INTERNATIONAL CONFERENCE. New horizons for the ozone therapy - *Pontevedra. June 5th, 2009.*

Ozone therapy and oxidative stress evaluation

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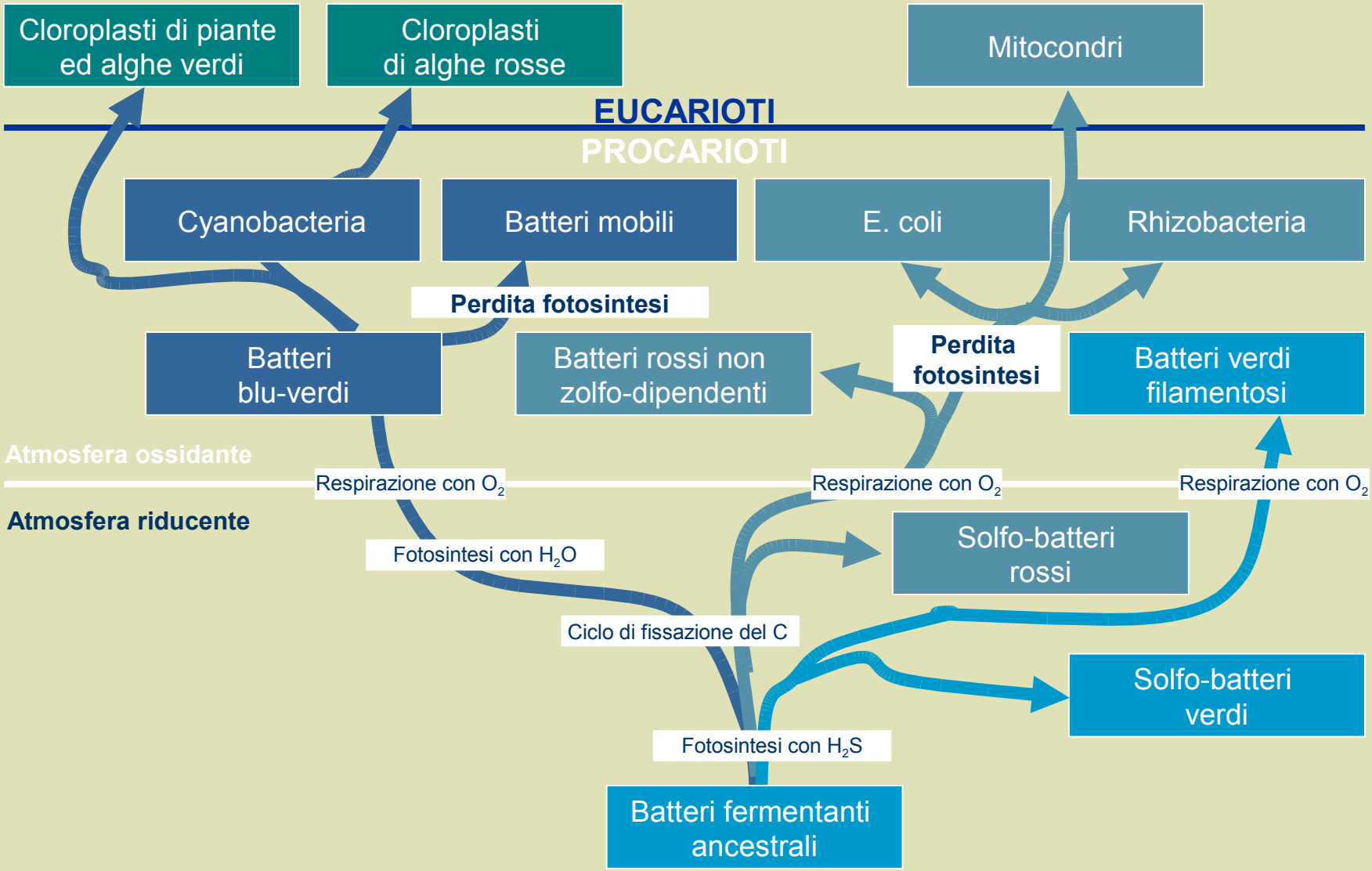
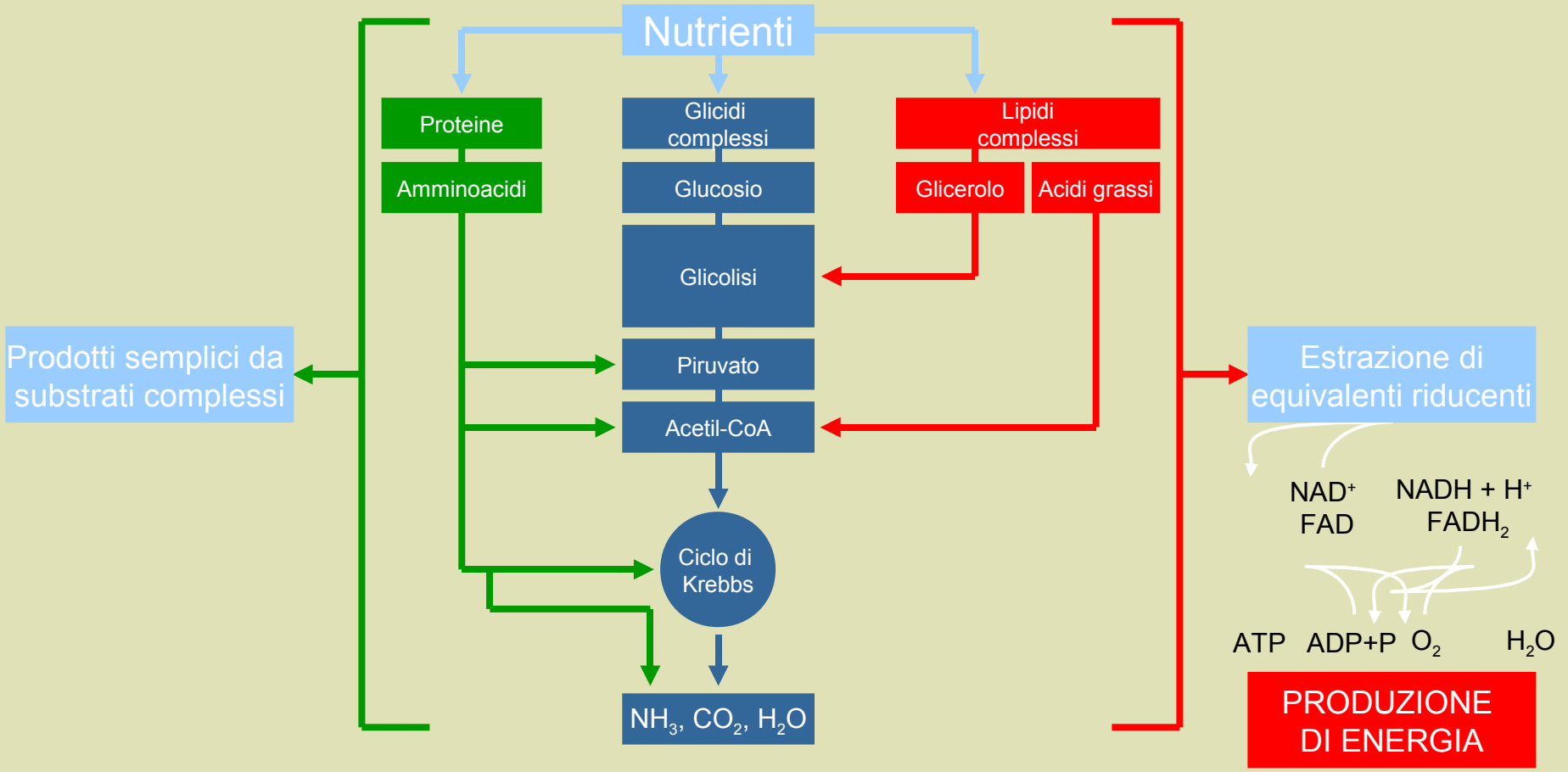
Evolution of living organisms and redox status

