INTERNATIONAL MEETING OF OZONE THERAPY SCHOOLS

National Royal Academy of Medicine
Madrid 3-4 June, 2010

aepromo
Asociación Española de Profesionales Médicos en Ozonoterapia

REDOX DIAGNOSTIC AS MAIN TOOL IN OZONE THERAPY

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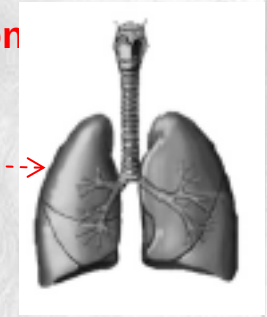
Is O_3 Toxic ???????

"Poison is in everything, and no thing is without poison. **The dosage makes it either a poison or a remedy**"

Paracelsus (1493–1541)

Ozone **Chronic exposition (0.7-0.77 mg / d)**



----> **Toxicity**

Ozone **Acute exposition (1-10 mg / d)**

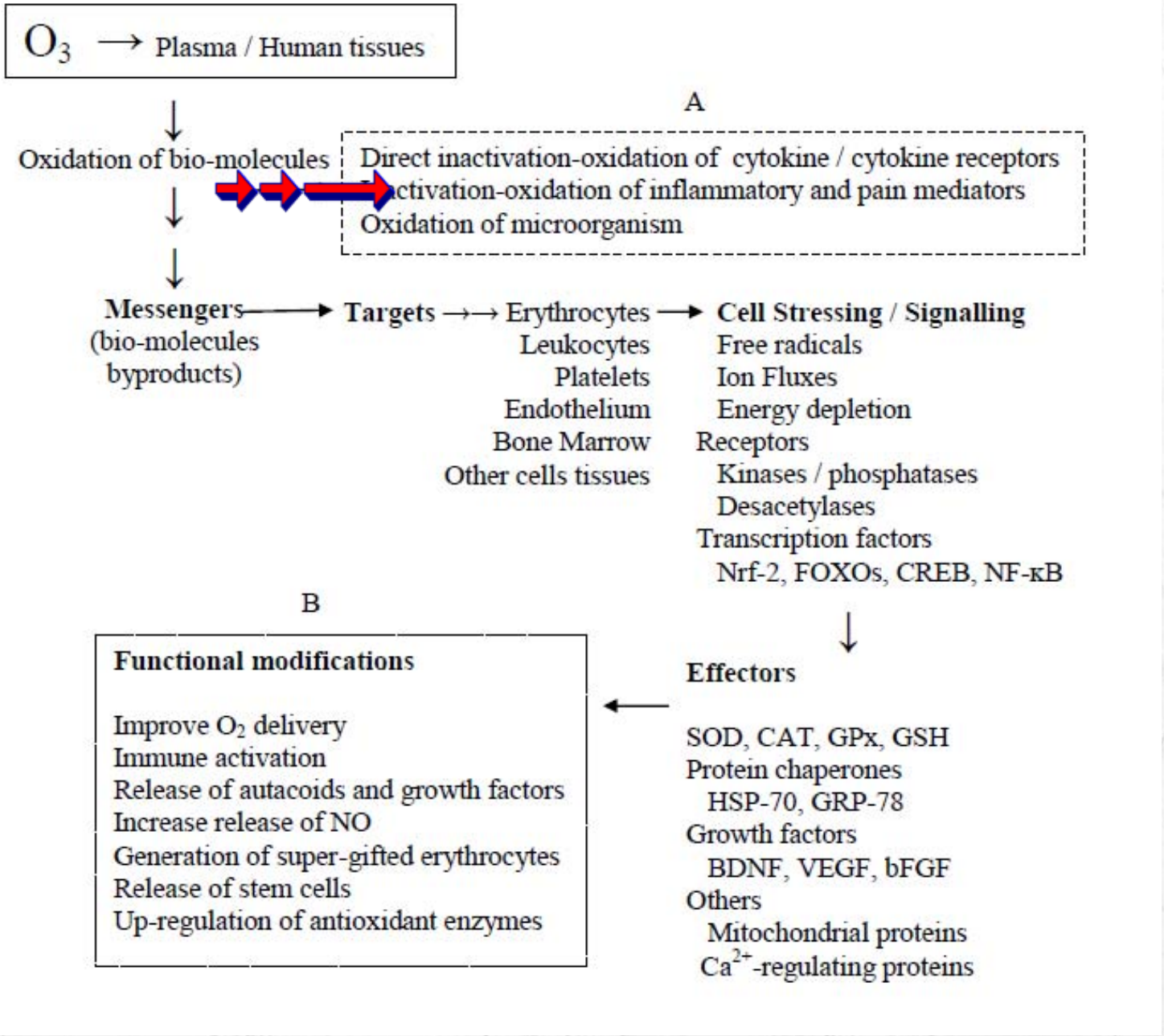
Blood or Target Organs

Doses + Target + Methods = Therapeutic efficacy

V. Bocci. Toxicology and Applied Pharmacology 216 (2006) 493–504

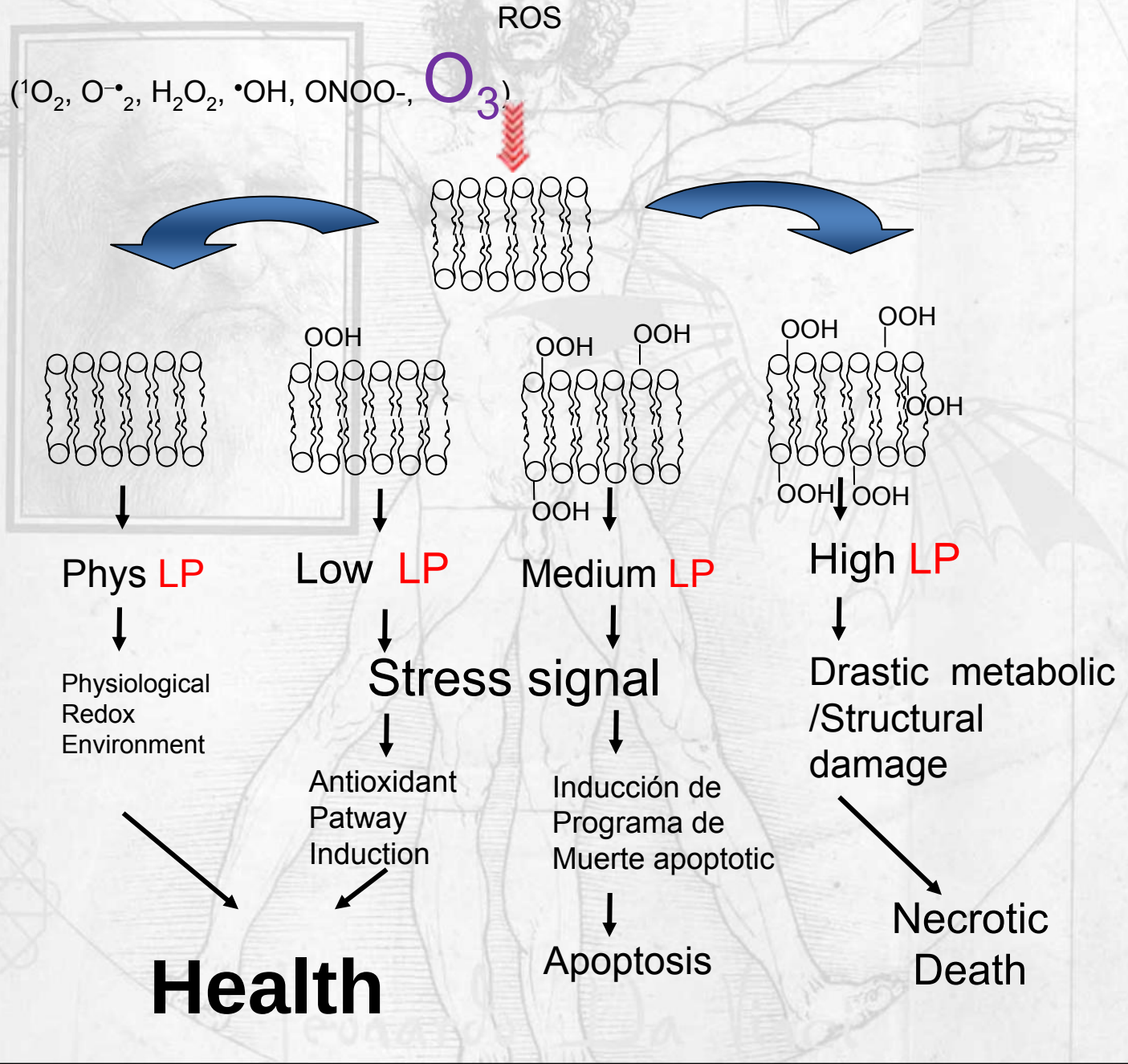
Paracelsus

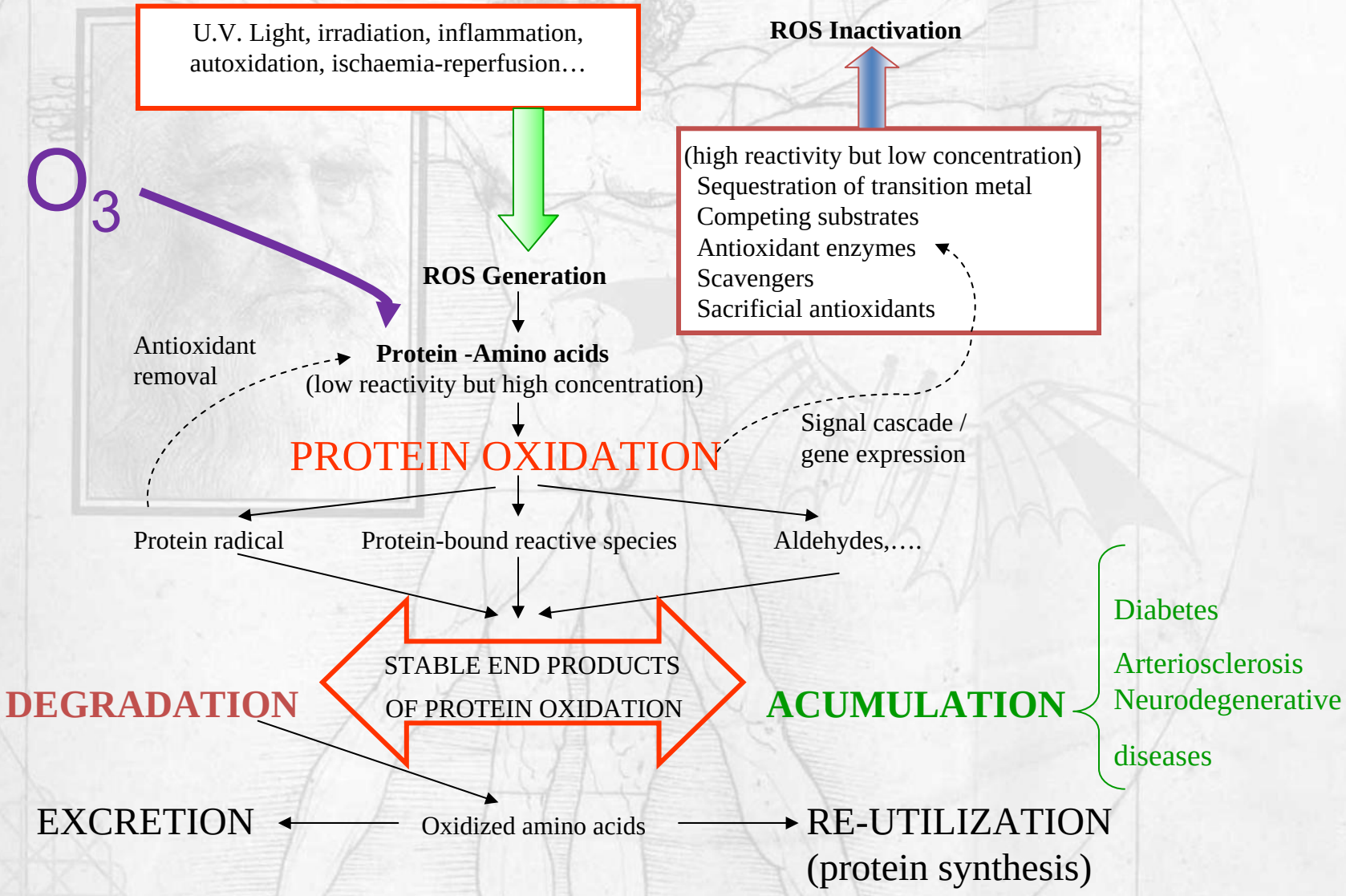
"What is it that is not poison? All things are poison and none without poison. Only the dose determines that a thing is not poison."



