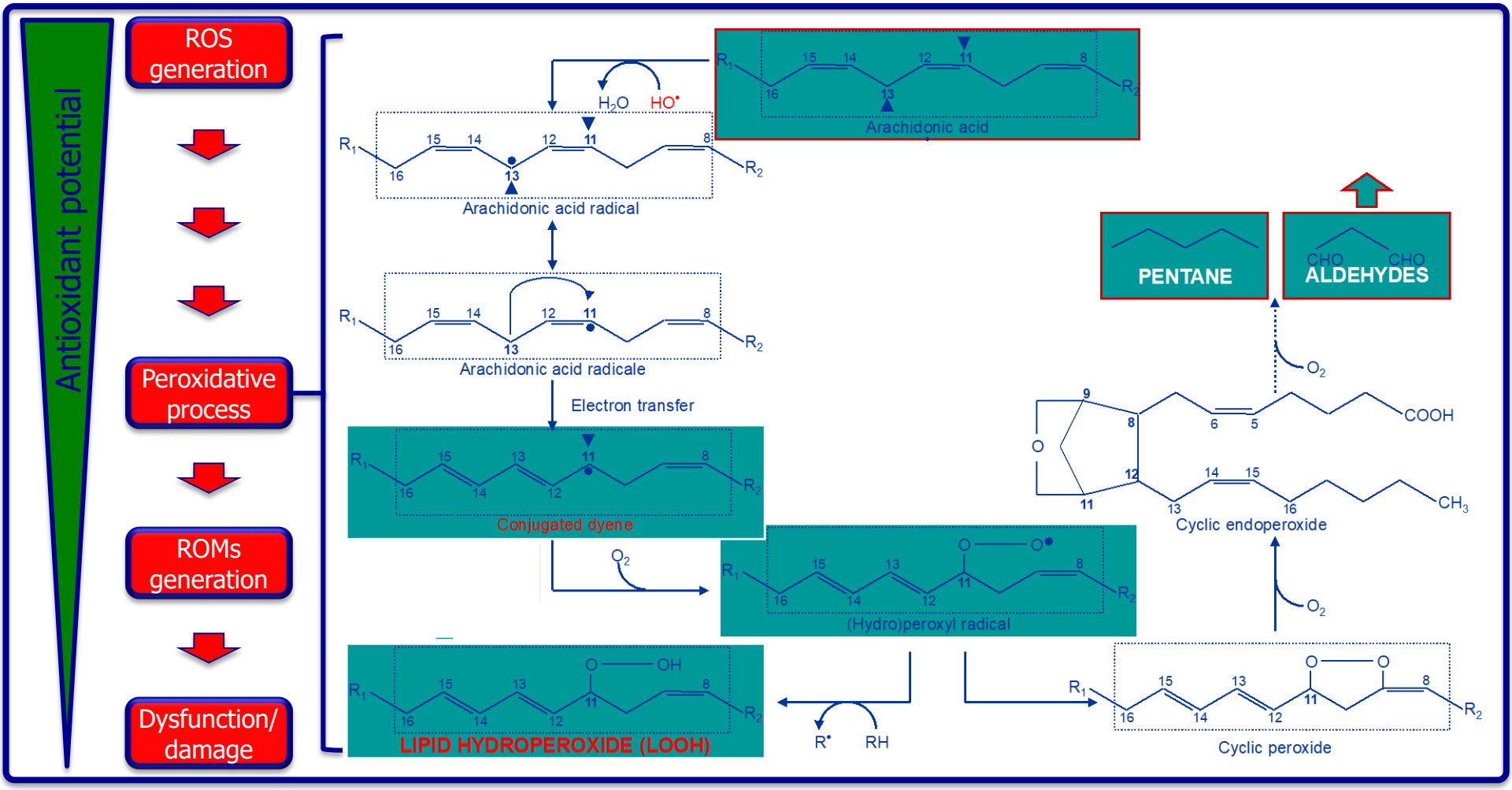
. . . to the TAO REDOX!

Oxidative di-stress and diseases



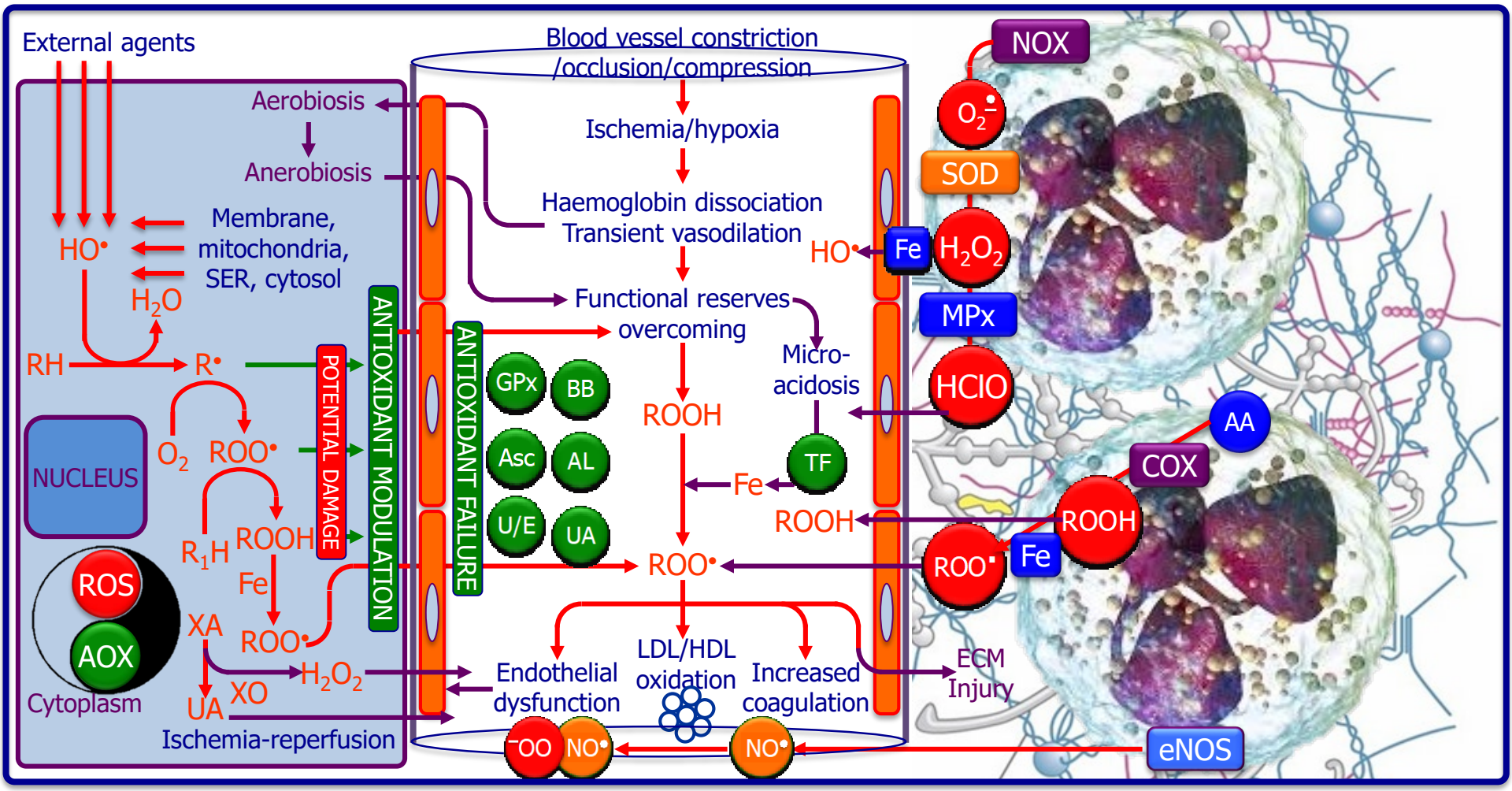
Oxidative di-stress can be either the cause or the effect of diseases but it must be identified as early as possible

Oxidative di-stress is a multistep processes with many possible variables.



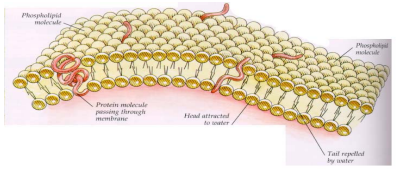
The peroxidative process, a model for oxidative stress understanding.

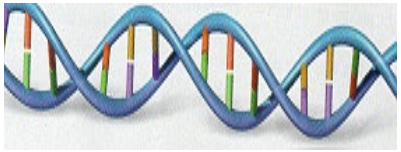
Let's go inside the tissues

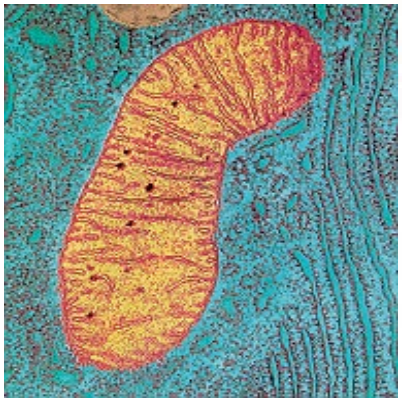


Cellular and biochemical basis of oxidative stress

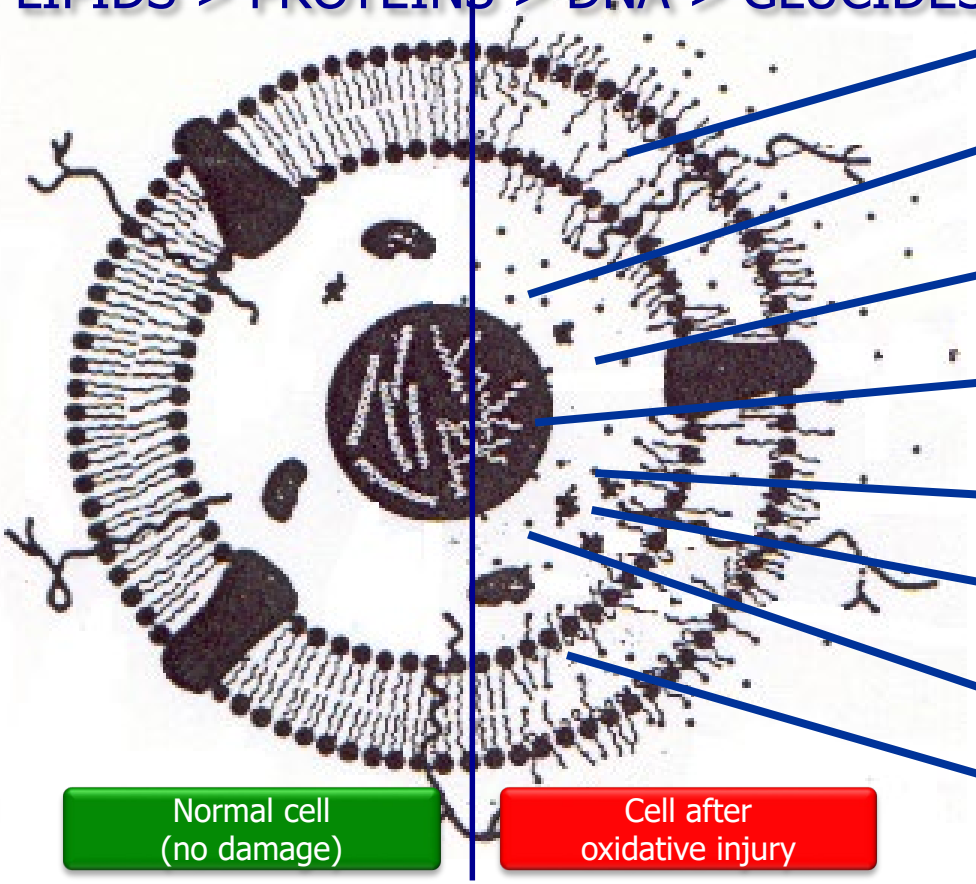
The primary targets of oxidative di-stress: the cell.







LIPIDS > PROTEINS > DNA > GLUCIDES.



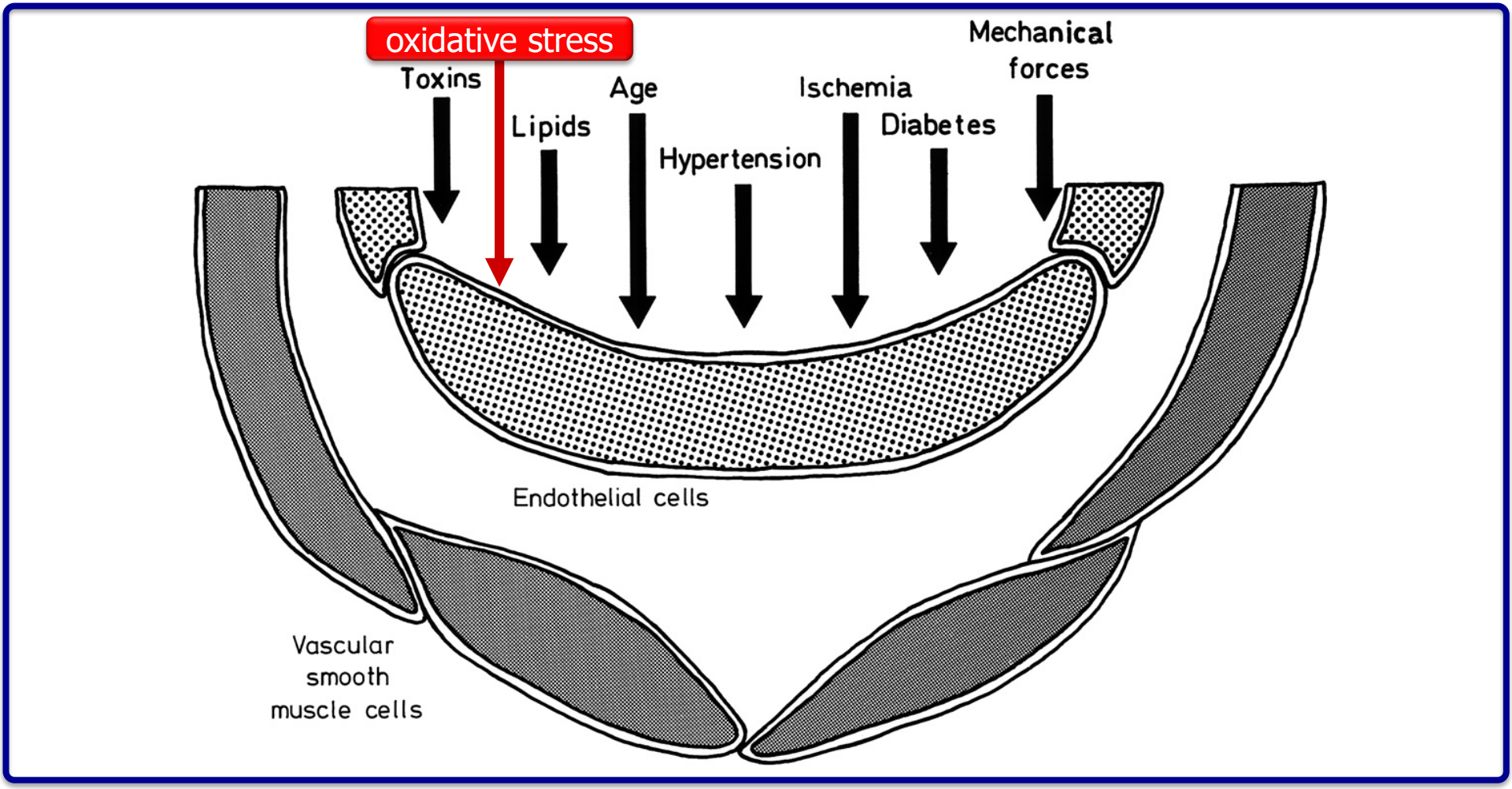
- Lipids peroxidation
- Enzymatic changes
- AAs and proteins (per)oxidation
- DNA damage
- Carbohydrate peroxidation
- Protein glycation
- Ion homeostasis impairment
- Disturbed signalling

Normal cell (no damage)

Cell after oxidative injury

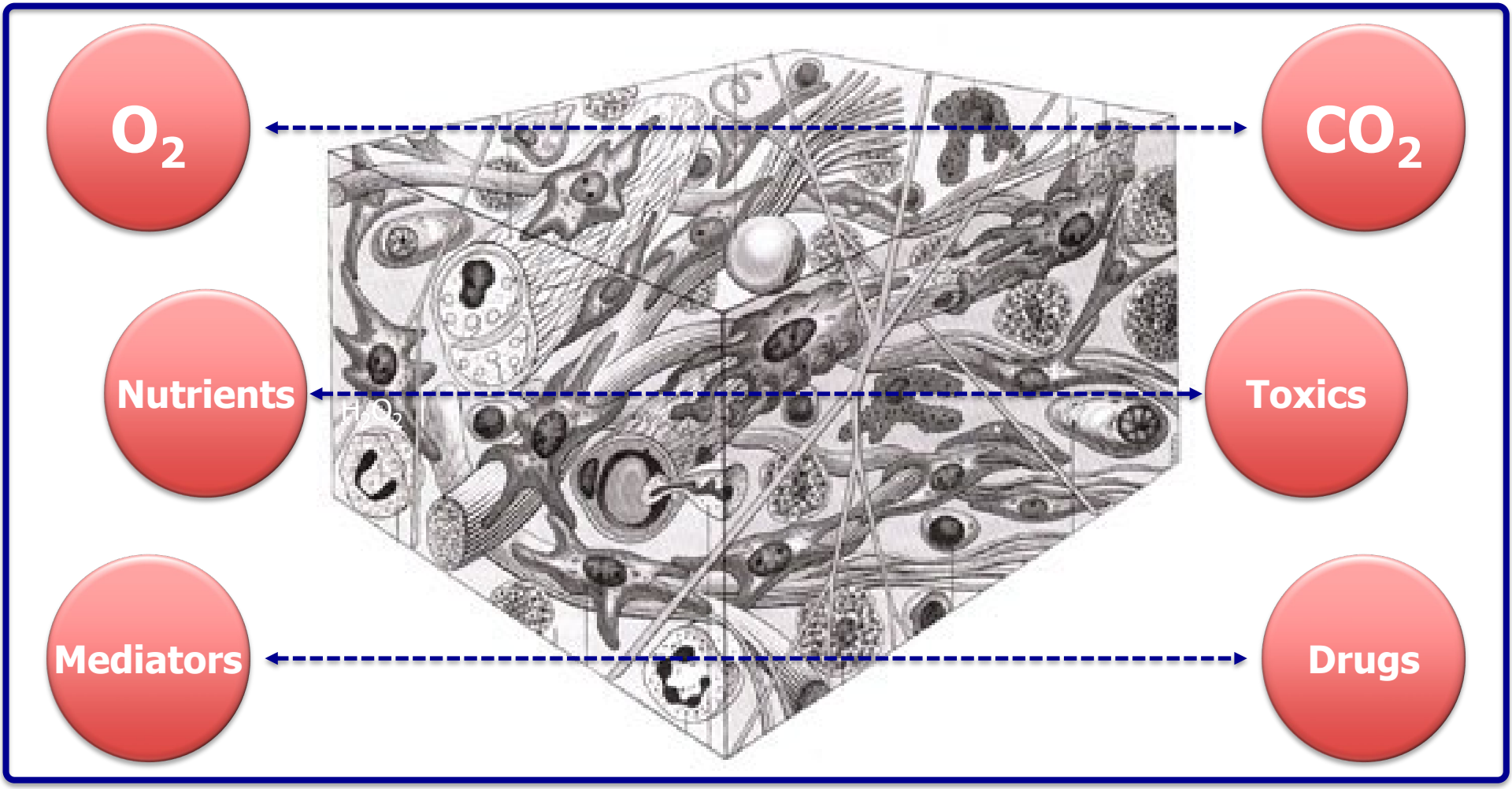
Oxidants disturbs cell signalling/transduction and can hit non only lipids but also proteins and nucleic acids

Other primary targets of oxidative di-stress: the blood vessels endothelium system.



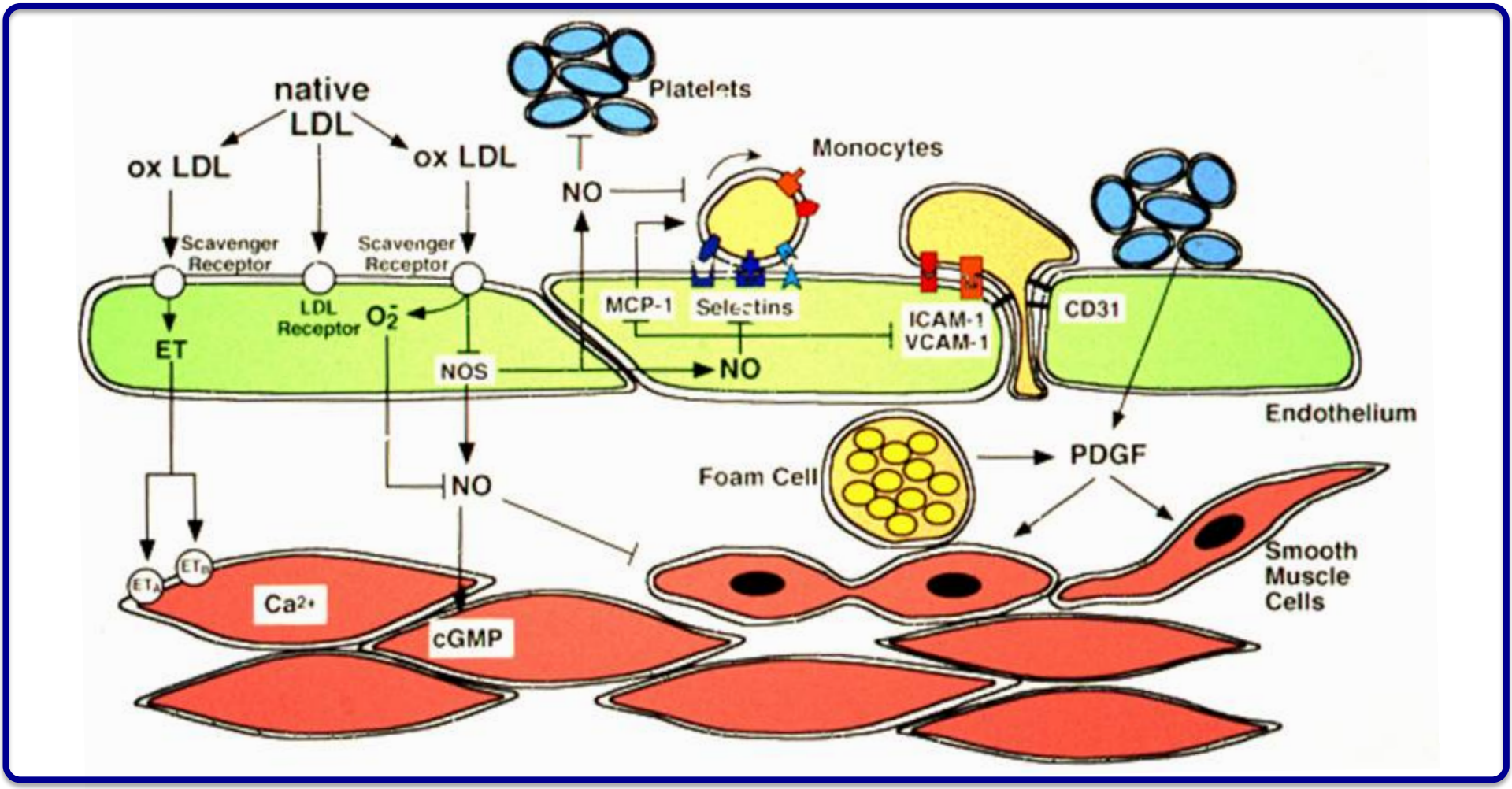
Nitrosative stress leads to endothelial dysfunction thus triggering/worsening atherosclerosis process

Other primary targets of oxidative di-stress: the extracellular matrix (non only that one of skin).



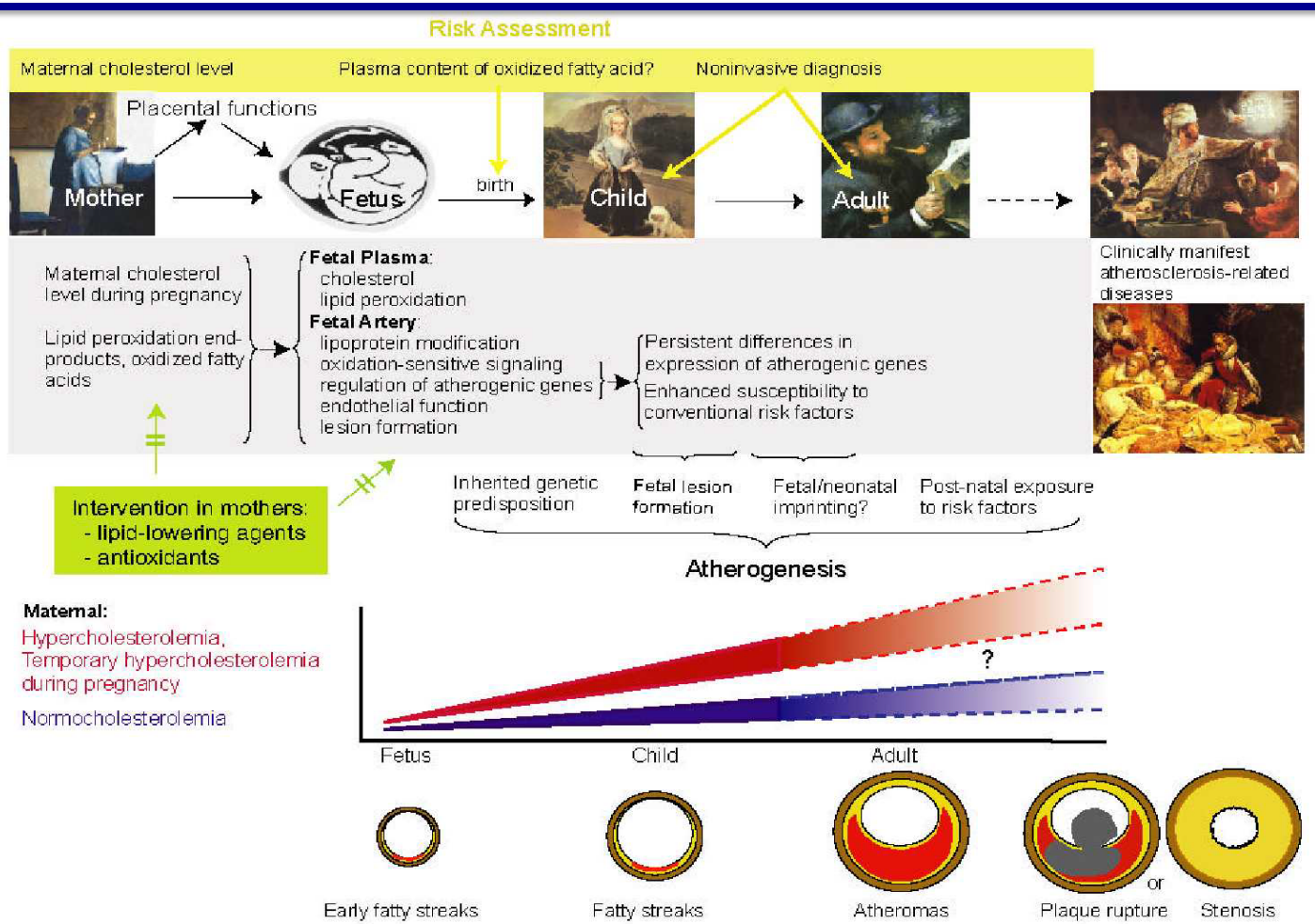
Oxidative di-stress may impair both metabolic and informational flow between the blood and the cells

Biochemical bases of atherogenesis



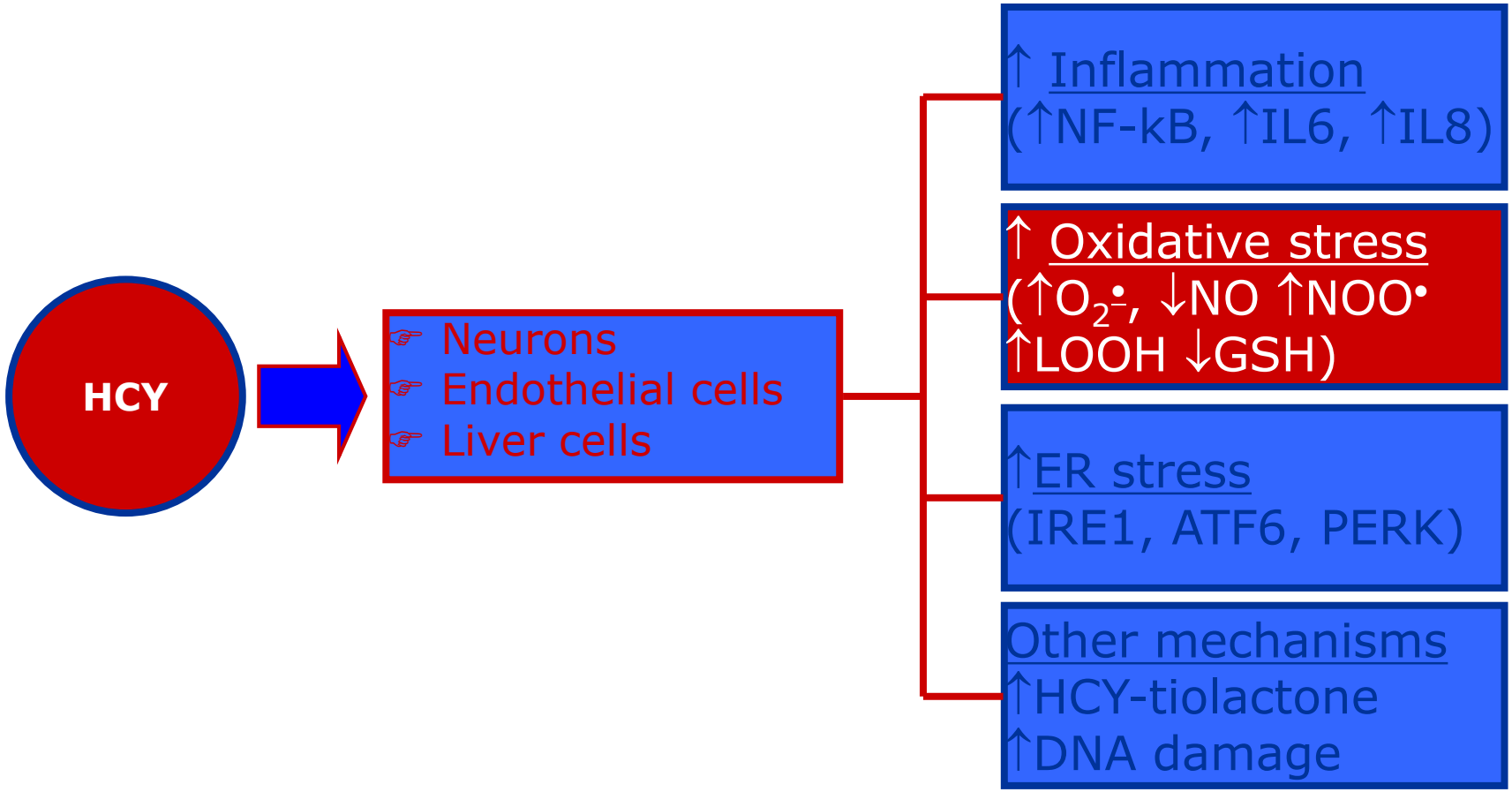
The role of oxidative stress and dyslipidemia

The atherogenetic process



Its stars during pregancy

Homocysteine and oxidative stress



Oxidative stress is on of the 4 pathophysiological mechanisms involved in HCT-induced cell damage

Targets of oxidative stress: the nervous systems.

☞ Reactive species, particularly ROS, were shown implicated in the pathology of **a number of neurological disorders**.

☞ Oxidative stress in the brain **is likely to occur as the brain uses up to 20% of the body's inspired oxygen**, yet only accounts for 2% body weight.

☞ The brain (neuronal plasmamembranes) also houses **large concentrations of polyunsaturated fatty acids**, which may undergo **trans-conversion** and **lipid peroxidation** in such an oxygen rich environment.

☞ Brain **catecholamines easily undergo auto-oxidation phenomena**, thus generating ROS.