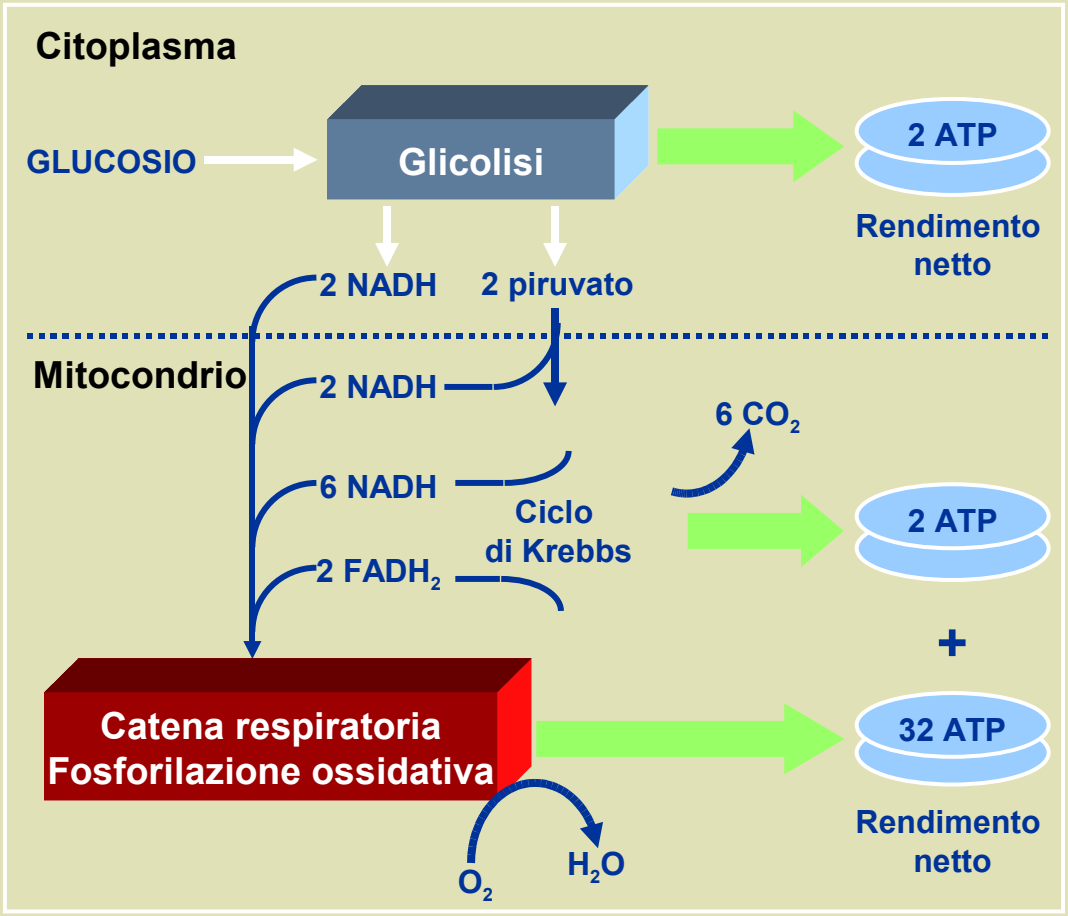


General metabolic pathways



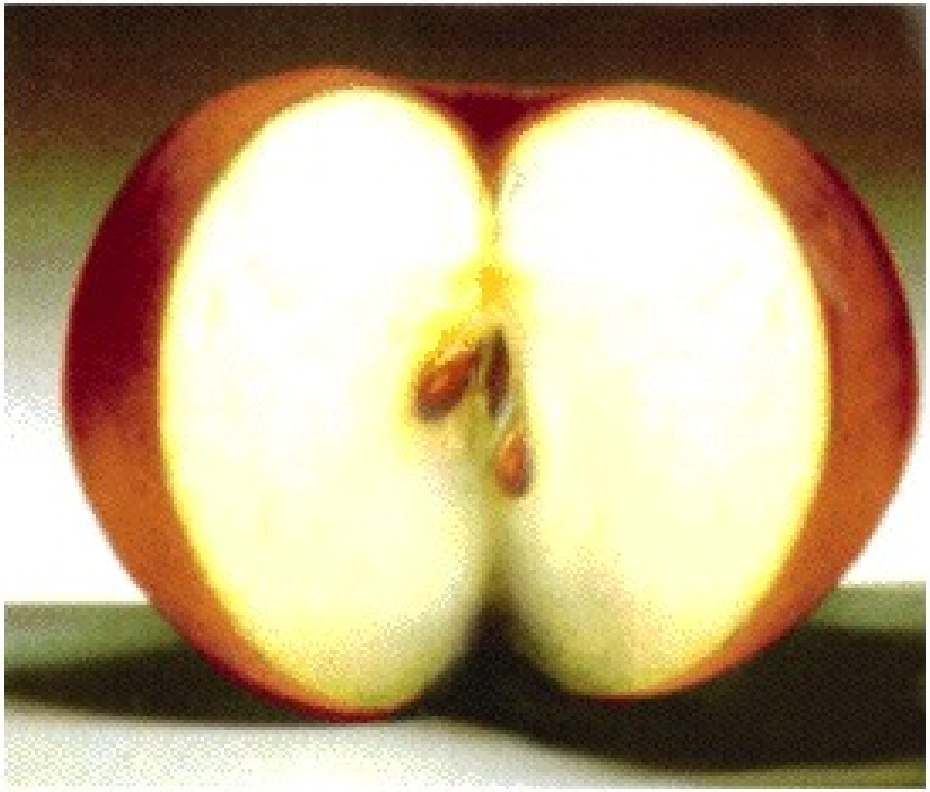
From oxidative processes to energy and live

Comparison between anaerobiosis and aerobiosis



Much more energy from aerobic oxidative processes

The other face of oxidation

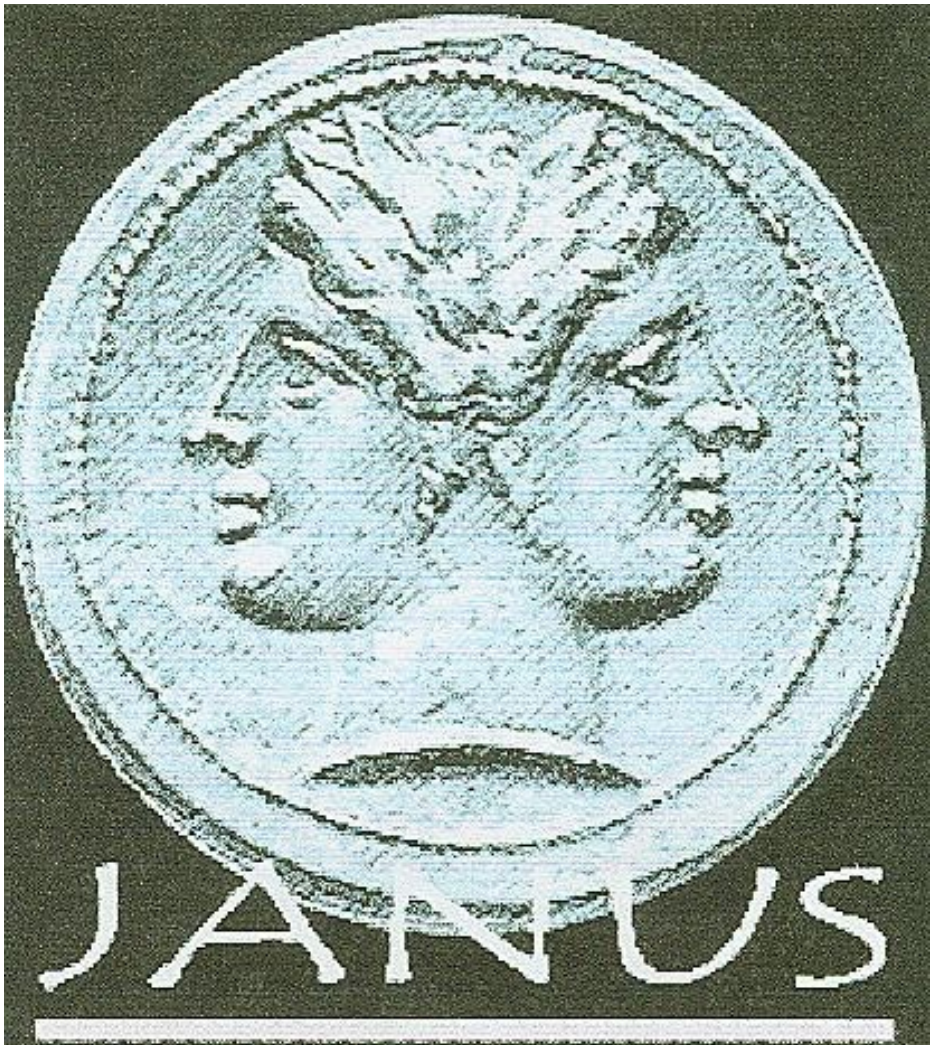


Oxygen and living organisms

1940-1950	Prime evidenze sperimentali in animali da laboratorio sul possibile ruolo tossico dell'iperossia
⇩	Disturbi respiratori fino a comparsa di lesioni alveolari dopo prolungata esposizione ad O_2 puro
1954-1955	La fibroplasia retrolenticolare è provocata dall'esposizione dei neonati ad elevata pO_2
⇩	Molte lesioni *in vivo* sono provocate dalle specie reattive dell'ossigeno
1960	Alcune specie reattive sono utili (fagocitosi)

The discovery of oxygen toxicity

The two sides of oxygen



What is OXIDATIVE STRESS?

The oxidative stress is a particular kind of chemical stress induced by the presence, in a living organism, of an **excess of oxidant chemical species (OCS)** that results from an **increased production** of OCS and/or a **decreased efficacy** of antioxidant enzymatic and/or non enzymatic defence system.