L Re, Martínez-Sánchez G. Drug Response (2010) in press

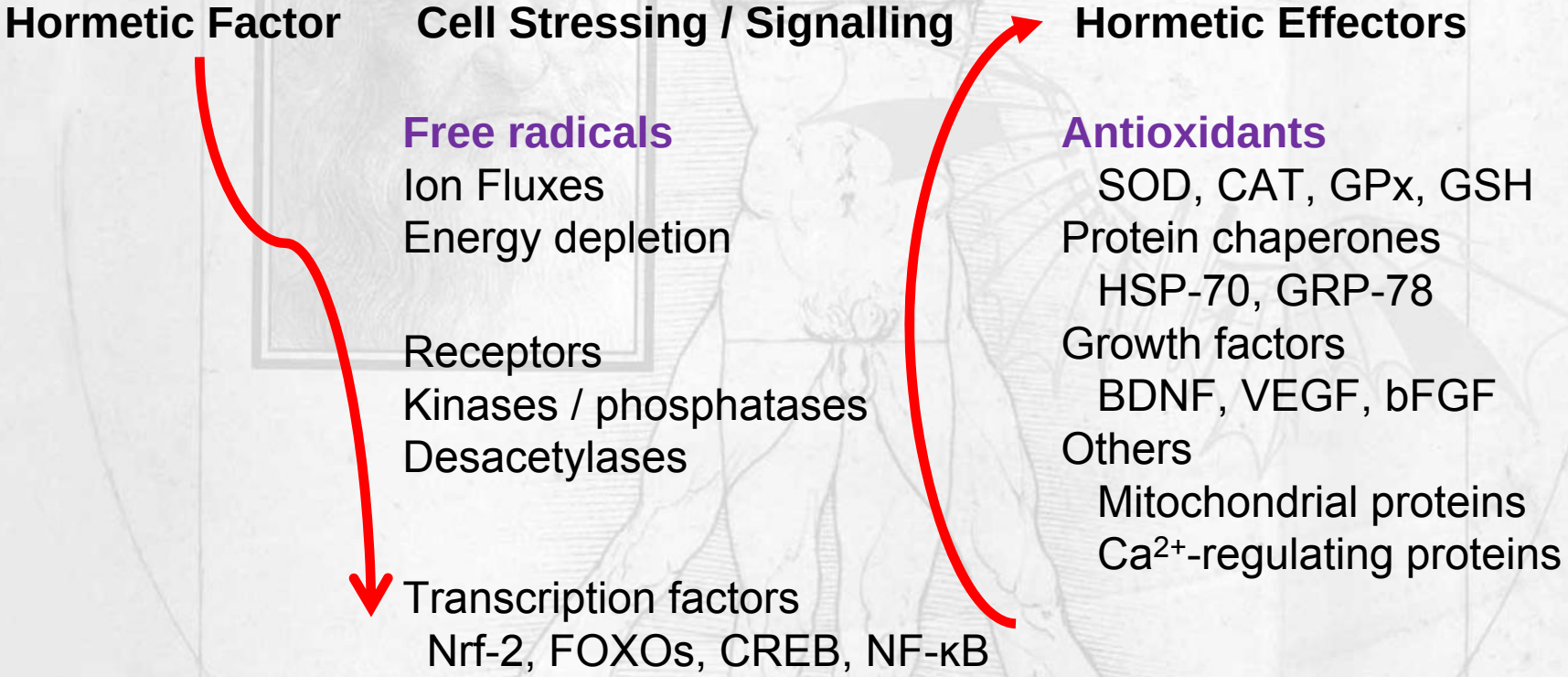
LIPID PEROXIDATION (LP) AND SIGNAL TRASDUCTION MECHANISM





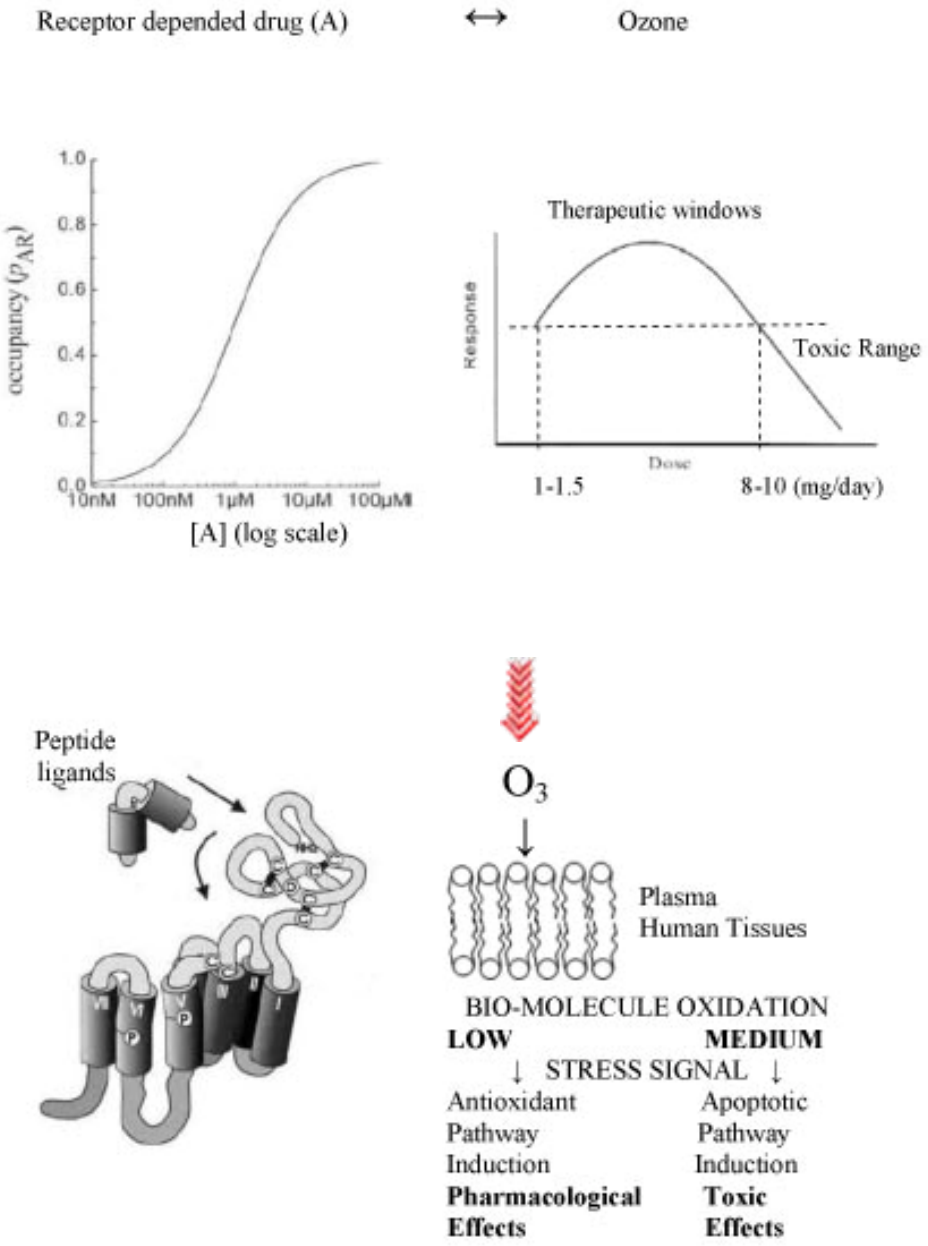
Gregorio M S, Redox Report. (2005)**10**(4):174-184

The cellular and molecular information flow hat mediates hormesis in organisms and cells



Modified from: M.P. Mattson. *Ageing Research Reviews* 7(2008)1–7

Fig 1.



L Re, Martínez-Sánchez G. Drug Response (2010) in press

nature

February 13, 2003

Dangerous levels of toxins miscalculated

Potential pollutants and poisons may be beneficial in low doses.

Science

October 17, 2003

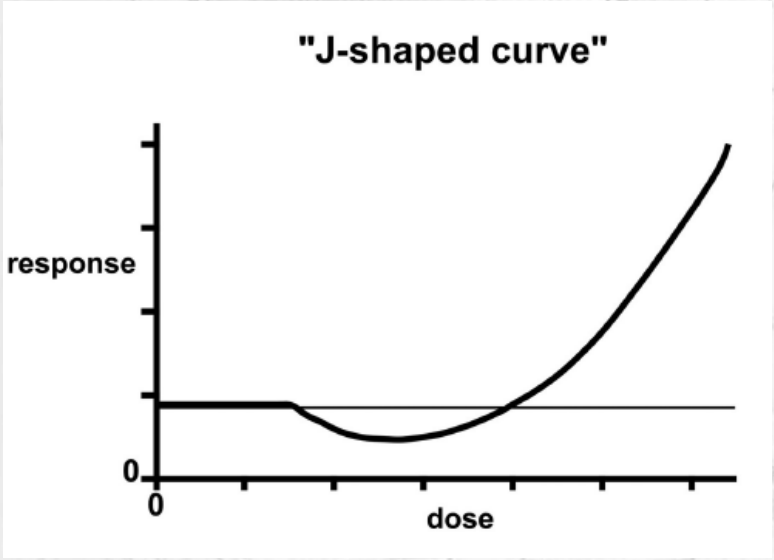
HORMESIS: Sipping From a Poisoned Chalice

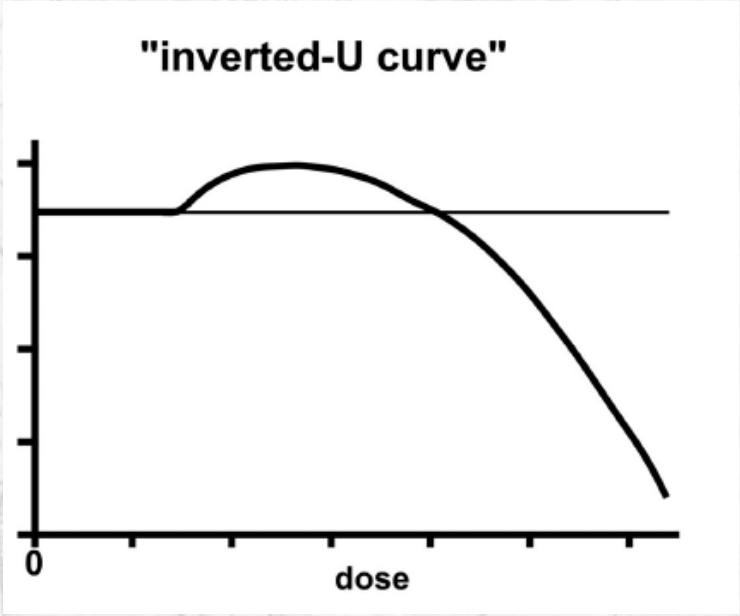
Edward Calabrese,
Professor in the School of Public Health and Health Sciences at the University of Massachusetts Amherst

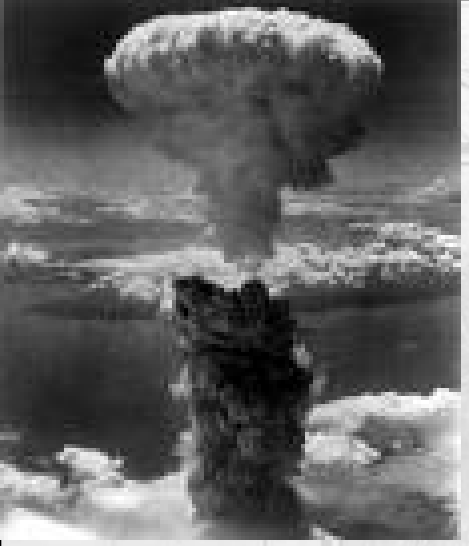
May 1, 2009
Receives Marie Curie Prize for Work on:

Hormesis, Low-Dose Radiation and Health

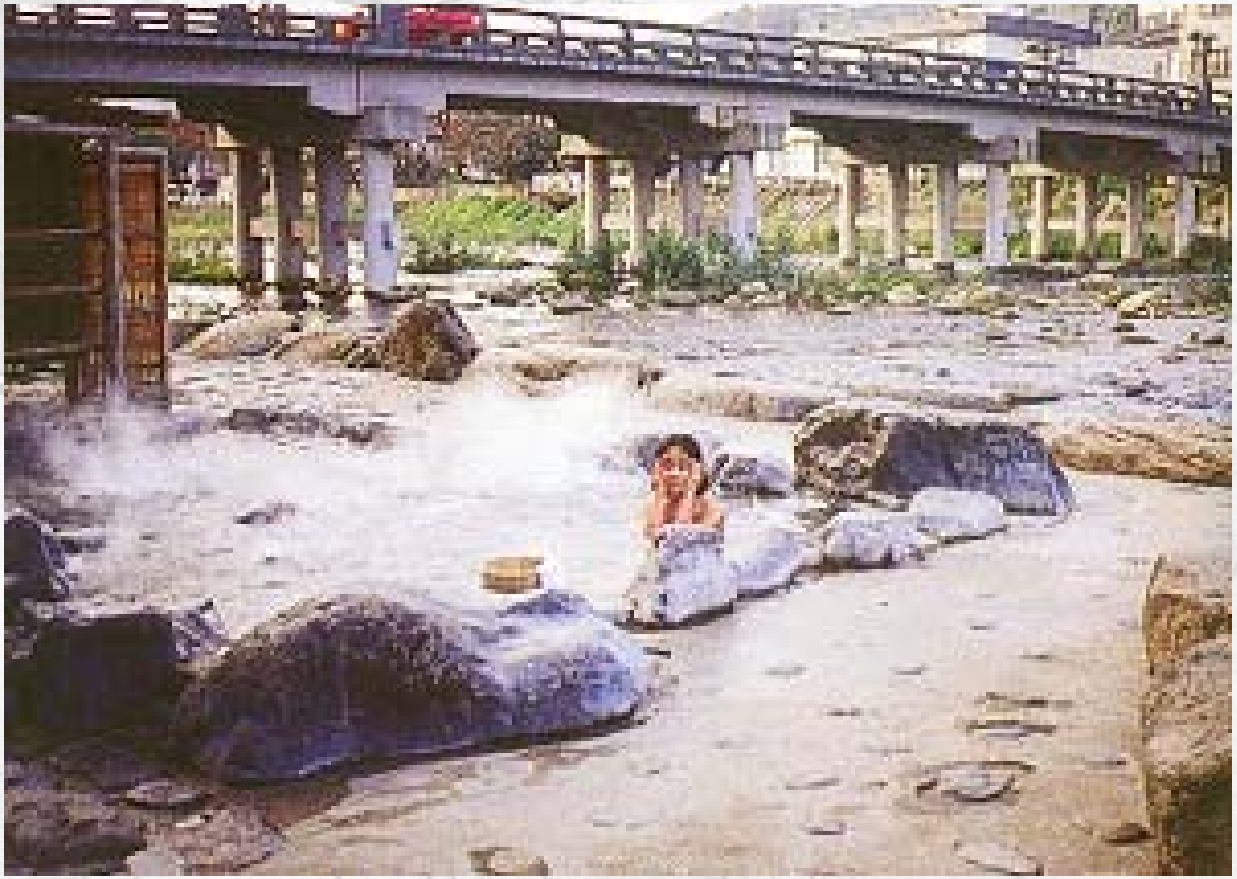
Hormesis is a dose response relationship in which effects at low doses are opposite to those at high doses. As a consequence, hormetic dose response curves are biphasic rather than being monotonic.