☞ Brain contains **conspicuous amounts of iron** (a powerful catalyst of free radical generation), although in a inactive form (chelated).

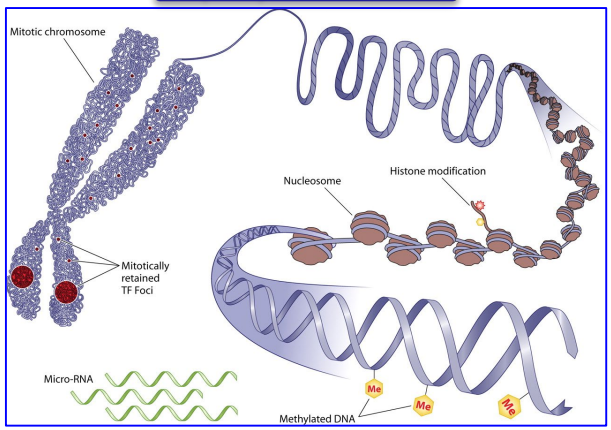
☞ Physiologically, brain exhibits low antioxidant defences (vitamin C, vitamin E, glutathione, and superoxide dismutase). Moreover, **reduced levels of antioxidants** such as vitamin E and C have been reported in many neurological conditions.

☞ The **supplementation of Vitamin E in deficient individuals** has been reported to either prevent or at least slow the progression of some neurological features.

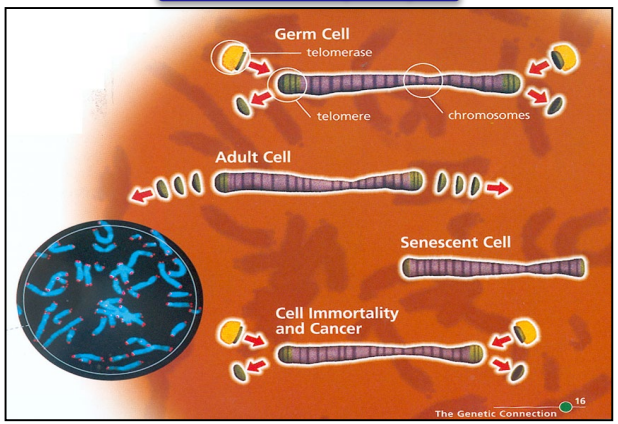
Neurons are particularly prone to oxidative stress

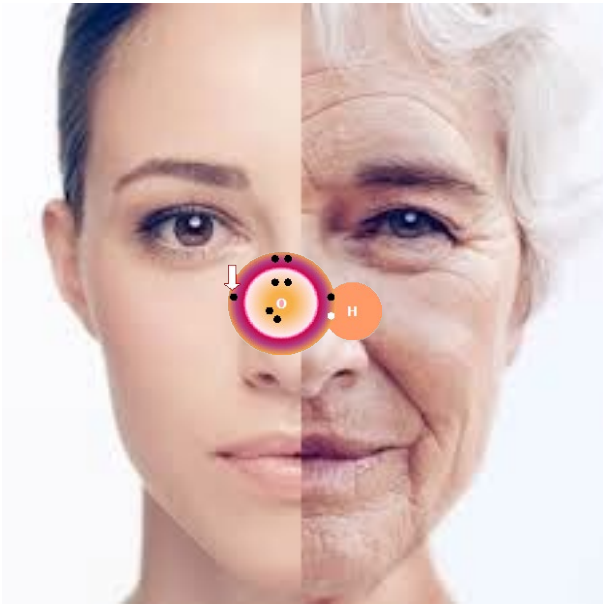
Perché s'invecchia?

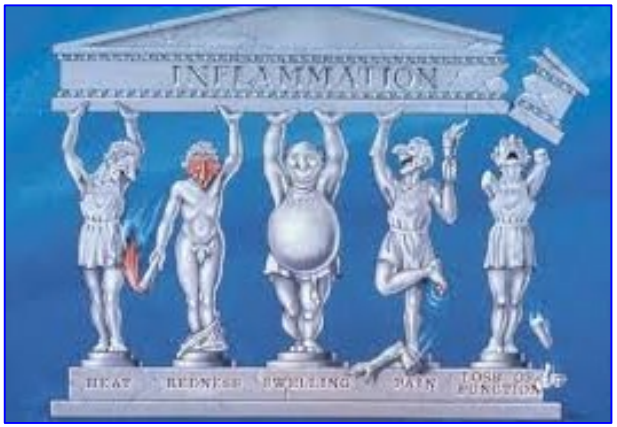
(EPI)GENETICS



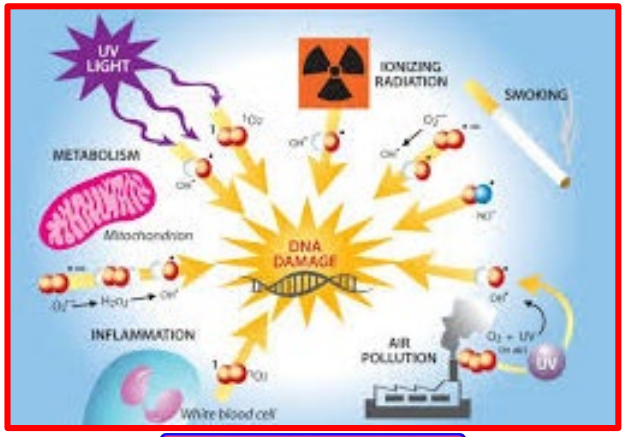
TELOMER(ASE)







(INFLAM)MAGING



OXIDATIVE STRESS

Quattro ipotesi … stress ossidativo come un possibile trait-d'union?

La teoria REDOX è l'unica in grado di spiegare in maniera esaustiva l'invecchiamento

☞Tutte le modificazioni di rilievo che caratterizzano l'invecchiamento si verificano a seguito dell'attivazione dei sistemi di esposizione, memoria e sviluppo codificati nel genoma. L'esecuzione dei programmi di sviluppo e le risposte alle esposizioni alla dieta ed altre condizioni ambientali alterano la metilazione del DNA e i tratti epigenetici che controllano l'espressione genica.

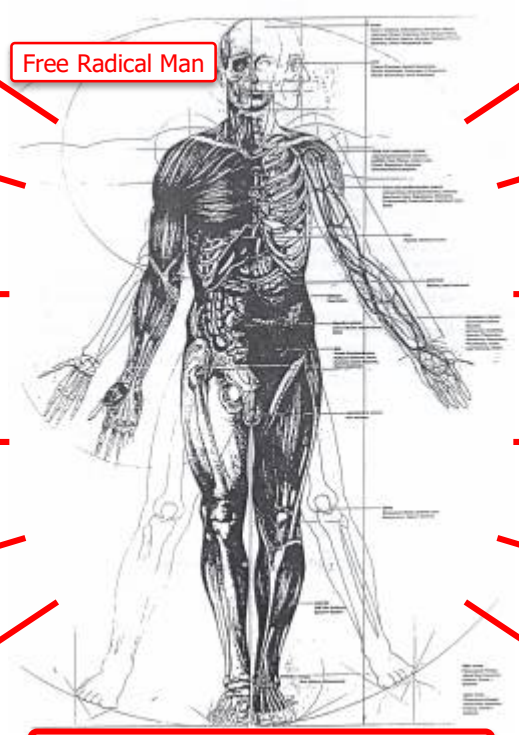
☞Programmi di sviluppo, esposizioni alimentari ed ambientali determinano funzione dei sistemi proteostatici, che coinvolgono la sintesi ed il catabolismo delle proteine, insieme a modificazioni epiproteomiche. Risposte permanenti agli eccessi alimentari e carenze, nel contesto delle risposte precocemente impresse alla dieta ed alle esposizioni, deregolamentano il rilevamento delle sostanze nutritive ed il loro utilizzo. Sistemi di memoria per la disponibilità, qualità e utilizzo del cibo causano disfunzione mitocondriale. Programmi di differenziazione, esposizioni permanenti cumulative ed esecuzione di programmi di risposta conducono a senescenza cellulare.

☞L'accorciamento dei telomeri è un sistema di memoria di differenziazione e di esposizione per gli organismi complessi. L'esaurimento delle cellule staminali è una conseguenza del sistema evoluto di memoria di esposizione e differenziazione. L'alterata comunicazione intercellulare è la conseguenza dell'esecuzione dei programmi di differenziazione e di risposte cumulative del sistema adattativo della memoria.

☞Si verifica instabilità genomica probabilmente come espressione del fallimento dei sistemi di differenziazione e di memoria esposizione, ma dell'esecuzione dei meccanismi genetici o delle funzioni trasposoni-dipendenti ancora ampiamente sconosciute.

Jones, 2015.

The free radical man



- Alzheimer's disease
- Stroke
- Atherosclerosis
- Pancreatitis
- Myocardial infarction
- Obesity
- Parkinsons' disease
- Eye diseases
- Rheumatoid arthritis
- Chron's disease
- Diabetes mellitus
- Cancer
- AGING
- INFERTILITY

Early aging and more than 100 diseases are related to oxidative stress

The REDOX system dysfunction (oxidative di-stress) is related to early aging and more than 100 diseases

Aceruloplasminemia	Aceruloplasminemia	Aceruloplasminemia
Acute/chronic alcoholic liver disease	Acute/chronic alcoholic liver disease	Acute/chronic alcoholic liver disease
Acute autoimmune myocarditis	Acute autoimmune myocarditis	Acute autoimmune myocarditis
Acute chest syndrome of sickle cell disease	Acute chest syndrome of sickle cell disease	Acute chest syndrome of sickle cell disease
Acute pancreatitis	Acute pancreatitis	Acute pancreatitis
Acute Respiratory Distress Syndrome	Acute Respiratory Distress Syndrome	Acute Respiratory Distress Syndrome
Alcoholic liver disease	**Alcoholic liver disease**	**Alcoholic liver disease**
Alzheimr's disease	Alzheimr's disease	Alzheimr's disease
Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis
Arterial/systemic hypertension	**Arterial/systemic hypertension**	**Arterial/systemic hypertension**
Asbestosis	Asbestosis	Asbestosis
Asthma	Asthma	Asthma
Ataxia telangiectasia	Ataxia telangiectasia	Ataxia telangiectasia
Atherosclerosis	**Atherosclerosis**	**Atherosclerosis**
Atopic dermatitis	Atopic dermatitis	Atopic dermatitis
Brain ischemia	**Brain ischemia**	**Brain ischemia**
Bronchopulmonary dysplasia	Bronchopulmonary dysplasia	Bronchopulmonary dysplasia
Burns	Burns	Burns
Cancer (several kinds)	**Cancer (several kinds)**	**Cancer (several kinds)**
Cardiopulmonary bypass	Cardiopulmonary bypass	Cardiopulmonary bypass
Cardiovascular diseases	**Cardiovascular diseases**	**Cardiovascular diseases**
Cataract	**Cataract**	**Cataract**
Cellulitis	Cellulitis	Cellulitis
Chemoterapy side-effect	**Chemoterapy side-effect**	**Chemoterapy side-effect**
Chronic fatigue syndrome	Chronic fatigue syndrome	Chronic fatigue syndrome
Chronic hepatitis C	Chronic hepatitis C	Chronic hepatitis C
Chronic kidney disease	Chronic kidney disease	Chronic kidney disease
Chronic Obstructive Pulmonary Disease	Chronic Obstructive Pulmonary Disease	Chronic Obstructive Pulmonary Disease
Chronic renal failure	Chronic renal failure	Chronic renal failure
Colitis	**Colitis**	**Colitis**
Coronary artery disease	**Coronary artery disease**	**Coronary artery disease**
Creutzfeldt–Jakob disease	Creutzfeldt–Jakob disease	Creutzfeldt–Jakob disease
Crohn disease	**Crohn disease**	**Crohn disease**
Cutaneous leishmaniasis	Cutaneous leishmaniasis	Cutaneous leishmaniasis
Cystic fibrosis	Cystic fibrosis	Cystic fibrosis
Diabetes mellitus type 1	Diabetes mellitus type 1	Diabetes mellitus type 1
Diabetes mellitus type 2	**Diabetes mellitus type 2**	**Diabetes mellitus type 2**
Dislipidemia	**Dislipidemia**	**Dislipidemia**

Most of these diseases are related to life style (Dalle Donne, 2006)

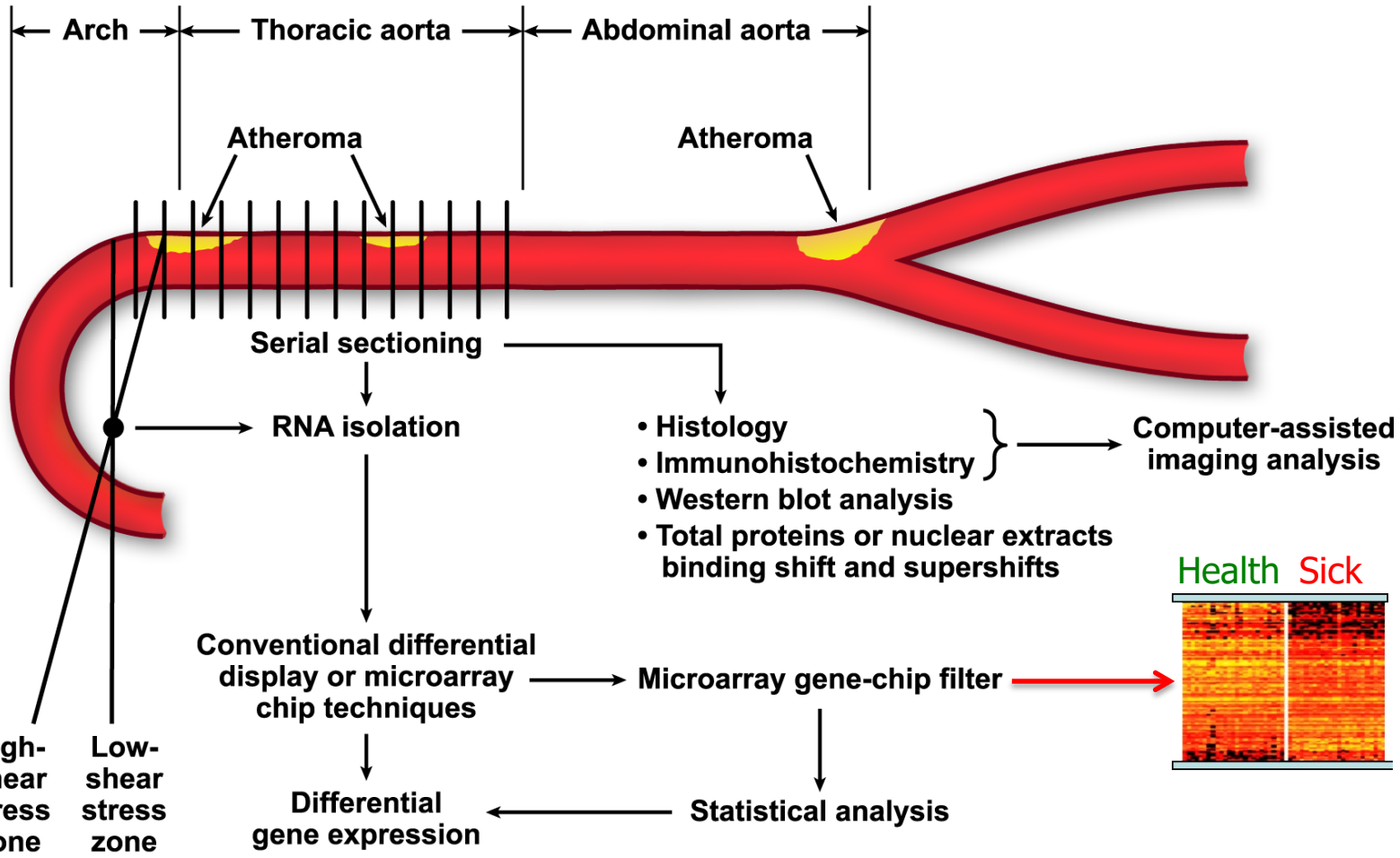
The oxidative di-stress can explode like dynamite at any time!

Oxidative di-stress does not show any specific clinical picture but can be identified only by laboratory tests

WARNING! Oxidative stress does not show any specific clinical picture!

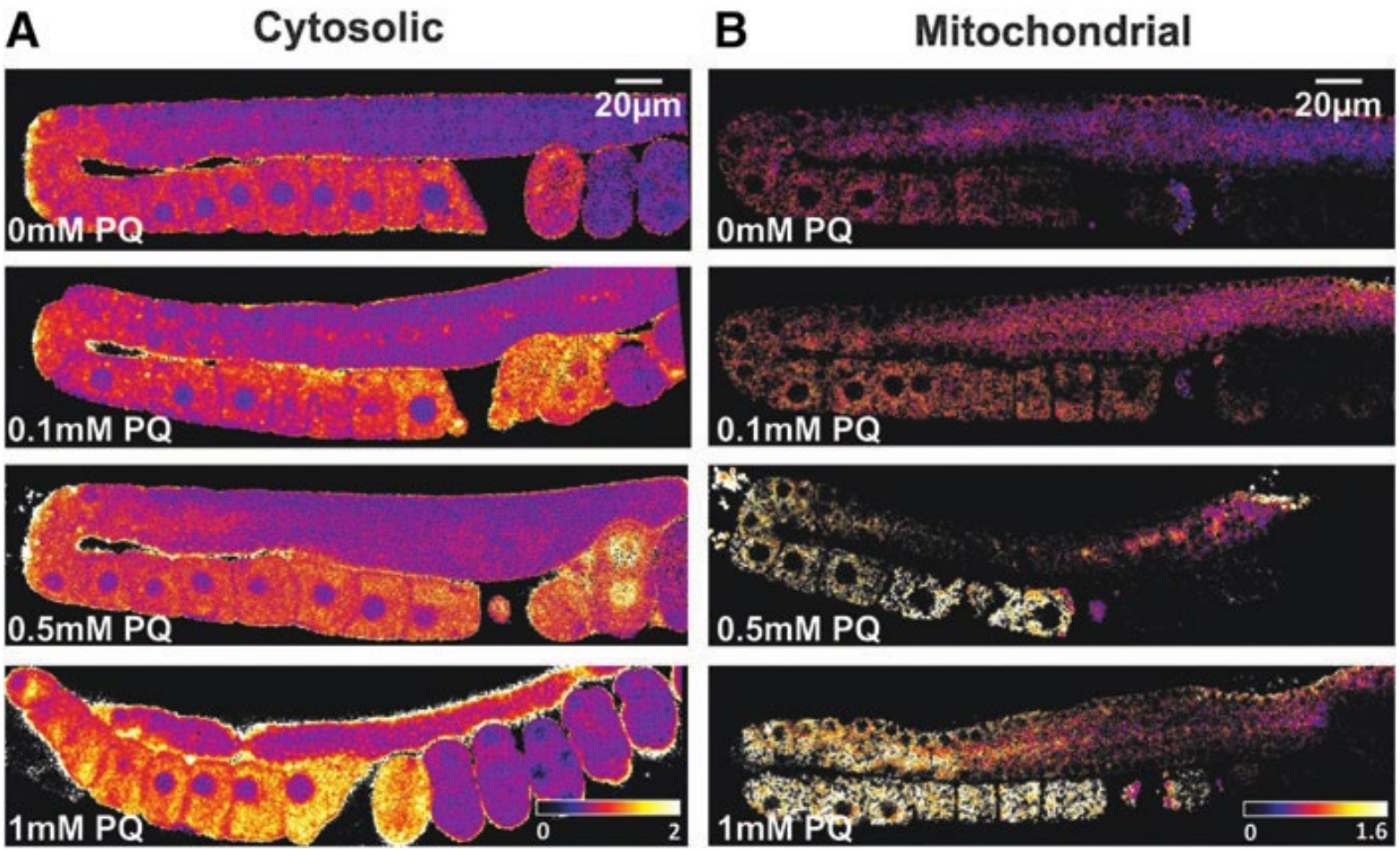
Oxidative stress can be identified and measured only by means of specific and suitable biochemical tests!

The challenge of new technologies.



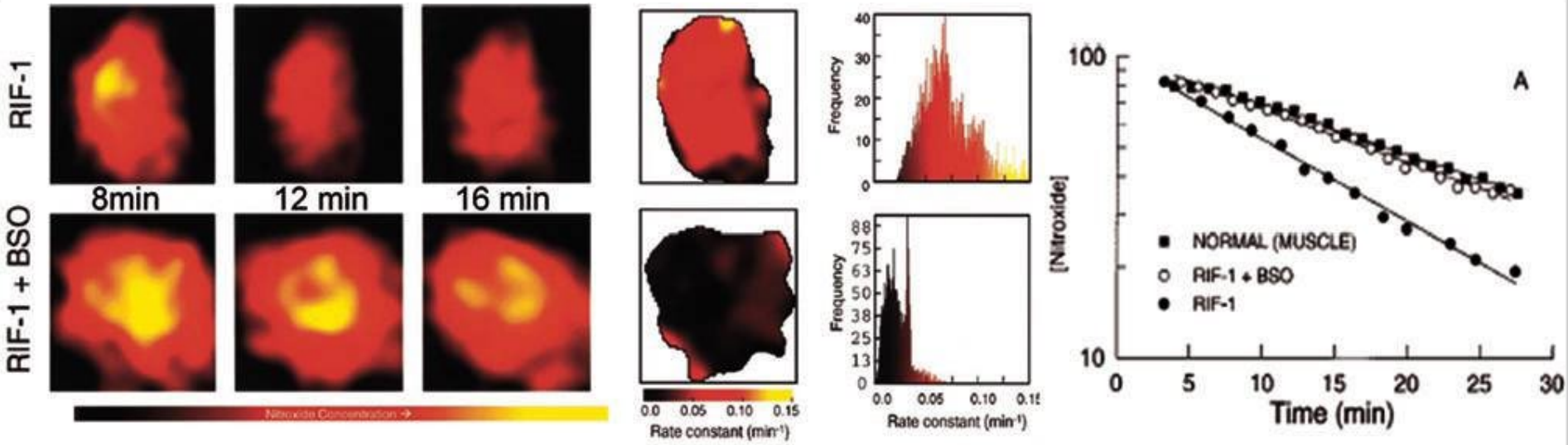
Napoli et Al. Heart. 2003. 89: 597–604.

Hydrogen peroxide mapping in vivo in Caenorhabditis elegans



Braeckman et Al. Antioxid Redox Signal. 2016. 25.

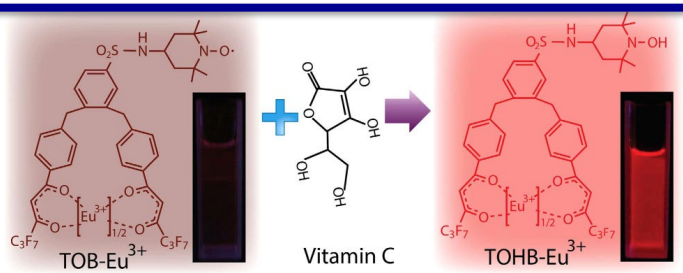
Reactive species (NO) can be detected *in vivo*: redox mapping of tumours is now available.

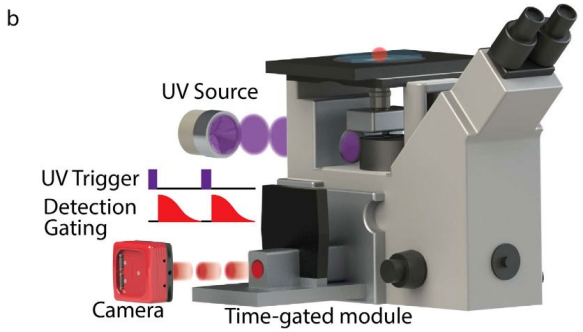


EPRI of the thigh of mouse with implanted RIF-1 tumour. **Left**: selected EPR images of clearance of 3CP nitroxide in untreated and BSO-induced (agent for glutathione synthesis) tumours. **Middle**: *redox mapping of the tumour; two dimensional mapping of pseudo-first-order rate constants and frequency plot of 3CP reduction rate constants*. **Right**: the semilog plot showing the whole tissue clearance of nitroxide in tumors and normal muscle of contra lateral leg. Images of tumour and muscle used for the measurement of pharmacokinetic data were collected simultaneously on the same animals.

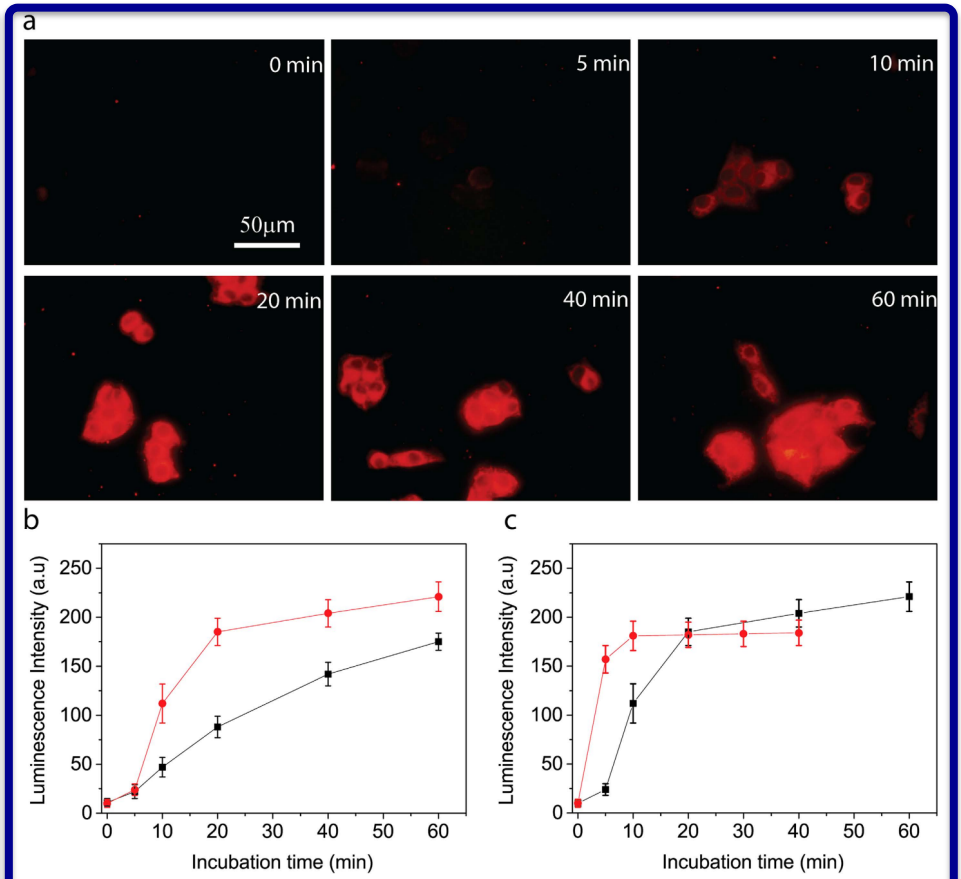
Maulucci et Al. Antioxid Redox Signal. 2016. 24.

Vitamin C can be detected in vivo





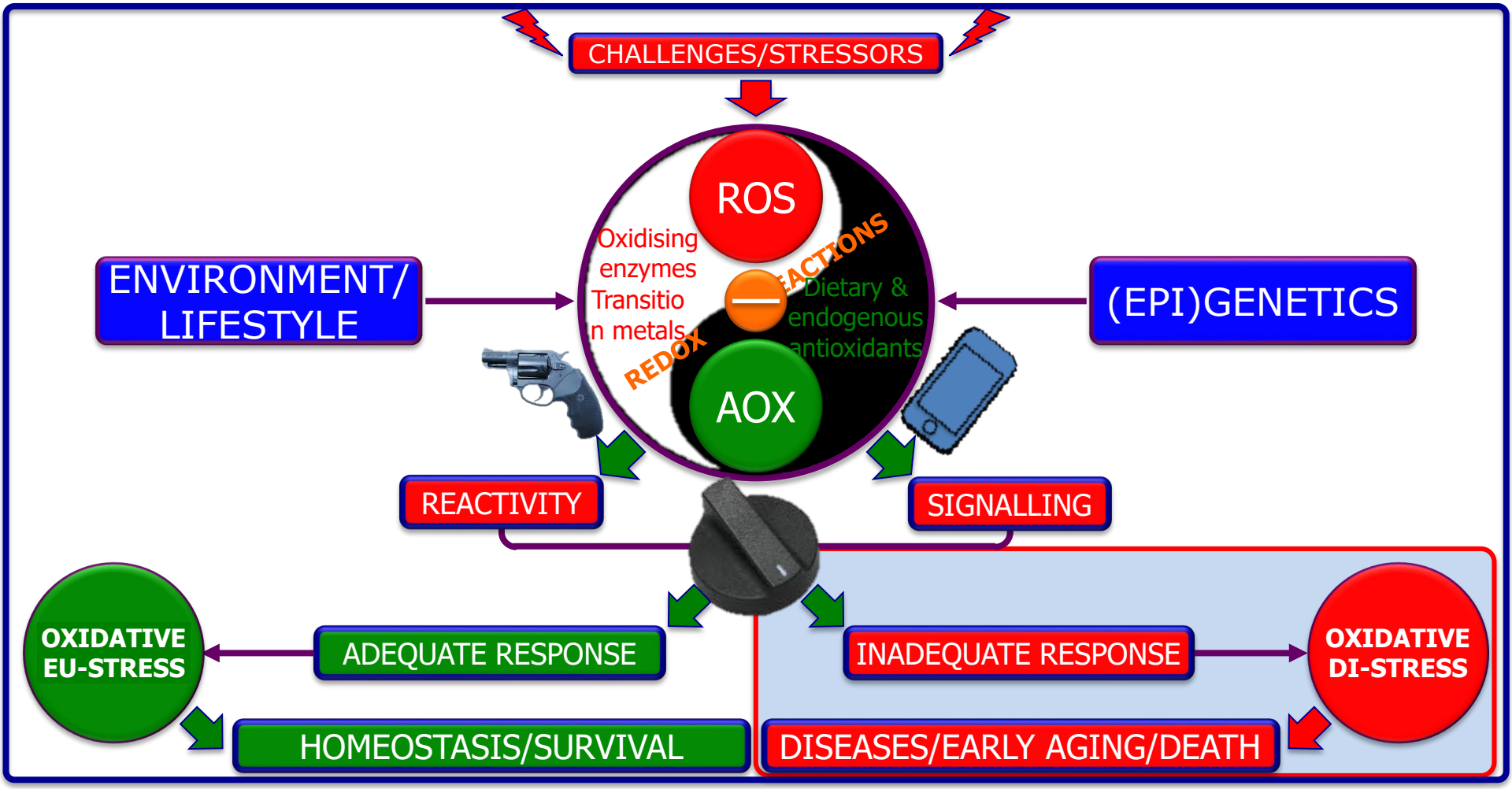
Schematic diagram illustrates the design of a "turn-on" molecular probe specific to vitamin C with long-lived luminescence suitable for time-gated luminescence microscopy (TGLM) system. (a) The luminescence probe TOB-Eu3+ turns on in its hydroxylamine derivative form of TOHB-Eu3+ in the presence of vitamin C (photographs show luminescence colours of the complex solutions under a 365 nm UV lamp). (b) The TGLM system employs a rapid-switching flash lamp synchronized to a time-gated optical chopper in front of the camera. The camera is externally shut off during the excitation pulse period, and a short time delay is given to allow the short-lived autofluorescence to vanish before the chopper opens for long-lived luminescence detection in absence of optical backgrounds.



Time-gated luminescence imaging and real-time quantifications of the extracellular vitamin C uptake and intracellular production of vitamin C in living cells (HepG). Vitamin C was used at 1 mM concentration.