The breaking of a balance

Ageing and almost 100 diseases are related to OXIDATIVE STRESS

Alzheimer's disease

Atherosclerosis

Kidney diseases

Pancreatitis and liver diseases

Stroke, miocardial infarction

COPD

AGEING

Parkinson's disease

Maculopathy, cataract

Rheumatoid arthritis

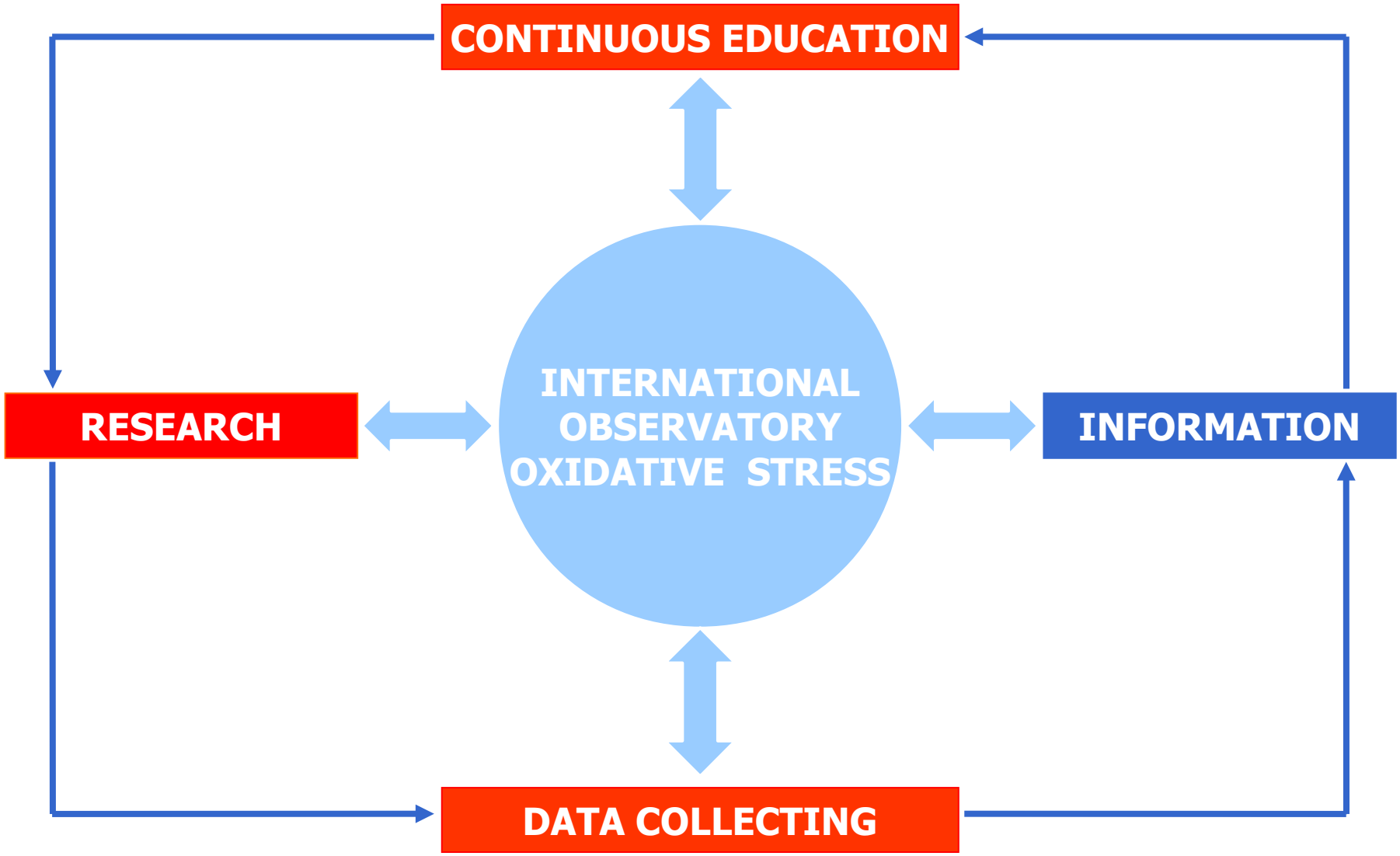
Crohn's disease

Diabetes, obesity

Cancer

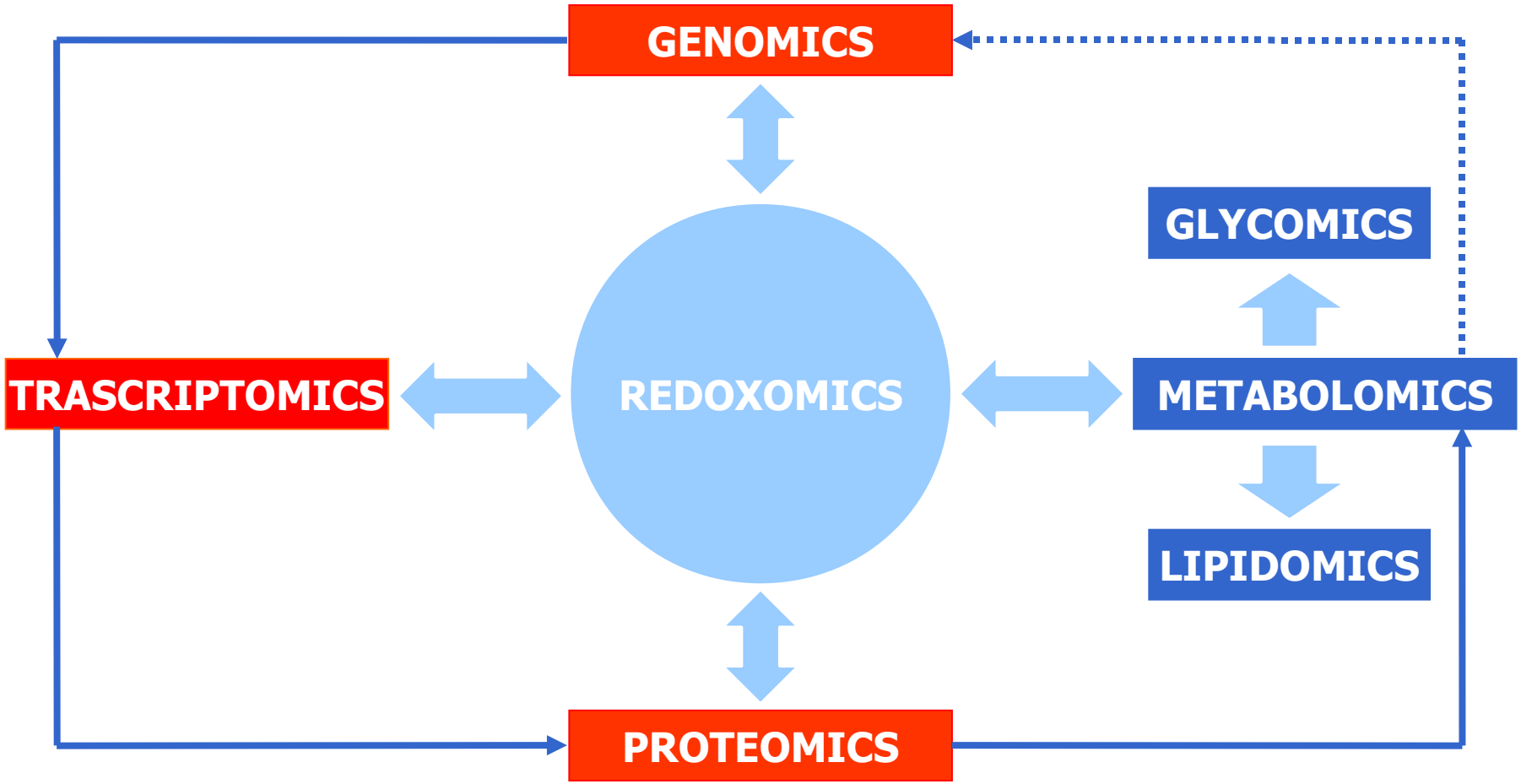
"The free radical man"

THE INTERNATIONAL OBSERVATORY OF OXIDATIVE STRESS ACTIVITIES



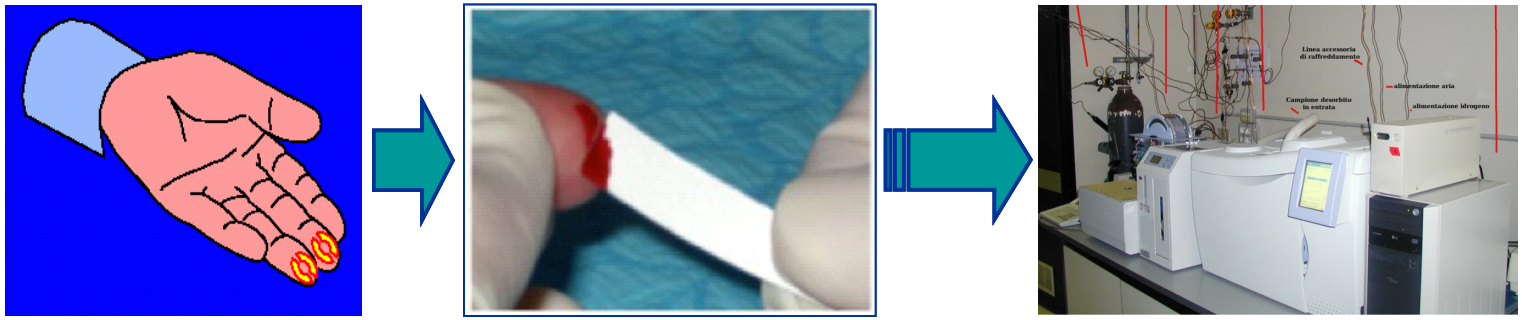
AN OXI.GEN LAB VIEW

The REDOXOMICS. A common *trait d'union*.



AN OXI.GEN LAB VIEW

FAST TEST®: THE LIPIDOMICS ENTERS IN TO THE POPULATION STUDIES.