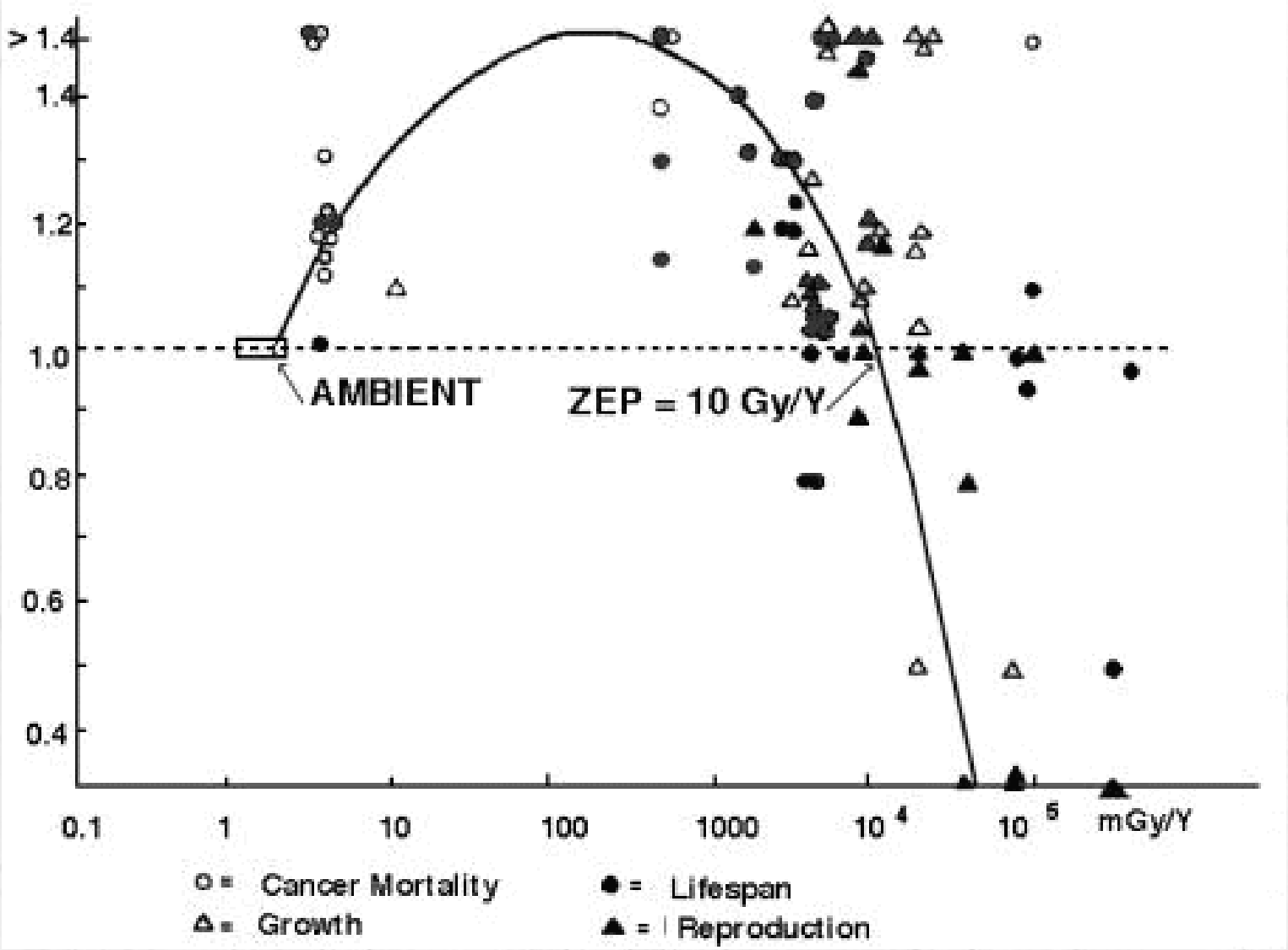




HORMESIS and RADIATION

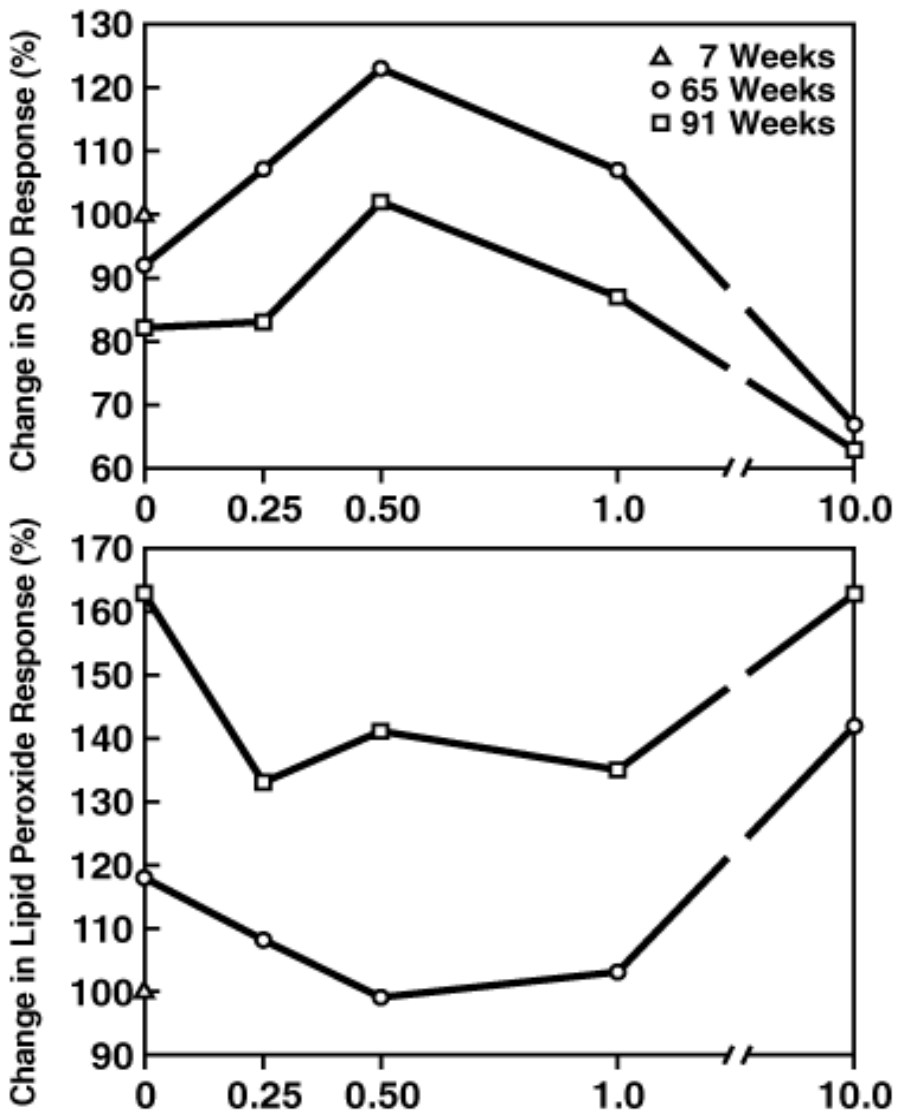


Misasa hot springs, Japan



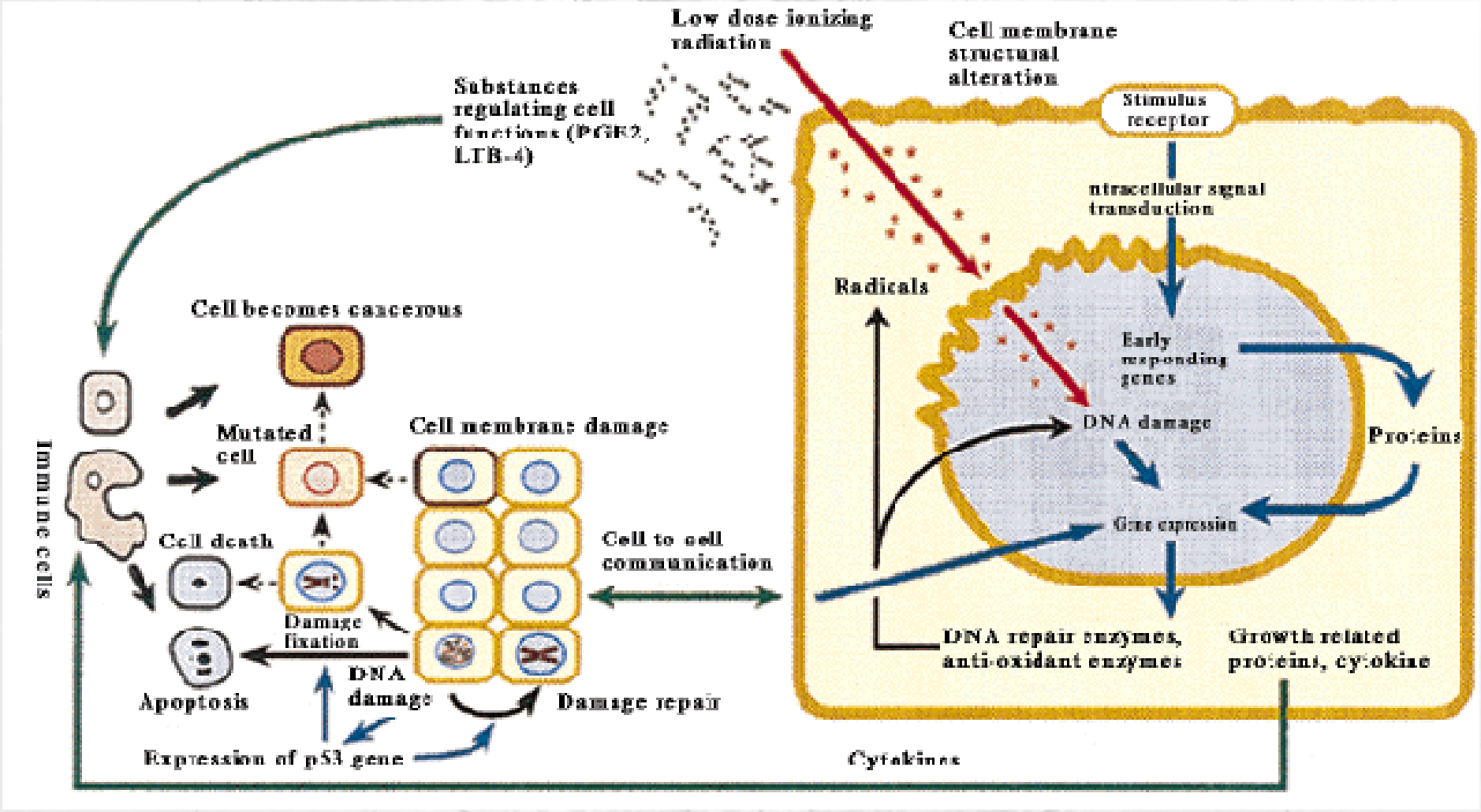
Jpn J Cancer Res. 1994 Nov;85(11):1063-6.