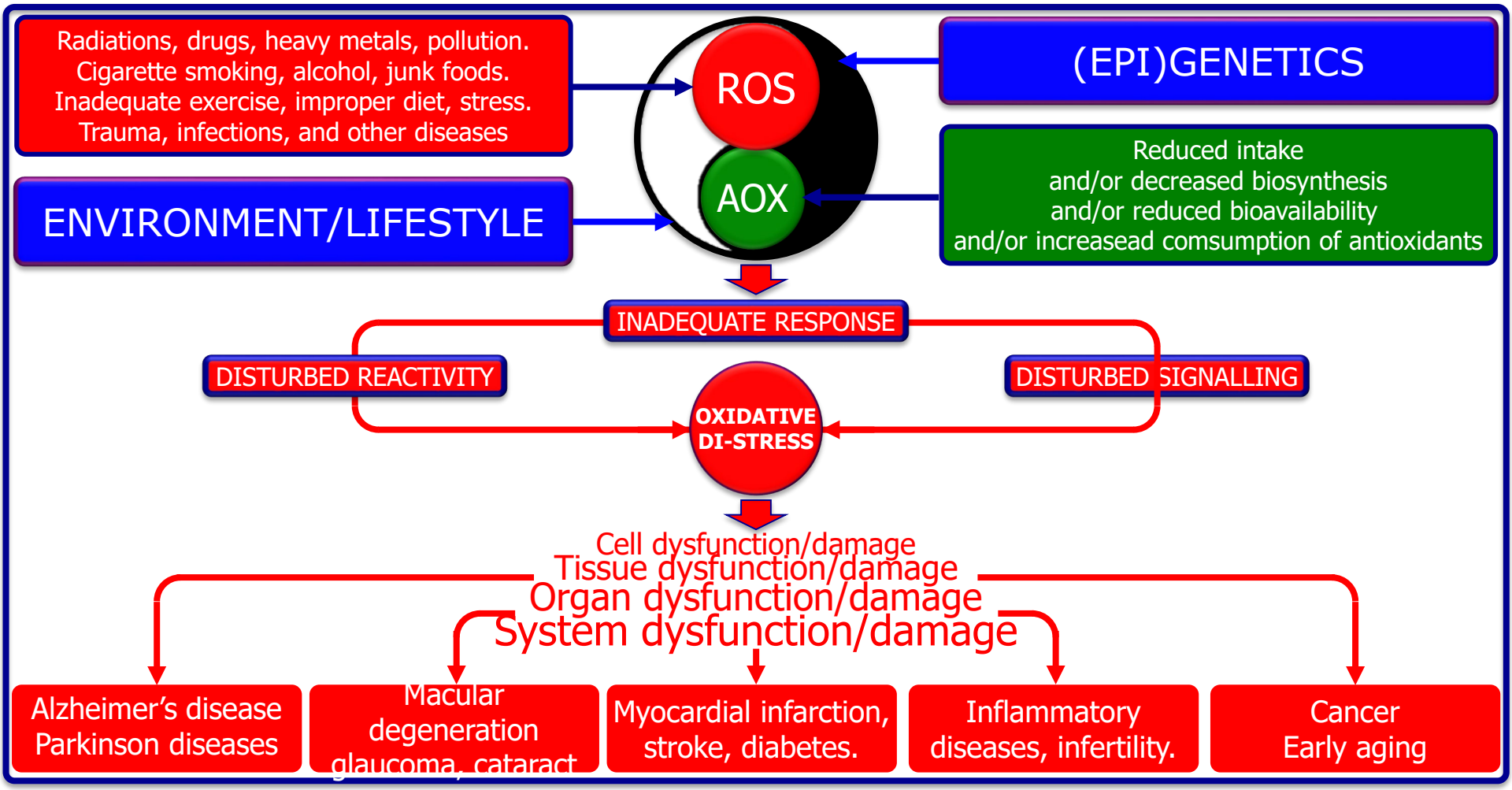
Song et Al. Sci Rep. 2015. 5: 14194.

While waiting direct measurements . . .



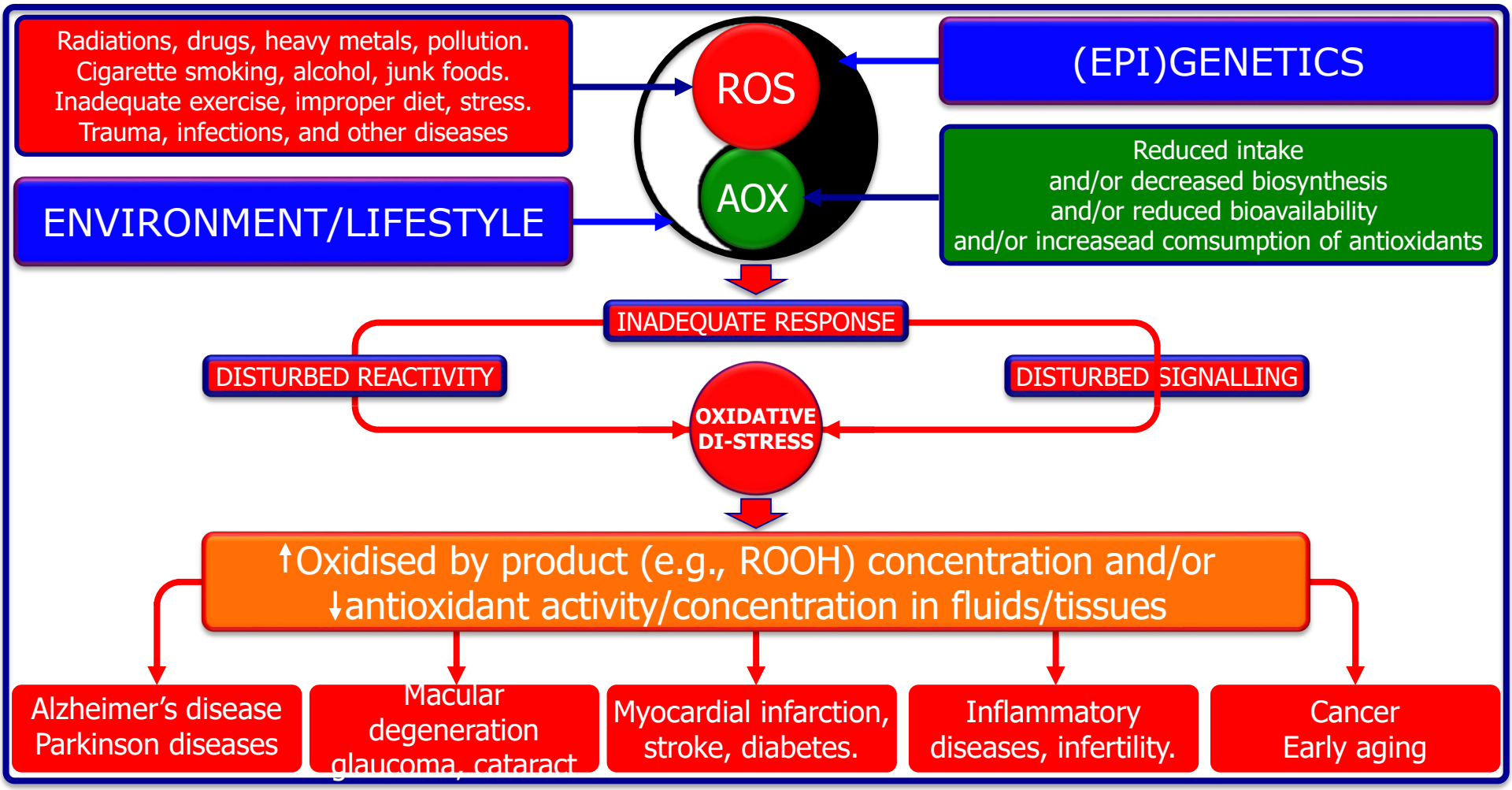
. . . take a step back!

Oxidative di-stress and diseases



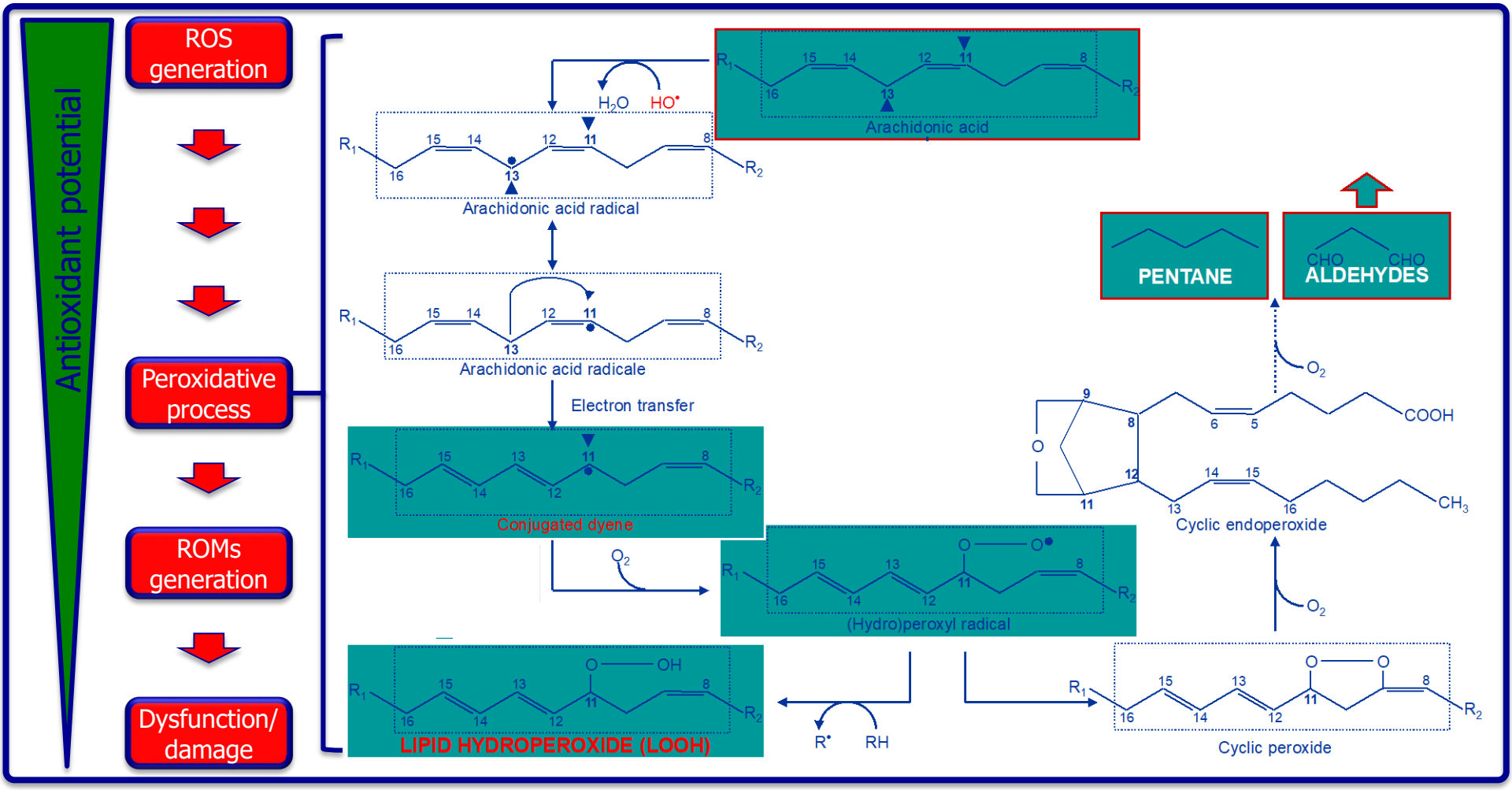
Oxidative di-stress can be either the cause or the effect of diseases but it must be identified as early as possible

Oxidative di-stress and diseases



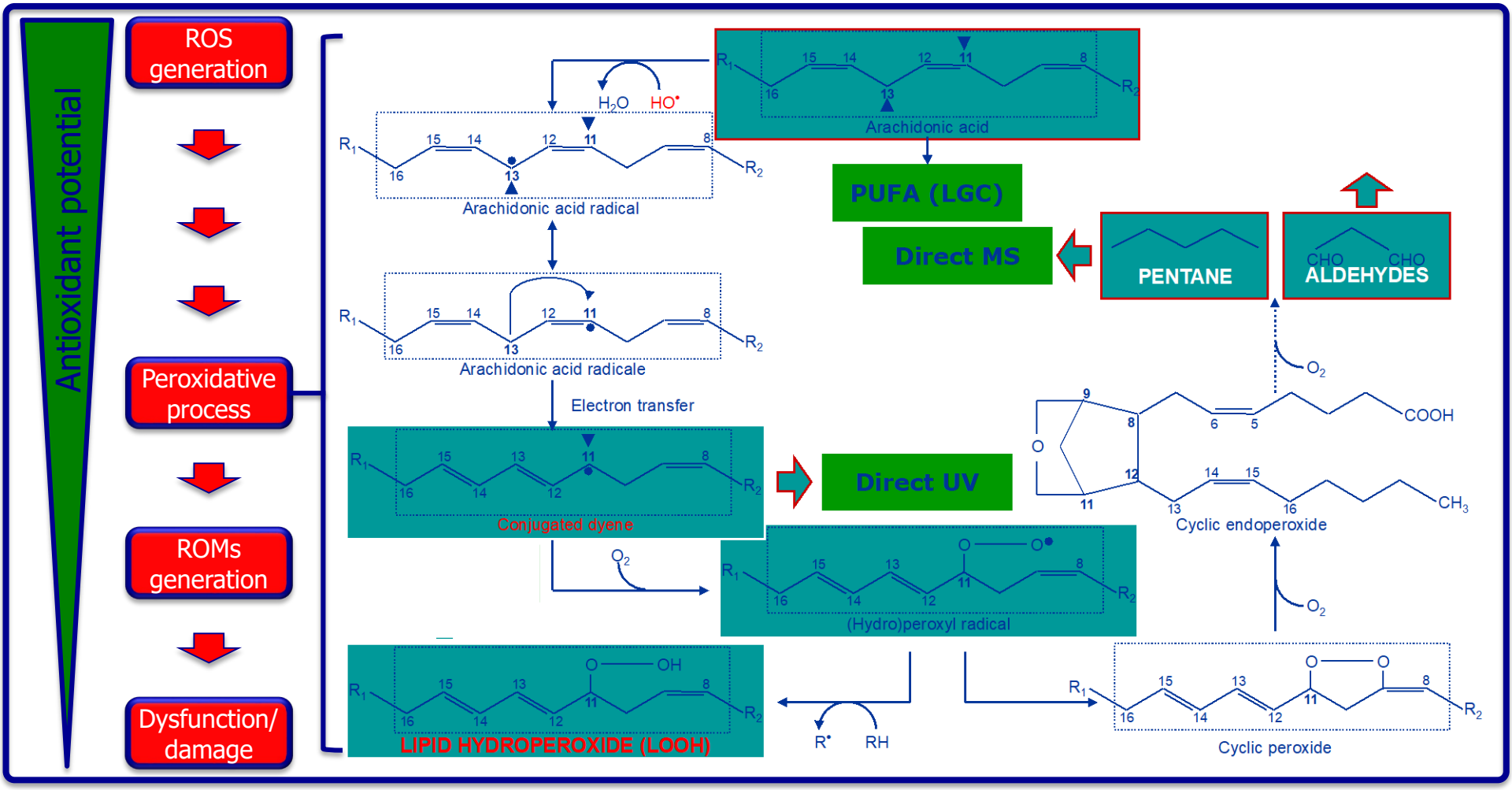
Oxidative di-stress can be either the cause or the effect of diseases but it must be identified as early as possible

The peroxidative process



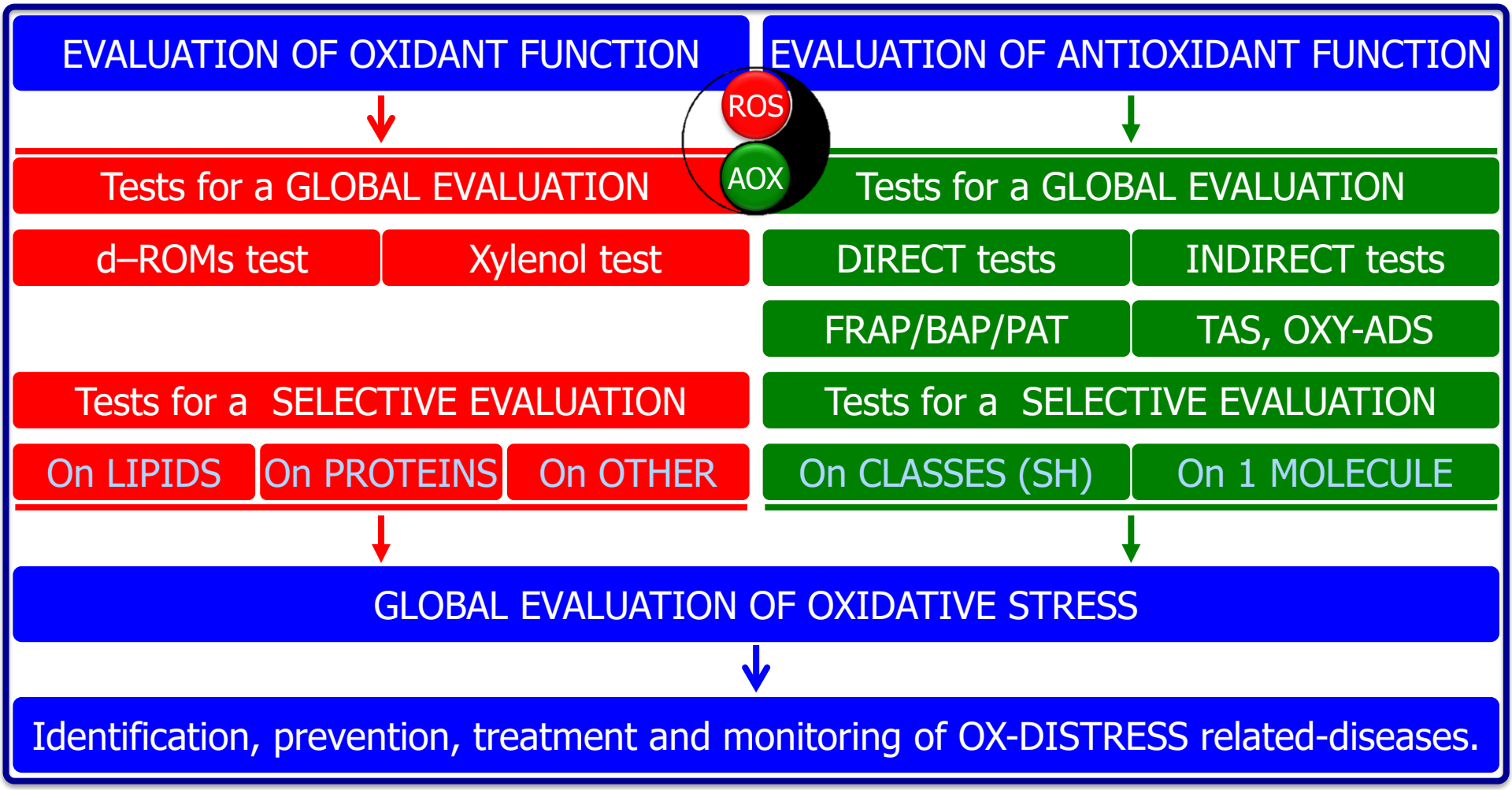
A multi-step process

The peroxidative process, a model for oxidative stress evaluation.



From cell biochemistry to laboratory assessment

The assessment of oxidative stress as balance between oxidant and antioxidant functions of redox system



First-line and second-line tests to measure either the production or the breakdown of reactive oxidant species

Features of the "ideal" test to measure oxidative stress

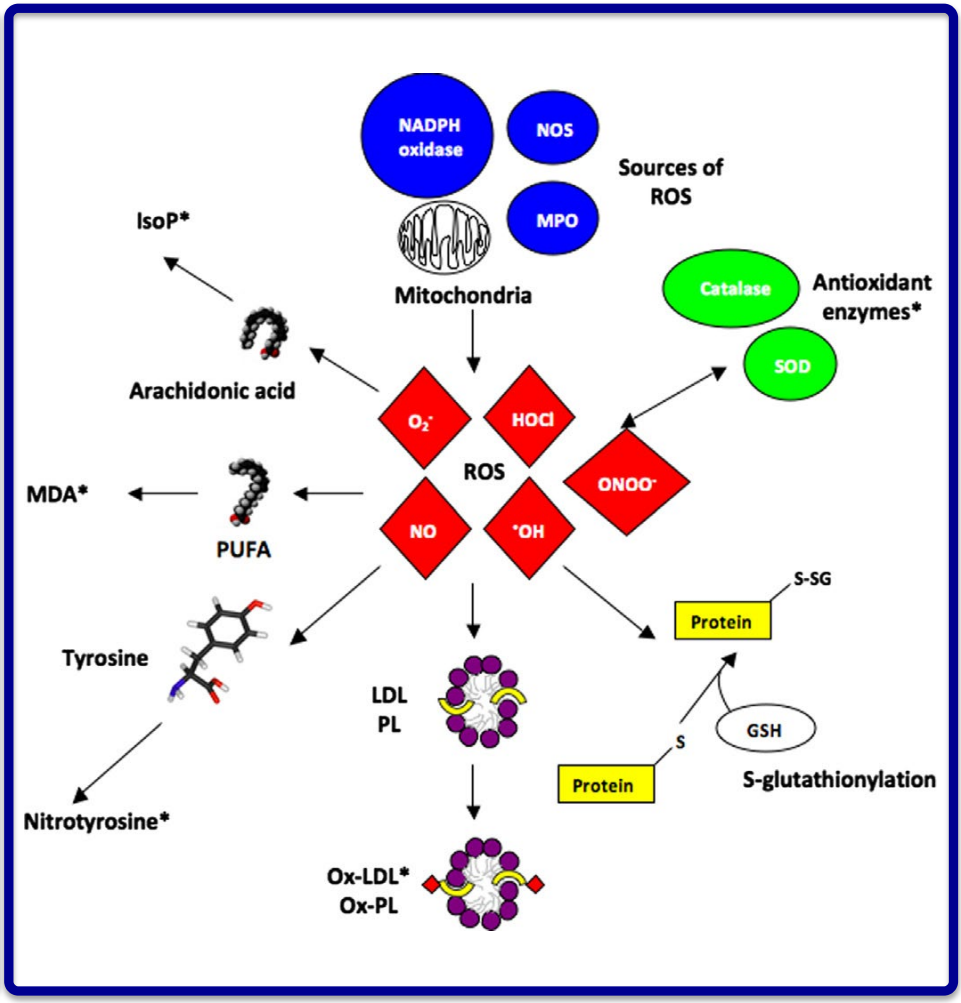
- ☞ Referred to chemically stable species with high sensitivity, specificity, precision…
- ☞ Validated by means of golden standard techniques (e. g. ESR spectrometry).
- ☞ Able to measure suitably the "level" of oxidative stress.
- ☞ Able to provide reliable information even in a early stage of the disease.
- ☞ Able to anticipate the progression of disease during a systematic monitoring.
- ☞ Modifiable with adequate sensitivity after medical/surgery/antioxidant treatments.
- ☞ Minimally invasive, highly compliant, fast, with optimal cost/benefit ratio.

The ideal test is not yet available and more than one test must be performed!

Bio-markers of oxidative stress: applications to cardiovascular research and practice.

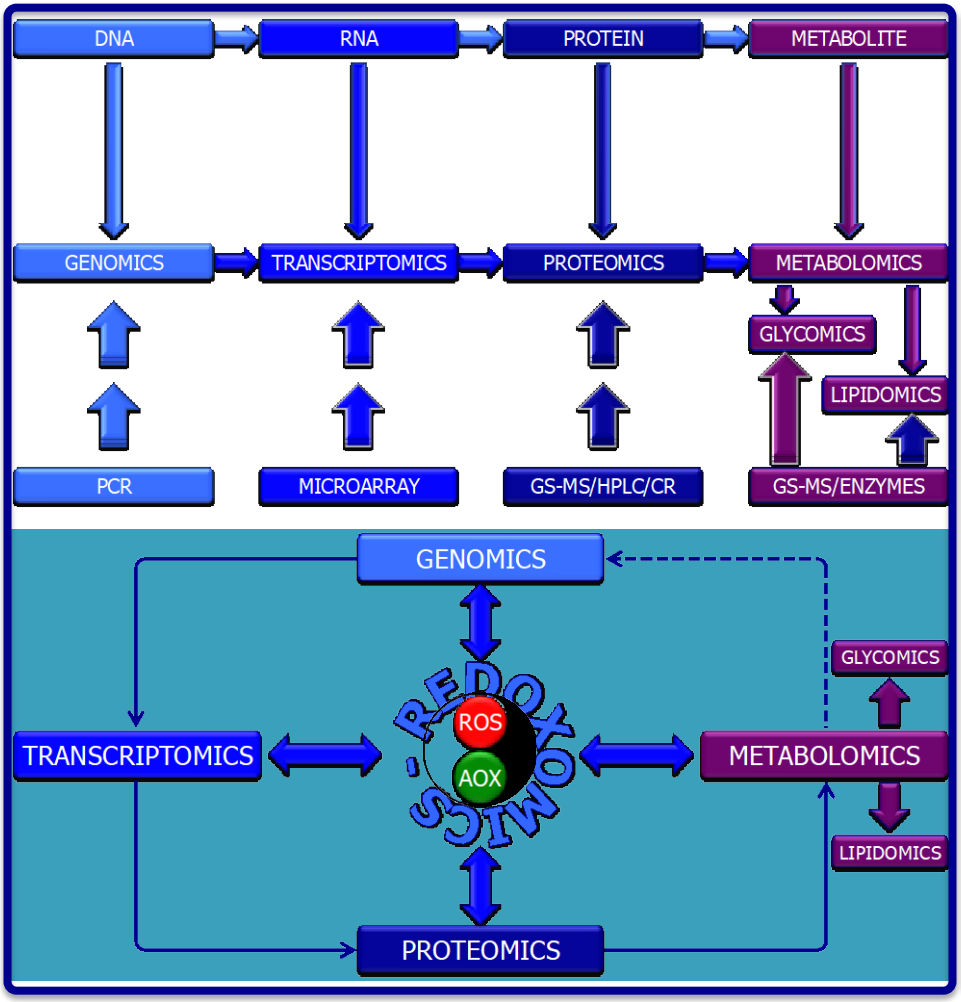
ELSEVIER

Oxidative stress is a common mediator in pathogenicity of established cardiovascular risk factors. Furthermore, it likely mediates effects of emerging, less well-defined variables that contribute to residual risk not explained by traditional factors. Functional oxidative modifications of cellular proteins, both reversible and irreversible, are a causal step in cellular dysfunction. Identifying markers of oxidative stress has been the focus of many researchers as they have the potential to act as an "integrator" of a multitude of processes that drive cardiovascular pathobiology. One of the major challenges is the accurate quantification of reactive oxygen species with very short half-life. Redox-sensitive proteins with important cellular functions are confined to signalling microdomains in cardiovascular cells and are not readily available for quantification. A popular approach is the measurement of stable by-products modified under conditions of oxidative stress that have entered the circulation. However, these may not accurately reflect redox stress at the cell/tissue level. Many of these modifications are "functionally silent". Functional significance of the oxidative modifications enhances their validity as a proposed biological marker of cardiovascular disease, and is the strength of the redox cysteine modifications such as glutathionylation. We review selected biomarkers of oxidative stress that show promise in cardiovascular medicine, as well as new methodologies for high-throughput measurement in research and clinical settings. Although associated with disease severity, further studies are required to examine the utility of the most promising oxidative biomarkers to predict prognosis or response to treatment.



Ho et Al. Redox Biol. 2013. 483–491.

Oxidative stress can be measured and managed



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Redoxomics. An integrated and practical approach to genomics, metabolomics and lipidomics to manage oxidative stress

[1] Eugenio Luigi Iorio, MD, PhD, [2] Maria Grazia Marin, BD, PhD

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In all living organisms a delicate equilibrium between the production and the elimination of oxidant chemical species (OCS) takes place (1). The production of OCS – which include either radical or non-radical oxygen-, nitrogen-, carbon-, chlorine-, sulphur-centred species – can depend on both exogenous agents (e. g. radiations, chemicals, bacteria) and endogenous phenomena (e. g. activation of specific enzymatic or non-enzymatic reactions) (2). The level/activity of antioxidants systems – which include either endogenous (e. g. antioxidant enzymes, like catalases and peroxidases) or exogenous agents (e. g. vitamins and vitamin-like compounds) devoted against OCS – is related to genetic and environmental factors (lifestyle mainly) (3). An increased production of OCS and/or a decreased efficacy of antioxidant systems can lead to the breakdown of oxidative balance thus generating the so-called oxidative stress (OS) (4).

Oxidative stress is generally recognised to play a pathogenic role either in early ageing or in several inflammatory and/or degenerative diseases, including atherosclerosis and hypertension (and their consequences, such as stroke, myocardial infarction, etc.), Alzheimer's disease, Parkinson's disease, cancer and so on (5).

Unfortunately, OS is not a "disease", according to the traditional sense of this word. Indeed, OS is the unwanted effect of the breakdown of a biochemical equilibrium. Therefore it can impact, often deceitfully, upon the onset and/or the course of several basic diseases. As it is not a classical disease, OS does not exhibit a specific clinical picture, but it hides itself behind the symptoms and the signs of the basic disease. Therefore, OS can be found only if the clinician submits the patient to specific biochemical tests.

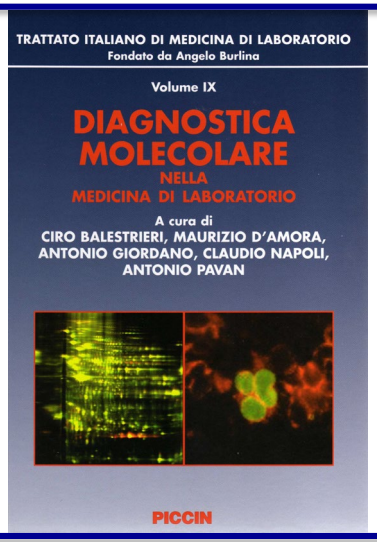
At long last, our laboratory (OXIGENLAB, Brescia, Italy) in cooperation with the International Observatory of Oxidative Stress (Salerno, Italy) now offers clinicians the opportunity to manage the oxidative stress by means of REDOXOMICS, an integrated approach where genomics and metabolomics (mainly lipidomics) interact with a innovative panel of oxidative balance biomarkers in order to offer to the patients a personalised analysis aimed to prevent oxidative damage, to diagnose and to monitor oxidative stress and, finally to evaluate the indication and the effectiveness of antioxidant supplementations and/or therapeutic interventions (6, 7).

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 2nd Edn, Clarendon Press, Oxford. 1989.
2. Gardes-Albert M. Physico-chemical aspects of reactive oxygen species. Ann Pharm Fr. 2006. 64(6):365–372.
3. Valko M, Leibfritz D, Moncol J, et Al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007. 39(1):44–84.
4. Delattre J. Introduction: from molecular oxygen to oxidative stress and radical biochemistry. Ann Pharm Fr. 2006. 64(6):363.
5. Favier A. Oxidative stress in human diseases. Ann Pharm Fr. 2006. 64 (6): 390–396.
6. Ridker PM, Brown NJ, Vaughan DE, et Al. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation. 2004. 109 (Suppl. IV): IV-6 –IV-19.
7. Iorio EL, Cinquanta L, Pisano R. A diagnostic algorithm to manage oxidative stress. Australasian J Cosmet Surg. 2006. 2 (1) : 26-30.

The new field of Redoxomics

References range of most common blood tests to measure oxidative stress in the clinical practice

Tests to measure the RS oxidant function		Tests to measure RS antioxidant function	
d-ROMs test	250-300 U CARR	Anti-RoMs test	>200/1000 μEq/L
8-isoprostans	<60 pg/mL	BAP test	> 2200 μmol/L
AOPP	< 29 μmol/L	TAS	> 1.54 μmol/L
Chemiluminescence	0.10 – 0.32 cps/cell	OXY-Adsorbent test	>350 mmol/L
		-SHp test	450-650 μmol/L
		Erythrocyte SOD	750-1150 UI/gHb
		Erythrocyte GPx	38-64 UI/gHb
		Erythrocyte GRx	47-79 U/L
		Erythrocyte catalase	>0.9 MU/gHb




28. LO STRESS OSSIDATIVO

E.L. Iorio, M.L. Balestrieri

PREMESSE FISIOPATOLOGICHE

In tutti gli organismi viventi esiste un delicato equilibrio fra la produzione e l'eliminazione delle cosiddette specie chimiche ossidanti (SCO) (1). Sotto questa denominazione si riassume una folta categoria di atomi, singoli o raggruppati, ovvero di molecole in grado di sottrarre, in determinate condizioni, uno o più equivalenti riducenti (atomi di idrogeno e/o elettroni) ad altre specie chimiche, per converso, riducenti (Fig. 1) (1).