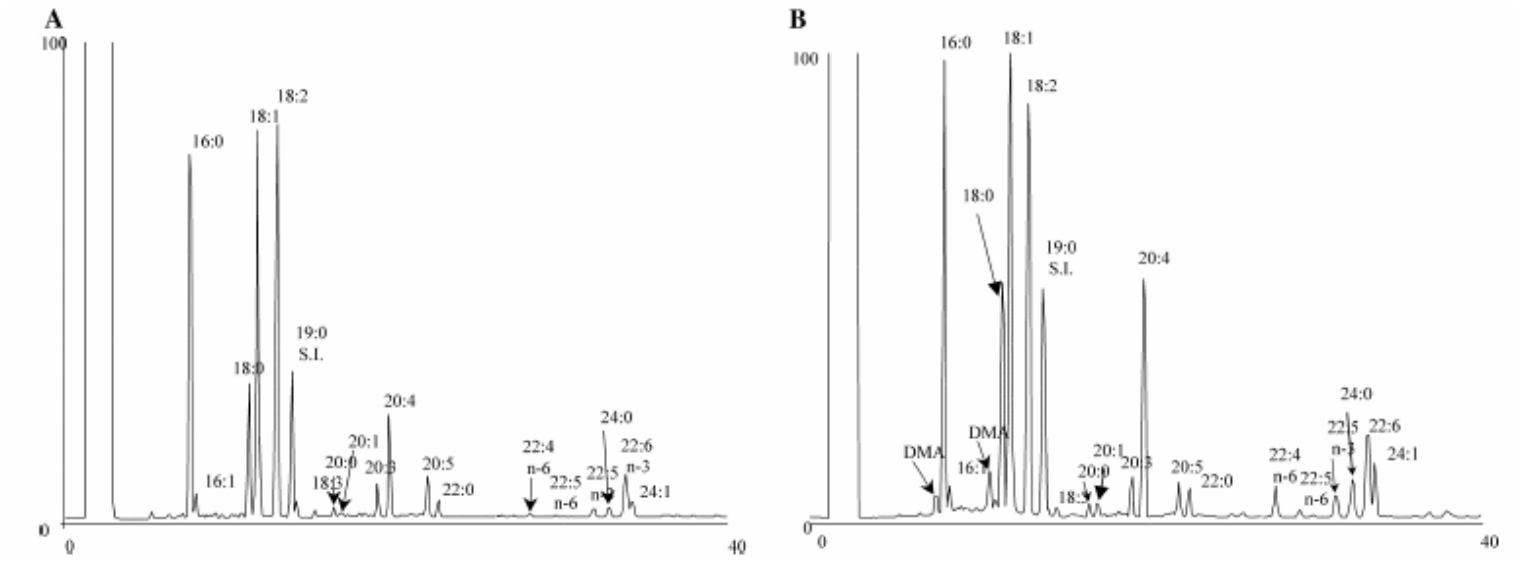


Fig. 1. Gas chromatographic profiles of FAME of plasma (A) and whole blood (B) lipids after direct transmethylation.

From the clinic to the laboratory

O. S. biochemical assessment

INCREASED OXIDANT STATUS

Inflammation/infection markers (ESV, CRP, AST, leukocytes)

Normal

Abnormal

O. S. by abnormal reactivity

Metabolism/respiration markers (BMI, thyroid hormones,LDH,lactate)

Normal

Abnormal

O. S. by metabolic/respiratory deficit

Cardiovascular markers (cholesterol, HCY, nitrates?)

Normal

Abnormal

O. S. by pO_2 changes

Toxicity/exposure markers (AST, ALT, alcohol, drugs, chemicals)

Normal

Abnormal

O. S. by microsomial induction

O. S. by indefinite causes

EMPIRIC APPROACH

DECREASED ANTOX STATUS

INTAKE/ABSORP/AVAILABILITY

AO ENZ

LP/HS AS

MINER

FURTHER INVESTIGATIONS

EZIOLOGIC DIAGNOSIS

SPECIFIC TREATMENT

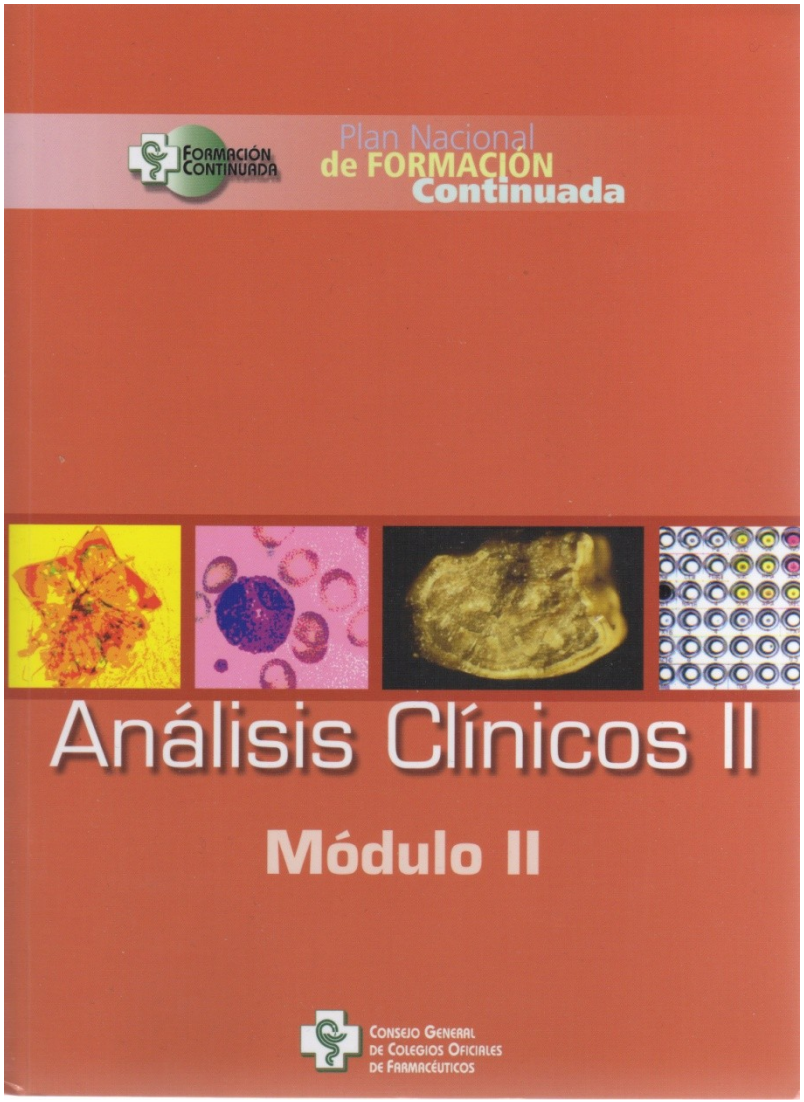
O. S. MONITORING

RECOVERY

O. S. MONITORING

RELAPSES PREVENTION

THE PROJECT FOR SPAIN PHARMACISTS



capítulo 1

El laboratorio en el estudio del estrés oxidativo

Eugenio Luigi Iorio

Oxidant Chemical Species (OCS). The Free Radicals.

An atom of Neon
Only paired electrons

Atom (stable)

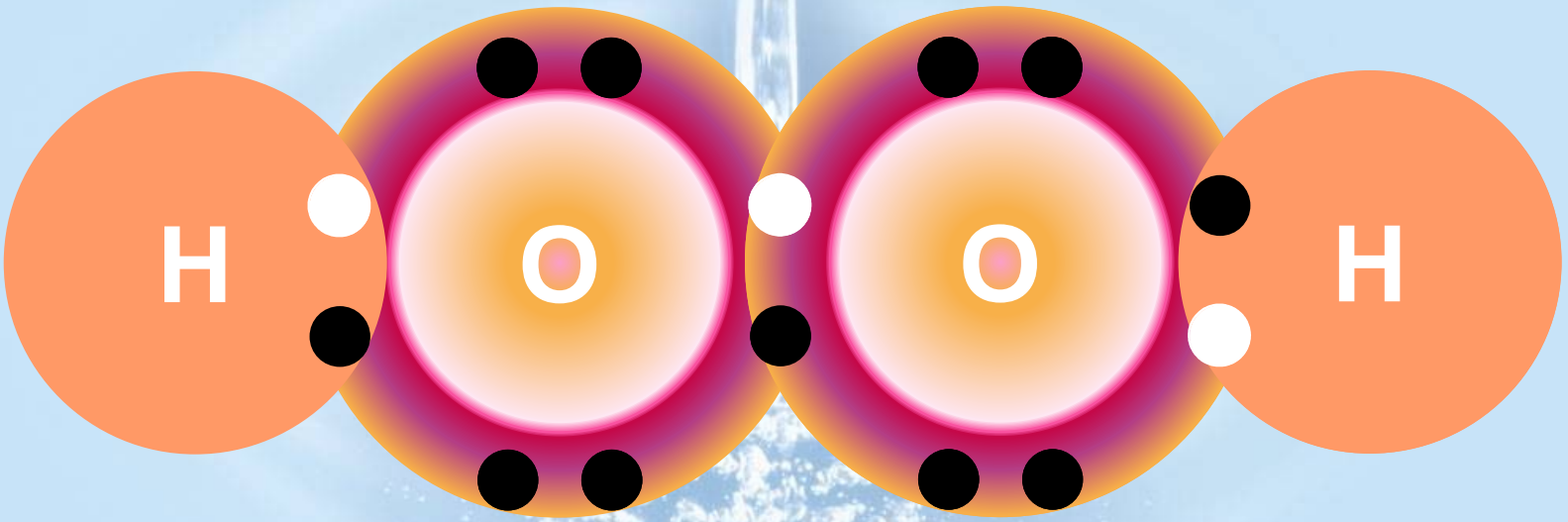
An atom of Oxygen
Two unpaired electrons

The hydroxyl radical ($HO^{\cdot}$)
One unpaired electron

Oxygen Free Radicals (unstable)