Dose response of SOD and LIPOX to radiation in rat brain cortex



Yamaoka 1991

Proposed mechanisms of radiation hormesis



Radiation, which is non-physiological stimulus at a low dose can result in:

-increased antioxidant enzyme activity
-increased activity of DNA repeair enzymes
-decreased oxidative damage
-decreased incidence of cancer
-increased life-span
-increased generation of free radicals

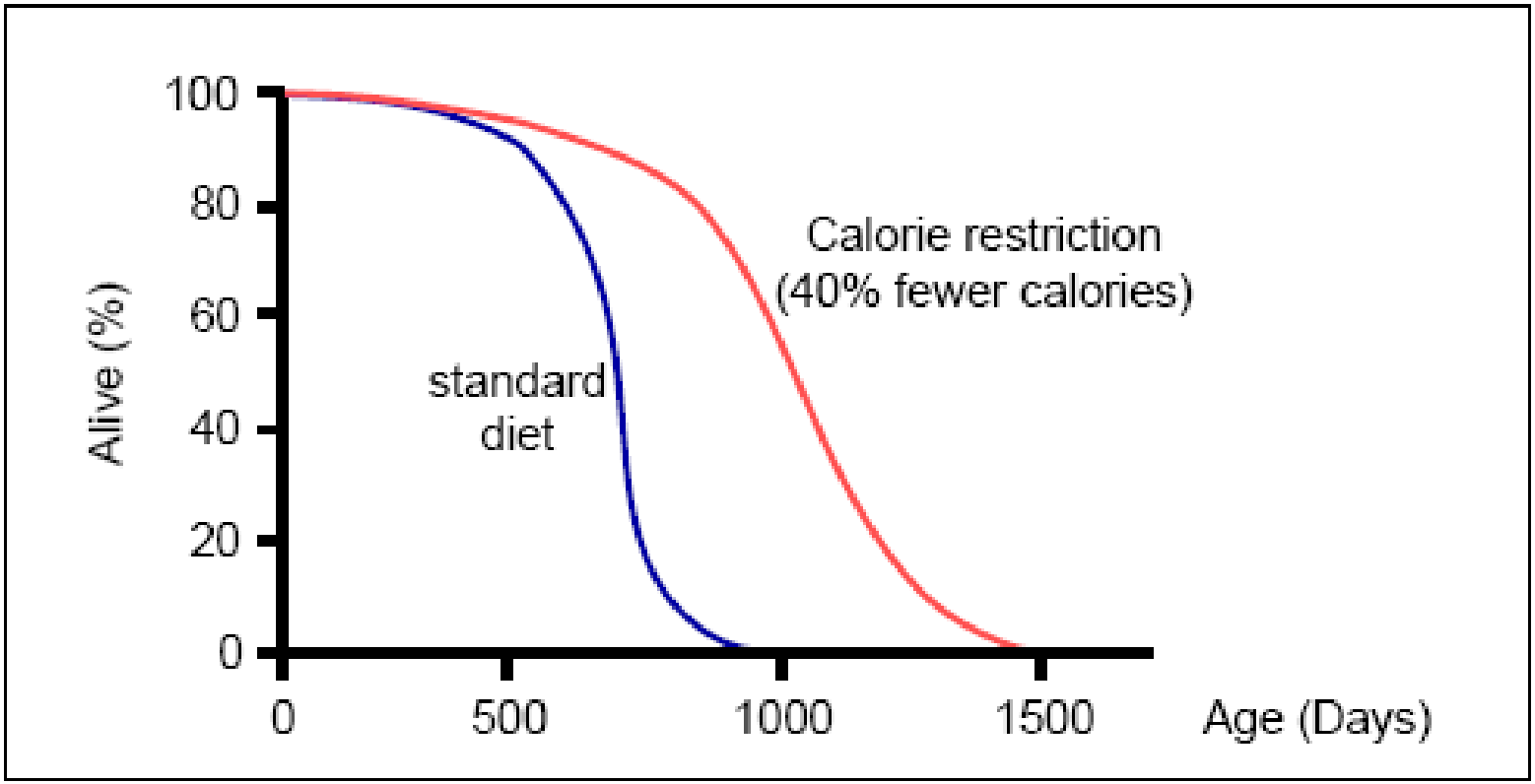
HORMESIS, DIET and AGEING

Calorie Restriction

Calorie Restriction

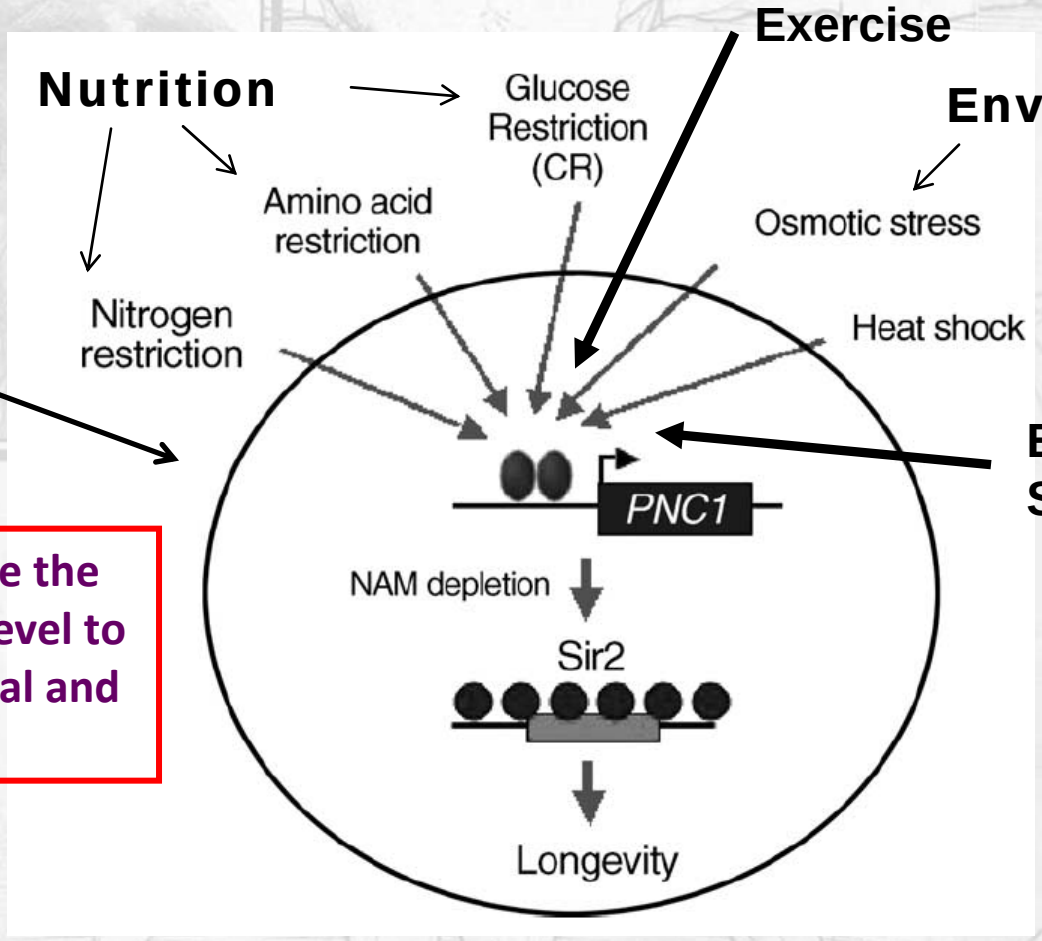
McCay CM, et al: The affect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr 10, 63-79, 1935.

Original 1935 study in rats



Stressors Can Alter Gene Function

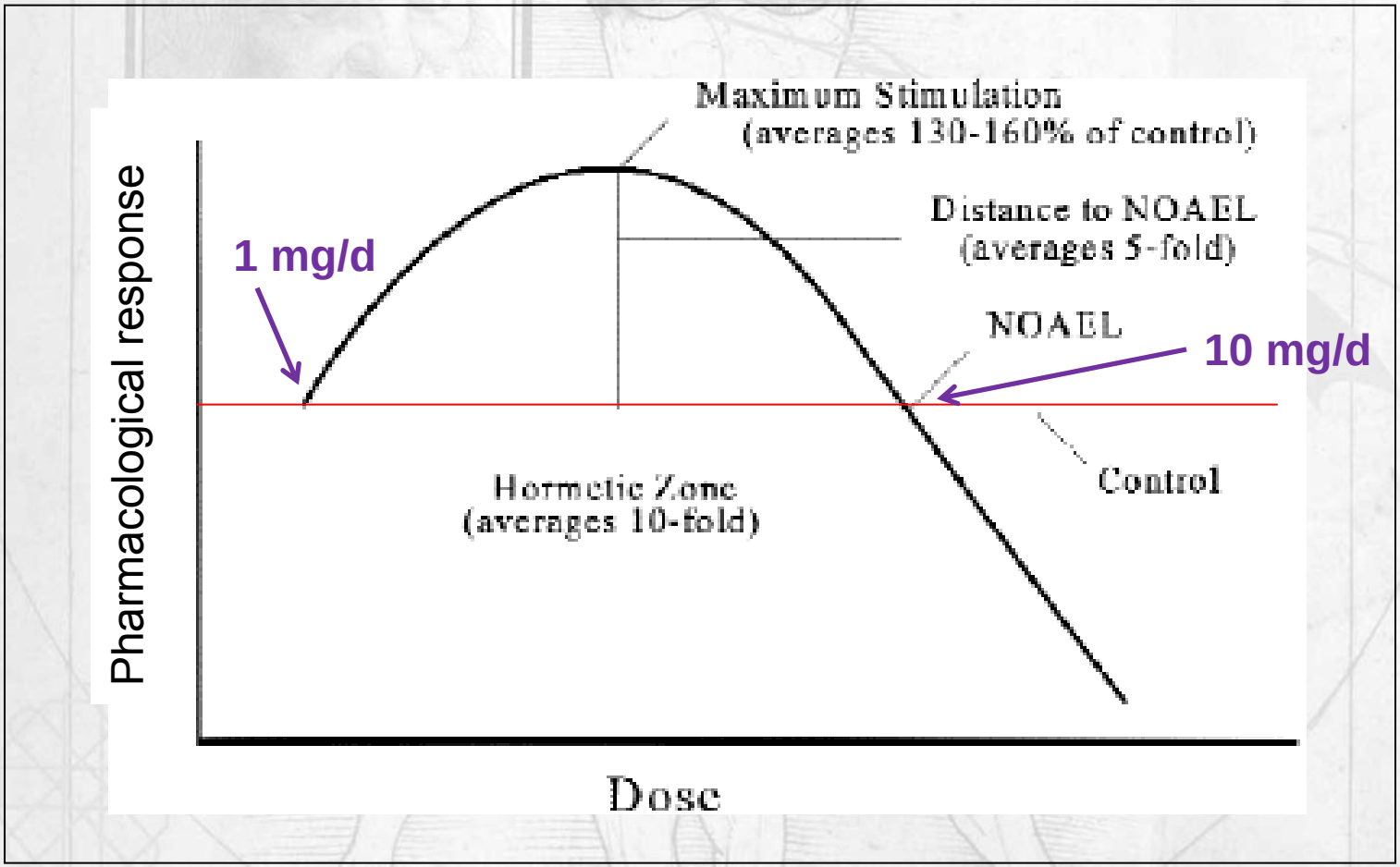
Oxidative stress



Stressors act inside the cell at the genetic level to enhance cell survival and longevity

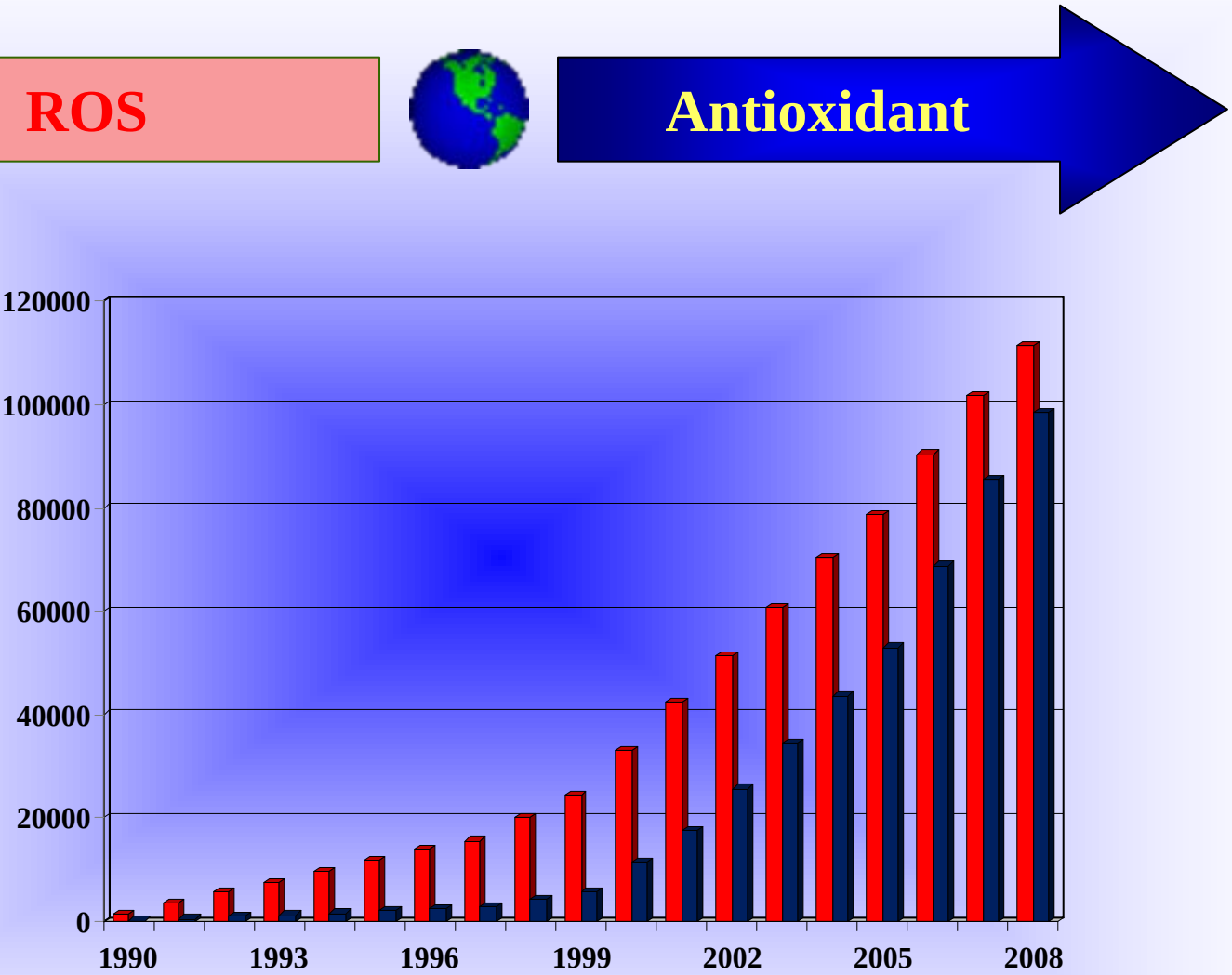
David A. Sinclair, Toward a unified theory of caloric restriction and longevity regulation Mechanisms of Ageing and Development Volume 126, Issue 9 , September 2005, Pages 987-1002