Le SCO vengono generalmente suddivise in due grandi categorie, varietà non radicaliche e varietà radicaliche, quest'ultime essendo caratterizzate dal possedere almeno un elettrone spaiato in uno degli orbitali più esterni (Tabella I) (1,2).

In rapporto all'elemento il cui atomo partecipa direttamente all'azione ossidante si distinguono specie chimiche ossidanti o reattive centrate sull'ossigeno (reactive oxygen species, ROS), sull'azoto (reactive nitrogen species, RNS), sul carbonio (reactive carbon species, RCS), sullo zolfo (reactive sulphur species, RSS) e sugli alogeni, in particolare sul cloro (RAS-RCIS) (1,2). Va rilevato che non tutte le suddette specie si sono ugualmente reattive, per cui è più corretto usare il termine "ossidanti" che sicuramente le accomuna tutte e li hanno una brevissima emivita, alcune di esse potendo addirittura essere isolate (es. trifenilmetile) (3).

Le SCO non rappresentano "schegge impazzite" ma normali prodotti del metabolismo cellulare che giocano un ruolo rilevante nell'economia generale dell'intero organismo (1,3,4). Infatti, alcune di esse, quali l'anione superossido ($O_2^{\bullet}$), il perossido di idrogeno (H_2O_2) e il radicale idrossile ($HO^{\bullet}$), sono comunemente utilizzate dai leucociti polimorfonucleati per distruggere i batteri (killing fagocitario) (1,5). Altre, come l'ossido di azoto ($NO^{\bullet}$), svolgono un ruolo determinante nella regolazione della pressione arteriosa e nella modulazione dei fenomeni mentivi (6). Altre ancora, come l'acido ipocloroso (HClO) costituiscono una valida risorsa per la distruzione di cellule tumorali (7,8).

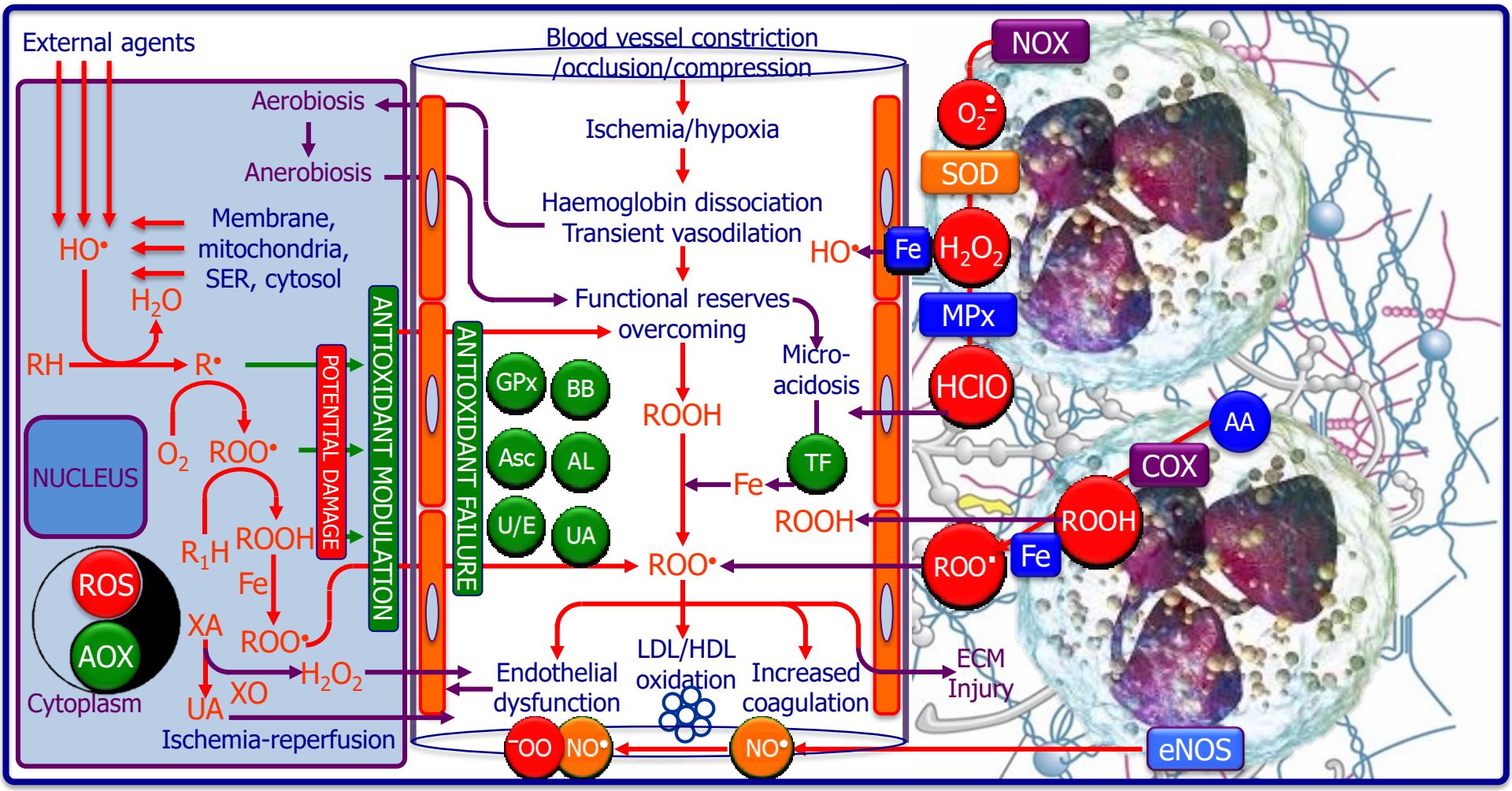
Purtroppo, in determinate condizioni, aumentando di livello in determinati distretti (es. apparato cardiovascolare, sistema nervoso, articolazioni, intestino, bronchi, cavo orale etc.), le SCO possono, proprio per la loro capacità ossidante, attaccare e, quindi, danneggiare molecole endogene, come quelle di lipidi, glicidi, acidi nucleici e proteine, con conseguenti lesioni disperma circoscritte e poi via via più diffuse, fino ad un coinvolgimento sistemico (9). Si parla in tali casi, di stress ossidativo o ossidante, un fattore emergente di rischio per la salute (9).

Per prevenire o, comunque, rallentare questi processi indesiderati di ossidazione, nel corso di millenni di evoluzione, gli organismi viventi si sono "adattati" a convivere con la presenza delle SCO, difendendosi dalla potenziale lesività di queste ultime grazie ad un complesso sistema di difesa, costituito dagli antiossidanti: (10). Questi comprendono una serie di enzimi (superossidodismutasi, catalasi, perossidasi) e di agenti non enzimatici, di provenienza sia esogena (vitamine, polifenoli) che endogena (acido urico, bilirubina). È ovvio che quando li-

Fig. 1. Esempio di azione ossidante dei radicali liberi. L'attacco ad un doppio legame.

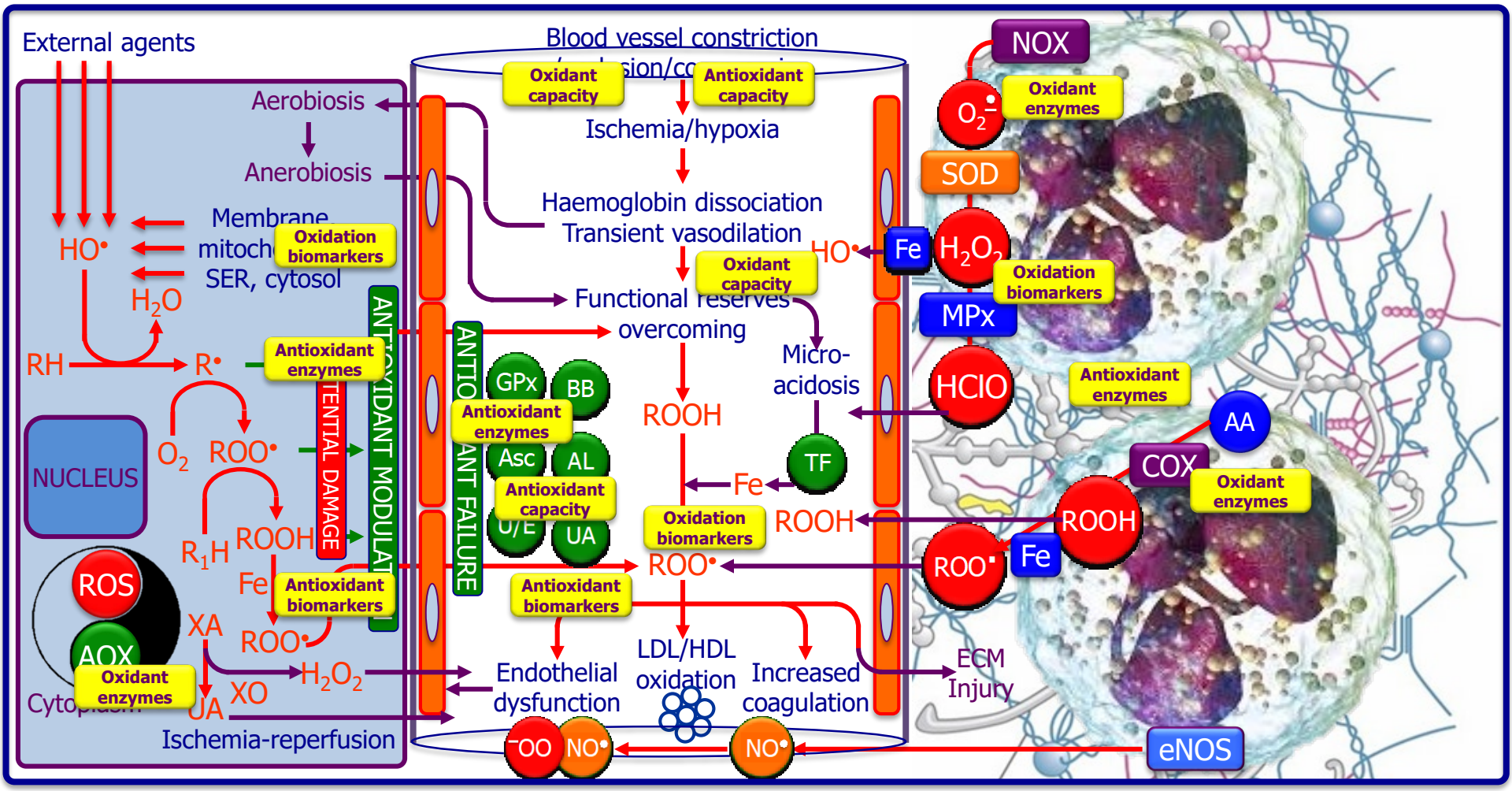
Iorio EL. The oxidative stress evaluation. Italian Treatise of Laboratory Medicine. 2009. Padua, Italy.

Let's go inside the tissues



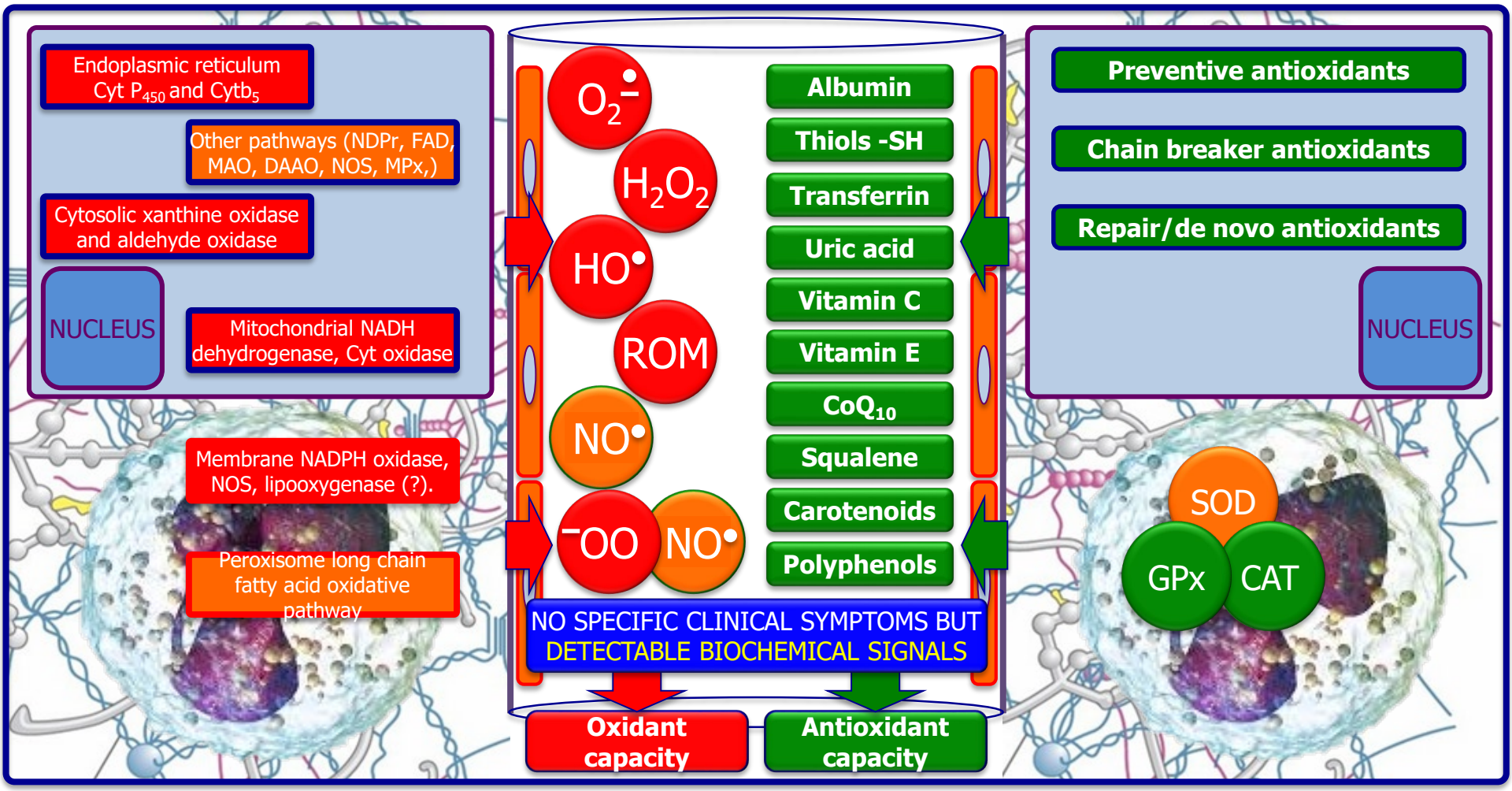
Cellular and biochemical basis of oxidative stress

The cell biochemistry of redox function



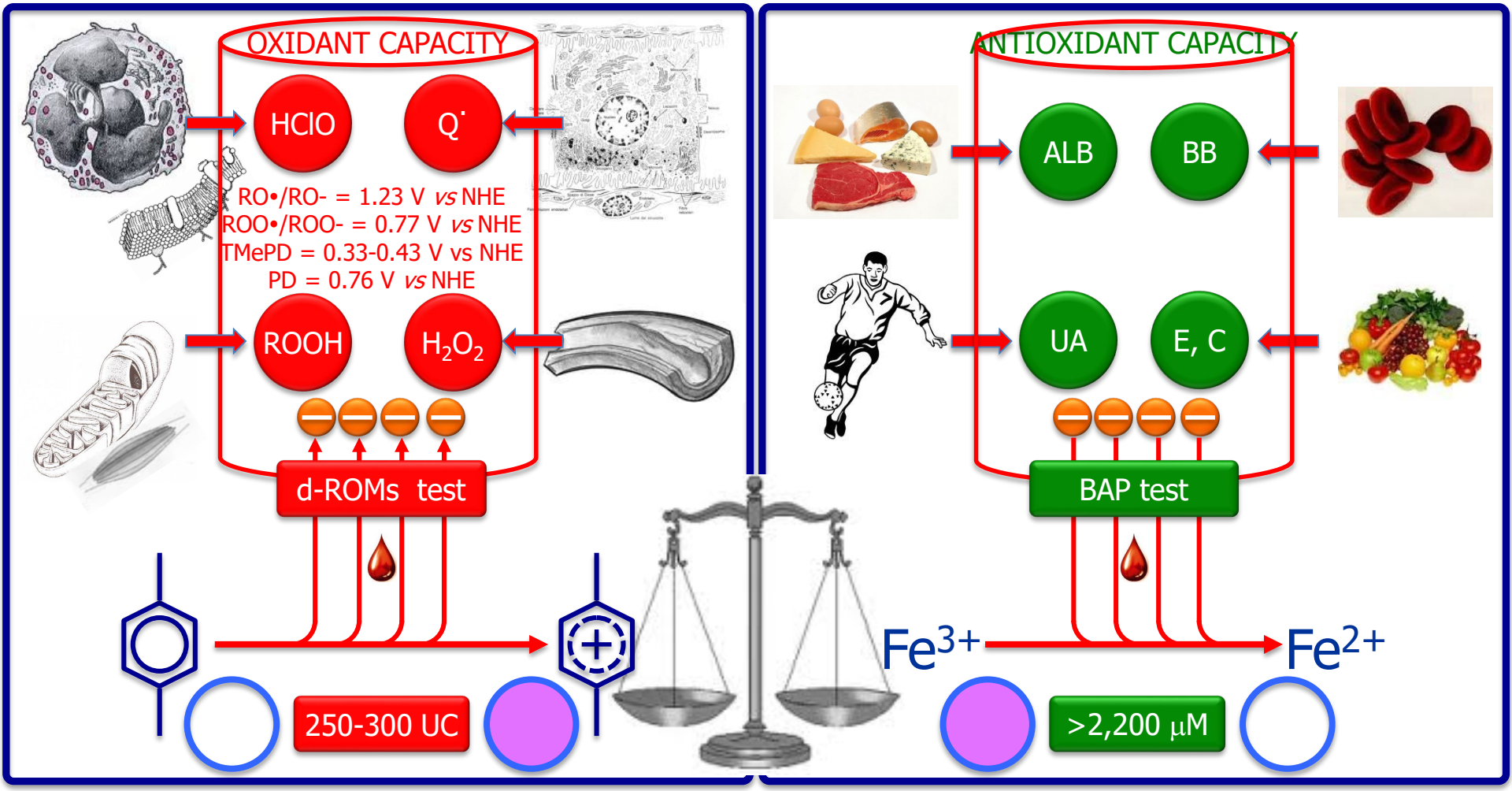
A whole panel to evaluate oxidative stress

Body redox system generates biochemical signals/ /biological activities changes in blood serum/plasma



Iorio EL. The oxidative stress evaluation. Italian Treatise of Laboratory Medicine. 2009. Padua, Italy.

A blood plasma/serum sample can provide a suitable specimen to measure body oxidative balance



Iorio EL. The oxidative stress evaluation. Italian Treatise of Laboratory Medicine. 2009. Padua, Italy.

The oxidative stress measurement in clinical practice

Global oxidative stress evaluation, may be after body temperature, blood pressure and pH measurements, is the most easy, minimally invasive and cost/benefit suitable tool to monitor periodically health status of every people.

A minimally invasive tool to monitor our health status

Blood plasma/serum total oxidant capacity measurement

1. The total oxidant capacity/potential of a blood plasma/serum sample can be evaluated by exploiting the ability of N,N-diethyl-paraphenylendiamine (DPPD) to give electrons (oxidation) after reacting with a biological sample. The newly generated radical cation can be detected – thus providing a suitable measure of oxidant capacity – either by evaluating photometrically the absorbance change at 505 nm (the solution become pink to red from transparent) or the specific spin resonance signal.
2. The blood plasma/serum of apparently healthy peoples (and that one of many Animal species) is able to oxidise the DPPD in a precise range of absorbance change as a function of either genetic or environment factors (age, gender, race, physiological conditions like pregnancy, and so on) and according to a Gauss-like curve of distribution.
3. The blood plasma/serum of apparently healthy peoples which are exposed to factors that are classically able to induce a condition of oxidative stress shows a total oxidant capacity constantly and significantly higher that one found in apparently healthy non-exposed peoples.
4. Patients suffering from diseases classically related to oxidative stress show significantly higher levels of blood plasma/serum total oxidant capacity compared to those one found in apparently healthy controls.
5. Sometime an increased blood plasma/serum total oxidant capacity correlates with the severity of the underlying disease.
6. Specific medical/surgical treatments may reduce significantly blood plasma/serum total oxidant capacity and sometime improve symptoms/health status compared to baseline (before the treatment).
7. Antioxidant supplementation may reduce significantly blood plasma/serum total oxidant capacity and sometime improve symptoms/health status compared to baseline (before treatment).
8. Peoples even apparently healthy showing high blood plasma/serum total oxidant capacity may show also significantly increased rates of morbidity/mortality and/or abnormal response to some therapies (predictive value)

Paradigmatic evidence from the scientific literature

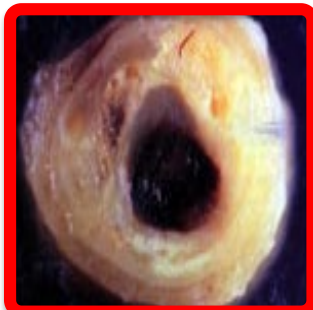
From Delphi's Oracle to Predictive Medicine: the role of oxidative stress measurement.

The Predictive Medicine

- Predictive medicine is the emerging field of medicine that entails predicting the probability of disease and taking proactive steps to either prevent the disease altogether or significantly decrease its impact upon the patient (such as by preventing mortality or limiting morbidity).
- Aside from genetic testing, predictive medicine utilizes a wide variety of tools to predict health and disease, including assessments of exercise, nutrition, spirituality, quality of life, and OXIDATIVE STRESS.

Biesecker LG. Genome Research 2013. 23: 1051–1053.

The dark side of cardiovasculas diseases



Artery section of a 45-years old man who died for acute myocardial infarction.

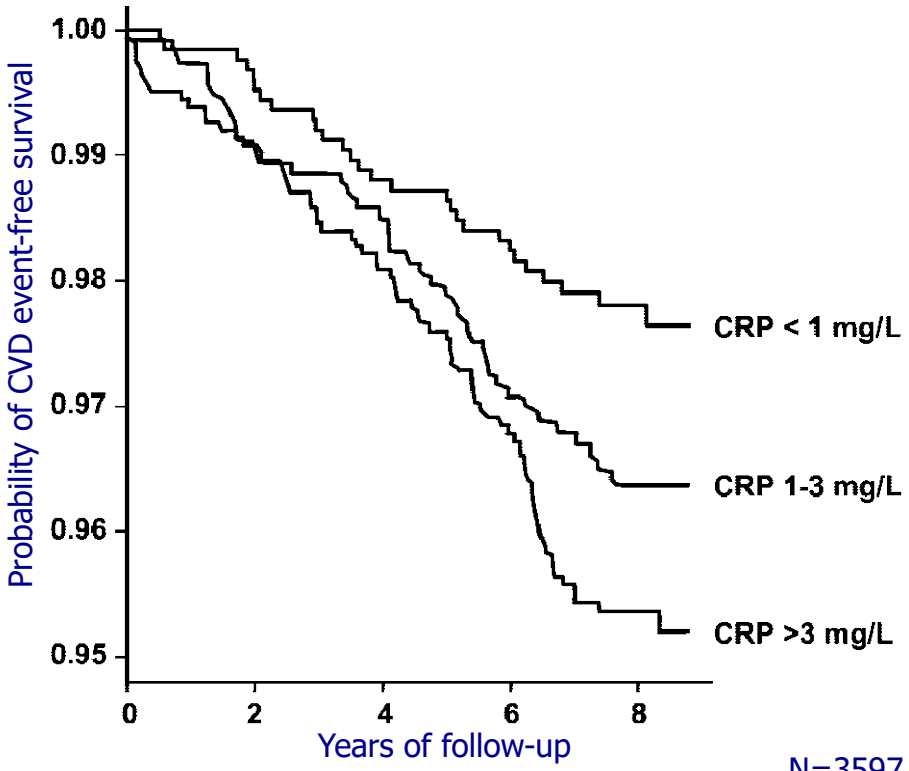
Blood cholesterol 200 mg/dL

- In the current Adult Treatment Panel guidelines for cardiovascular risk detection, the plasma-based markers recommended for use in global risk assessment or in the definition of the metabolic syndrome are low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, and triglycerides.
- It is widely recognized, however, that more than half of all future vascular events occur in individuals without overt hyperlipidemia. For example, in a recent large-scale analysis of >27 000 healthy American women, 77% of all future events occurred in those with LDL-C levels <4.14 mmol/L (<160 mg/dL) and 45% of all events occurred in those with LDL-C values<3.36 mmol/L (<130 mg/dL).
- Although risk-scoring systems that additionally evaluate traditional risk factors such as smoking, hypertension, and diabetes greatly improve risk prediction, multiple studies demonstrate that 20% to 25% of all future events occur in individuals with only 1 of these factors. Moreover, the prevalence of traditional risk factors is almost as high in those without disease as in affected individuals.

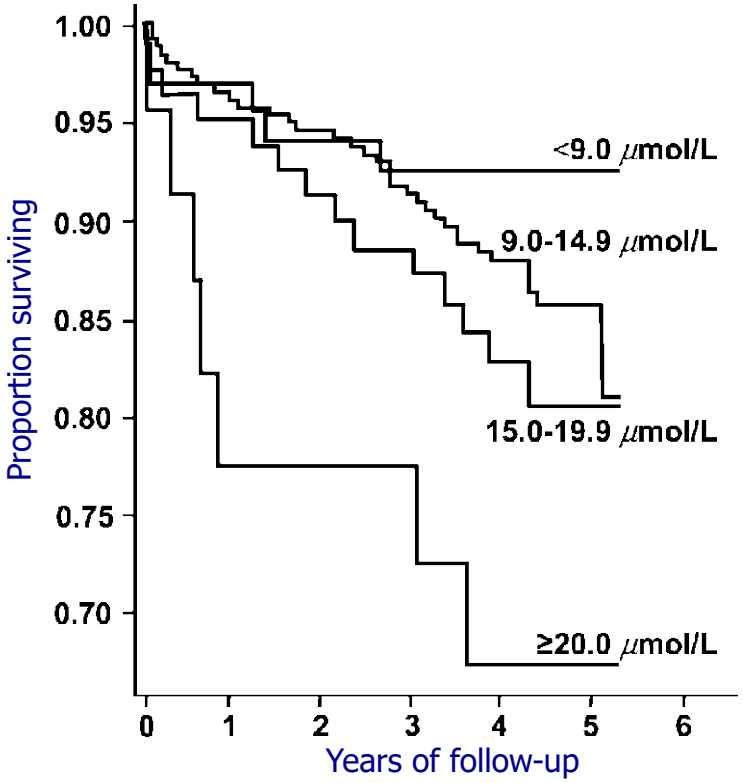
Riedker et Al. Circulation. 2004. 109: IV6–IV19.

Recognised biomarkers

Prognostic value of HS-CRP in individual with metabolic syndrome at the study entry

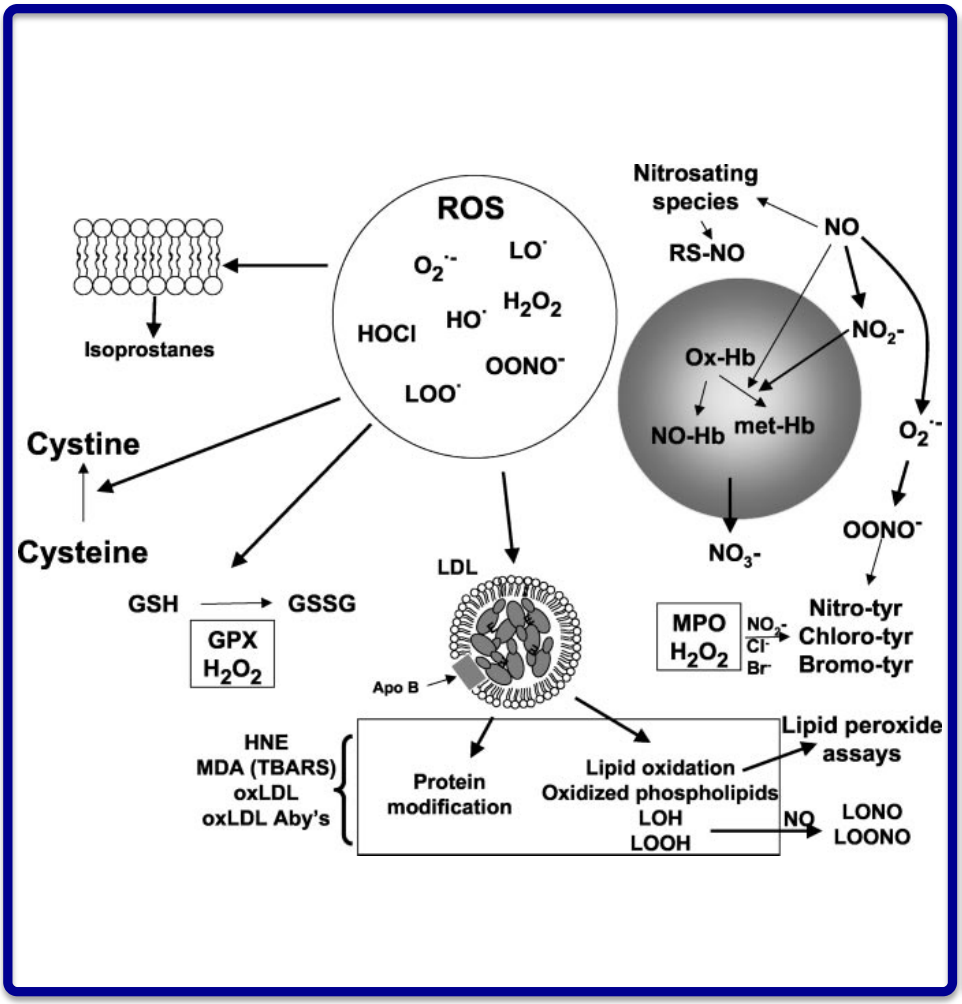


CAD event-free survival in individuals undergoing HCY evaluation for CAD



C-Reactive Protein and homocysteine

Emerging plasma biomarkers for CVD



Oxidant Stress Marker	Previous Use	Method of Measurement	Advantages	Disadvantages
F2-isoprostanes[147]	↑ in smokers, diabetes, COPD, hypercholesterolemia, scleroderma	GS/mass spectroscopy; ELISA (new kits need widespread validation); Urine and plasma	Best characterized	GC/mass spectroscopy method impractical for large studies; ELISA kit measurements promising but need validation; Currently not well-accepted; Plasma measurements of questionable value
Thiobarbituric acid reactive substances (TBARS)[148]	↑ in a variety of systemic illnesses (multiple sclerosis, hemodialysis, malaria, diabetes), after hyperbaric oxygen exposure	Spectrophotometric reaction between malondialdehyde (end product of lipid oxidation) with TBA	Simple assay; Extensively used in basic studies.	Spectrophotometric method is nonspecific and may detect other aldehydes; HPLC modification is more specific but impractical for large studies
Oxidized LDL (ox-LDL),[146] antibodies to ox-LDL[150]	Ox-LDL ↑ in acute coronary syndromes, heart failure, after MI; ox-LDL antibodies correlate inversely with endothelial function in transplantation subjects and coronary artery disease	ELISA and related antibody-based assays; ox-LDL can be measured with murine antibody EO6	Relatively straightforward assays may be applied to large number of subjects	Specificity of ox-LDL measurements questionable and may reflect oxidized fatty acids that have been exchanged with the LDL particle; ox-LDL rapidly cleared from plasma, low levels may not reflect level of the underlying disease; Exercise ↑ LDL oxidation acutely
d-ROMs test	↑ in peripheral vascular disease, can be ↓ by antioxidants; ↑ in hypertension and can be ↓ by antihypertensive treatment; ↑ by heavy alcohol use	Measure of lipid peroxides and lipid alcohols; Depends on Fenton-like reaction leading to formation of lipid peroxy and alkoxy- radicals that in turn react with a chromogenic substrate	Simple assay can be completed in minutes	Specificity not established; Probably not suitable for samples stored for prolonged periods; EDTA and EGTA interfere with assay
8-Hydroxy 2′ deoxyguanosine (8OHdG)[152–154]	↑ in smokers, implicated in carcinogenesis; ↑ in blood and mononuclear cells in hypertensive subjects	Oxidation of guanine at the C8 position leads to a G to T substitution; Can be measured using HPLC or ELISA; Urine samples most commonly used	Potentially very important mechanism underlying oxidative modification of gene expression	Can be altered by gene excision rather than oxidation; Increased by enhanced metabolic rate. ELISA may not be specific for 8OHdG
Protein carbonyls[155–157]	↑ in tissues and plasma in aging, Alzheimer disease, cystic fibrosis, cataracts, Parkinson disease, and in muscle after exercise; Plasma levels ↓ by antioxidant treatment	Formed by oxidation of side chains of lysine, praline, arginine, and threonine; also by reactions with hydroxynonenal (product of lipid oxidation); Colorimetric reaction with 2,4 dinitrophenylhydrazine; Antibody tests also available	Simple assays; May reflect oxidation of both proteins and lipid oxidation; Pathophysiologically relevant targets	Tissue levels may not be reflected in plasma samples; Not specific for cardiovascular disease
Modified tyrosines (nitrotyrosine, chlorotyrosine, bromotyrosine)[158,159]	Nitrotyrosine levels ↑ in coronary artery disease; Statins ↓ nitrotyrosine levels	Most accurate measurement requires quadrapole GC/mass spectroscopy; ELISA assays also available for nitrotyrosine	May reflect generation of peroxynitrite or reactions of peroxidases with hydrogen peroxide	Not widely available; ELISA assays need validation
Plasma glutathione levels; Ratio of oxidized to reduced glutathione[160]	↑ in hypertension, experimental models of atherosclerosis	Glutathione is most prevalent intracellular thiol; Oxidation may occur on direct reaction with oxidants or may reflect reaction of glutathione peroxidase and H_2O_2; Requires HPLC	Physiologically very relevant; May reflect oxidative status in nonlipid compartments (eg, cytoplasma, intracellular space)	Measurement difficult; Samples must be collected in specific buffer

The role of oxidative stress biomarkers

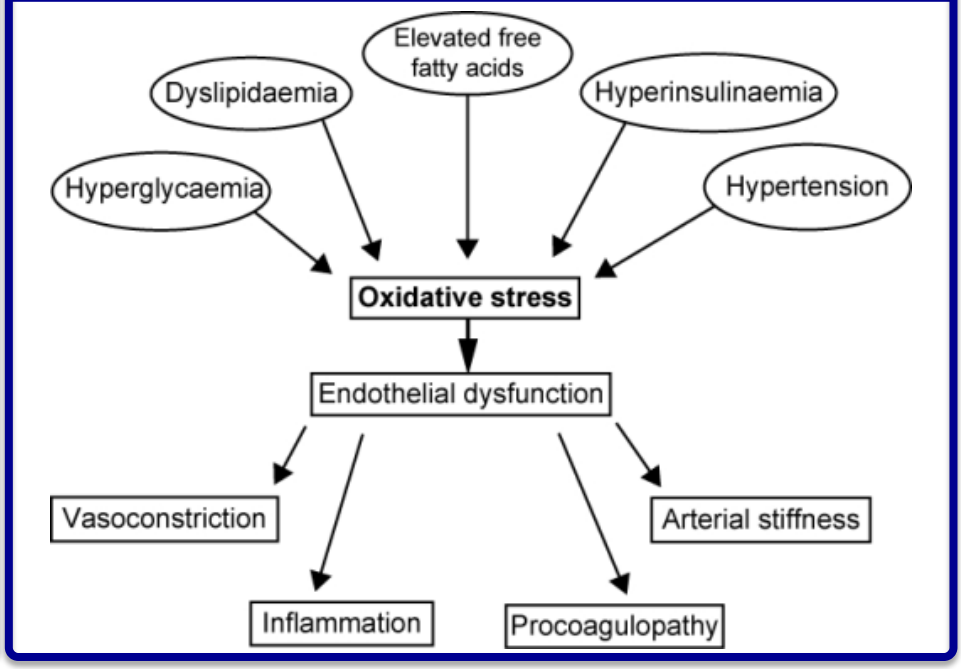
High d–ROMs test values as early markers of endothelial dysfunction?