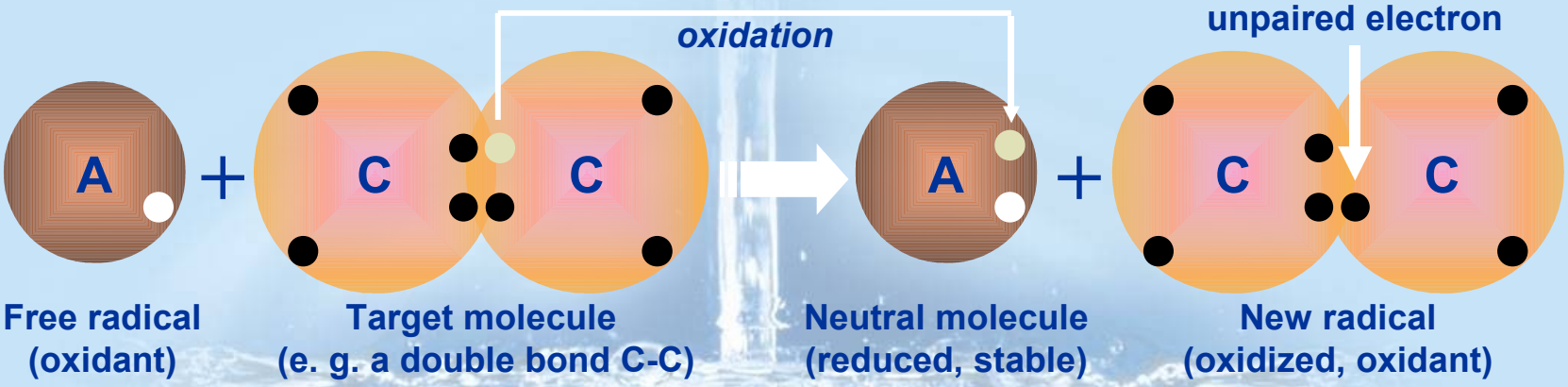
Free Radicals or Radicals, instead of most atoms, share *always* one or more unpaired electron(s)