Hormetic respond of O_3



Except during inhalat. administration

ROS

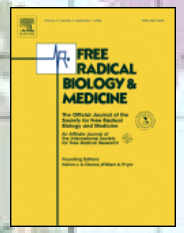


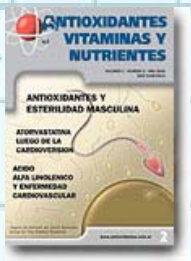
Antioxidant

Accumulative

Sources PubMed February 2009

Educacional Programs





Canada

USA

México

Cuba

Europe

Rusia

China:

Japan

India

Brazil

Uruguay

Chile

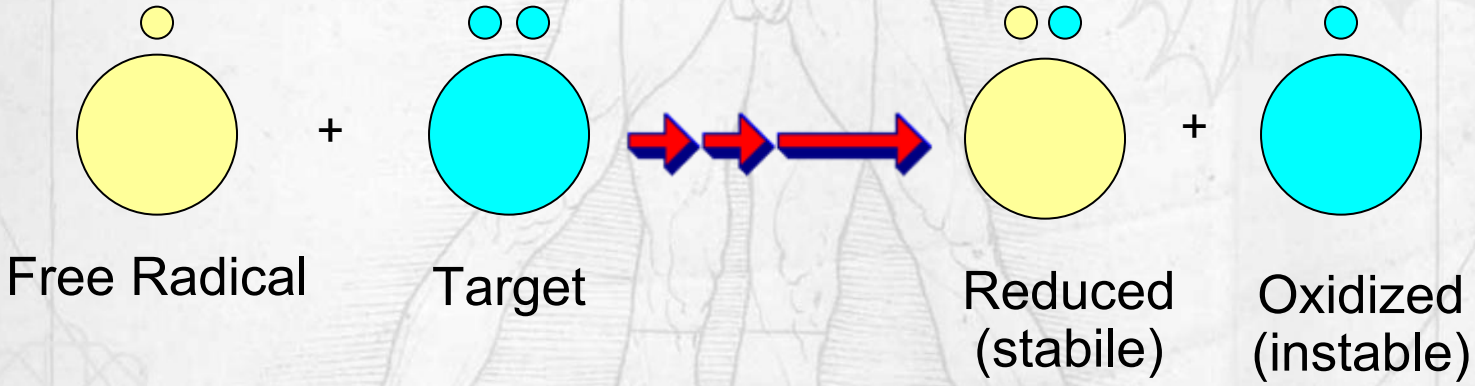
Argentina

South Africa

Active:

¿Free Radicals?

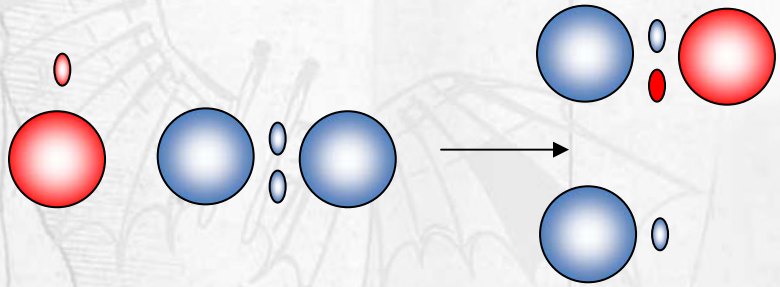
Free radical are atoms, molecules, or ions with unpaired electrons on an open shell configuration. (Halliwell & Aruoma, 1989).



Steps of the free radicals reactions

Initiation: $R{:}R \rightarrow R\bullet + R\bullet$

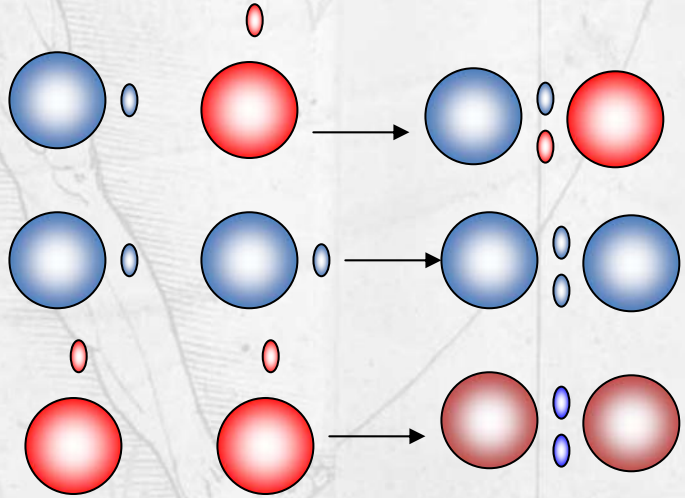
Propagation $R\bullet + H{:}H \rightarrow H\bullet + R{:}H$



Termination $H\bullet + R\bullet \rightarrow R{:}H$

$H\bullet + H\bullet \rightarrow H{:}H$

$R\bullet + R\bullet \rightarrow R{:}R$



¿What mean Reactive Oxygen Species (ROS)?

Free radicals are atoms, molecules, or ions with unpaired electrons on an open shell configuration

Free Radical		t ½
Superoxide anion	$O_2^{\bullet -}$	Enzymatic = 10^{-9} Spontaneous= 10^{-5}
Hydroxyl radical	$^{\bullet}OH$	10^{-9} s (10^{-7}-10^{-10})
Lipid radical	$L^{\bullet}$	10^{-8} s
Nitric oxide	$NO^{\bullet}$	3-5 s
Alkoxyl radical	$RO^{\bullet}$	10^{-6} s
Alkylperoxyl radical	$ROO^{\bullet}$	7 s

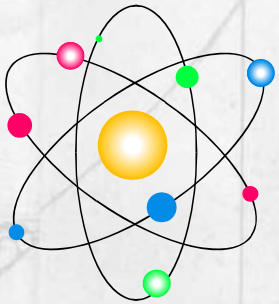
Non radical, with high chemical reactivity

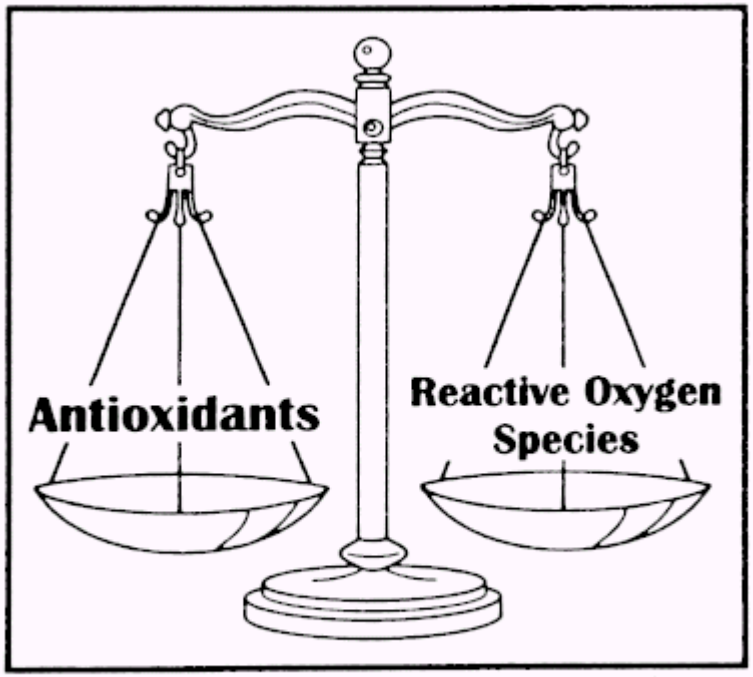
		t ½
Hydrogen peroxide	H_2O_2	Depend of enzyme
Peroxynitrite	ONOO-	0,05-1 s
Hypochlorous acid	HClO	10^{-6} s

ANTIOXIDANT

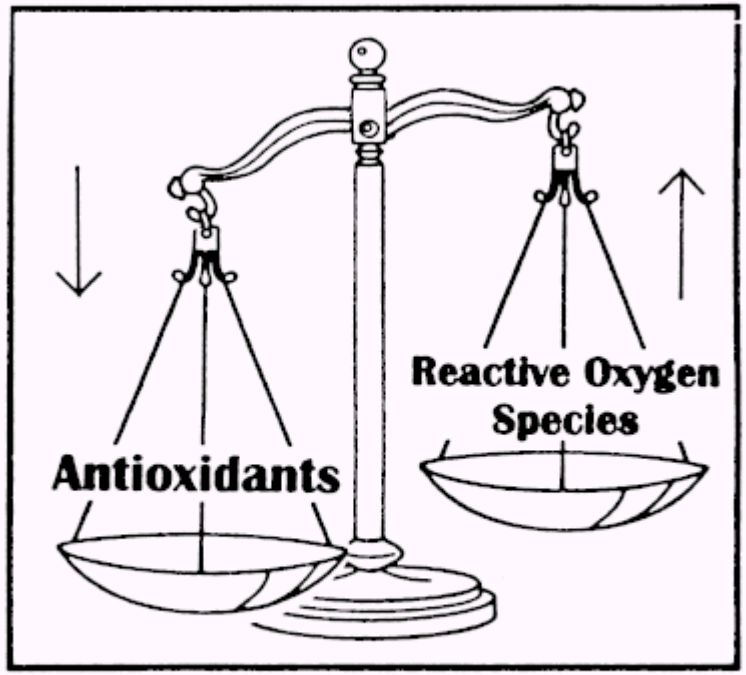
Any substance that present in low concentrations compared to the oxidized substance, prevent or reduce it oxidation

B. Halliwell 1990 **Free Rad. Res.Comms** 9





Minimal oxidative damage



Increased oxidative damage-aging, age-related diseases

OXIDATIVE STRESS

Disruption between the antioxidant defense system and the generation of oxidants that happen acute or chronically. Can be consequences of a decrease in antioxidant, increase of generation of pro-oxidant or both.

- H.K.Bieslaski. (1997) **Clin. Nut.** 16: 151-155

New concepts of oxidative stress

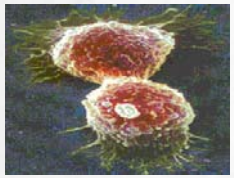
H Sies 1985

Oxidative stress: imbalance between the production of oxidants and the occurrence of cell antioxidant defenses…….

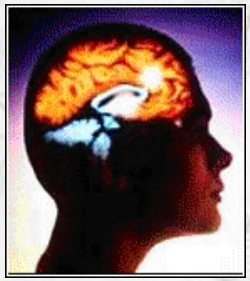
Jones 2006: a disruption of redox signalling and control that recognizes the occurrence of compartmentalized cellular redox circuits.

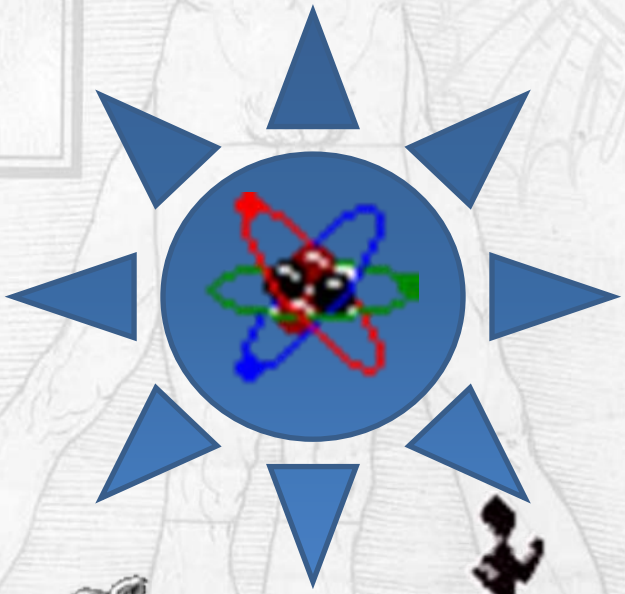
Cardiovascular diseases

Cancer



CNS diseases





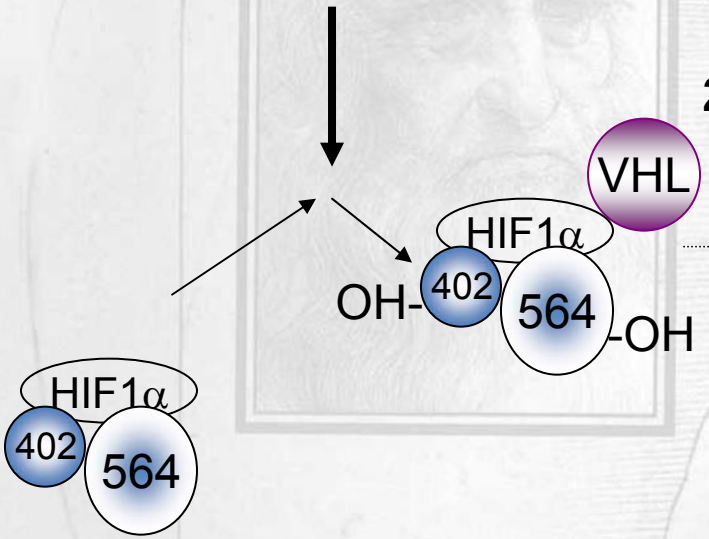
Inflammation

Fisical activ.