Background & Aims. There are a lot of works showing the strong relation between oxygen free radicals, nitric oxide consumption and endothelial dysfunction. Aim of our study was to quantify the serum levels of oxygen free radicals in obese and non-obese subjects.

Subjects and Methods. We analysed 97 volunteers (age between 18 and 35 years) that were subdivided into two groups according to their BMI): group A formed by 25 thin subjects with BMI = 22 ± 2 and mean age = 26 ± 4 years, group B formed by 72 fat subjects with BMI = 36 ± 8 and mean age = 27 ± 4 years. In all the volunteers were carried out the following measurements: (1) Echo-color Doppler measurement of intima-media thickness (IMT) of common carotid artery; (2) d-ROMs test; (3) Insulin tolerance test (KITT); (4) Insulinemia.

Results. The results of our study show in fat subjects a higher oxidative stress and insulin-resistance compared to thin subjects despite a non significant intima-media thickness difference.

Conclusions. Our study shows that obese subjects have an oxidative stress and an insulin resistance that are more evident than in thin subjects. Moreover there is a non-significant difference of intima-media thickness between the two groups. The insulin resistance and the increased oxidative stress in young obese subjects may represent an early diagnostic element of endothelial dysfunction in spite of intima-media thickness.

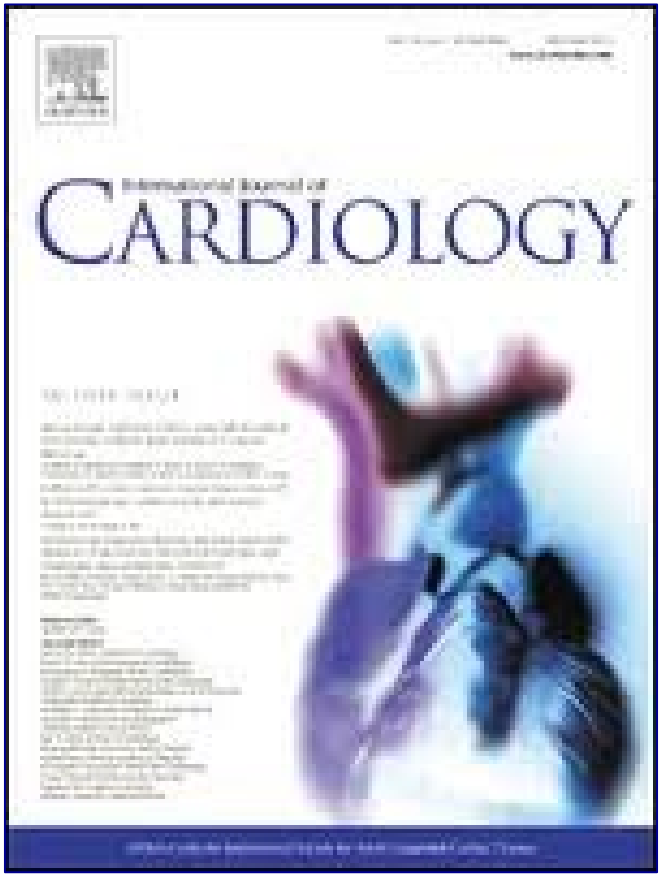


Ciccone et Al. Clinical Hemorheol Microcircul. 1999. 21: 341–342.

Predictive role of d-ROMs test in cardiovascular diseases

Elevated hydroperoxide levels as a prognostic predictor of mortality in a cohort of patients with cardiovascular disease

Cristina Vassalle*, Claudio Boni, Pietro Di Cecco, Patrizia Landi



☞The aim of this study was to evaluate whether d-ROMs test, an index of oxidative stress, predict cardiac and total death in patients with cardiovascular disease.

☞d-ROMs test values were measured in 157 consecutive inpatients, followed during a mean follow-up of 20±0.3 months.

☞Elevated oxidative stress resulted in an independent predictors for cardiac death, suggesting that hydroperoxide evaluation could provide an adjunctive estimate in the evaluation of prognosis in the cardiovascular clinical setting.

Multivariate Cox predictive model of cardiac and total mortality

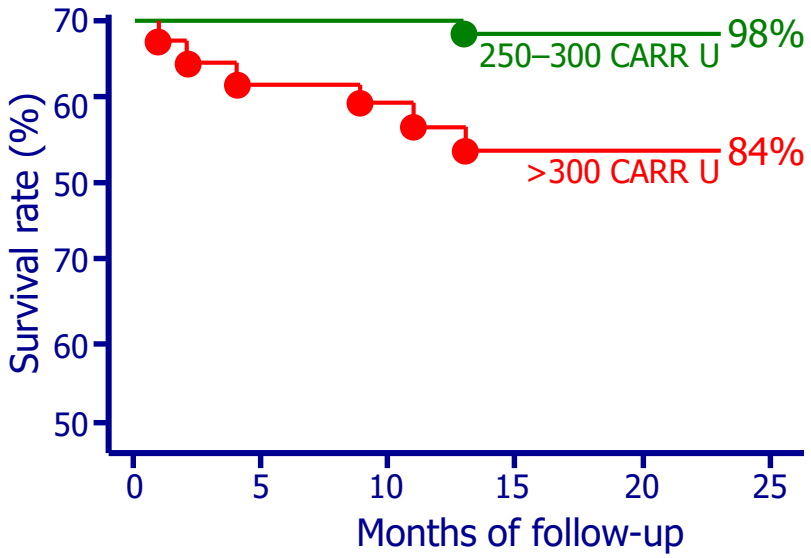
	Cardiac mortality			Total mortality		
	Odds ratio	95% CI	*p*	Odds ratio	95% CI	*p*
Age				1.1	1.0–1.2	0.09
Prior infarction	1.66	0.5–9.1	0.56	1.6	0.5–5.2	0.42
d-ROMs test 75th percentile	8.6	1.5–50.2	0.016	2.8	0.8–9.3	0.1
Ejection fraction <40%	5	1–25.9	0.05	3.4	1.1–11.2	0.036
Diabetes	2.4	0.5–9	0.55	2.3	0.7–7.6	0.11

CI=confidence interval.

Vassalle et Al. Int J Cardiol. 2006. 110: 415-416.

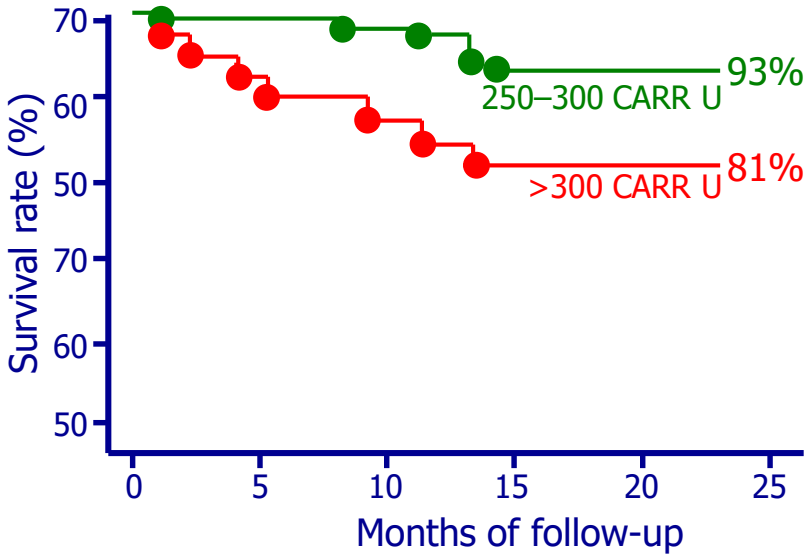
High d-ROMs test values are predictive for cardiac death and total mortality.

Cardiac death



Kaplan–Meier survival curves according to 75th d-ROMs test percentiles

Total mortality



Kaplan–Meier survival curves according to 75th d-ROMs test percentiles

Vassalle et Al. Int J Cardiol. 2006. 110: 415–416.

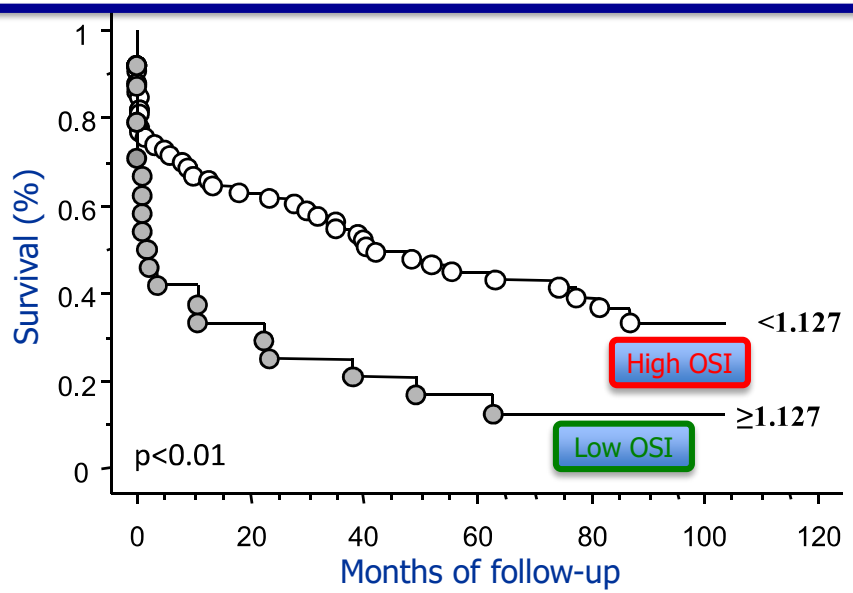
High d-ROMs test values (as OSI with OXY-ADS test) are predictive for major CV events in CAD patients.

Elevated Levels of Oxidative Stress as a Prognostic Predictor of Major Adverse Cardiovascular Events in Patients with Coronary Artery Disease

	number (%)
Age > 60 years (> 50th percentile)	46 (47)
Males	78 (80)
Hypertension	56 (58)
Type 2 Diabetes	32 (33)
Dyslipidemia	51 (53)
Smoking habit	36 (37)
Current smokers	9 (9)
Ex smokers	27 (28)
Ejection fraction < 40%	36 (37)
Coronary angiography	
One-vessel disease	41 (42)
Multi-vessel disease	56 (58)
Previous myocardial infarction	50 (52)
Oxy-Adsorbent Test (> 75th percentile)	24 (25)

Characteristic	no (n = 31)	yes (n = 66)	no (n = 31)	yes (n = 66)	p values
Age > 60 years (> 50th percentile)	18 (58)	35 (53)	18 (58)	35 (53)	0.1
Males	26 (84)	52 (79)	26 (84)	52 (79)	0.5
Hypertension	18 (58)	38 (58)	18 (58)	38 (58)	0.9
Type 2 Diabetes	6 (19)	26 (39)	6 (19)	26 (39)	0.0
Dyslipidemia	15 (48)	36 (54)	15 (48)	36 (54)	0.5
Smoking habit (current smokers)	10 (32)	26 (39)	10 (32)	26 (39)	0.5
Ejection fraction < 40%	9 (29)	27 (41)	9 (29)	27 (41)	0.2
Coronary angiography					0.02
One-vessel disease	20 (65)	21 (32)	20 (65)	21 (32)	0.0
Multi-vessel disease	11 (35)	45 (68)	11 (35)	45 (68)	0.0
Previous myocardial infarction	12 (39)	38 (58)	12 (39)	38 (58)	0.0
Oxy-Adsorbent Test (> 75th percentile)	10 (32)	21 (32)	10 (32)	21 (32)	0.9

Data are number (%) of patients



Aims. To evaluate the prognostic significance of OS on the rate of major adverse cardiovascular events (MACEs: cardiac and all-cause death, nonfatal myocardial infarction, coronary revascularization-PTCA/CABG) in CAD.

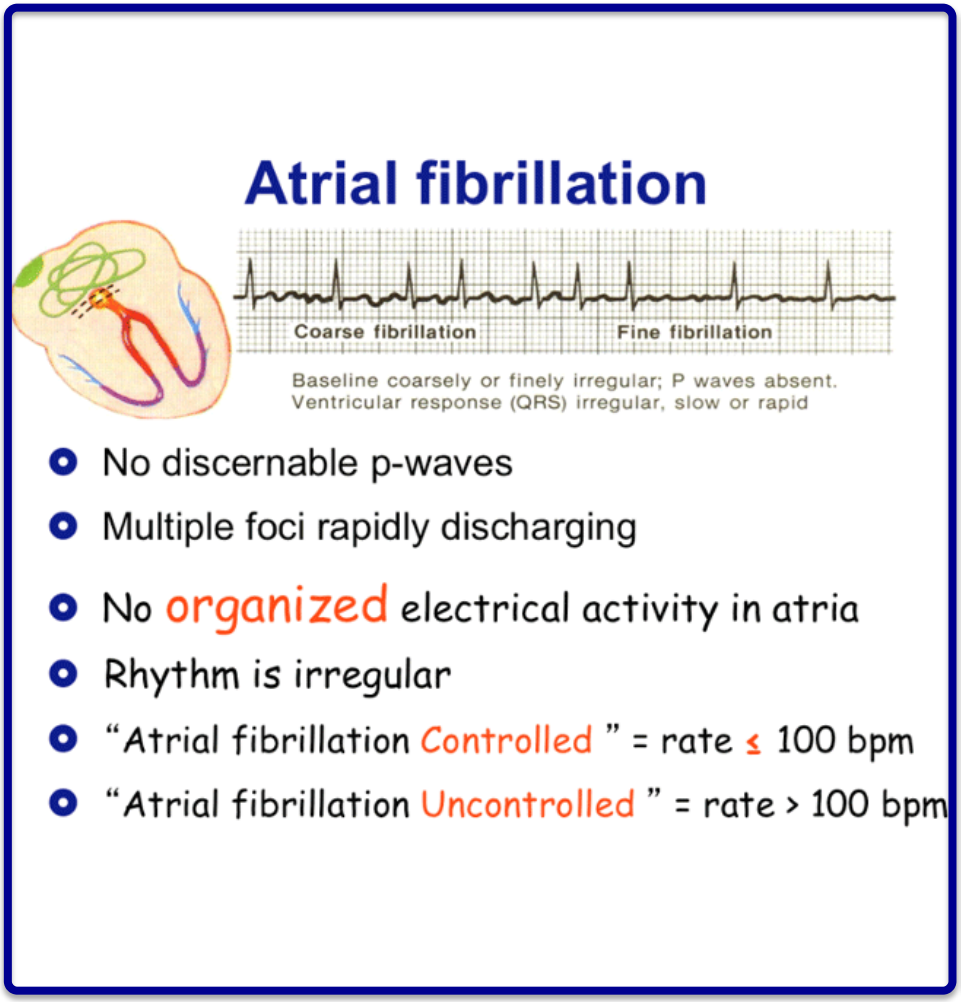
Methods. We studied 97 angiographically proven CAD patients (78 males, age: 67±11 years, mean± SD). ROMs and total antioxidant status, assessed by commercially assays (d-ROMs and OXY-Adsorbent Test; Diacron, Grosseto, GR, Italy), were used to calculate the oxidant/antioxidant balance. Patient data were collected from the Institute's electronic databank, which saves demographic, clinical, instrumental and follow-up data of all patients admitted to our department.

Results. Kaplan-Meier survival estimates showed a significantly worst outcome in patients presenting with elevated oxidative stress levels (>75th percentile, p<0.01). Multivariate Cox models showed that a higher level of oxidative stress was an independent predictor of developing MACEs (hazard ratio=2.1, confidence intervals 1.2-3.6, p<0.01).

Conclusion. *Oxidative stress may represent a useful additional tool in the prediction of MACE in CAD.*

Vassalle et Al. J Atheroscler Thromb. 2012. 19: 712–727.

Increased d-ROMs test values are associated with atrial conduction disturbance in patients with atrial fibrillation.



Background & Aims. Oxidative stress (OS) stress is associated with atrial fibrillation (AF) but little is known about the relationship between OS biomarkers and electrical activity in AF patients. The aim of this study was to investigate the potential association between these markers and atrial remodeling in paroxysmal or persistent AF.

Methods. As index of OS we measured serum d-ROMs test in consecutive 306 AF patients receiving radiofrequency catheter ablation (RFCA): 225 paroxysmal AF patients and 81 persistent AF patients. As index of inflammation we meaaured high sensitive C-reactive protein (HsCRP). Aa index of heart electric acticity we measured filtered P wave duration (FPD) by P waves signal averaged electrocardiogram (P-SAECG).

Results. Patients were followed up for 1.2±0.8 years. d-ROMs levels in patients with persistent AF (342± 85 CARR U were significantly higher (P<0.001) compared with paroxysmal AF (305±778 CARR U). d-ROMs levels in persistent AF patients showed a tighter, positive correlation with FPD (r=0.56, p<0.001) than those in all AF patients (r=0.13, p<0.05). d-ROMs levels also showed a weaker but significant correlation with HsCRP in patients with AF. The Kaplan-Meier analysis revealed that the highest quartile of basal d-ROMs levels exhibited a significantly higher AF recurrence rate after RFCA in patients with paroxysmal AF (p<0.01).

Conclusions. Serum d-ROMs test test values reflects atrial conduction disturbance and predicts AF recurrence after RFCA in paroxysmal AF patients. It could serve as biomarker for predicting risk of AF recurrence following RFCA.

Shimano et Al. Heart Rhythm. 2009. 6: 935–940.

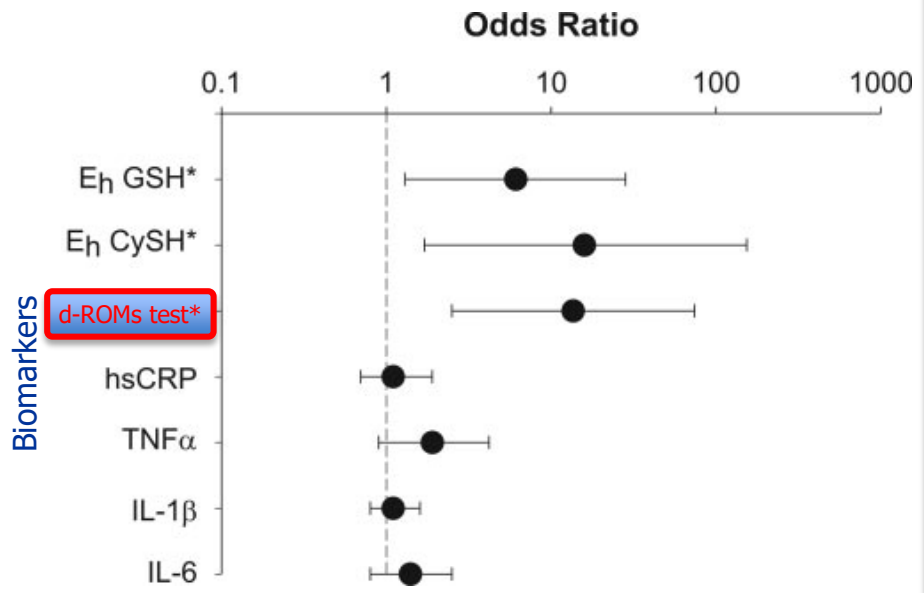
High d-ROMs test values are associated with persistent atrial fibrillation

Background. Atrial fibrillation (AF) has been associated with myocardial oxidative stress, and antioxidant agents have demonstrated antiarrhythmic benefit in humans. We compared serum markers of oxidation and associated inflammation in individuals with or without AF.

Methods. Serum markers of oxidative stress and inflammation were compared in a cross-sectional, case-control design study of 40 male individuals, with or without persistent or permanent AF, who were matched for age, sex, diabetes, and smoking status, known confounding variables for the measurement of oxidative stress. We used d-ROMs test and ratios of oxidized to reduced glutathione (Eh GSH) and cysteine (Eh CySH) to quantify oxidative stress. We also measured inflammatory markers, including high-sensitivity C-reactive protein, interleukins 1b and 6, and tumor necrosis factor a.

Results. Univariate, conditional logistical regression analysis showed that oxidative stress but not inflammatory markers were statistically associated with AF ($P < 0.05$). The increase in the odds ratios for AF for Eh GSH, Eh CySH, and d-ROMs were 6.1 (95% CI, 1.3–28.3; $P = 0.02$), 13.6 (95% CI, 2.5–74.1; $P = 0.01$), and 15.9 (95% CI, 1.7–153.9; $P = 0.02$), respectively. There was a stronger correlation between Eh GSH and Eh CySH ($r = 0.66$) than between Eh GSH and d-ROMs test ($r = 0.41$). In multivariate analysis corrected for statins and other AF isk factors differing between the groups, the association of AF and oxidative stress remained significant.

Conclusions. These data suggest that oxidative stress markers may have predictive value in AF management.



The univariate ODD RATIOS for AF as a function of an interquartile increase in various markers. *E_h GSH, E_h CySH and d-ROMs significant at $p \leq 0.02$.

Neuman t Al. Clin Chem. 2007. 53: 1652–1657.

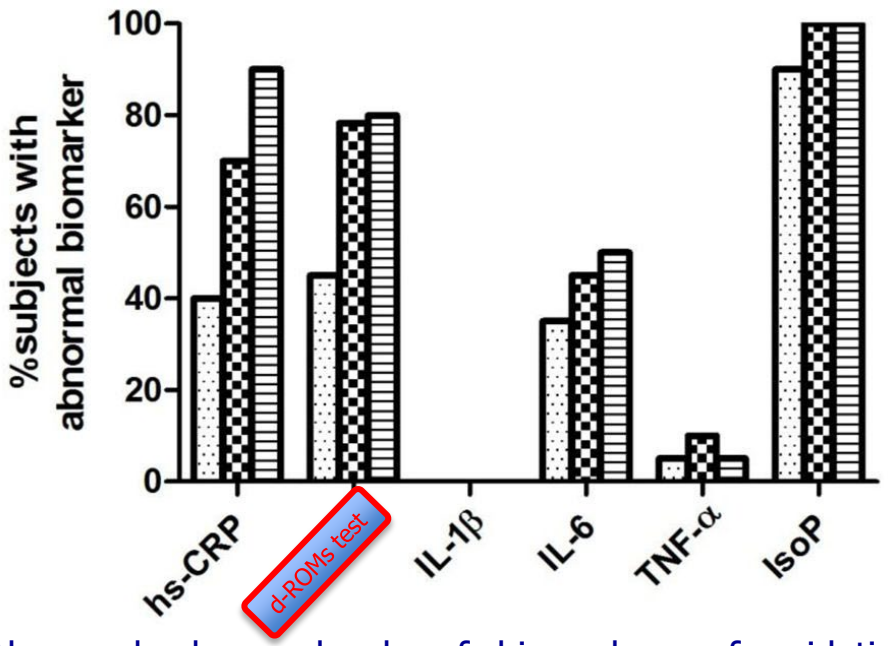
The combination d-ROMs test and HS-CRP correlates well with CHADS-2 risk score in chronic atrial fibrillation

Background. Inflammation and oxidative stress have been linked to the origin and persistence of atrial fibrillation (AF). CHADS-2 scoring system is a risk stratification schema well validated in prognostication of stroke in AF. We evaluated the association of markers of oxidative stress and inflammation with CHADS-2 scores in chronic AF patients.

Methods. CHADS-2 scores were calculated for 64 subjects with chronic AF. Serum markers of inflammation [C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α)] and of oxidative stress [Derivatives of reactive oxygen metabolites (d-ROMs) and isoprostanes (IsoPs)] were measured.

Results. Twenty subjects were categorized as 0 (no risk), 24 as 1 (intermediate risk) and 20 as 2 (severe risk) based on their CHADS-2 scores. High sensitivity-CRP (CHADS-2 0=40.0%, 1=70.0%, 2=90.0%; p=0.003) and d-ROMs (CHADS-2 0=45%, 1=78%, 2=80%; p=0.04) were positively associated with the CHADS-2 risk score. Subjects with intermediate to severe CHADS-2 risk retained significant associations with abnormal HS-CRP (OR: 5.3, 95%CI: 1.1– 25.0) and d-ROMs (adjusted OR: 6.7, 95%CI: 1.2–38.8) after adjusting for gender and hypertension. In a multiple logistic interaction model, there was no significant interaction between HS-CRP and DROMs in their association with CHADS-2 risk categories (p=0.64). A biomarker risk-model, combining HS-CRP and d-ROMs, correlated well with the CHADS-2 risk categories (r= 0.49, p<0.001).

Conclusions. A biomarker risk-model using a combination of HS-CRP and d-ROMs correlates well with CHADS-2 risk scores in chronic AF. Either or both of these markers may add predictive power to future stroke risk prediction models.



Abnormal plasma levels of biomarkers of oxidative stress and inflammation across risk categories by CHADS-2 scores (HS-CRP, high sensitivity C-reactive protein; d-ROMs, derivatives of reactive oxygen metabolites; IL-1β, Interleukin Iβ; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor-α; Iso-P, Isoprostanes).

Neuman t Al. Clin Chem. 2007. 53: 1652–1657.

d-ROMs test values are inversely related to global cognition and verbal fluency in early old age (HAPIEEs)

Background & Aims. Oxidative stress is involved in Alzheimer disease pathology, but its impact on cognitive function in community-dwelling older adults remains unknown. We estimated associations between serum oxidative stress markers and cognitive function in early old age.

Methods. Subjects aged 45-69 years recruited in urban centers in Central and Eastern Europe had memory, verbal fluency, and processing speed assessed at baseline (2002-2005) and 3 years later. Derivatives of reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and total thiol levels (TTLs) were measured at baseline in a subsample. Linear regression was used to estimate associations of biomarkers with cognitive test scores cross-sectionally (**n = 4,304**) and prospectively (**n = 2,882**).

Results. Increased d-ROMs levels were inversely associated with global cognition and verbal fluency cross-sectionally and in prospective analysis; observed effects corresponded to 3-4 years' higher age. TTL was inconsistently associated with memory. BAP was not related to cognitive function.

Conclusion. This study found modest evidence for a relationship between serum d-ROMs and cognitive function in a population sample of older adults.

Horvat et Al. Dement Geriatr Cogn Disord. 2016. 42 (5–6): 297–309.

d-ROMs test values are related to serum uric acid in the Japanese general population

Background & Aim. Increased production of reactive oxygen species is a condition that is associated with, and plays a role in the progression of, various disorders such as hypertension, atherosclerosis, and diabetes. To assess in vivo oxidative stress levels and antioxidant potential and to analyze the relationship with serum uric acid (UA) levels.

Subjects and Methods. Oxidative stress levels (derivatives of reactive oxygen metabolites, d-ROMs) and antioxidant potential (biological antioxidant potential, BAP) were measured in individuals who underwent a general health screening test, and data were analyzed from **8,025 individuals (2,953 women and 5,072 men)** who were free from UA-lowering medication.

Results. Higher serum UA levels were associated with increased levels of d-ROMs in both genders, and this trend was more prominent in women. In addition, higher UA levels were also associated with higher BAP in both genders, although the dose dependence was not apparent in men. These associations remained statistically significant after adjusting for age, blood pressure, renal function, albuminuria, C-reactive protein, and insulin resistance index.

Conclusions. In individuals who underwent general health screening, serum UA levels were positively associated with both d-ROMs and BAP levels. Whether lowering of UA by lifestyle modification or by medication alters d-ROM/BAP levels awaits further investigations.

Yamakado M et Al. Nephron Clin Pract. 2014. 128: 49–56.

High d-ROMs test values may predict renal function deterioration in renovascular diseases. 4 yrs. follow-up.

Background & Aims. There is no consensus about the renal function outcome after revascularization with stenting in atherosclerotic renovascular disease. In the present study, the outcome in BP control and renal function in patients with renovascular disease treated with percutaneous angioplasty and stent placement is compared with the outcome in patients with renovascular disease treated with medical treatment only. Additionally, the impact of oxidative stress and eosinophil count in peripheral blood as predictors of renal function deterioration in renovascular disease irrespective of treatment is investigated.

Subjects and Methods. Eighty-two patients with renovascular disease were enrolled into a follow-up study (47.5±35.4 months). Thirty-six patients (group 1) underwent revascularization and stenting, and 46 patients (group 2) were on medical treatment only. In all patients, serum creatinine concentration, eosinophil count (EO) in peripheral blood, and estimation of oxidative stress with d-ROMs test were determined before and at the end of the followup.