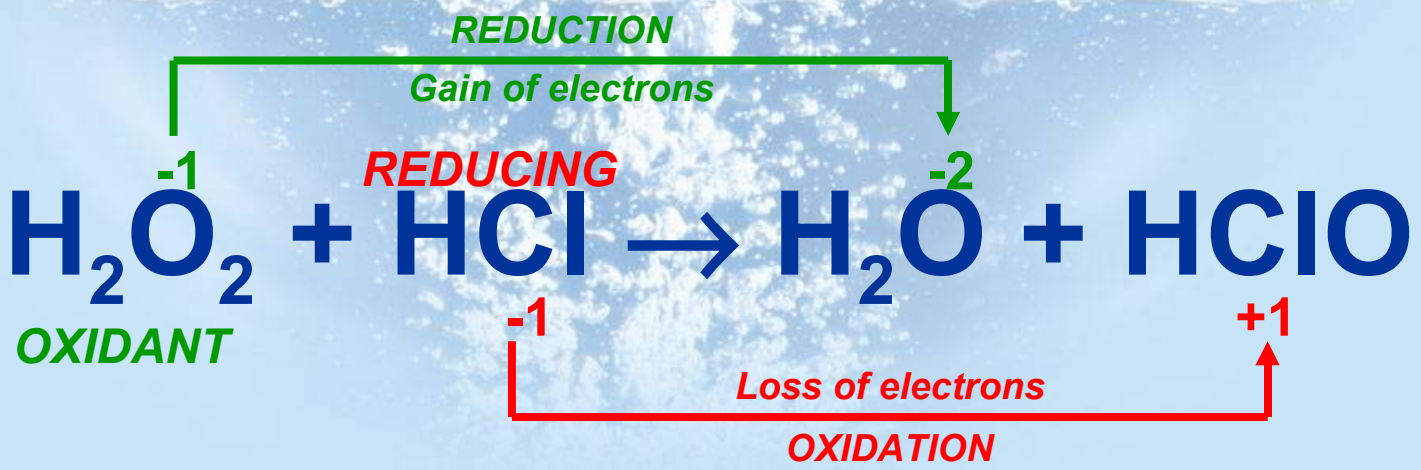
Non-Radical Oxidant Chemical Species



Non-Radical OCS, like hydrogen peroxide, share *always* paired electrons

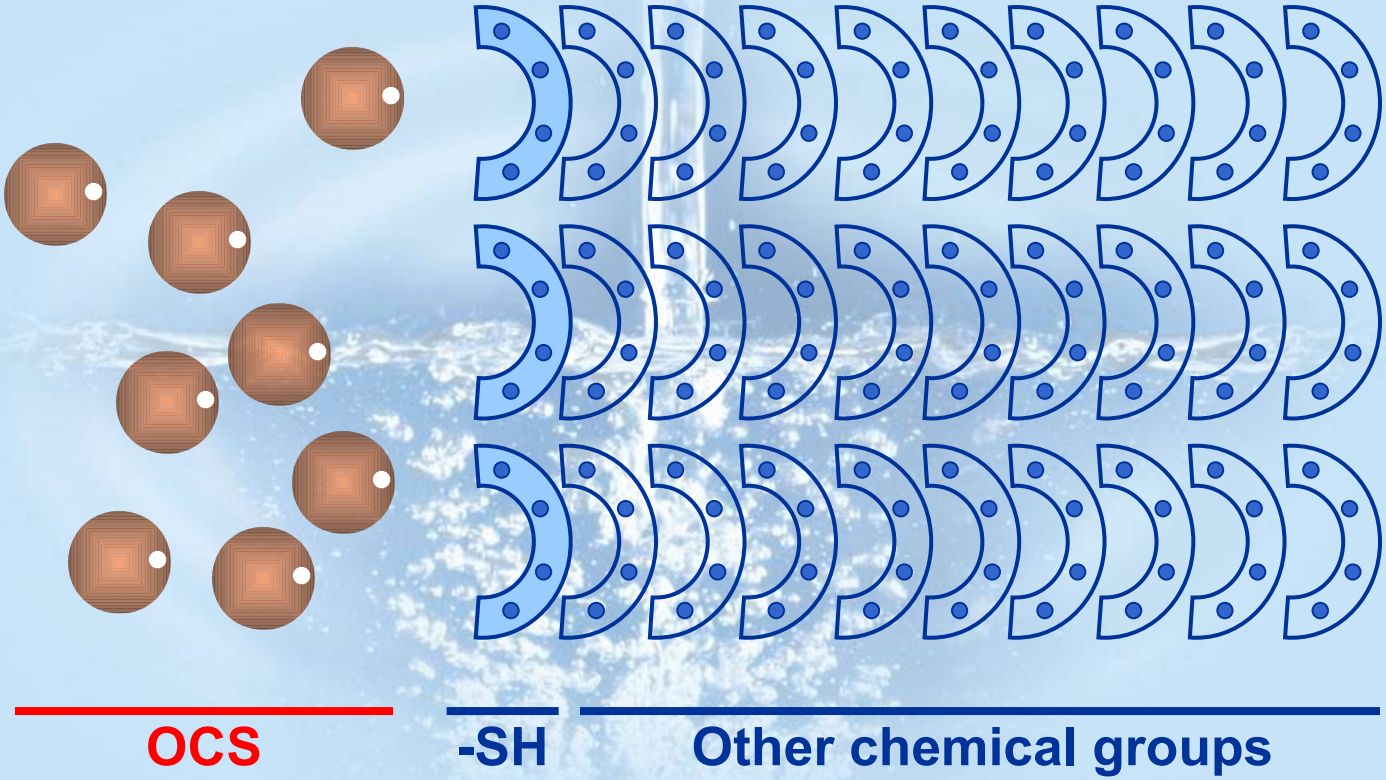
The REDOX process





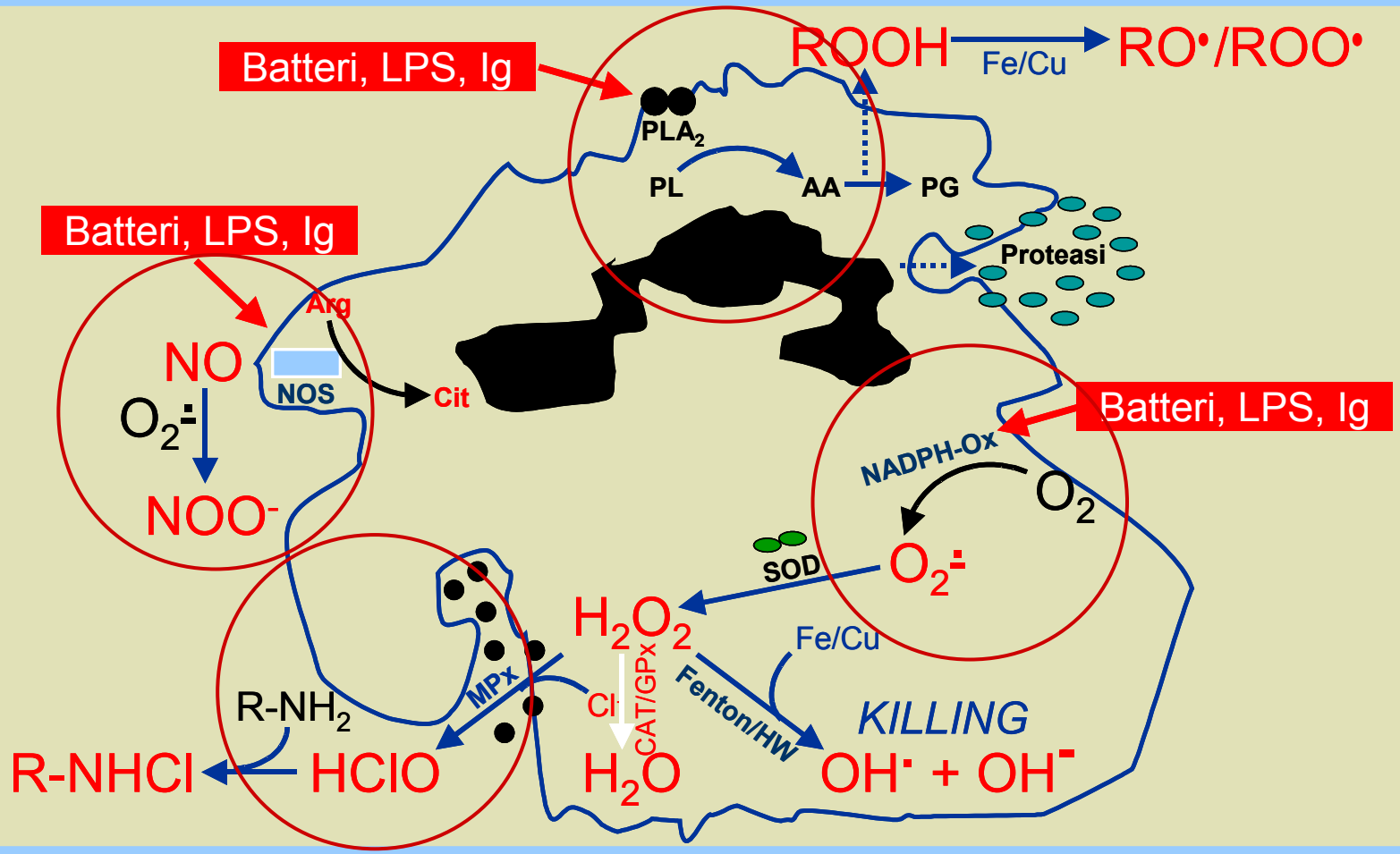
The chemical basis of REDOXOMICS

Plasma antioxidant barrier prevents cellular oxidative damage by blocking OCS in the blood



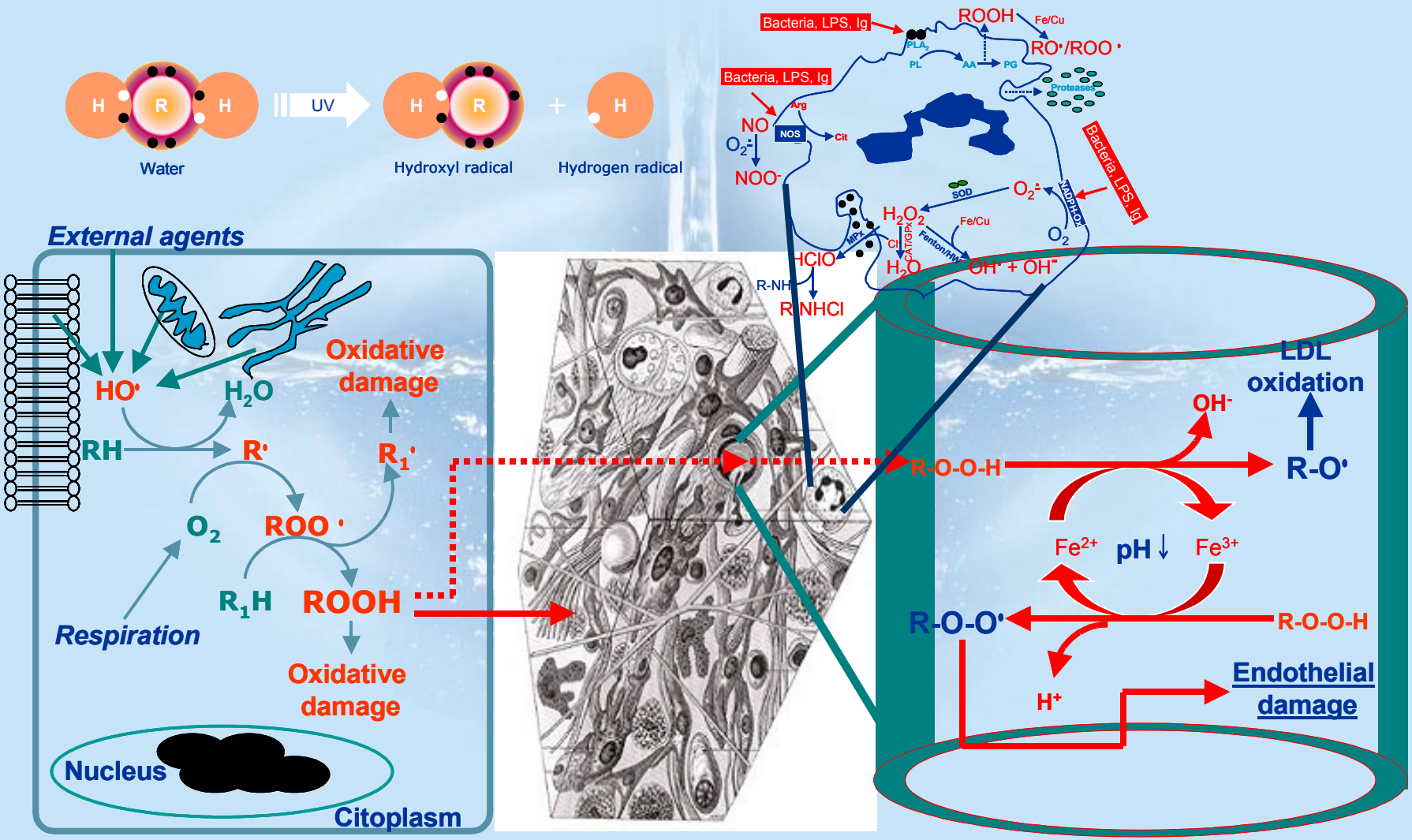
Thiol groups (-SH) are qualitatively important components of plasma antioxidant barrier