HIF-1α is constitutively made and degraded *via* VHL.

1. Prolyl hydroxylase

NORMOXIA ~6% O_2 40 mmHg



2. Binding Hippel-Lindau tumor suppressor (VHL)

HIF1α

CYTOPLASM

3. Ubiquitinylation

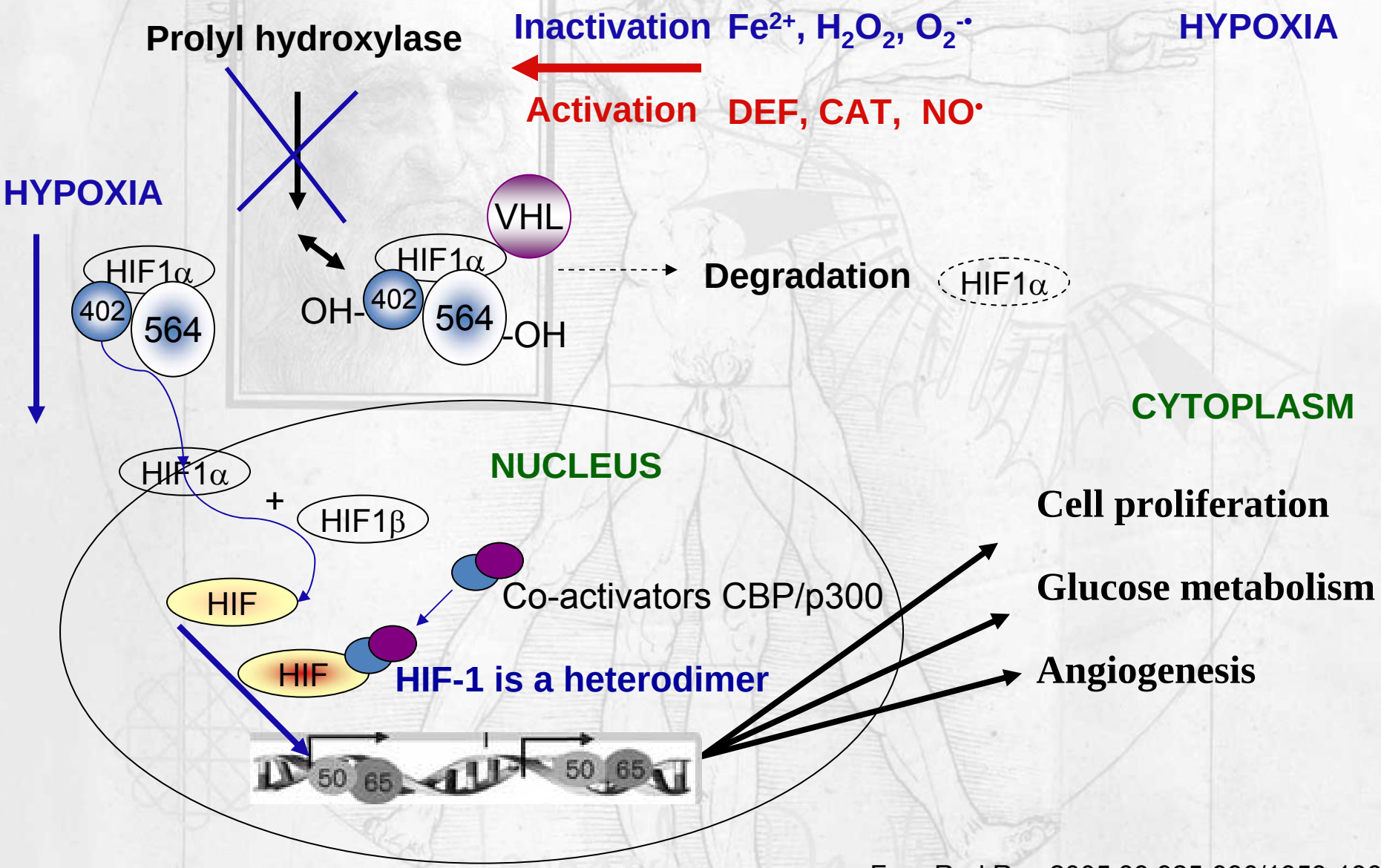
26S

4. Degradation by proteasome

NUCLEUS

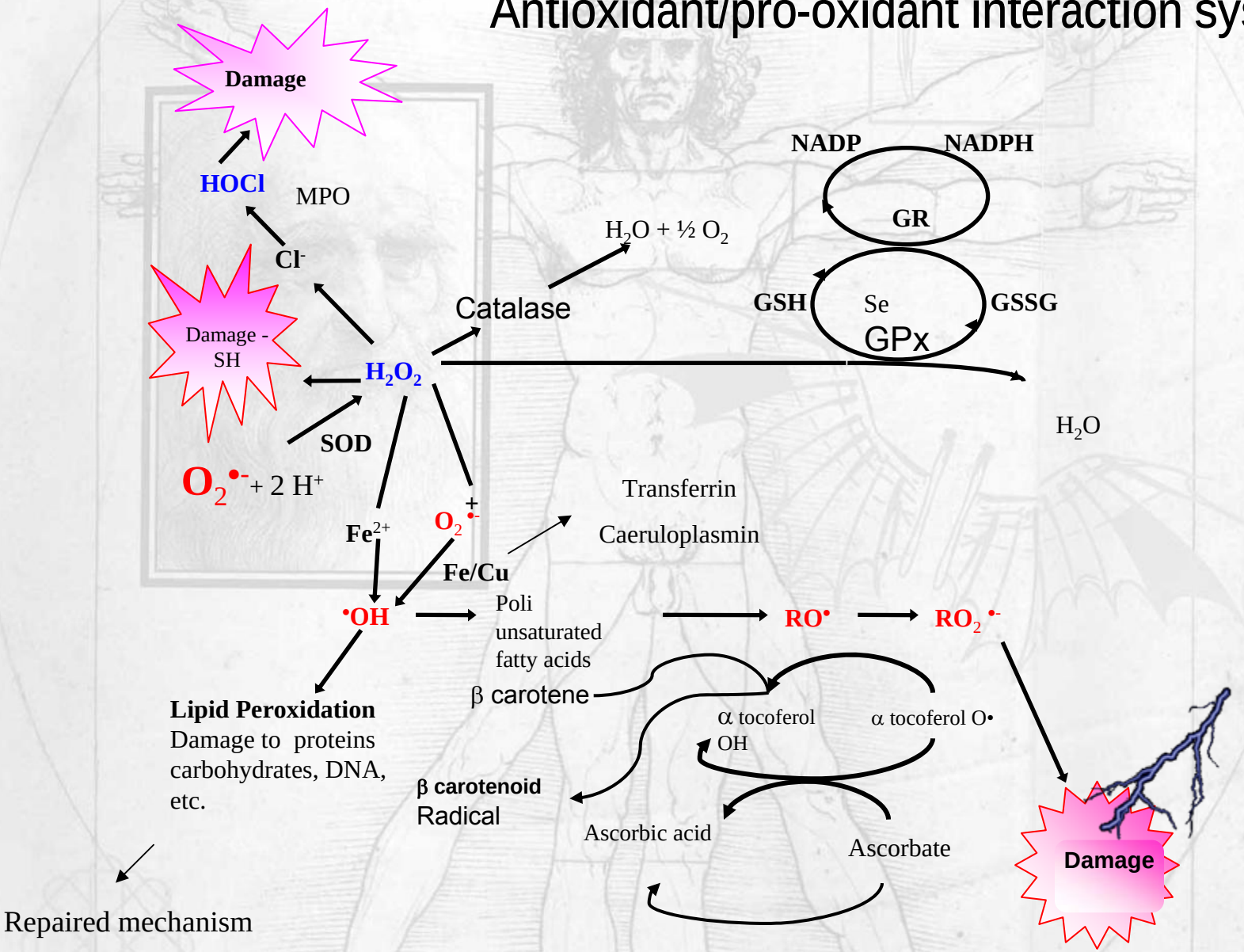
Bruick RK. (2002) *Science*. 295:807-808

Prolyl hydroxylase is O_2-dependent



Free Rad Res 2005 39:925-936/1353-1361

Antioxidant/pro-oxidant interaction system



SOURCES OF OXIDATIVE STRESS IN HUMAN PHATOPHYSIOLOGY

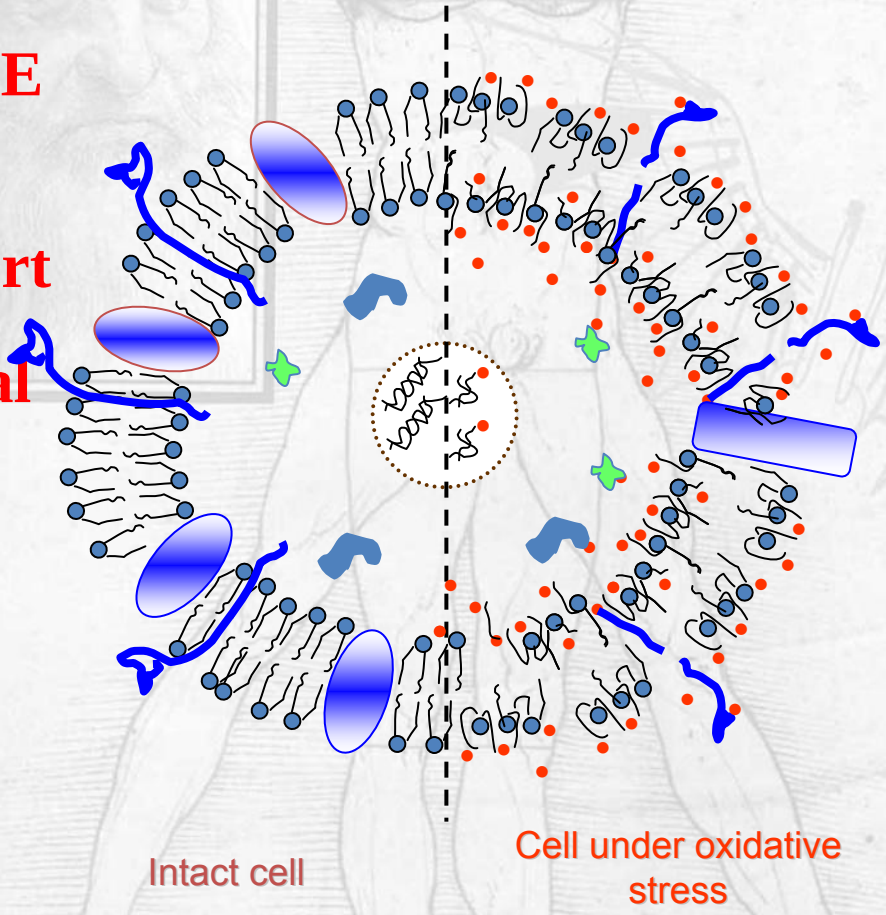
UNAVOIDABLE

Mitochondrial electron transport

Transition metal ions

Inflammation

Enzymes



AVOIDABLE

Drug metabolism

Xenobiotic

Cigarette smoke

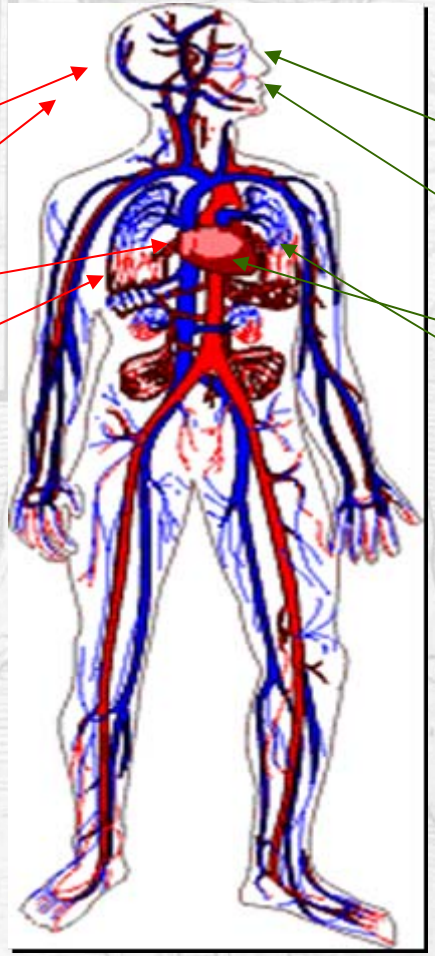
Pollution

Radiations

Diseases where an imbalance of oxidative stress and anti-oxidant defense has been implicated in the pathophysiology

ACUTE

- Trauma
- Stroke
- Ischaemia Reperfusion injury
- Adult respiratory distress syndrome
- Acute rejection
- Acute inflammation
- Paracetamol (overdose)
- Xenobiotic
- Acute exercise