Results. In revascularized patients (group 1), hypertension was cured in 11.1% and improved in 66.6%. Renal function improved in 30.5% and worsened in 36.2% of patients. In the medical treatment arm (group 2), hypertension improved in 71.4% of the patients. Renal function remained stable in 69.8% of patients and worsened in 30.2%. Cox regression analysis showed that higher levels of eosinophil count and higher levels of d-ROMs test, irrespectively of mode of treatment, were associated with renal function deterioration (i. e. serum creatinine increase more than 20% during follow-up).

Conclusions. Revascularization was not superior to medical treatment in renal survival but had a greater positive impact on blood pressure control. Eosinophil count and d-ROMs test values were the stronger predictive factors for serum creatinine increase.

Ziakka et Al. Renal Failure. 2008. 30: 965–970.

d-ROMs test and CRP values are closely related to frailty in an elderly German population (ESTHER cohort study)

Background & Aims. Oxidative stress (OS) and inflammatory biomarkers have been postulated to be important factors in the development of age-related diseases. While causes of frailty are complex and multidimensional based on the interaction of genetic, biological, physical, and environmental factors, the biological basis of frailty has been difficult to establish. In this study, we aimed to assess the possible association between different OS and inflammatory biomarkers and frailty.

Methods. This cross-sectional analysis was performed among **2,518** subjects participating in a large population-based cohort study on aging conducted in Germany. Frailty was assessed as proposed by Fried et al. [J Gerontol A Biol Sci Med Sci 2001;56:M146-M156]. OS biomarkers, biological antioxidant potential (BAP), derivate of reactive oxygen metabolites (d-ROMs test) and total thiol levels (TTL), and an established biomarker of inflammation C-reactive protein (CRP) were measured by spectrophotometry and immunoturbidimetry. Logistic regression models were performed to assess the relationship between the OS biomarkers and frailty status. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to quantify the associations.

Results. Mean levels of d-ROMs, TTL, and CRP differed between frail and non-frail participants (p values <0.0001). Comparing highest and lowest quartiles of the biomarkers, statistically significant positive associations with frailty were observed for d-ROMs (OR: 2.02, 95% CI: 1.25-3.25) and CRP (OR: 3.15, 95% CI: 2.00-4.96), respectively, after controlling for age and sex. An inverse statistically significant association with frailty was observed for TTL (OR: 0.42, 95% CI: 0.25-0.69).

Conclusion. The strong associations with OS biomarkers and CRP support a major role of OS and inflammation in the development of frailty, which should be followed up in further longitudinal studies on frailty.

Saum et Al. Gerontology. 2015. 61 (5): 407–415.

Increased d-ROMs test values are related to morbidity in older peoples (German cohort)

Background. Imbalances in metabolic, inflammatory and redox homeostasis play an important role in the leading theories of age-related morbidity, but no large-scale epidemiological study has been conducted so far assessing their associations with total morbidity and multi-morbidity in the same model.

Subjects and Methods. Analyses were conducted in **2,547** participants of an established population-based cohort study from Germany. The participants' median age was 70 years (range: 57-84) and 51.9% were women. End points were total somatic morbidity and multi-morbidity, assessed by the Cumulative Illness Rating Scale-Geriatric version.

Results. Overall, 251 study participants had multi-morbidity (9.9%). Except for the redox marker 'total thiol levels of proteins', all other assessed metabolic (obesity, diabetes, dyslipidaemia and hypertension), inflammatory (C-reactive protein) and oxidative stress markers (d-ROMs test) were significantly associated with total somatic morbidity and multi-morbidity if assessed individually. If modelled jointly, effect estimates were attenuated but remained statistically significant for the outcome 'total morbidity' and for low weight, obesity, insufficiently controlled diabetes and derivatives of reactive oxygen metabolites with respect to the outcome 'multi-morbidity'.

Conclusions. Results from this large sample of older adults support hypotheses that relate imbalances in metabolic, inflammatory and redox homeostasis to age-related morbidity. Despite over adjustment for closely related metabolic, inflammatory and oxidative stress conditions in the full model, independent associations of the markers with total morbidity and/or multi-morbidity were observed. Therefore, adverse metabolic, inflammatory and oxidative stress conditions may all play important roles in the pathogenesis of age-related morbidity, which should be investigated further in future longitudinal studies

Schottker et Al. Age Ageing. 2016. 45 (1): 127–135.

High d-ROMs test values are related to all-cause mortality at older age (population-based cohort study)

Background. The free radical/oxidative stress theory of aging has recently received much attention but the association of oxidative stress markers with all-cause mortality was not yet assessed in humans.

Subjects and Methods. We measured derivatives of reactive oxygen metabolites (d-ROMs) as a proxy for the reactive oxygen species concentration and total thiol levels (TTL) as a proxy for the redox control status in **2,932** participants of a population-based cohort study from Germany.

Results. The median age of the population was 70 years and 120 (4.1%) study participants died during a mean follow-up of 3.3 years. Compared with the bottom tertiles, the top tertiles of d-ROMs and TTL concentrations were both associated with all-cause mortality in models adjusted for age, sex, education, smoking, physical activity, and alcohol consumption (hazard ratios and 95% confidence intervals: 1.63 [1.01; 2.63] and 0.68 [0.53; 0.87], respectively). Adding diseases, the inflammatory marker C-reactive protein or a cumulative somatic morbidity index did not alter the results for TTL. However, the association of d-ROM and mortality was attenuated and no longer statistically significant after adding C-reactive protein and the somatic morbidity index to the model.

Conclusions. This study adds epidemiological evidence to the free radical/oxidative stress theory of aging. Both d-ROM and TTL were associated with mortality at older age. For TTL, this association was independent of baseline health status. Inflammation and higher general morbidity could be intermediate states on the pathway from high d-ROMs test levels to mortality. This hypothesis should to be explored by future studies with repeated measurements.

Schottker et Al. J Gerontol. 2015. 70 (4): 518–524.

High d-ROMs test values are associated to all-cause and CVD mortality (meta-analysis from CHANCES study)

Background. The free radical/oxidative stress theory of ageing has received considerable attention, but the evidence on the association of oxidative stress markers with mortality is sparse.

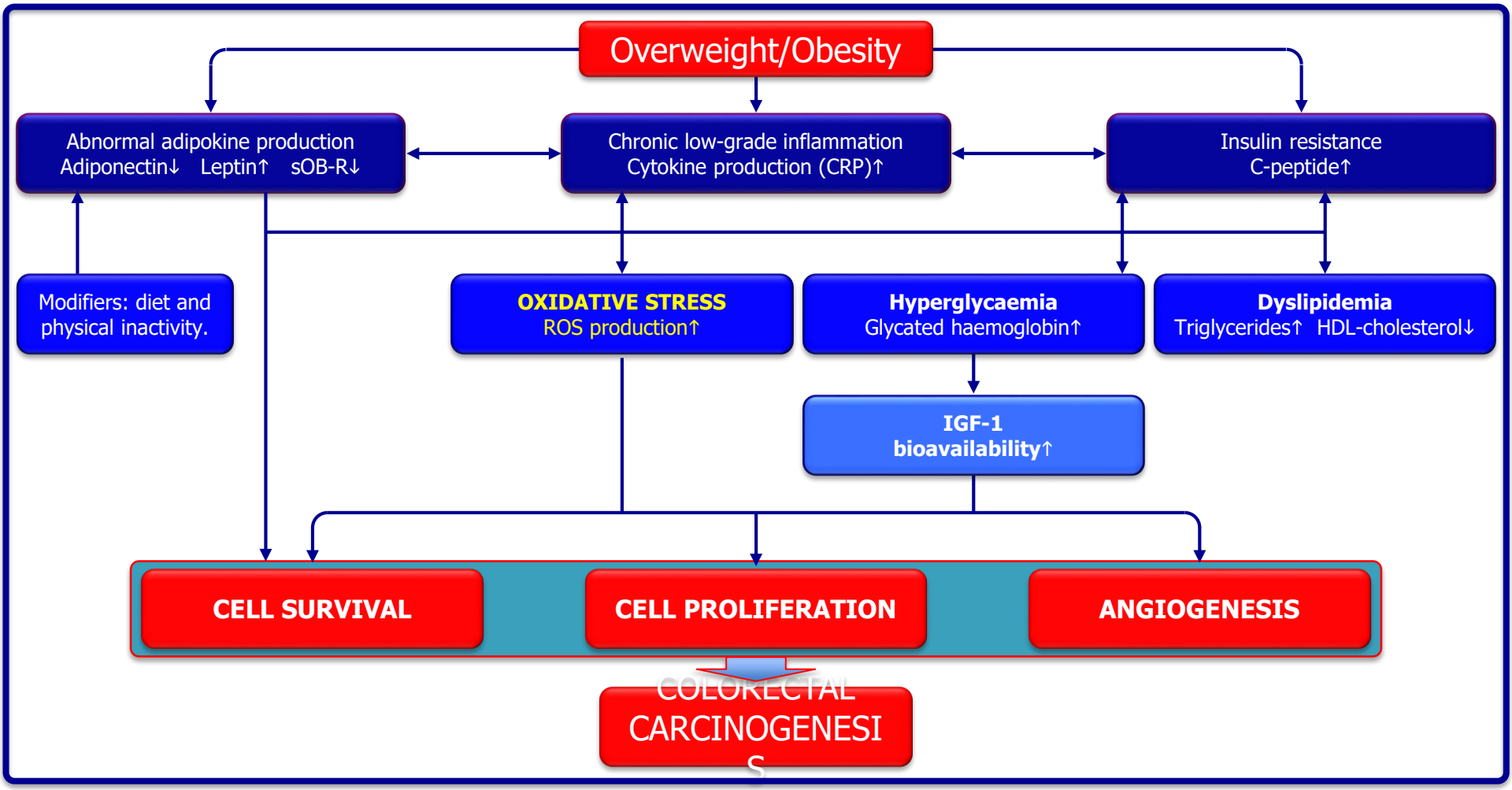
Subjects and Methods. We measured derivatives of reactive oxygen metabolite (d-ROMs) levels as a proxy for the reactive oxygen species concentration and total thiol levels (TTL) as a proxy for the redox control status in **10,622** men and women (age range, 45-85 years), from population-based cohorts from Germany, Poland, Czech Republic, and Lithuania, of whom 1,702 died during follow-up.

Results. Both oxidative stress markers were significantly associated with all-cause mortality independently from established risk factors (including inflammation) and from each other in all cohorts. Regarding cause-specific mortality, compared to low d-ROMs levels (≤ 340 CARR U), very high d-ROM levels (>500 CARR U) were strongly associated with both cardiovascular (relative risk (RR), 5.09; 95 % CI, 2.67-9.69) and cancer mortality (RR, 4.34; 95 % CI, 2.31-8.16). TTL was only associated with CVD mortality (RR, 1.30; 95 % CI, 1.15-1.48, for one-standard-deviation-decrease). The strength of the association of TTL with CVD mortality increased with age of the participants (RR for one-standard-deviation-decrease in those aged 70-85 years was 1.65; 95 % CI, 1.22-2.24).

Conclusions. In these four population-based cohort studies from Central and Eastern Europe, the oxidative stress serum markers d-ROMs and TTL were independently and strongly associated with all-cause and CVD mortality. In addition, d-ROMs levels were also strongly associated with cancer mortality. This study provides epidemiological evidence supporting the free radical/oxidative stress theory of ageing and suggests that d-ROMs and TTL are useful oxidative stress markers associated with premature mortality.

Schottker et Al. BMC Med. 2015. 13 (300).

Oxidative stress and colorectal carcinogenesis



Aleksandrova et Al. Eur J Epidemiol. 2014. 29 (4): 261–275.

High d-ROMs test values are predictive for small colorectal cancer after a 3 years-follow-up.

Background. Oxidative stress has been shown to play an important role in carcinogenesis, but prospective evidence for an association between biomarkers of oxidative stress and colorectal cancer (CRC) risk is limited.

Subjects and methods. We investigated the association between pre-diagnostic serum levels of oxidative stress indicators (i.e., d-ROMs and FRAP) and CRC risk. This was examined in a nested case-control study (**1,064 CRC cases, 1,064 matched controls**) in the European Prospective Investigation Into Cancer and Nutrition cohort (**EPIC**) (1992–2003). Incidence rate ratios and 95% confidence intervals were calculated using conditional logistic regression analyses.

Results. d-ROMs values were associated with overall CRC risk (highest tertile vs. lowest: adjusted incidence rate ratio (IRRadj) 1/4 1.91, 95% confidence interval (CI): 1.47, 2.48), proximal (IRRadj 1/4 1.89, 95% CI: 1.06, 3.36) and distal (IRRadj 1/4 2.31, 95% CI: 1.37, 3.89) colon cancer, and rectal cancer (IRRadj 1/4 1.69, 95% CI: 1.05, 2.72). When results were stratified by tertile of follow-up time, the association remained significant only in participants with less than 2.63 years of follow-up (IRRadj 1/4 2.28, 95% CI: 1.78, 2.94; P-heterogeneity < 0.01). FRAP was not associated with CRC risk.

Conclusion. Pre-diagnostic serum ROM levels were associated with increased risk of CRC. However, this association was seen only in subjects with relatively short follow-up, suggesting that the association results from production of reactive oxygen species by preclinical tumours.

	No. of Cases	No. of Controls	d-ROMs test Mean (SD)	d-ROMs test Median	Crude[a] IRR	Crude[a] 95% CI	Multivariate-Adjusted[b] IRR	Multivariate-Adjusted[b] 95% CI
COLORECTUM								
Tertile of serum ROM level[c]								
1 (90–348 U/mL)	246	336	306 (36)	317	1.00		1.00	
2 (349–409 U/mL)	362	360	381 (17)	382	1.43	1.14, 1.79	1.40	1.10, 1.77
3 (410–735 U/mL)	445	357	464 (46)	452	1.92	1.51, 2.45	1.91	1.47, 2.48
P_{trend}								<0.01
Continuous (per 1-SD increase[d])	1,053	1,053			1.34	1.21, 1.48	1.33	1.19, 1.49
COLON								
Tertile of serum ROM level								
1	142	206	306 (37)	316	1.00		1.00	
2	219	234	381 (17)	383	1.43	1.07, 1.90	1.34	0.98, 1.84
3	304	225	466 (49)	453	2.28	1.67, 3.11	2.15	1.53, 3.01
P_{trend}								<0.01
Continuous (per 1-SD increase)	665	665			1.38	1.22, 1.57	1.35	1.18, 1.54
PROX. COLON								
Tertile of serum ROM level								
1	51	73	306 (39)	317	1.00		1.00	
2	89	103	381 (17)	383	1.23	0.79, 1.92	0.95	0.56, 1.62
3	136	100	473 (52)	460	2.25	1.38, 3.66	1.89	1.06, 3.36
P_{trend}								0.02
Continuous (per 1-SD increase)	276	276			1.46	1.20, 1.78	1.40	1.12, 1.76
DISTAL. COLON								
Tertile of serum ROM level								
1	70	103	306 (38)	316	1.00		1.00	
2	112	111	381 (18)	382	1.58	1.04, 2.39	1.90	1.15, 3.13
3	143	111	461 (46)	449	2.12	1.37, 3.27	2.31	1.37, 3.89
P_{trend}								<0.01
Continuous (per 1-SD increase)	325	325			1.32	1.10, 1.58	1.33	1.07, 1.64
RECTUM								
Tertile of serum ROM level								
1	104	130	307 (34)	318	1.00		1.00	
2	143	126	380 (18)	380	1.46	1.01, 2.09	1.69	1.10, 2.61
3	141	132	458 (39)	450	1.42	0.96, 2.11	1.69	1.05, 2.72
P_{trend}								0.04
Continuous (per 1-SD increase)	388	388			1.25	1.05, 1.49	1.35	1.09, 1.67

Incidence rate ratios for colorectal cancer and its sub-sites, continuously and by tertile of d-ROMs test results.

Leufkens et Al. Am J Epidemiol. 2012. 175: 653–663.

44 Researchers – more than 2,000 subjects monitored for 5 years – 10 different European Countries involved

The European Prospective Investigation Into Cancer and Nutrition (EPIC) was designed to prospectively investigate the relation between diet and various lifestyle factors and the risk of cancer. The study was initiated in 1991 and included 521,448 participants, approximately 70% women and mostly aged 35–70 years. Participants were recruited at 23 regional or national centres in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom). In the majority of study centres, individuals were selected from the general population of a specific town or province.

Multivariate-adjusted continuous incidence rate ratios for colorectal, colon, and rectal cancer according to a 1-SD increase in d-ROMs test values, by tertile of follow-up time.

Tertile of Follow-up Time[c]	No. of Cases[d]	No. of Controls[d]	d-ROMs test values (CARR U)				Colorectum (1,053 Cases, 1,053 Controls)		Colon (665 Cases, 665 Controls)		Rectum (388 Cases, 388 Controls)	
			Cases		Controls							
			Mean	Range	Mean	Range	IRR	95% CI	IRR	95% CI	IRR	95% CI
1	348	348	412	133–639	374	90–633	2.28	1.78, 2.94	2.35	1.69, 3.27	3.07	1.50, 6.03
2	368	368	390	191–581	384	91–735	1.14	0.95, 1.38	1.14	0.89, 1.47	0.93	0.58, 1.50
3	337	337	386	134–643	380	177–639	1.11	0.89, 1.38	1.08	0.82, 1.43	1.60	0.93, 2.74
P for heterogeneity[e]							<0.01		<0.01		0.02	

Abbreviations. CI, confidence interval; IRR, incidence rate ratio; d-ROMs, reactive oxygen metabolites.
[a]Conditioned on matching factors and adjusted for smoking status/dose/duration, physical activity, educational level, month of blood collection, weight, height, waist circumference, intake of red meat, processed meats, alcohol, fruit, vegetables and fish.
[b]A 1-unit increase in SD was the same for all cancer sites: 74.4 U/mL.
[c]Cutoff points for follow-up time were the same for all cancer sites: <2.63 years and <4.81 years.
[d]In the total data set.
[e]P for heterogeneity across tertiles, calculated using the heterogeneity statistic derived from the inverse variance method.

Leufkens et Al. Am J Epidemiol. 2012. 175: 653–663.

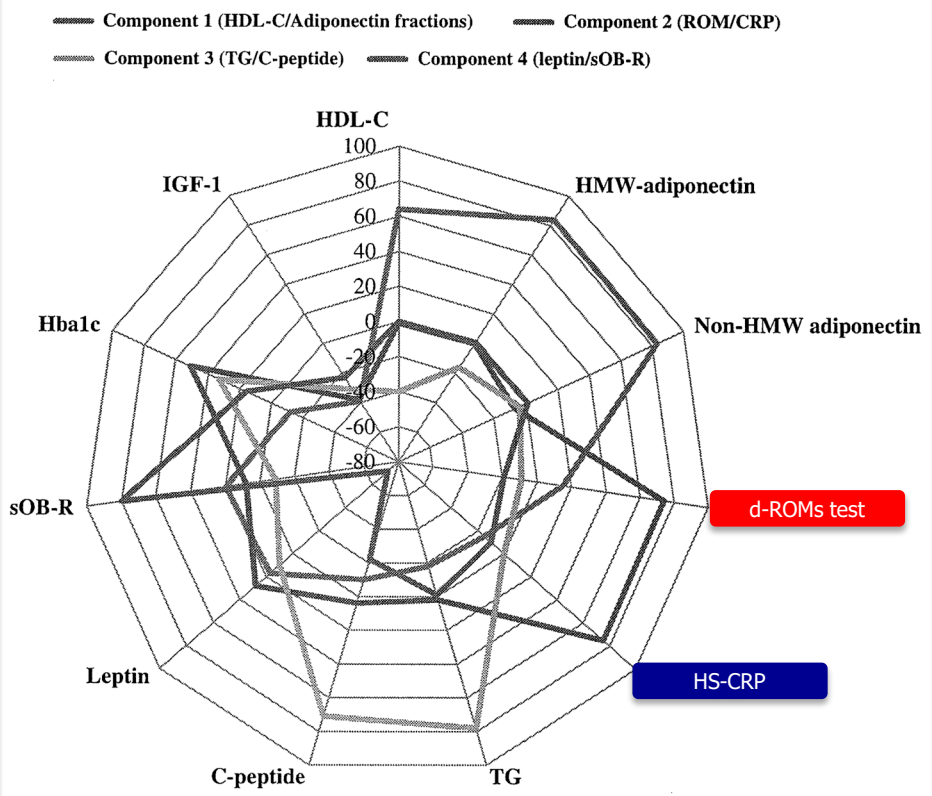
High d-ROMs test values coupled to high CRP are associated with increased risk of colorectal cancer

Background & Aims. A number of biomarkers of inflammatory and metabolic pathways are individually related to higher risk of colorectal cancer (CRC); however, the association between biomarker patterns and CRC incidence has not been previously evaluated. Our study investigates the association of biomarker patterns with CRC in a prospective nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC).

Subjects and Methods. During median follow-up time of 7.0 (3.7–9.4) years, **1,260 incident CRC cases occurred and were matched to 1,260 controls using risk-set sampling**. Pre-diagnostic measurements of C-peptide, glycated hemoglobin, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), d-ROMs test, insulin-like growth factor 1, adiponectin, leptin and soluble leptin receptor (sOB-R) were used to derive biomarker patterns from principal component analysis (PCA). The relation with CRC incidence was assessed using conditional logistic regression models. We identified four biomarker patterns 'HDL-C/Adiponectin fractions', 'ROM/CRP', 'TG/C-peptide' and 'leptin/sOB-R' to explain 60 % of the overall biomarker variance.

Results. In multivariable-adjusted logistic regression, the 'HDL-C/Adiponectin fractions', 'ROM/CRP' and 'leptin/sOB-R' patterns were associated with CRC risk [for the highest quartile vs the lowest, incidence rate ratio (IRR) = 0.69, 95 % CI 0.51–0.93, *P*-trend = 0.01; IRR = 1.70, 95 % CI 1.30–2.23, *P*-trend = 0.002; and IRR = 0.79, 95 % CI 0.58–1.07; *P*-trend = 0.05, respectively]. In contrast, the 'TG/C-peptide' pattern was not associated with CRC risk (IRR = 0.75, 95 % CI 0.56–1.00, *P*-trend = 0.24). After cases within the first 2 follow-up years were excluded, the 'ROM/CRP' pattern was no longer associated with CRC risk, suggesting potential influence of preclinical disease on these associations.

Conclusion. By application of PCA, the study identified 'HDL-C/Adiponectin fractions', 'ROM/CRP' and 'leptin/sOB-R' as biomarker patterns representing potentially important pathways for CRC development.



Incidence rate ratios for colorectal cancer and its sub-sites, continuously and by tertile of d-ROMs test results.

Aleksandrova et Al. Eur J Epidemiol. 2014. 29 (4): 261–275.

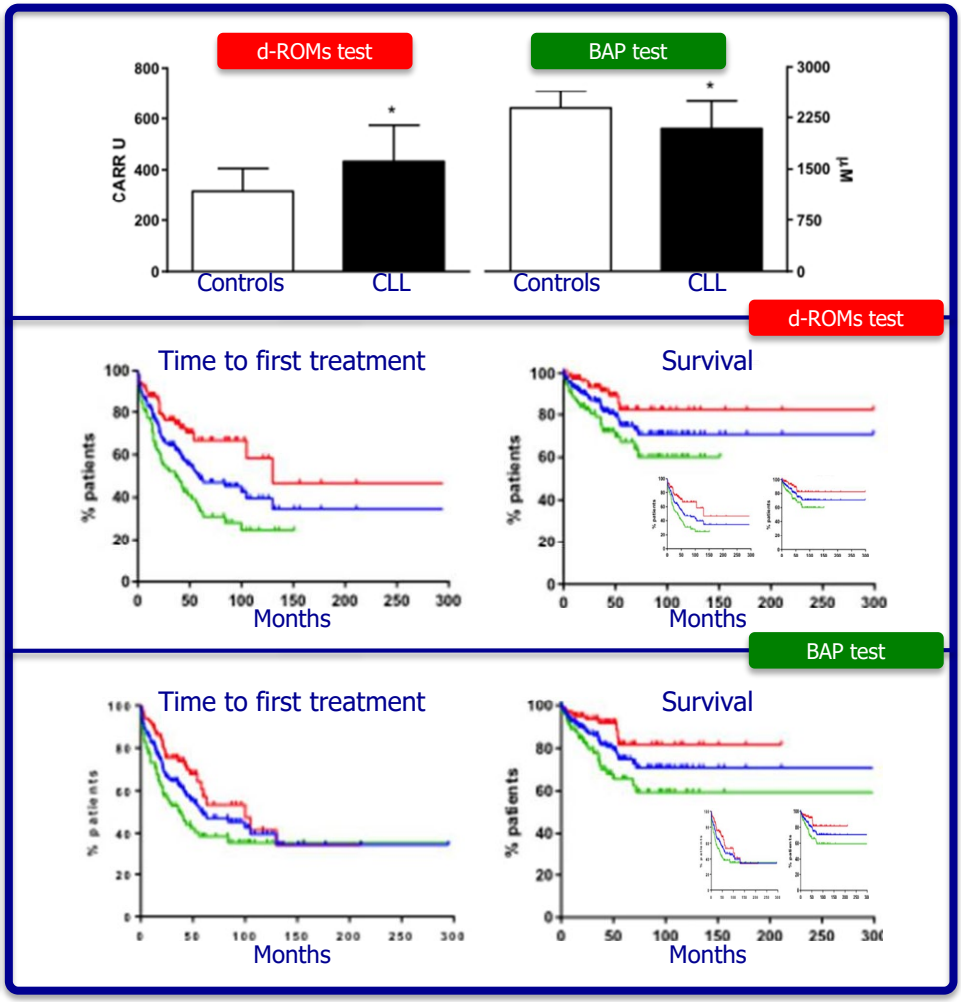
Predictive value of d-ROMs test in chronic lymphocytic leukaemia

Background & Aims. To evaluate the prognostic significance of oxidative stress (OS) and antioxidant defense status measurement in patients with chronic lymphocytic leukemia (CLL).

Subjects and Methods. d-ROMs test and BAP test were evaluated at diagnosis of 165 patients with CLL and correlated with clinical-biological features and prognosis.

Results.
An increased oxidative damage (d-ROMs test) and a reduced antioxidant potential (BAP test), were found in CLL patients than normal controls (p <0.0001). CLL patients with higher d-ROMs values had higher number of circulating white blood cells and lymphocytes, and higher values of β2 -microglobulin. Higher d-ROMs values were found in female (p=0.0003), in patients with unmutated IgVH (p=0.04), unfavourable cytogenetics (p=0.002), and more advanced clinical stage (p=0.002). Higher BAP test values were found in patients expressing CD49d (p=0.01) and with more advanced clinical stage (p=0.004). At a median follow-up of 48 months, CLL patients with d-ROMs ≥418 CARR U were found to have a shorter time to first treatment (TFT) (p=0.0002), and a reduced survival (p=0.006) than others. CLL patients with BAP test values ≥2,155 μM had a shorter TFT (p 0.008) and a shorter survival (p 0.003).

Conclusion. OS can affect CLL patients by concomitantly increasing reactive oxygen metabolites production and decreasing antioxidant defences.



D'Arena, Iorio et Al. Eur J Haematol. 2017. doi: 10.1111/ejh.12918

Hepatocellular carcinoma patients with increased d-ROMs test are prone to recurrence after curative treatment

Hepatocellular carcinoma patients with increased oxidative stress levels are prone to recurrence after curative treatment: a prospective case series study using the d-ROM test

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Journal of Cancer Research and Clinical Oncology

ISSN 0171-5216
Volume 139
Number 5

J Cancer Res Clin Oncol (2013)
139:845-852
DOI 10.1007/s00432-013-1389-1

Springer

Background & Aims. Oxidative stress plays an important role in liver carcinogenesis. To determine the impact of oxidative stress on the recurrence of stage I/II hepatocellular carcinoma (HCC) after curative treatment, we conducted a prospective case series analysis.

Subjects and Methods. This study included 45 consecutive patients with stage I/II HCC, who underwent curative treatment by surgical resection or radiofrequency ablation at Gifu Municipal Hospital from 2006 to 2007. In these 45 cases, recurrence-free survival was estimated using the Kaplan-Meier method. The factors contributing to HCC recurrence, including the serum levels of derivatives of reactive oxygen metabolites (d-ROMs) as an index of oxidative stress, were subjected to univariate and multivariate analyses using the Cox proportional hazards model.

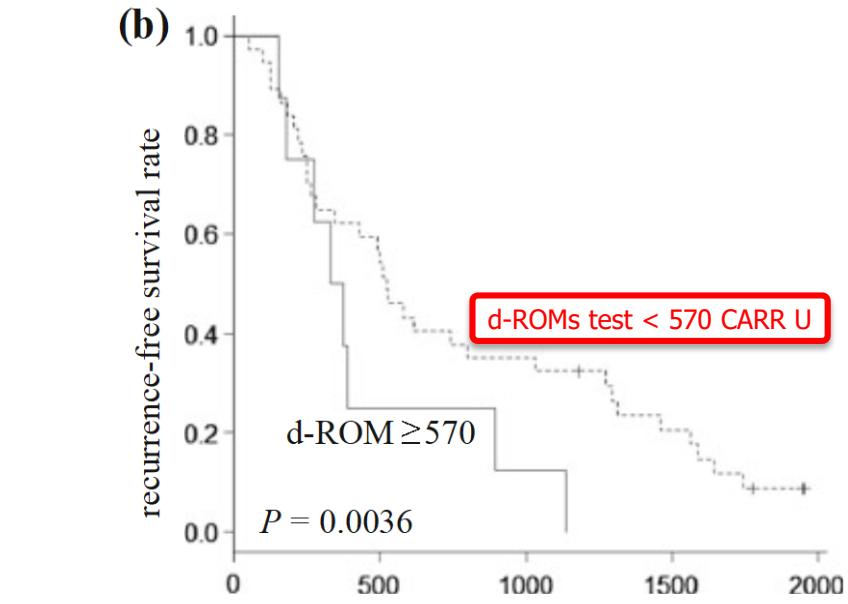
Results. The serum levels of d-ROMs (P = 0.0231), α-fetoprotein (AFP, P = 0.0274), and fasting plasma glucose (P = 0.0400) were significantly associated with HCC recurrence in the univariate analysis. Multivariate analysis showed that the serum levels of d-ROMs (hazard ratio [HR] 1.0038, 95 % confidence interval [CI] 1.0002-1.0071, P = 0.0392) and AFP (HR 1.0002, 95 % CI 1.0000-1.0003, P = 0.0316) were independent predictors of HCC recurrence. Kaplan-Meier analysis showed that recurrence-free survival was low in patients with high serum d-ROMs (≥570 CARR U, P = 0.0036) and serum AFP (≥40 ng/dL, P = 0.0185) levels.

Conclusions. The serum levels of d-ROMs and AFP can be used for screening patients with a high risk for HCC recurrence. Patients who show increased levels of these factors require careful surveillance.

Suzuki et Al. J Cancer Res Clin Onc. 2013. 139: 845–852.