The production of free radicals from leukocytes

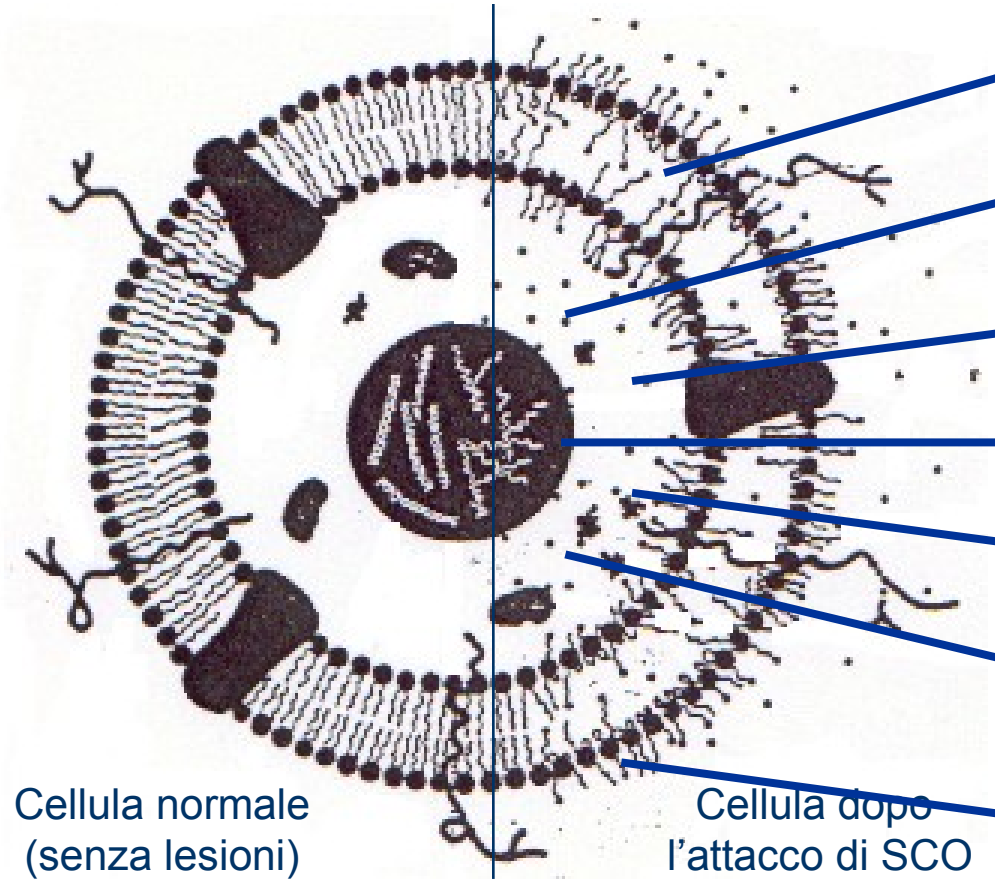


Relevance of oxidative stress

Basic cell mechanisms of oxidative tissue damage



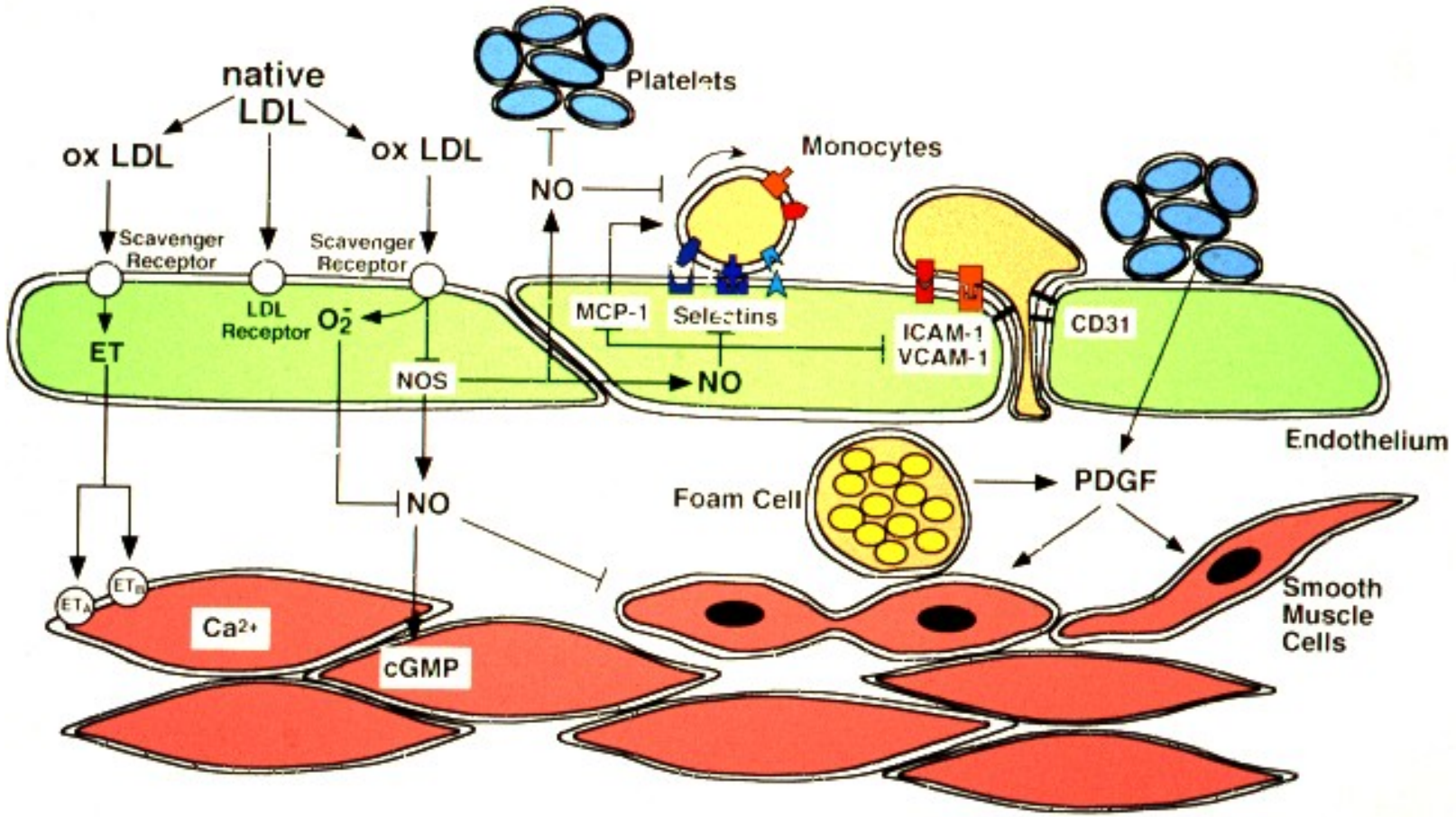
Disastrous effects of free radical excess on the cell



- Perossidazione di lipidi
- Modificazioni enzimatiche
- Perossidazione amminoacidi e proteine
- Lesioni del DNA
- (Per)ossidazione di carboidrati
- Rottura di proteine
- Alterazioni dell' omeostasi ionica

Every biomolecule can be affected by OCS

Biochemical basis of atheromatous lesion

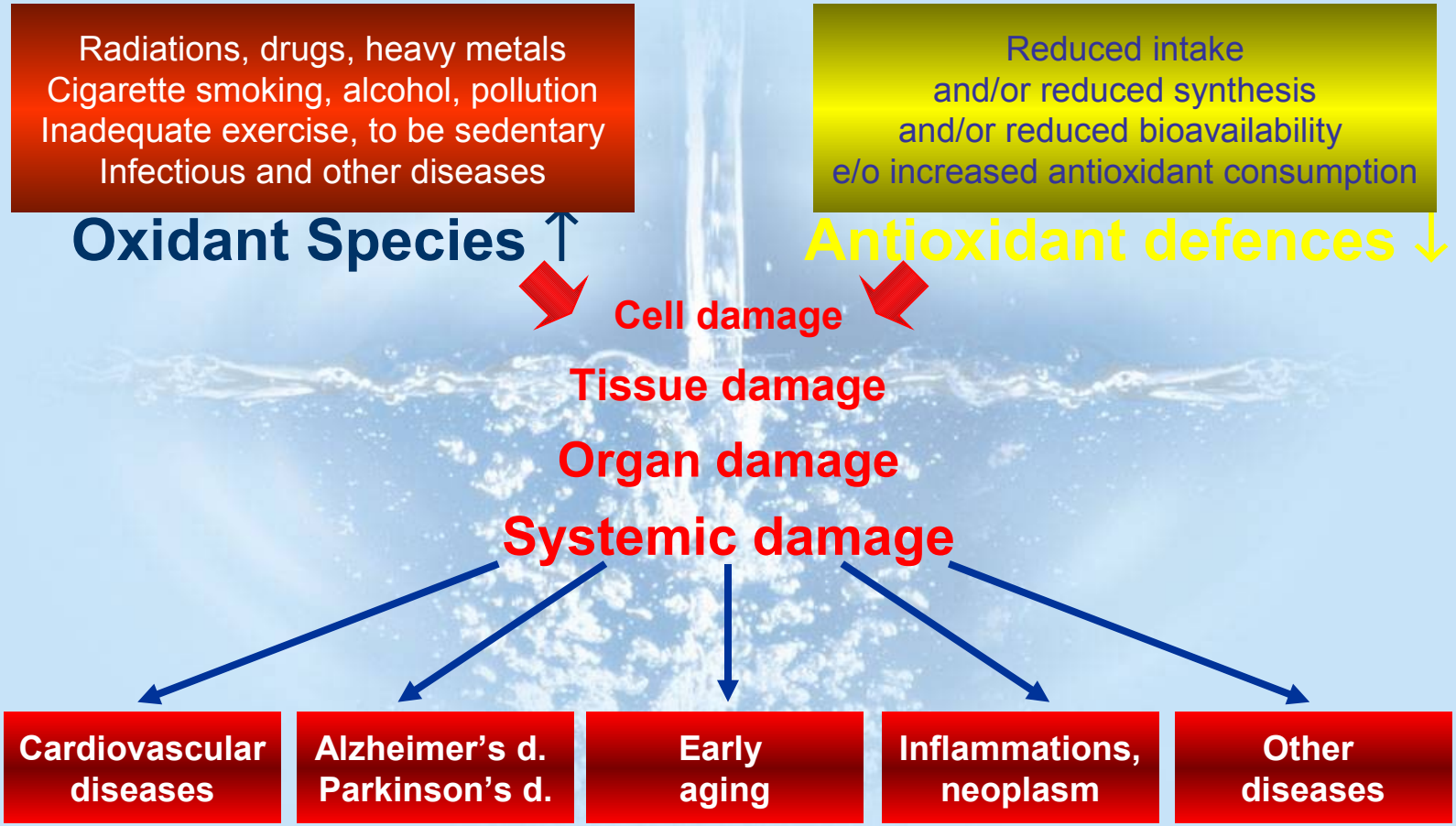


The role of dyslipidemia and oxidative stress

The discovery of NITRIC OXIDE as signalling molecule

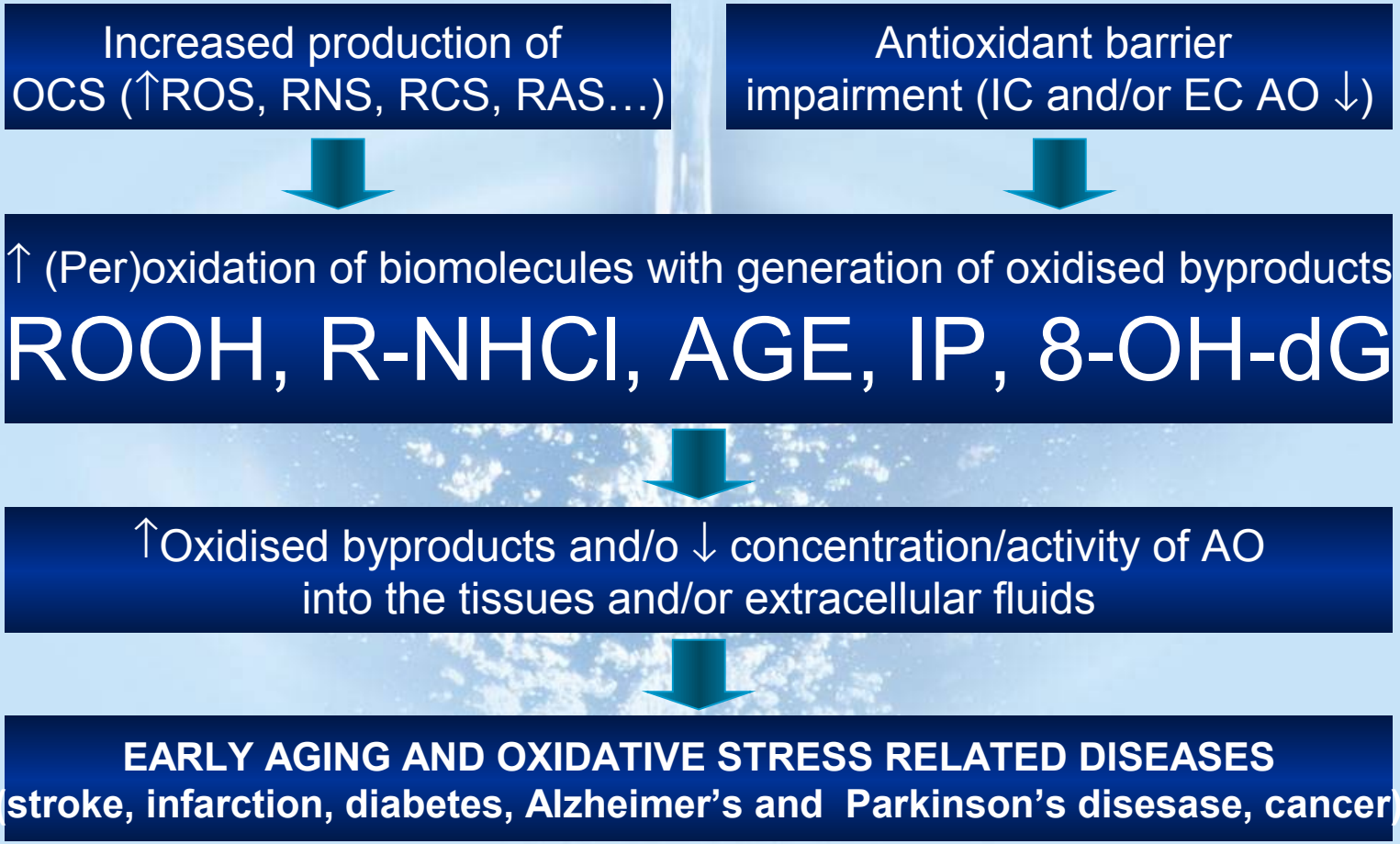
Together with the Nobel Prize, Louis Ignarro.