CHRONIC

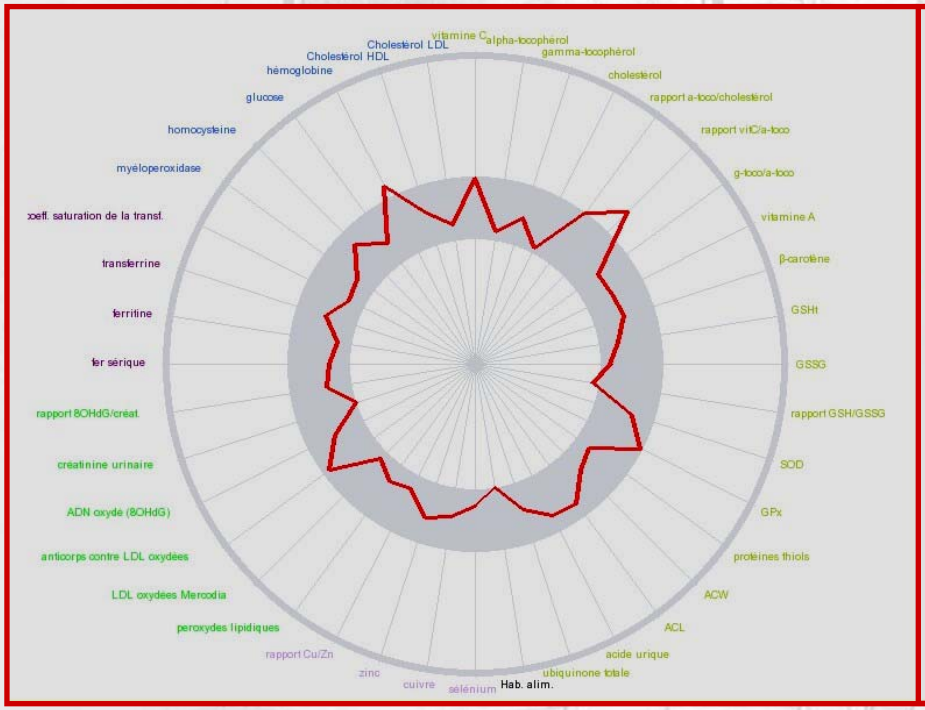
- Parkinson
- Alzheimer's disease
- Hypertension, Atherosclerosis
- Cystic Fibrosis
- Chronic rejection
- Chronic Inflammation
- Cancer
- Ageing
- Chronic training

Biochemical evaluation of the OS balance

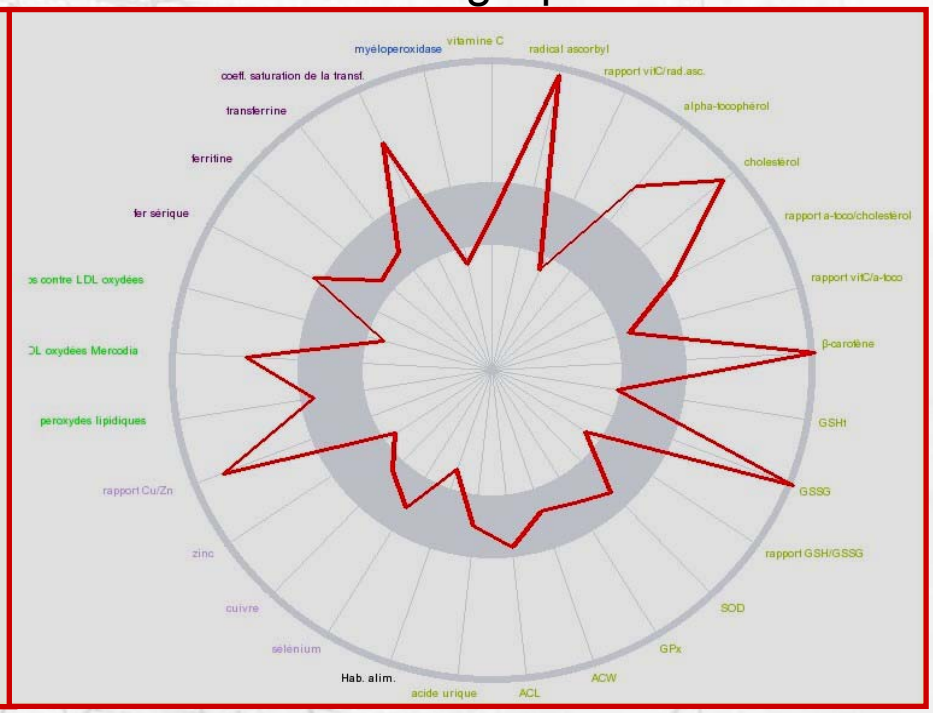
• Anti-oxidants	• Total anti-oxidant capacity	• Photochem, ORAC
	• Vitamin C	• Colorimetric
	• α-tocopherol	• HPLC
	• γ-tocopherol	• HPLC
	• β-carotene	• HPLC
	• Glutathion	• Enzymatic
	• Glutathion peroxidase (GPx)	• Enzymatic
	• Uric acid	• Enzymatic
	• Ubiquinone	• HPLC
• Oligo-elements	• Selenium	• ICP/MS or Atom abs
	• Copper	• ICP/MS or Atom abs
	• Zinc	• ICP/MS or Atom abs
• Oxidative stress markers	• Lipid peroxides	• Colorimetric
	• Oxidized LDL	• Immunologic
	• Anti-oxLDL antibodies	• Immunologic
	• Oxidized DNA (8-OH-dG)	• Immunologic
	• Protein thiols	• Immunologic
• Iron metabolism	• Iron	• Colorimetric or Atom Abs
	• Ferritin	• Immunologic
	• Transferrin	• Immunologic
• Sources of OS	• Myeloperoxidase	• Immunologic
	• Homocystein	• Immunologic

Biochemical tests → oxidative stress profile

Normal profile



Pathologic profile



The oxidative stress rosette: a snapshot of the OS status of the patient

Integrated Clinic Diagnostic of Oxidative Status

Ultra micro analytic system make possible fast analysis, low cost, data acquisition automation.

Involves analysis of:

Total Antioxidant Status,

Enzymes,

Biomolecules damage indicators,

Low molecular weight antioxidants

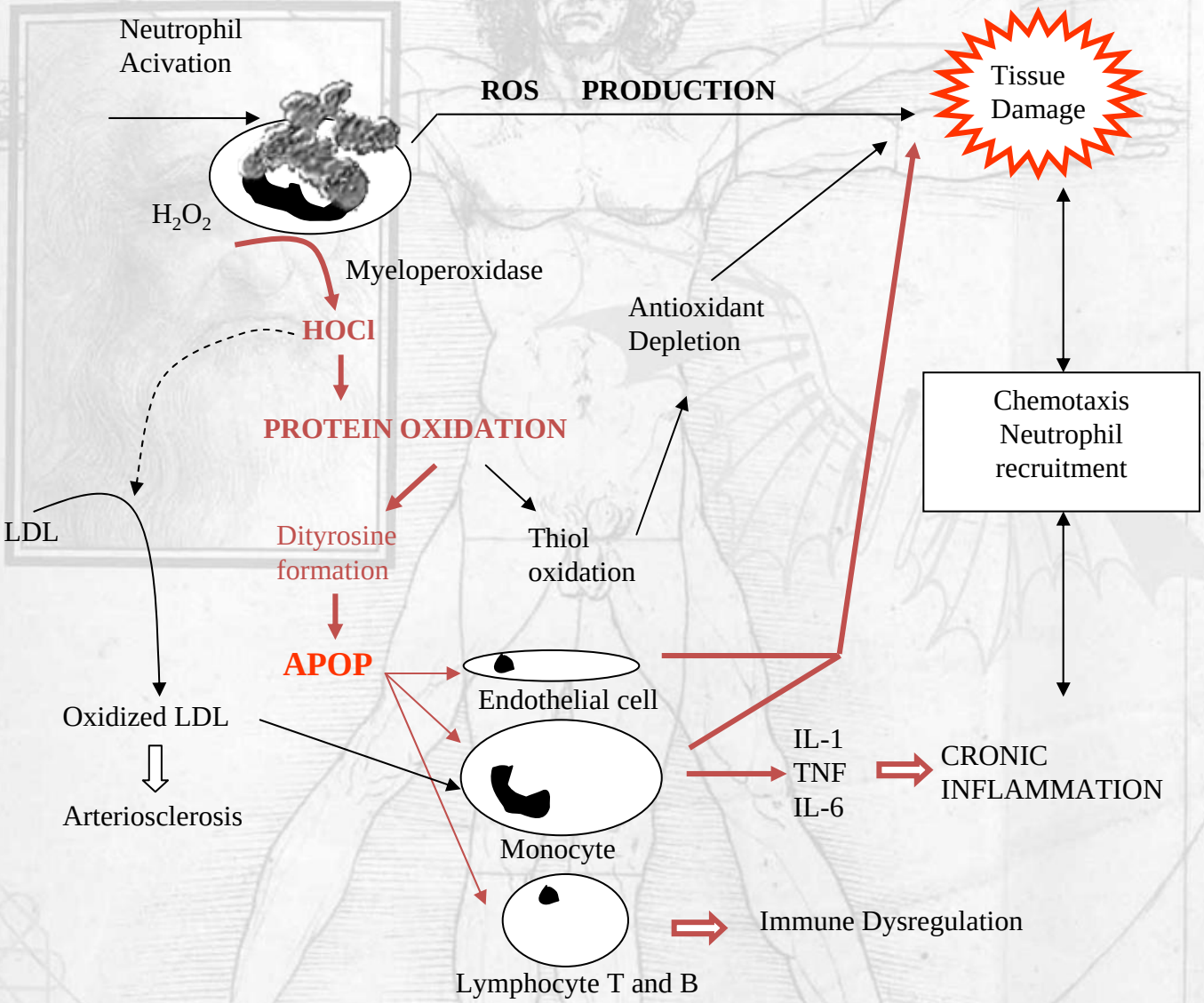
Useful for:

Clinical diagnostic of groups of patients

Clinical diagnostic of individual patients

Monitoring nutritional intervention

Monitoring pharmacological intervention



Gregorio M S, Redox Report. (2005)**10**(4):174-184

ADVANCED PROTEIN OXIDATION PRODUCTS

Status	APOP μM
Normal	**12.13±0.93**
Diabetes	**19-21***
Colitis	**29***
Bronchitis	**95****
Ataxia	**119****

Clinical diagnostic of groups of patients

ACADEMIC PRESS

Pharmacological Research 47 (2003) 217–224

Pharmacological research

www.elsevier.com/locate/yphrs

Contribution to characterization of oxidative stress in HIV/AIDS patients

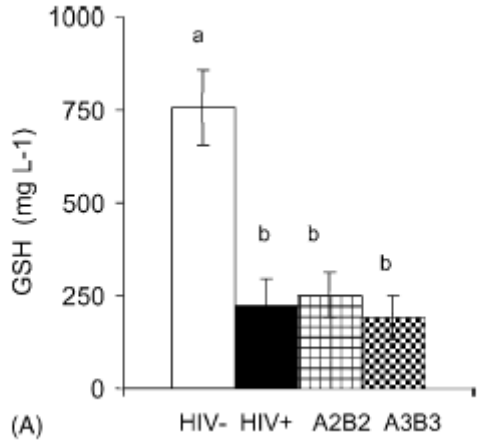
Lizette Gil [a,*], Gregorio Martínez [b], Ivón González [b], Alicia Tarinas [a], Alejandro Álvarez [a], A. Giuliani [c], Randelis Molina [a], Rolando Tápanes [a], Jorge Pérez [a], Olga Sonia León [b]

[a] *Department of Clinical Pharmacology, Institute of Tropical Medicine "Pedro Kouri" (IPK), P.O. Box 601, Marianao 13, Ciudad de La Habana, Havana, Cuba*

[b] *Center for Research and Biological Evaluations, Institute of Pharmacy and Food, Havana University, Havana, Cuba*

[c] *Department of Chemistry and Medical Biochemistry, University of Milan, Via Saldini, 50-20133 Milan, Italy*

Accepted 25 November 2002



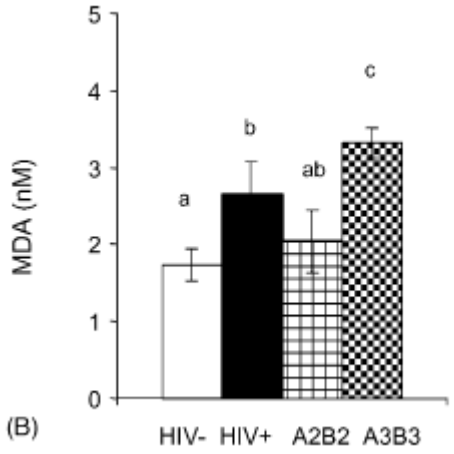


Table 2
Correlation coefficients (Pearson coefficients) between redox indices and CD4+, CD95+, and CD8+ relative count in VIH seropositive patients

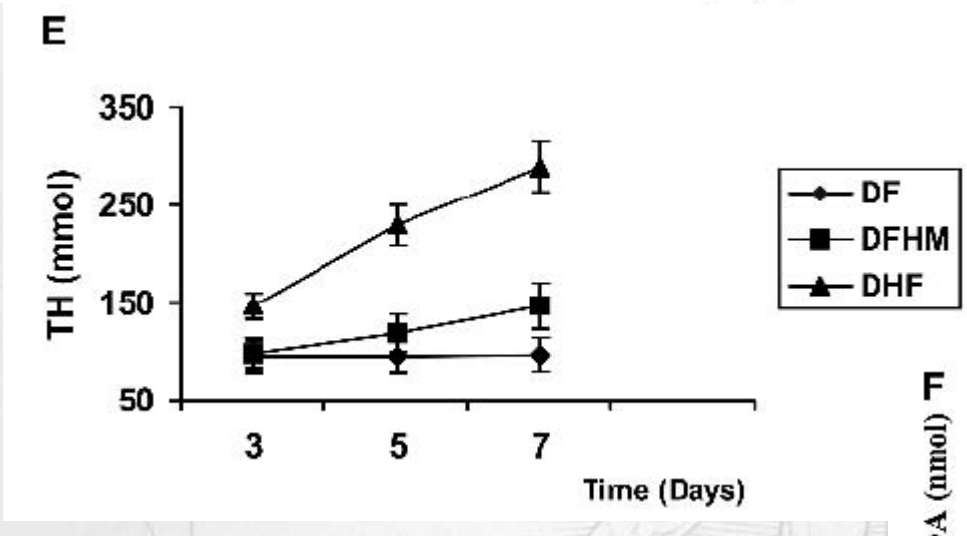
Redox index/immunological markers	CD4+	CD95+	CD8+
MDA*	−0.90	+0.89	+0.73
SOD*	−0.81	+0.72	+0.67
PP*	−0.79	+0.71	+0.64
TAS*	+0.86	−0.80	−0.74
GSH	+0.21	−0.32	−0.19
GPx	+0.26	−0.29	−0.15
TH	−0.33	+0.51	+0.46
% DNA	−0.71	+0.42	+0.38