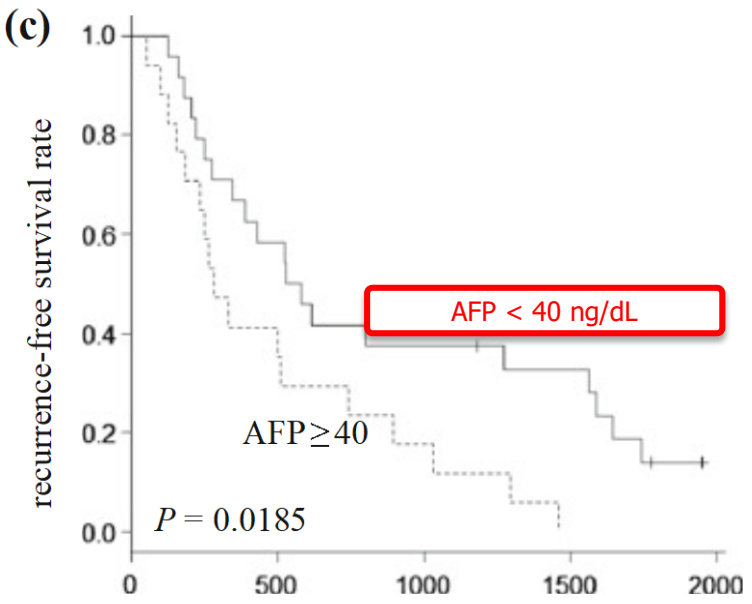
Longer recurrence-free increased survival with total oxidant capacity values <570 CARR U

d-ROMs test



No. at risk				
d-ROM < 570 36	20	13	7	0
d-ROM ≥ 570 9	2	1	0	0

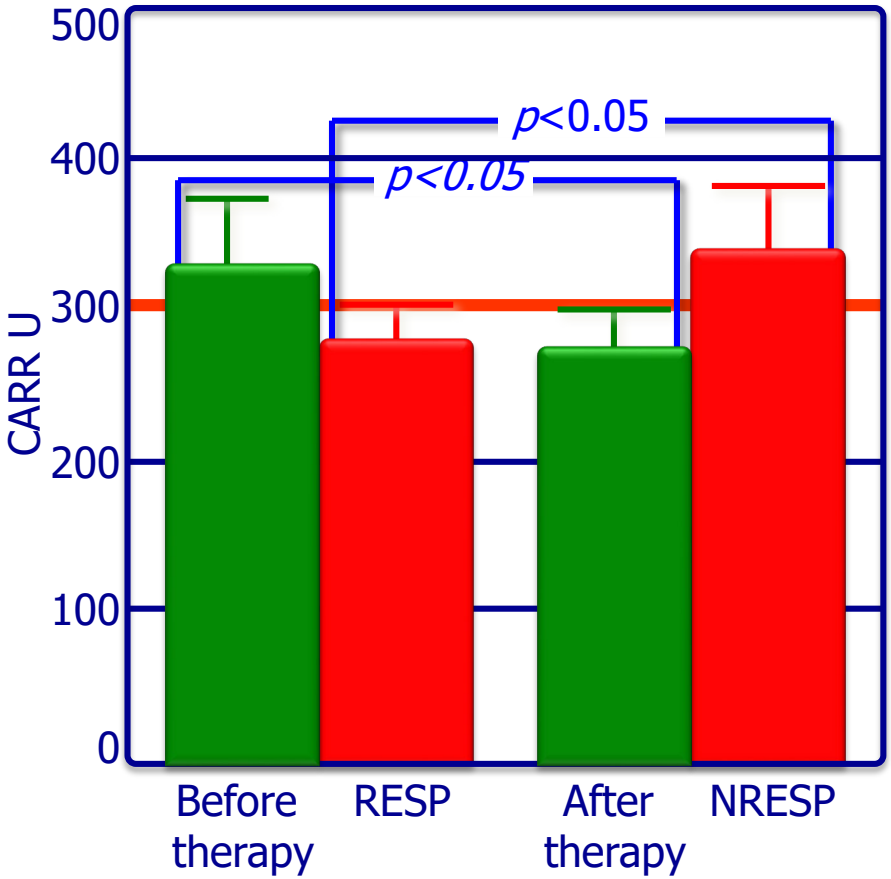
AFP

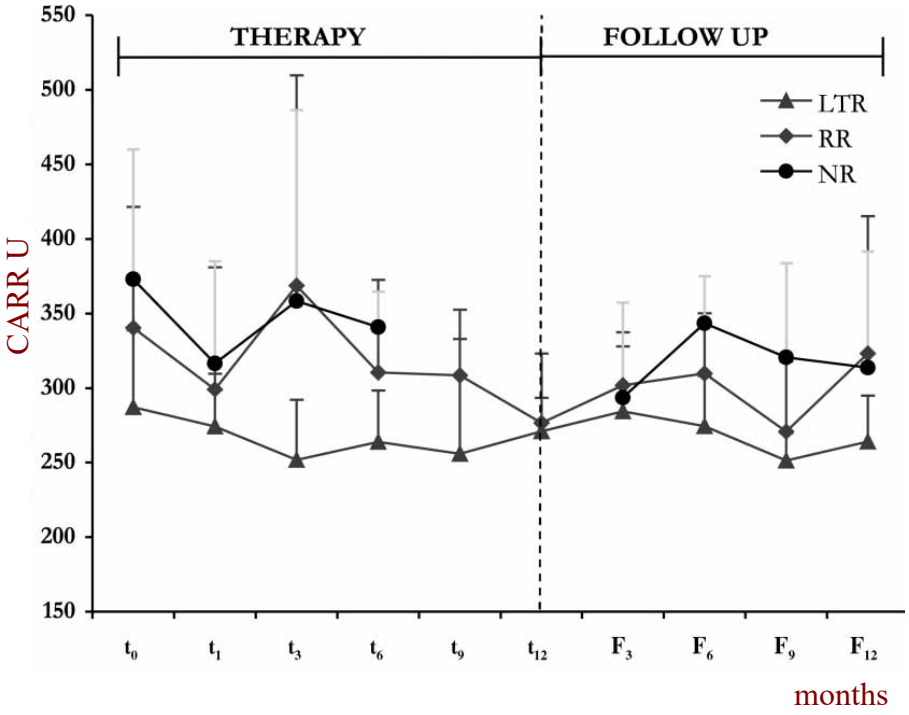


No. at risk				
AFP < 40	24	14	9	7	0
AFP ≥ 40	17	6	3	0	0

Longer recurrence-free increased survival with AFP values <40 ng/dL

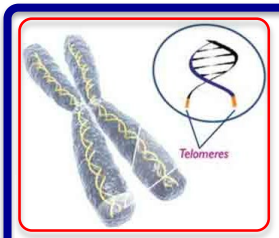
Low initial oxidant capacity is predictive of long-time response (LTR) to Ribavirin-IFN in chronic C hepatitis





Morisco et Al. Free Radical Research. 2004. 38: 573–580.

Telomeres and telomerase. Overview. 1.



TTAGG repeat-binding factor 1/2

Repressor-activator protein 1

TRF1 interacting nuclear factor-2

Tripeptidyl peptides 1

Protection of telomere β1

Catalytic subunit possessing reverse transcriptase activity (DNA from RNA)

RNA component

TERC

TIN2

RAP1

TPP1

TRF1

TRF2

POT1

TERT

NHP2

NOP10

GAR

Dyskerin

TELOMERE

G T G G G A G G G G T T A G G G T T A G G G T T A G G G 3'

C A C C C A U C C C A A U C C C A 5'

A A U C C C

RNA TEMPLATE

5′

3′

TELOMERASE COMPLEX
(telomeres elongation)

SHELTERIN COMPLEX
(telomere end protection)

The normal function of shelterin is essential for telomeric function; its suppression results in the development of degenerative pathologies.

☞The telomeric chromatin plays also other functions i. e. it contains negative regulators of telomeric lengths and telomeric recombination and is involved in the transcriptional silencing of genes located in vicinity of the telomeres ("subtelomeric silencing") and in the correct chromosome segregation during mitosis.

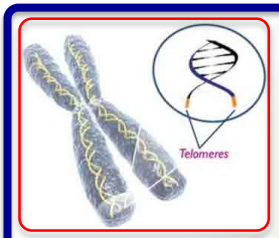
☞Mutations involving various telomerase components such as Tert, terc, Dkc1 and shelterin (Tin2) are linked to human genetic disorders such as dyskeratosis congenita and aplastic anemia. These diseases are associated with short or dysfunctional telomeres.

Shortening of telomeres below a threshold length and/or alterations in functioning of telomere-binding proteins result in loss of telomeric protection, which leads to "end-to-end chromosomal fusion," "arrest of cell cycle," and "apoptosis." Telomeric shortening is associated with cell division owing to inability of DNA polymerases to replicate ends of linear chromosomes. This is called "end replication problem."

The "telomerase" causes addition of TTAGG repeats at the chromosomal ends. Strong telomerase expression is detectable in stem cells and in early stages of embryogenesis.

Olovnikov. Exp Gerontol. 1996. 31(4): 443–448.

Telomeres and telomerase. Overview. 2.



CHROMOSOME

DNA

TELOMERE-TELOMERASE COMPLEX

During cell division, the cell copies the entire length of each chromosome within the nucleus except for the last few nucleotides that make up the telomere, the protective tip at the chromosome's end.

The enzyme telomerase bring in repeating base pairs to protect the telomere from getting shorter. The corresponding nucleotides, floating freely in the nucleus, find their way to the telomerase.

When a cell divides, so does its DNA, telomeres and telomerase not only ensure that the code is copied faithfully but also determine when cells can and can't divide.

When we're young, telomerase keeps out telomeres long so DNA can be copied many times without loosing its protective tips. But too much telomerase activity later in life can lead to uncontrolled cell division (cancer).

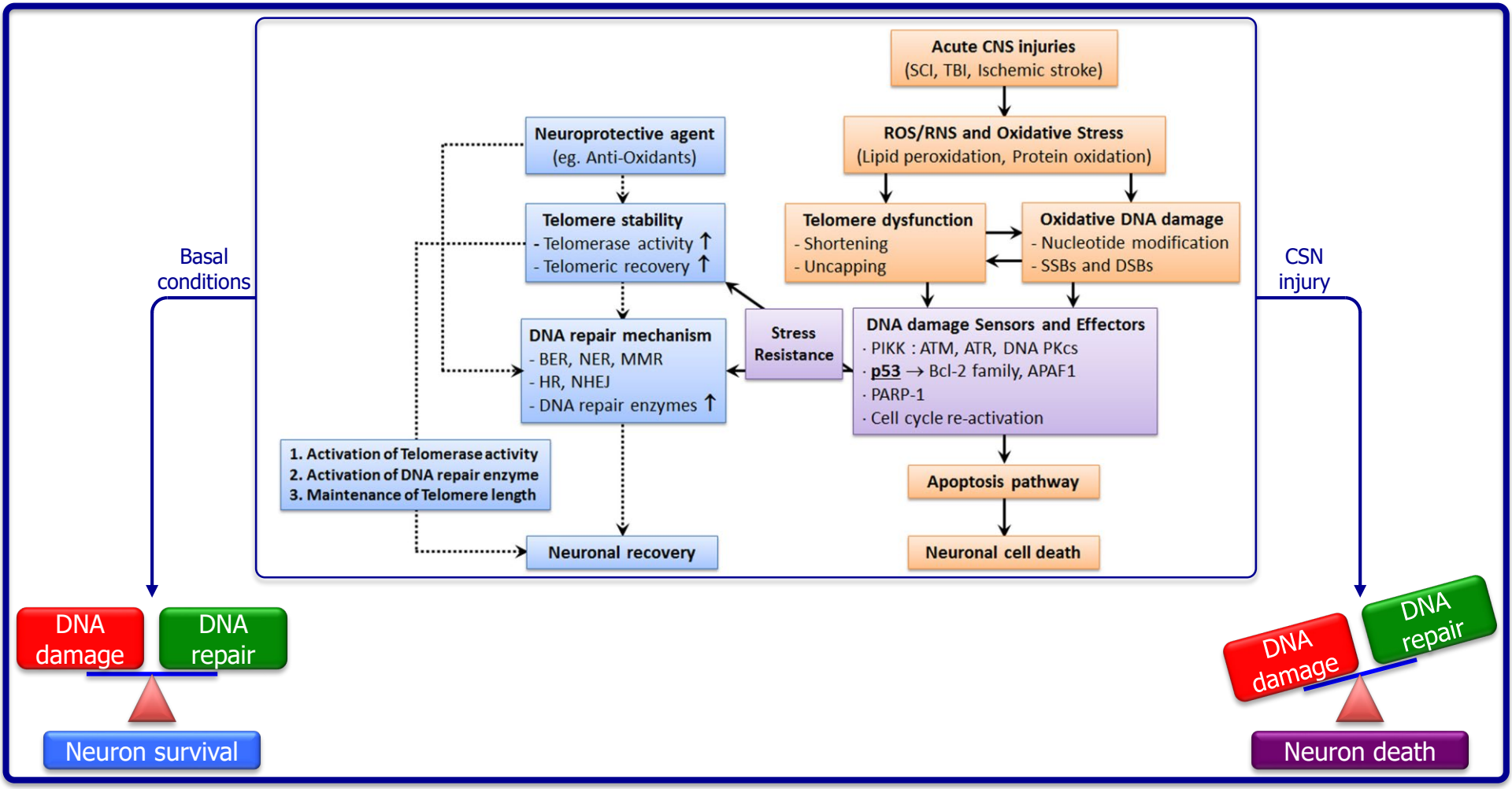
As we age, telomerase production drops off; telomeres shorten, sending cells into senescence.

Some researchers are looking to block telomerase as a way to prevent cancer.

Some researchers hope to boost telomerase to stave off aging.

Chatterjee. J Oral Maxillofac Pathol. 2017. 21(1): 87–91.

Telomeres, telomerase and oxidative stress: the neurodegeneration model.



Smith et Al. Neurochem Int. 2013. 62(5): 764–765.

Higher d-ROMs test values and shorter telomeres in fastly growing wild king penguins

Background & Aims. One of the reasons for animals not to grow as fast as they potentially could is that fast growth has been shown to be associated with reduced lifespan. However, we are still lacking a clear description of the reality of growth-dependent modulation of ageing mechanisms in wild animals. Using the particular growth trajectory of small king penguin chicks naturally exhibiting higher-than-normal growth rate to compensate for the winter break, we tested whether oxidative stress and telomere shortening are related to growth trajectories.

Animals and Methods. Parameters were measured at the beginning and at the end of the post-winter growth period in three groups of chicks (small chicks, which either passed away or survived the growth period, and large chicks).

Results, discussion and conclusion. Small chicks that died early during the growth period had the highest level of oxidative damage and the shortest telomere lengths prior to death. Here, we show that small chicks that grew faster did it at the detriment of body maintenance mechanisms as shown by (i) higher oxidative damage and (ii) accelerated telomere loss. This is the first evidence for a mechanistic link between growth and ageing rates under natural conditions.

Geiger et Al. Mol Ecol. 2012. 21(6): 1500–1510.

Oxidative stress, chronic inflammation, and telomere lenght in patients with periodontitis.

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Oxidative stress, chronic inflammation, and telomere length in patients with periodontitis

Stefano Masi [a,b], Klelia D. Salpea [c], KaWa Li [c], Mohamed Parkar [d], Luigi Nibali [d], Nikos Donos [d], Kalpesh Patel [d], Stefano Taddei [b], John E. Deanfield [a], Francesco D'Aiuto [d,*,1], Steve E. Humphries [c,e,1]

Background & Aims. The aim of this study was to determine leukocyte telomere length (LTL) in individuals with periodontitis and controls, exploring its relationship with systemic inflammation and oxidative stress.

Subjects and Methods. Five hundred sixty-three participants were recruited for this case–control study: 356 subjects with and 207 subjects without periodontitis. LTL was measured by a qPCR technique from leukocytes' DNA. Global measures of oxidative stress (reactive oxygen metabolites) and biological antioxidant potential in plasma were performed together with high-sensitivity assays for C-reactive protein (CRP). Leukocyte counts and lipid profiles were performed using standard biochemistry.

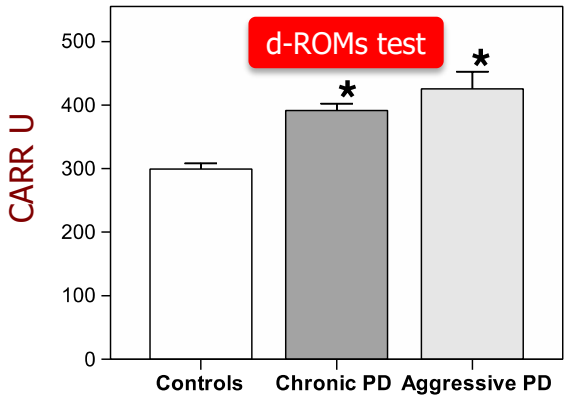
Results. Cases had higher levels of CRP (2.1 ± 3.7 mg/L vs 1.3 ± 5.4 mg/L, P b 0.001) and reactive oxygen metabolites (378.1±121.1 U Carr vs 277.4±108.6 U Carr, Pb0.001) compared to controls. Overall, cases had shorter LTL with respect to controls (1.23 ± 0.42 vs 1.12 ± 0.31 T/S ratio, P = 0.006), independent of age, gender, ethnicity, and smoking habit. When divided by subgroup of periodontal diagnosis (chronic, n = 285; aggressive, n = 71), only chronic cases displayed shorter LTL (P = 0.01). LTL was negatively correlated with age (P=0.001; R=−0.2), oxidative stress (P=0.008; R=−0.2), and severity of period- ontitis (P=0.003; R=−0.2) in both the whole population and the subgroups (cases and controls).

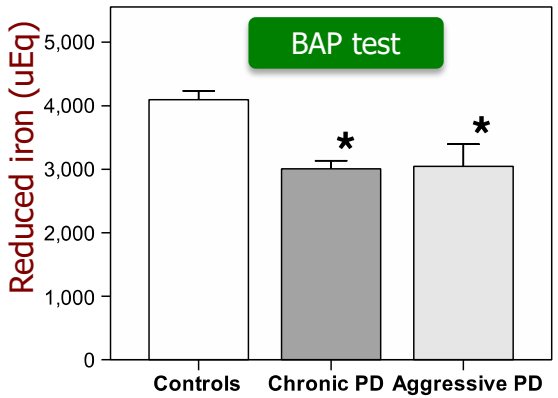
Conclusion. Shorter telomere lengths are associated with a diagnosis of periodontitis and their measures correlate with the oxidative stress and severity of disease.

Masi et Al. Free Radic Biol Med. 2011. 50: 730–735.

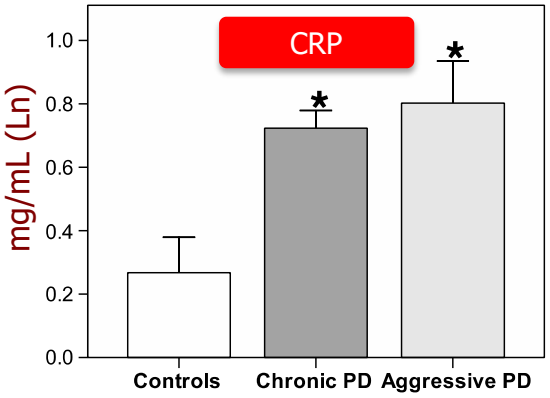
Higher levels of oxidative stress and inflammation and shorter telomeres in periodontitis patients *vs.* controls

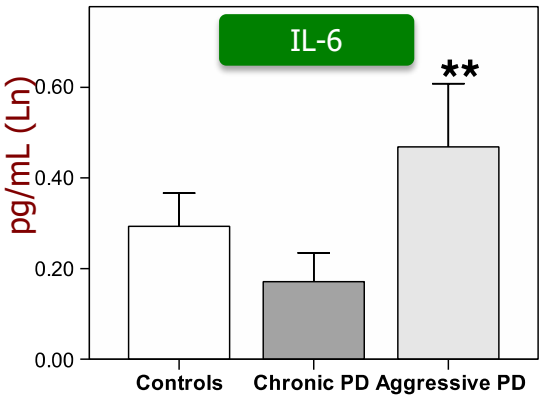
Oxidative balance



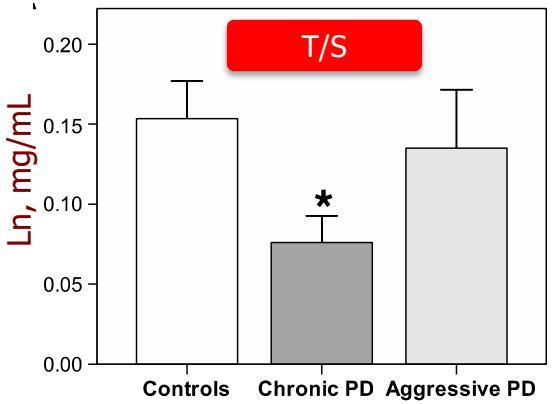


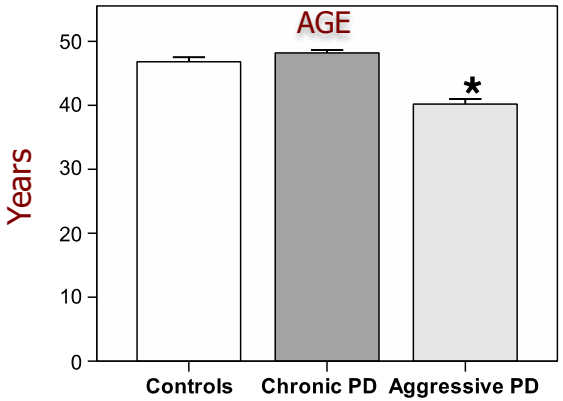
Inflammatory biomarkers





Telomeres lenght/age

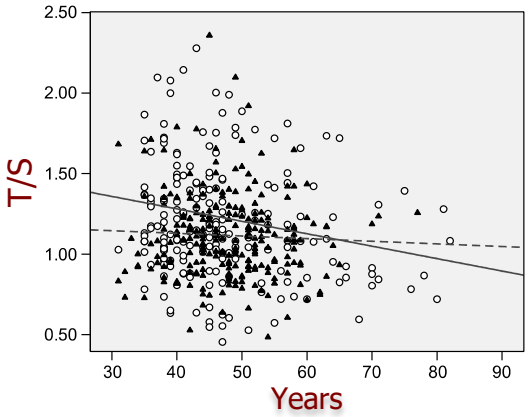




Masi et Al. Free Radic Biol Med. 2011. 50: 730–735.

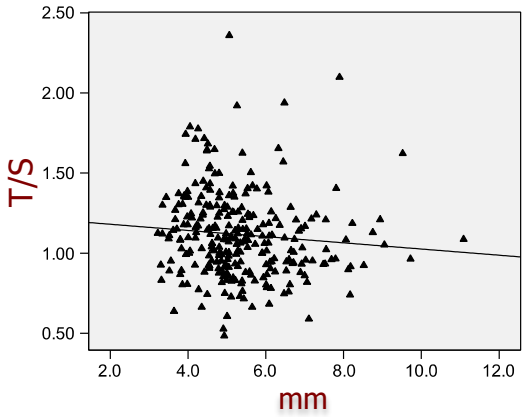
Statistically significant relationships between telomere lenght and age/PCAL/oxidative stress

Telomere lenght *vs.* age



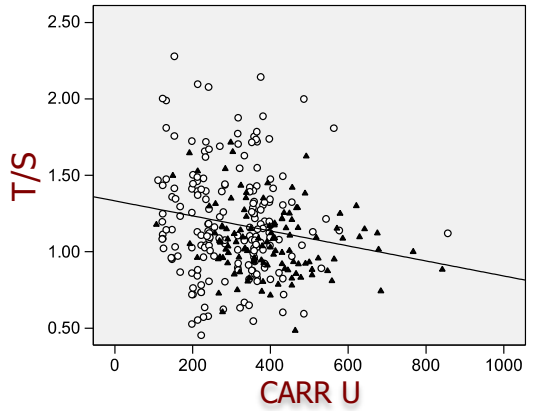
Scatter plot of predicted T/S ratios based on fully adjusted models (age, gender, ethnicity, smoking, lipids) by age in cases and controls. Controls are drawn as open circles and cases as filled triangles. Solid line corresponds to the slope of T/S by age in cases and dashed line to the slope of T/S by age in controls. P value of the statistical difference in slope between cases (slope=0.002) and controls (slope=0.035) is 0.001.

Telomere lenght *vs.* PCAL



Scatter plots of leukocyte T/S ratios against periodontal clinical attachment levels (R=−0.2, P=0.008), by Spearman rank correlation test. Controls are drawn as open circles and cases as filled triangles.

Telomeres lenght *vs.* d-ROMs



Scatter plots of leukocyte T/S ratios against d-ROM test serum levels (R = −0.2, P=0.001), by Spearman rank correlation test. Controls are drawn as open circles and cases as filled triangles.

Masi et Al. Free Radic Biol Med. 2011. 50: 730–735.

Embryonic exposure to cortisone increased oxidative stress (d-ROMs/OXY) and shortened telomeres in chicken

Background & Aims. Early embryonic exposure to maternal glucocorticoids can broadly impact physiology and behaviour across phylogenetically diverse taxa. The transfer of maternal glucocorticoids to offspring may be an inevitable cost associated with poor environmental conditions, or serve as a maternal effect that alters offspring phenotype in preparation for a stressful environment. Regardless, maternal glucocorticoids are likely to have both costs and benefits that are paid and collected over different developmental time periods. We manipulated yolk corticosterone (cort) in domestic chickens (Gallus domesticus) to examine the potential impacts of embryonic exposure to maternal stress on the juvenile stress response and cellular ageing.

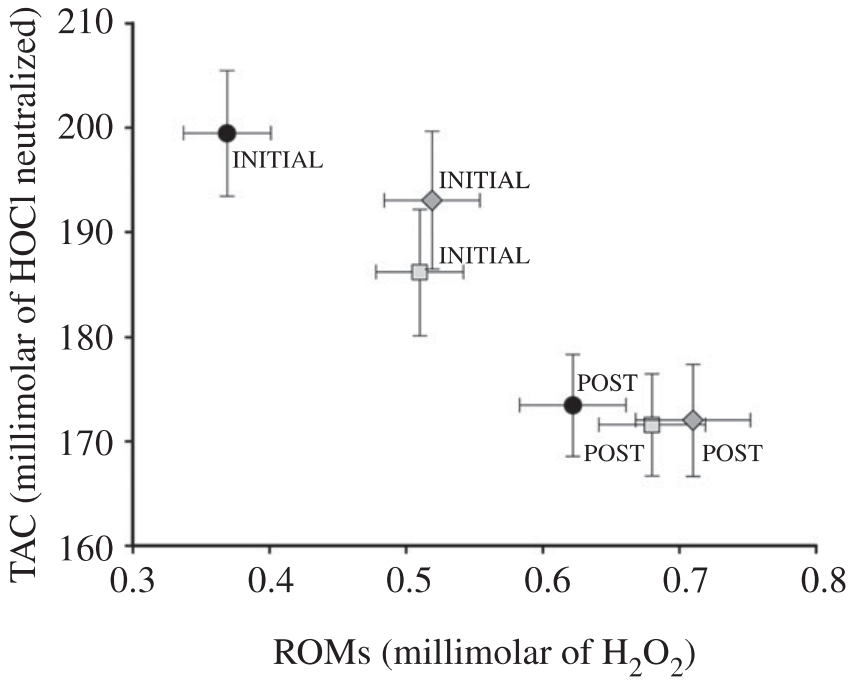
Results and discussion. Here, we report that juveniles exposed to experimentally increased cort in ovo had a protracted decline in cort during the recovery phase of the stress response. All birds, regardless of treatment group, shifted to oxidative stress during an acute stress response. In addition, embryonic exposure to cortisone resulted in higher levels of reactive oxygen metabolites and an over-representation of short telomeres compared with the control birds.

Conclusions. In many species, individuals with higher levels of oxidative stress and shorter telomeres have the poorest survival prospects. Given this, long-term costs of glucocorticoid-induced phenotypes may include accelerated ageing and increased mortality.

Haussmann et Al. Proceed R Soc B. 2012. 279: 1447-1456

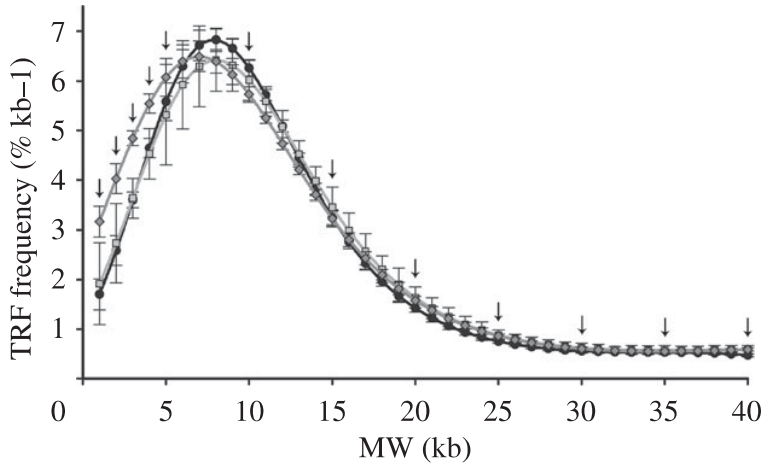
Embryonic exposure to cortisone increased oxidative stress (d-ROMs/OXY) and shortened telomeres in chicken

Redox balance



Relationships between reactive oxygen metabolites (ROMs) and total antioxidant capacity (TAC) within treatment groups at the initiation (INITIAL) and after (POST) an acute stressor. Least square groups, mean ± s.e.m. plotted (all groups, n=7). Filled circles, control; squares, low cort; diamonds, high cort.

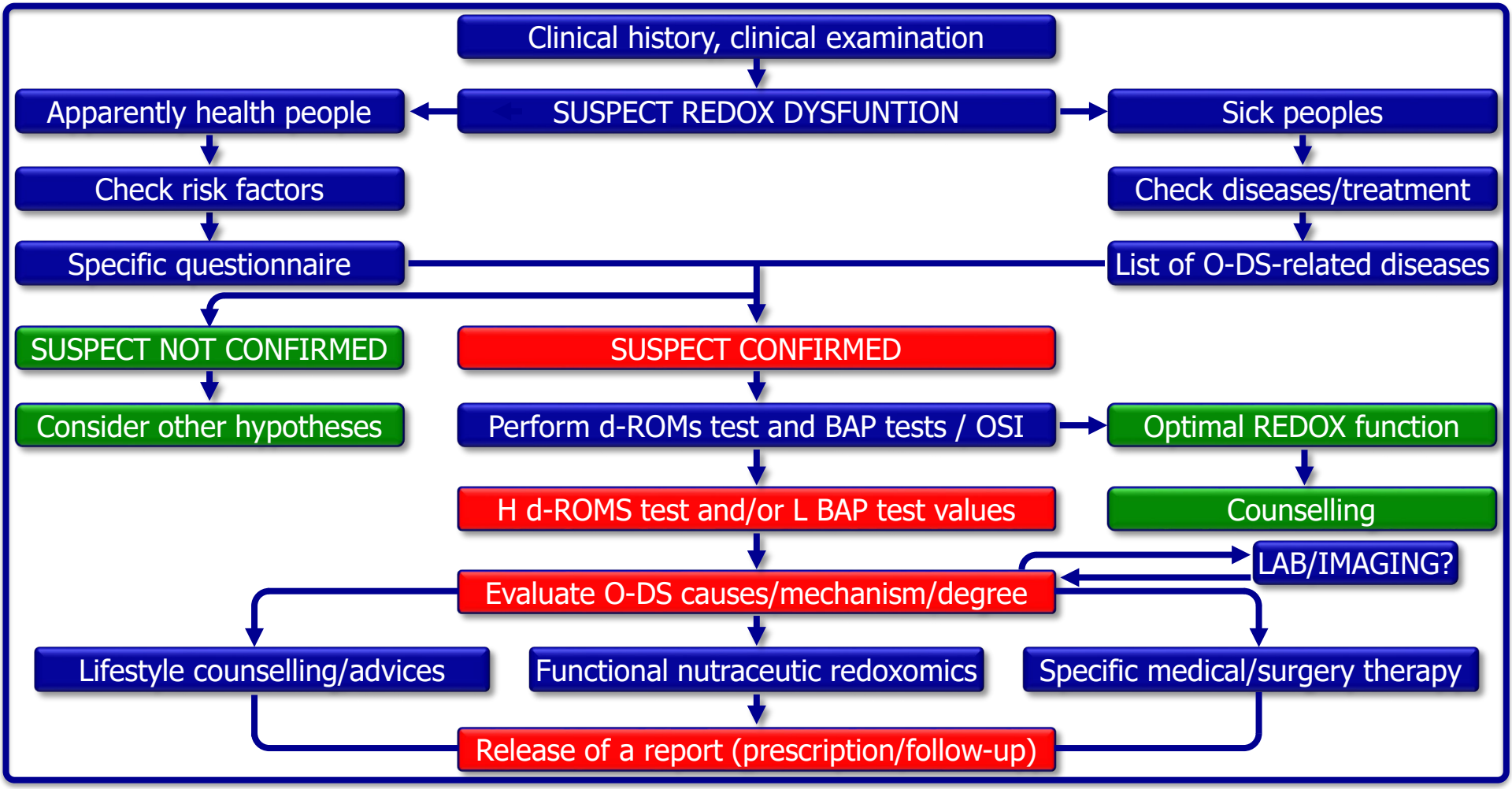
Telomeres



The effect of elevated embryonic corticosterone on telomere restriction fragment (TRF) length of juvenile chickens measured at 21 days of age. Plotted are mean (+ s.e.m.) TRF frequency distributions from chicken erythrocytes against molecular weight (MW). The data are log transformed and then fit by least-squares fourth-order polynomial regression using the equation: log(freq) 1/4 ß0 þ ß1 (MW) þ ß2 (MW 2 medianMW)2 þ ß3 (MW 2 medianMW)3 þ ß4 (MW 2 medianMW)4. Analysis was performed on the 12 TRF intervals denoted by the arrows (see text; all groups n1/47). Filled circles with solid, control; squares with solid line, low cort; diamonds with solid line, high cort.