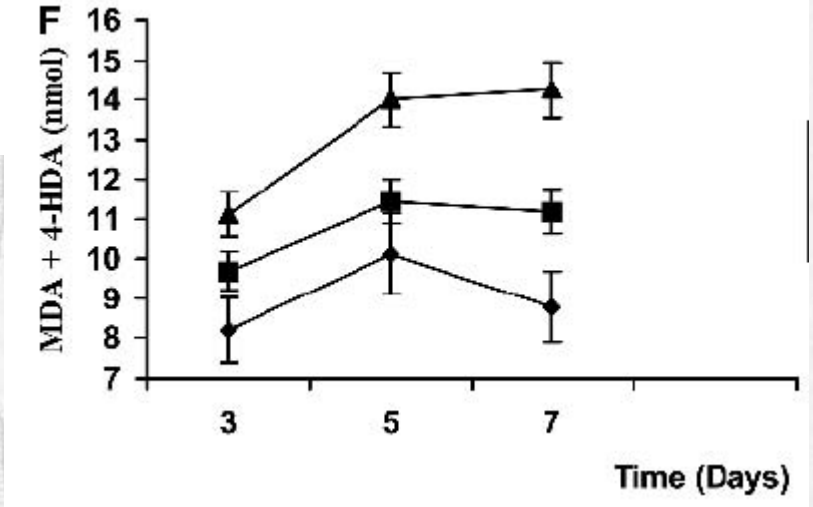
Clinical diagnostic of groups of patients

Am. J. Trop. Med. Hyg., 71(5), 2004, pp. 652–657

OXIDATIVE STRESS IN ADULT DENGUE PATIENTS

LIZETTE GIL, GREGORIO MARTÍNEZ, ROLANDO TÁPANES, OSVALDO CASTRO, DANIEL GONZÁLEZ, LIDICE BERNARDO, SUSANA VÁZQUEZ, GUSTAVO KOURÍ, AND MARÍA G. GUZMÁN





Patient with Fe overload

Weakness Syndrome

54 year old ♀

Total Antiox. Status

PP (7.32±0.56) 61.35 μM MDA

Enzyme

SOD (1.45±0.15) 13.75 U·mL $^{-1}$·min $^{-1}$

CAT (161.5±12.5) 1545 U·mL^{-1}·min $^{-1}$

CAT/SOD (0.11±0.02) 0.11

Biomolecules damage

MDA (1.74±0.27) 6.56 μM

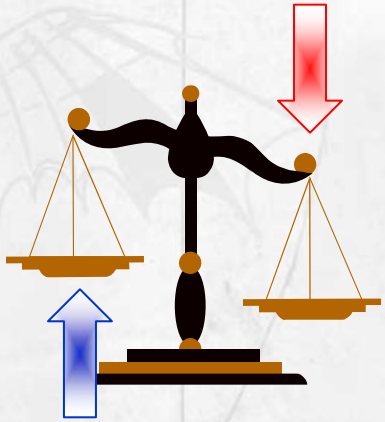
ROOH (103±17) 67.40 μM

PAOP (12.13±0.93) 58.21 μM

Low MW antioxidants

Vit. C (30-150) 3.18 μM

GSH (786-1146) 301.18 mg·L^{-1}



HC. *39 SHC*

Monitoring nutritional intervention

International Journal of Vitamin and Nutrition Research (2005). **75**(1):19-27.

Effects of increase micronutrients intake on oxidative stress indicators in HIV/AIDS patients.

Lidianis Luján, Lizette Gil, Gregorio Martínez, Attilia Giuliani, Ivón González, Alicia Tarinas, Alejandro Álvarez, Randelis Molina, Maite Robaina, Rolando Tápanes, Alberto Nuñez, Jorge Pérez

Table II: Oxidative stress indicator data during study period

Indicator	Time (months)	Mean ± SEM	P
TAS (mM Trolox)	control	1.69 ± 0.20	
	0	0.83 ± 0.18[a]	0.005
	3	1.03 ± 0.11[b]	
GPx (U/mL)	control	39.3 ± 5.5	
	0	20.4 ± 4.3[a]	
	3	28.6 ± 4.3[b]	
SOD (U/mL)	control	1.12 ± 0.05	
	0	1.58 ± 0.19[a]	0.000
	3	1.36 ± 0.39[b]	
HPO ((M)	control	249.9 ± 56.9	
	0	542.4 ± 277.5[a]	0.175
	3	401.9 ± 273.9[b]	
MDA ((M)	control	1.74 ± 0.05	
	0	2.98 ± 1.42[a]	0.071
	3	2.02 ± 1.00[b]	
GSH ((M	control	2464 ± 462	
	0	393 ± 240[a]	
	3	1045 ± 552[b]	
FDNA (%Fragment.)	control	6.30 ± 0.10	
	0	7.19 ± 1.16[a]	0.929
	3	7.29 ± 1.89[b]	

Legend: Controls are seronegative-HIV subjects

Monitoring therapeutic intervention

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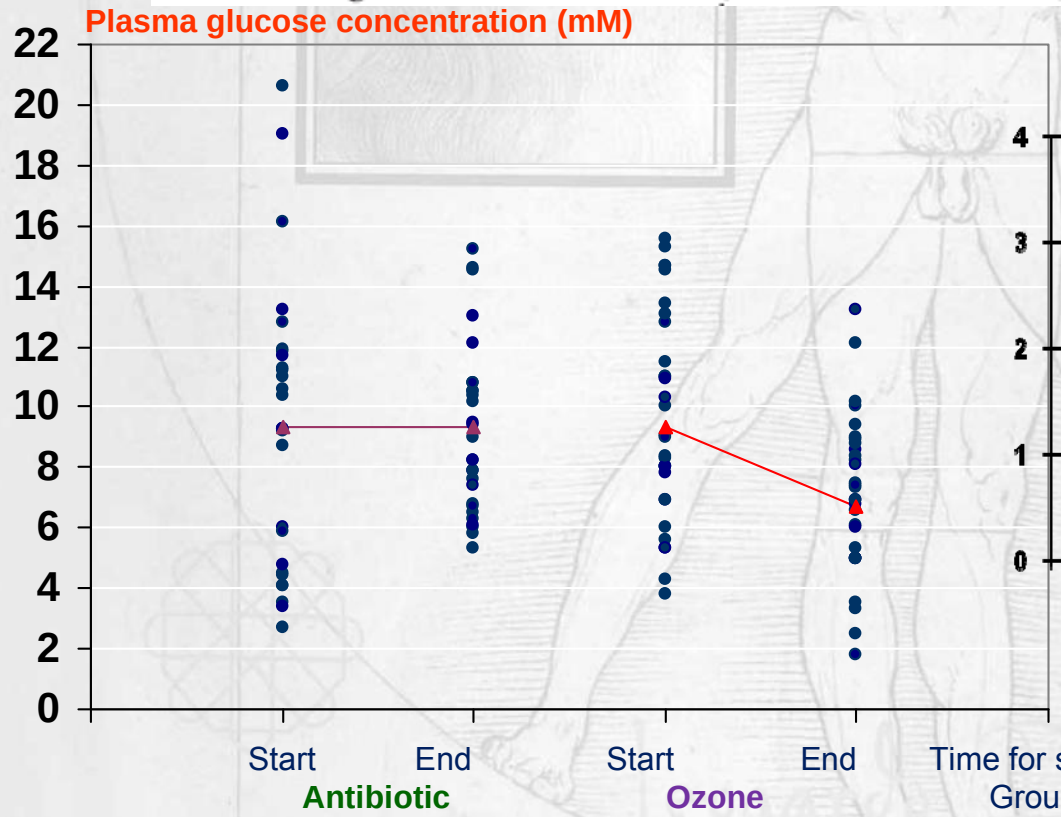
Available online at www.sciencedirect.com
SCIENCE DIRECT

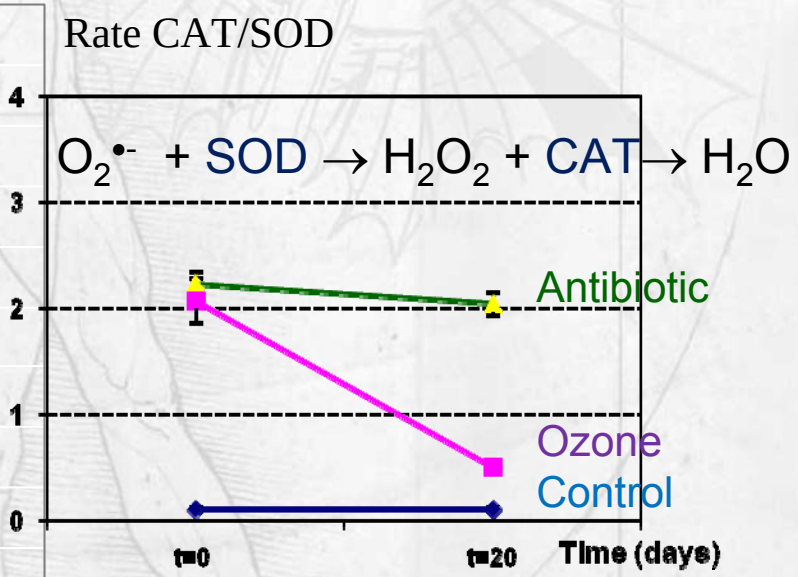
European Journal of Pharmacology 523 (2005) 151 – 161

ejp
www.elsevier.com/locate/ejphar

Therapeutic efficacy of ozone in patients with diabetic foot

Gregorio Martínez-Sánchez [a], Saied M. Al-Dalain [a], Silvia Menéndez [b], Lamberto Re [c],



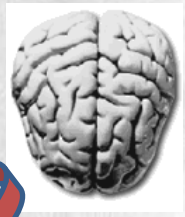


Future: Diagnostic in a software

Lab. Data

Algorithms

Damage	Eq. Variables
> 10%	± 10 %



SIMPLIFIED INTERPRETATION

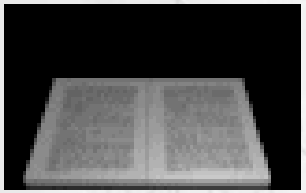
Near future

1 Normal

2 Moderate / Low

3 High

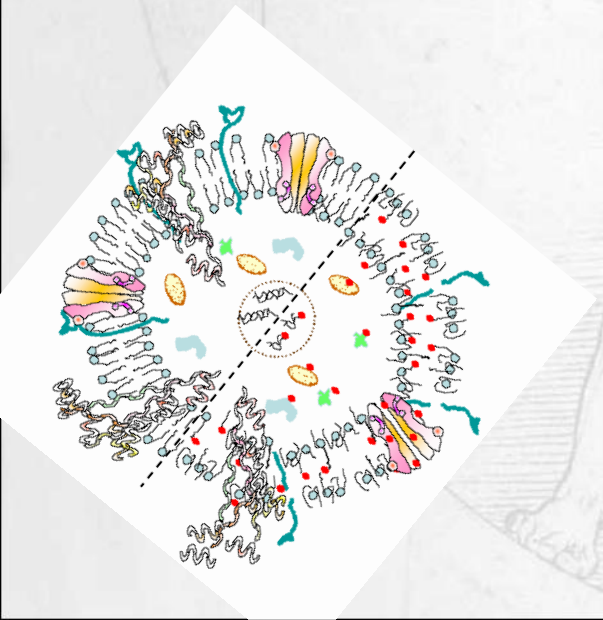
4 Critical



Clinical data

Prospective

Physicians recommendations



III INTERNATIONAL CONFERENCE OF OXIDATIVE STRESS

Havana Redox 2011

Havana (Cuba), January 27 - 29, 2011

http://www.scf.sld.cu

email: hr2011@cieb.sld.cu

Post: Dr.C. Gregorio Martínez Sánchez

Scientific Committee President

Apartado postal 4301,

Ciudad de La Habana 4,

Zona 10400, Cuba

Scientific Topics

- *Oxidative Stress and Diseases.*
- *Oxidative Stress and Aging.*
- *Biomarkers and Diagnostic Methods.*
- *Antioxidants.*
- *Redox Signaling Mechanisms.*
- *Pharmacological or Dietetic Interventions.*
- *Oxidative Damage, Formation, Repair and Biological Consequences.*
- *Special Symposium Hormesis and Oxidative Stress.*

Call for papers (short lectures, posters)

Short lectures (25 min. including discussion) and posters related to the congress topics are kindly requested. Contributions in other fields of redox research will be also welcomed.

❖ ❖ ❖

Please download instructions from the website.

Submission deadline for Abstracts: December 1, 2010.

We look forward to seeing you in Havana in January 2011

Questions

Feel free to contact:

Gregorio Martínez Sánchez, Pharm.D, Ph.D.

Senior Researcher

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