Haussmann et Al. Proceed R Soc B. 2012. 279: 1447-1456

Guide-lines to manage the results of oxidative stress evaluation



Flow diagram for apparently healthy or sick peoples

History application form to evaluate the risk factors for oxidative stress

Name	Family name
Place of birth	Day of birth
Marital status	Job
Address	Phone
Weight	Height
Living area	Cigarette smoke
Alcohol intake	Strong drinker
Drugs	Fruits (portions/day)
Exercise (hours/week)	Vegetable (portions/day)
Past clinical history	Recent clinical history
Total oxidant capacity	Total antioxidant capacity
Medical/surgical therapy	Note

To improve the patient's framing (Iorio, 2002)

The REDOX system dysfunction (oxidative di-stress) is related to early aging and more than 100 diseases

Aceruloplasminemia	Aceruloplasminemia	Aceruloplasminemia
Acute/chronic alcoholic liver disease	Acute/chronic alcoholic liver disease	Acute/chronic alcoholic liver disease
Acute autoimmune myocarditis	Acute autoimmune myocarditis	Acute autoimmune myocarditis
Acute chest syndrome of sickle cell disease	Acute chest syndrome of sickle cell disease	Acute chest syndrome of sickle cell disease
Acute pancreatitis	Acute pancreatitis	Acute pancreatitis
Acute Respiratory Distress Syndrome	Acute Respiratory Distress Syndrome	Acute Respiratory Distress Syndrome
Alcoholic liver disease	**Alcoholic liver disease**	**Alcoholic liver disease**
Alzheimr's disease	Alzheimr's disease	Alzheimr's disease
Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis
Arterial/systemic hypertension	**Arterial/systemic hypertension**	**Arterial/systemic hypertension**
Asbestosis	Asbestosis	Asbestosis
Asthma	Asthma	Asthma
Ataxia telangiectasia	Ataxia telangiectasia	Ataxia telangiectasia
Atherosclerosis	**Atherosclerosis**	**Atherosclerosis**
Atopic dermatitis	Atopic dermatitis	Atopic dermatitis
Brain ischemia	**Brain ischemia**	**Brain ischemia**
Bronchopulmonary dysplasia	Bronchopulmonary dysplasia	Bronchopulmonary dysplasia
Burns	Burns	Burns
Cancer (several kinds)	**Cancer (several kinds)**	**Cancer (several kinds)**
Cardiopulmonary bypass	Cardiopulmonary bypass	Cardiopulmonary bypass
Cardiovascular diseases	**Cardiovascular diseases**	**Cardiovascular diseases**
Cataract	**Cataract**	**Cataract**
Cellulitis	Cellulitis	Cellulitis
Chemoterapy side-effect	**Chemoterapy side-effect**	**Chemoterapy side-effect**
Chronic fatigue syndrome	Chronic fatigue syndrome	Chronic fatigue syndrome
Chronic hepatitis C	Chronic hepatitis C	Chronic hepatitis C
Chronic kidney disease	Chronic kidney disease	Chronic kidney disease
Chronic Obstructive Pulmonary Disease	Chronic Obstructive Pulmonary Disease	Chronic Obstructive Pulmonary Disease
Chronic renal failure	Chronic renal failure	Chronic renal failure
Colitis	**Colitis**	**Colitis**
Coronary artery disease	**Coronary artery disease**	**Coronary artery disease**
Creutzfeldt–Jakob disease	Creutzfeldt–Jakob disease	Creutzfeldt–Jakob disease
Crohn disease	**Crohn disease**	**Crohn disease**
Cutaneous leishmaniasis	Cutaneous leishmaniasis	Cutaneous leishmaniasis
Cystic fibrosis	Cystic fibrosis	Cystic fibrosis
Diabetes mellitus type 1	Diabetes mellitus type 1	Diabetes mellitus type 1
Diabetes mellitus type 2	**Diabetes mellitus type 2**	**Diabetes mellitus type 2**
Dislipidemia	**Dislipidemia**	**Dislipidemia**

Most of these diseases are related to life style (Dalle Donne, 2006)

Causes of increased production of reactive species and blood plasma/serum total oxidant capacity

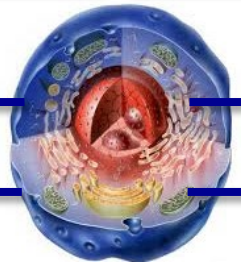
Environment	Pollution, ionising radiations.
Physiological status	Pregnancy, menopause.
Lifestyle	Nutrition, alcohol, cigarette smoke, exercise.
Psycology	Emotional stress, sleeping disorders.
Diseases	Trauma, inflammation, infections, ischemia-reperfusion damage, dementia, cancer.
Iatrogenic factors	Drugs, dialysis, stent/by-pass, rX exposure

Consider the different physiological and pathological factors able to stimulate reactive species production

Evaluating the mechanism

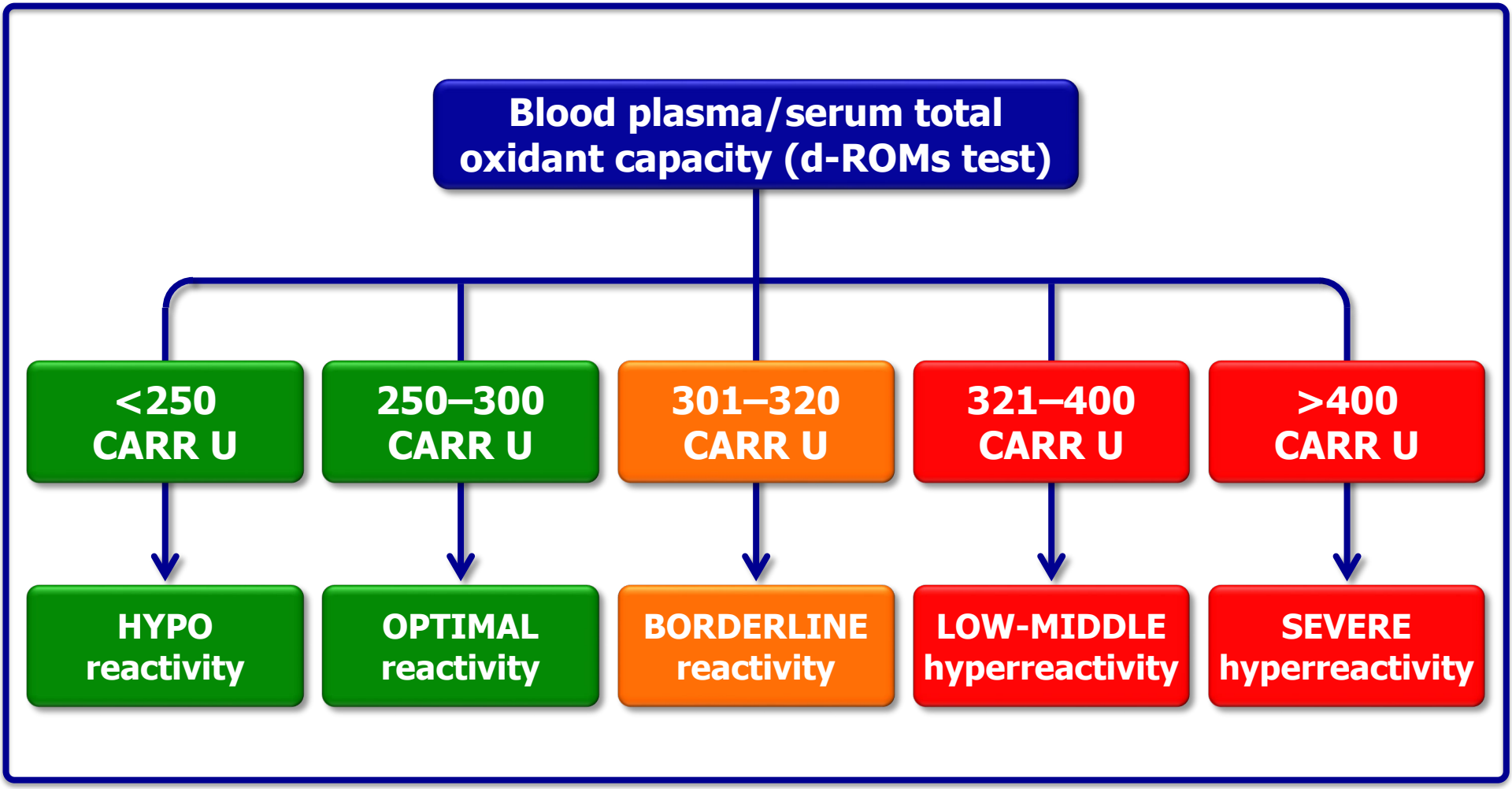
Increased blood plasma/serum total oxidant capacity

Oxidative stress by reactive changes of cell surface	Oxidative stress by abnormal changes of intracellular pO_2	Oxidative stress by pharmaco-metabolic induction	Oxidative stress by impaired cell respiration
NADPH oxidase Lipooxygenase Activation	Xanthine oxidase Aldehyde oxidase Activation	Cytochrome P_{450} Cytochrome b_5 Activation	NADH dehydrogenase Cytochrome oxidase Activation



Pathophysiological and clinical pictures

Evaluating the severity



Five different degrees

Causes of decreased antioxidant defences and blood plasma/serum total antioxidant capacity

Decrease intake of natural antioxidants by foods	Monotonous diets, hypovitaminosis, food processing and cooking
Decrease absorption of foods natural antioxidants	Malabsorption syndromes, celiac diseases
Decrease bioavailability of foods natural antioxidants	Antioxidant cell uptake and/or transport abnormalities
Antioxidant enzymes abnormalities	Genetic disorders, drugs, xenobiotics
Excessive consumption of antioxidants	Excessive production of reactive species (e. g. due to cigarette smoke or alcohol)
Chronic drugs intake/abuse	Microsome overload

Either genetic or environmental factors may contribute to reduce the effectiveness of antioxidant defence system

Evaluating the mechanism

Decreased blood plasma/serum total antioxidant capacity

Relative deficiency syndrome

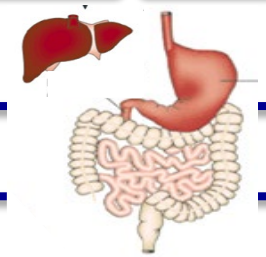
Absolute deficiency syndrome

Decreased food antioxidant intake

Decreased production of antioxidants

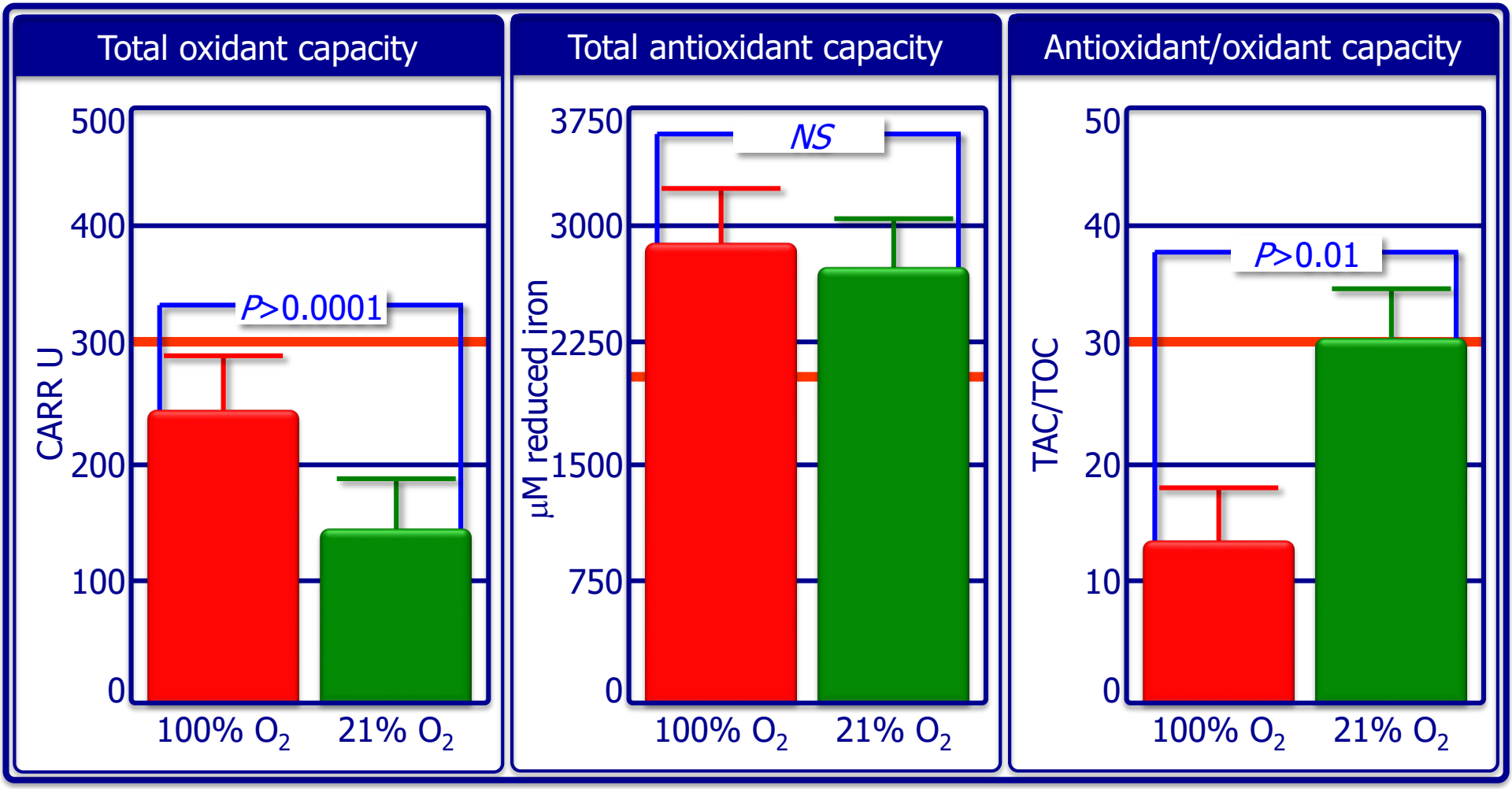
Antioxidant decreased bioavailability

Increased consumption of antioxidants



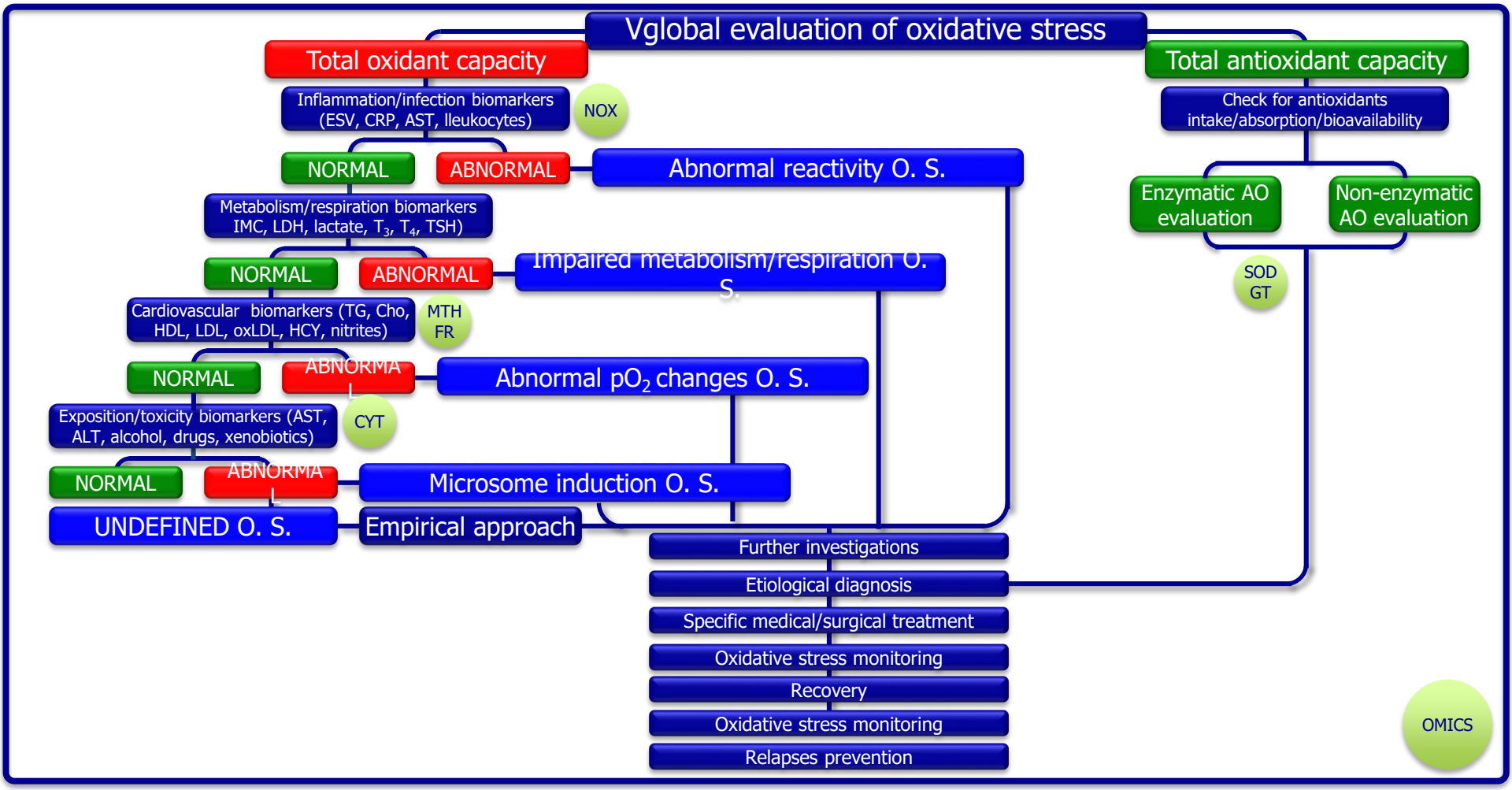
Pathophysiological and clinical pictures

Statistical significance recovery by combining total oxidant capacity with total antioxidant capacity



Ezaki et Al. Proceedings PSABZ 2007

An original algorithm to manage the results of a global evaluation of oxidative stress



Iorio EL, et Al. Austral J Cosmet Surg. 2006. 2 (1): 26–30.

Lifestyle change

☞Reduce the exposure of your body to environmental factors that induce the release of free radicals, taking care of the hygiene of living and working: limit contact with polluted air, avoid or reduce exposure to secondhand smoke, radiation and electromagnetic fields, etc.

☞Improve your lifestyle: reduce/eliminate cigarette smoking, reduce the consumption of alcoholic drinks and spirits, perform physical activity with low-intensity exercise (30' fast walking without interruption, three times a week, according to Cooper)

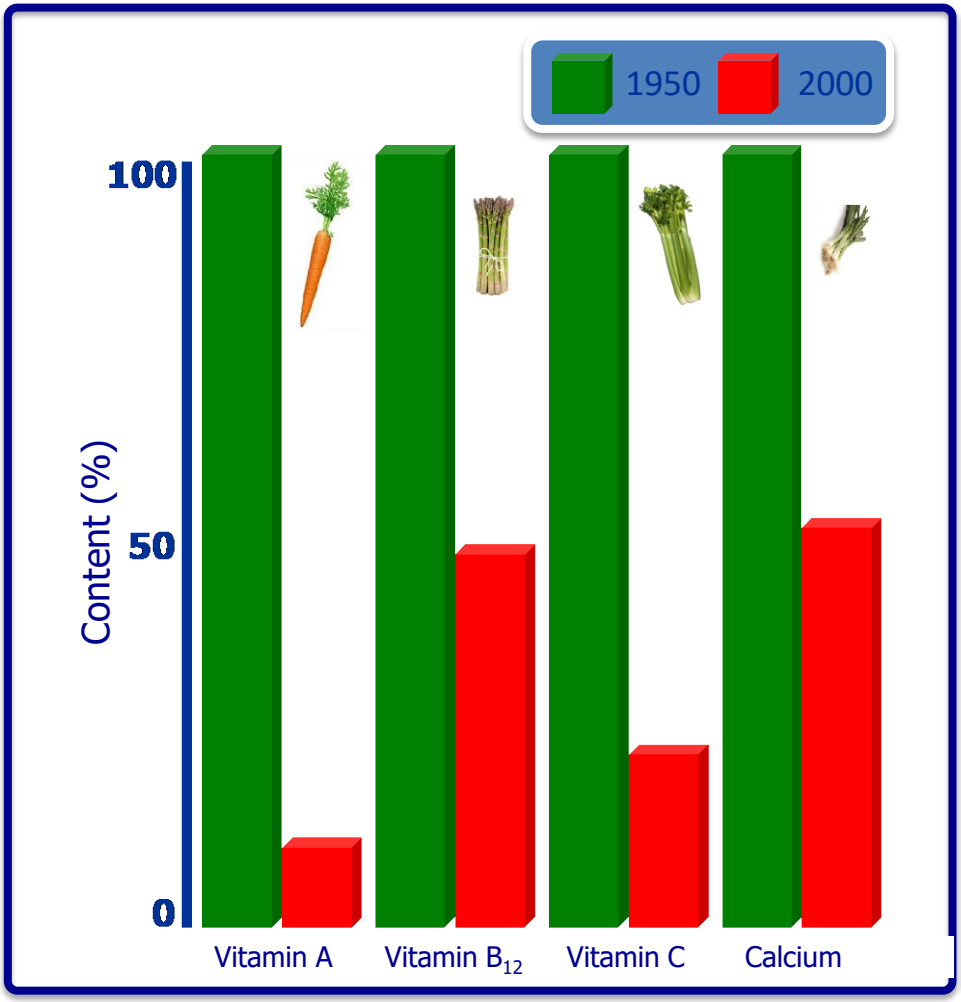
☞Cook and eat following the antioxidant way, favoring the food model "Mediterranean"; in particular consume bread and pasta and cruciferous vegetables (broccoli and cabbage), favoring some marriages food synergetic action (e. g. tomato and olive oil), take a lot of colorful fruit in season and wet ... possibly lunch with a glass of good red wine!

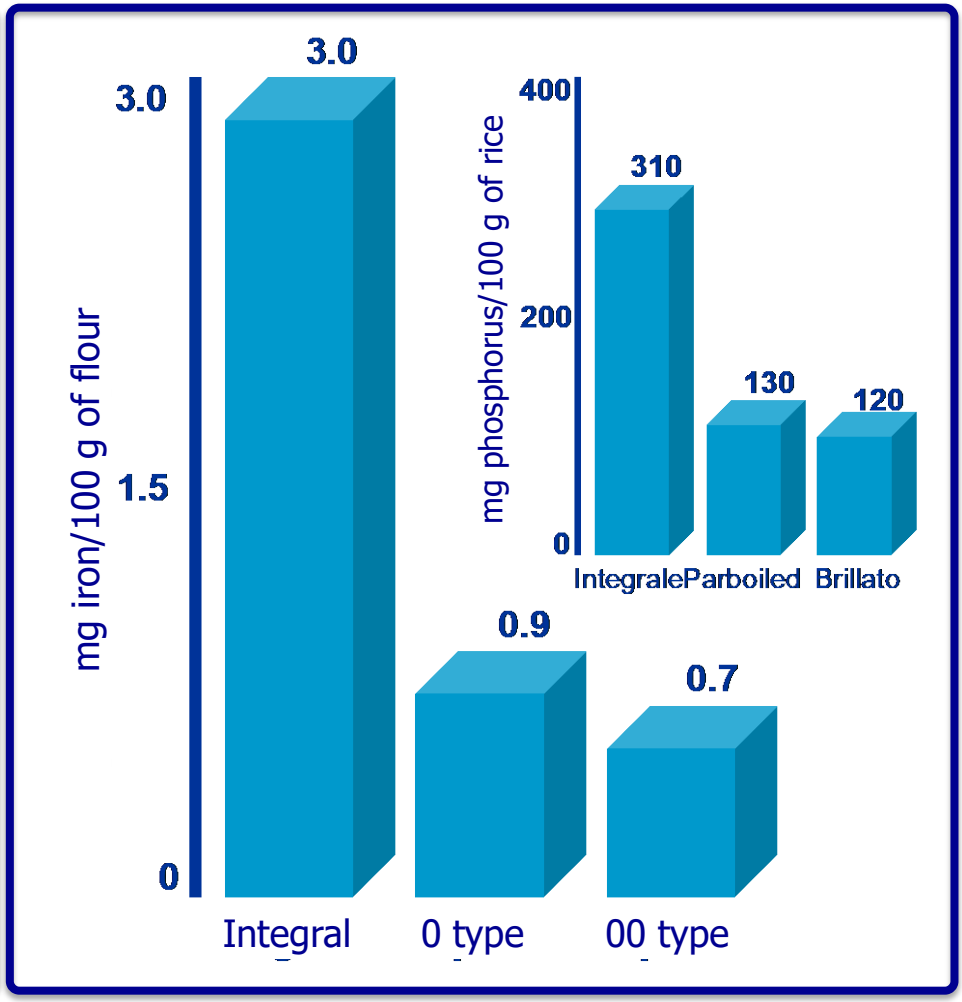
☞Integrate, when necessary, with physiological modulators, under the direct supervision of a doctor, and after careful biochemical evaluation of oxidative stress.

☞Check periodically your level of oxidative stress tests with simple, reliable and accurate.

The first strategy to prevent and to treat oxidative stress

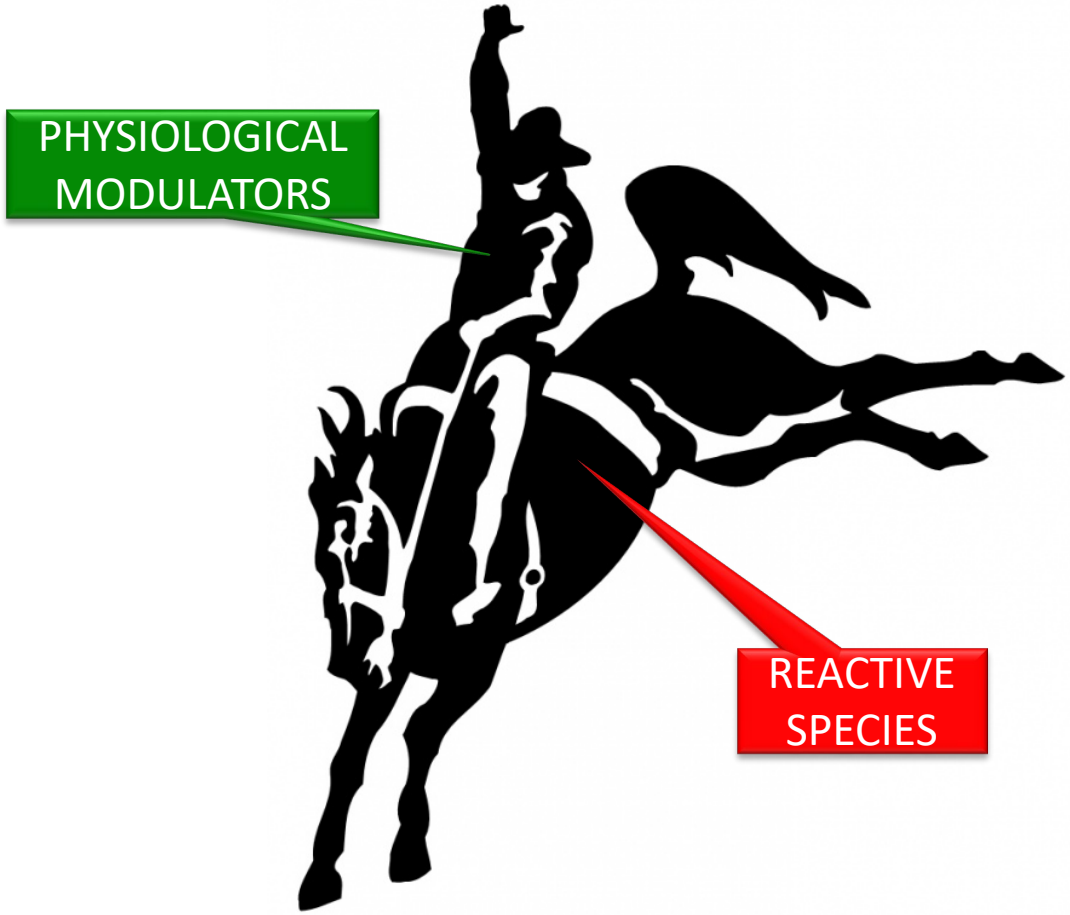
Plant vitamins and minerals content changes





Difficult to find, very easy to loose, Today.

Reactive species are essential for life



Any approach aimed to control oxidative stress must modulate instead of stop their action (like *antioxidants*)

Functional Modulators

☞The evolution of vitamins and food supplements into Physiological Modulators (PM) is a quantum leap. Vitamins and food supplements offer some generic protection. Physiological Modulators are a set of antioxidants in balanced quantities that offer a specific ORGAN protection.

☞Physiological Modulators are not simple antioxidants but modulators of the oxidation. Why? Because oxidation is necessary for life and physiological modulators act only where oxidation is excessive without altering its fundamental function.

☞Physiological Modulators reproduce what happens in nature and in the human body, where antioxidants are always produced and used at low concentrations and in association. This is what happens when we take in food: we absorb a pool of substances at low dosage. It is biologically necessary a coordinated action of several antioxidants in order to work harmonically.

☞Excessive dosages of antioxidants can be harmful and may lead to oxidative action, that is the opposite of why we take them. For this reason giving high dosages of a single antioxidant will not harmonize the oxidative balance. Physiological modulators have balanced dosages and they are able to control optimally the oxidative stress.

☞Oxidation must be modulated by acting in different ways on different body systems. Oxidative stress in a hypertensive person is different from the one in a woman in menopause or from the one in a patient with Alzheimer's Disease. All three are oxidative stress conditions, but they involve three different body systems (endothelium, skin and brain) and each body system has its "way" to enter into oxidative stress and its "way" to exit this condition.

A new concept

The functional modulators network

Vitamins	Vitamin A, vitamin E, vitamin C, nicotinamide, riboflavin, niacin.
Lipids	Squalene
Amino acids and thiol derivatives	Taurine, L-arginine, L-hystidine, glycine, L-cysteine, L-glutamine, L-methionine, N-acetylcysteine, S-adenosyl-L-methionine, L-lipoic acid.
Peptides	Carnosine, γ-glutamil-cysteiniy-glycine (GSH).
Proteins and enzymes	Albumin. Lactoferrin, transferrin, ceruloplasmin. Superoxidedismutase (SOD), peroxidases, thioredoxin, catalase.
Phytonutrients	Polyphenols (hydroxycinamic acid derivatives, hydroxybenzoic acid derivatives, flavonols[a], flavones[a], anthocyanidins[a], flavanols[a], isoflavons[a], flavanons[a], stilbens, lignans), glucosinolates, carotenoidis(α,β,γ,δ-carotene, lycopene, luteia, zeaxanthine, cantaxanthine), phytic acid, allicin.
Minerals	Zinc, iron, copper, selenium, chromion.
Miscellaneous	Uric acid, bilirubin.

A great natural resource

Functional modulators supplementation

- ☞ Pharmaceutical form, administration route and dose.
- ☞ Bioavailability
- ☞ Biological activity and its relationships with chemical structure
- ☞ Horizontal stratification (redox-dependent network, recycling)
- ☞ Vertical stratification (power hierarchy)
- ☞ Elective tissue target
- ☞ Scientific evidence

The quality parameters to be evaluated before to take

Thank you for attention!

A special thank to dr. Maria Somers.

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Italian Society of Lifestyle Medicine

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The European Journal of Aesthetic Medicine and Dermatology

London (UK). October 11th, 2018.