OXIDATIVE STRESS results by an unbalance between prooxidant and antioxidant systems



Oxidant Chemical species can be either the cause or the effect of OXIDATIVE STRESS

Oxidative stress. The break of a balance.



Evaluating to prevent and to monitor (OCS can be either the cause or the effect of damage)

Biomarker of oxidative stress and relative diseases

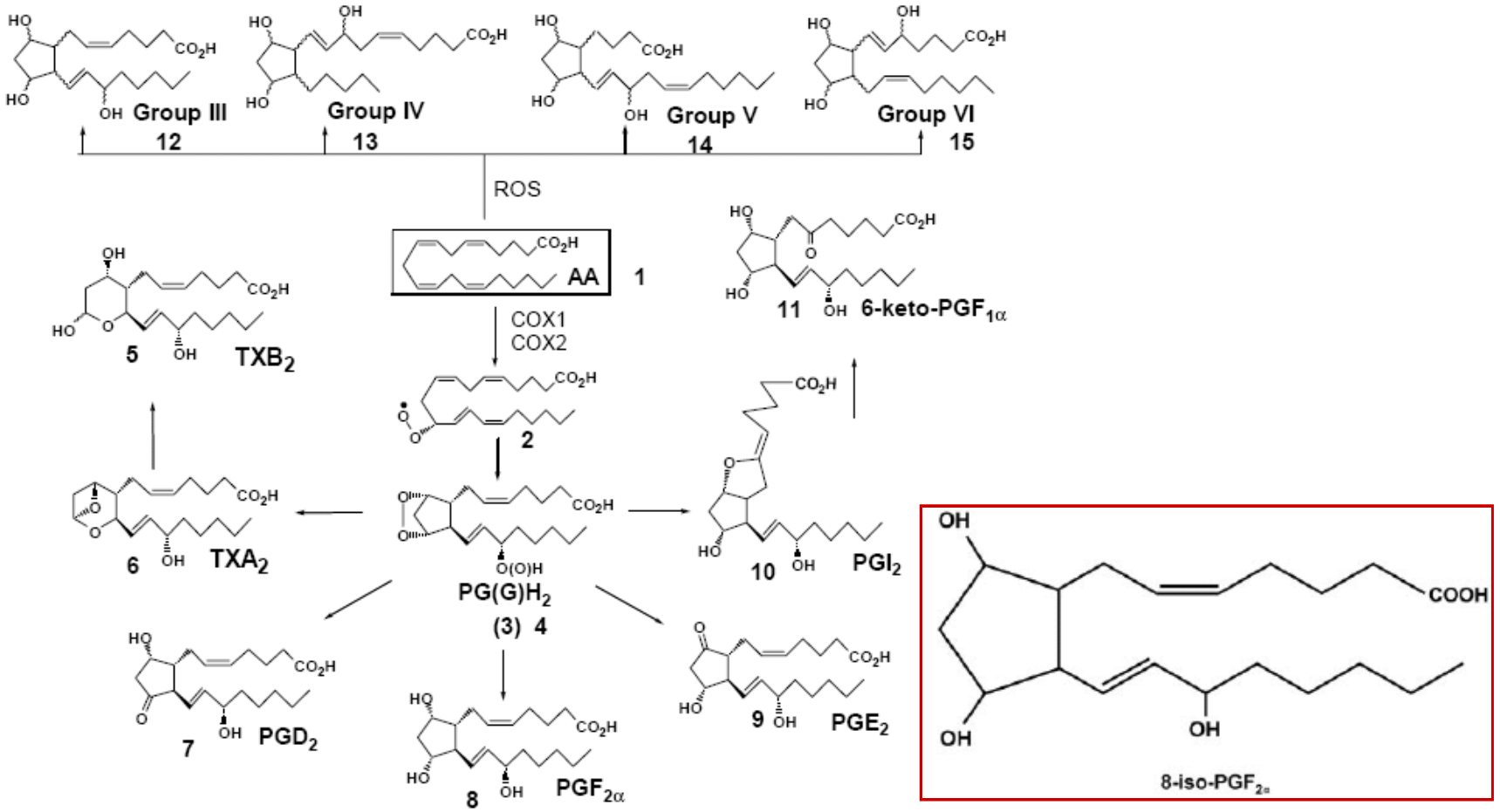
Table 1. Human diseases that were found to be associated with increased oxidative stress on the basis of (potential) biomarkers of oxidative damage.

Biomarker	Reference(s)
MDA	
AD	(11, 43)
ALS	(43)
Asthma	(42)
Atherosclerosis	(3, 40)
Cutaneous leishmaniasis	(176)
Diabetes mellitus	(39)
Preeclampsia	(41)
HNE	
AD	(5, 50, 51, 53)
Atherosclerosis	(3)
Cardiovascular disease	(57)
COPD	(59, 60)
Mild cognitive impairment	(53)
PD	(56)
Acrolein	
Atherosclerosis	(58)
Cardiovascular disease	(57)
Mild cognitive impairment	(53)
F_2-IsoPs	
ARDS	(23, 24, 64)
Acute and chronic alcoholic liver disease	(24)
Acute chest syndrome of sickle cell disease	(23)
AD	(23, 24, 64, 68, 83)
Asthma	(24, 42)
Atherosclerosis	(23, 24, 64)
Cardiopulmonary bypass	(64)
Cardiovascular disease	(80)
Chronic kidney disease	(147)
COPD	(5, 23, 24, 79)
Coronary artery disease	(24)
Creutzfeldt–Jakob disease	(23, 24)
Crohn disease	(23, 24)
Cystic fibrosis	(23, 24, 64, 78)
Diabetes (types 1 and 2)	(23, 24, 64)
Down syndrome	(23, 24)
Heart failure	(24, 64)
Hepatic cirrhosis	(23)
Huntington disease	(23, 24)
Hypercholesterolemia	(23, 24, 64)
Hyperhomocysteinemia	(23, 24)
Ischemia/Reperfusion injury	(24)
Interstitial lung disease	(23)
Multiple sclerosis	(24)
Myocardial infarction	(23)
Obesity	(24)
Osteoarthritis	(64)
Osteoporosis	(24)
Pancreatitis	(23)
Primary biliary cirrhosis	(24)
Psoriatic arthritis	(64)
Pulmonary hypertension	(23, 64)
Reactive arthritis	(23, 64)
Rheumatoid arthritis	(23, 64)
Sickle cell disease	(23, 64)
Spinal cord injury	(23, 64)
Systemic lupus erythematosus	(23)
Systemic sclerosis (scleroderma)	(23, 24, 64)
Unstable angina	(23)
Zellweger syndrome	(82)
Decrease in GSH concentration and/or GSH:GSSG ratio	
ARDS	(101)
Alcoholic liver disease	(90)
AD	(90)
ALS	(90)
Asbestosis	(101)
Asthma	(101)
Ataxia telangiectasia	(90, 93)
Cancer	(99)
Cardiovascular disease	(90, 98)
Cataract genesis	(90)
Diabetes mellitus (both types)	(5, 90, 93)
HIV-positive patients	(90, 93, 101)
Idiopathic pulmonary fibrosis	(101)
Ischemic brain	(97)
PD	(96)
Preeclampsia	(93)
Respiratory distress syndrome	(90)
Retinopathy of prematurity	(93)
Rheumatoid arthritis	(90)
Werner syndrome	(90)
S-Glutathionylated proteins	
Cataract genesis	(105, 177)
Diabetes (types 1 and 2)	(104)
Friedreich ataxia	(90)
HIV infections	(178)
Hyperlipidemia	(104)
Renal cell carcinoma	(104)
Spherocytosis	(5)
Uremia associated with hemodialysis or peritoneal dialysis	(104)
NO_2-Tyr	
ARDS	(5)
AD	(132)
ALS	(5, 132)
Asthma	(5)
Atherosclerosis	(5)
Cardiovascular disease	(125)
COPD	(5)
Coronary artery disease	(123–125)
Crohn disease	(5)
Cystic fibrosis	(107, 118)
Diabetes (types 1 and 2)	(179, 180)
Hypercholesterolemia	(5)
Lung cancer	(129–131)
Lung injury	(31)
Multiple sclerosis	(5)
Myocardial inflammation	(5)

Table 1. Continued

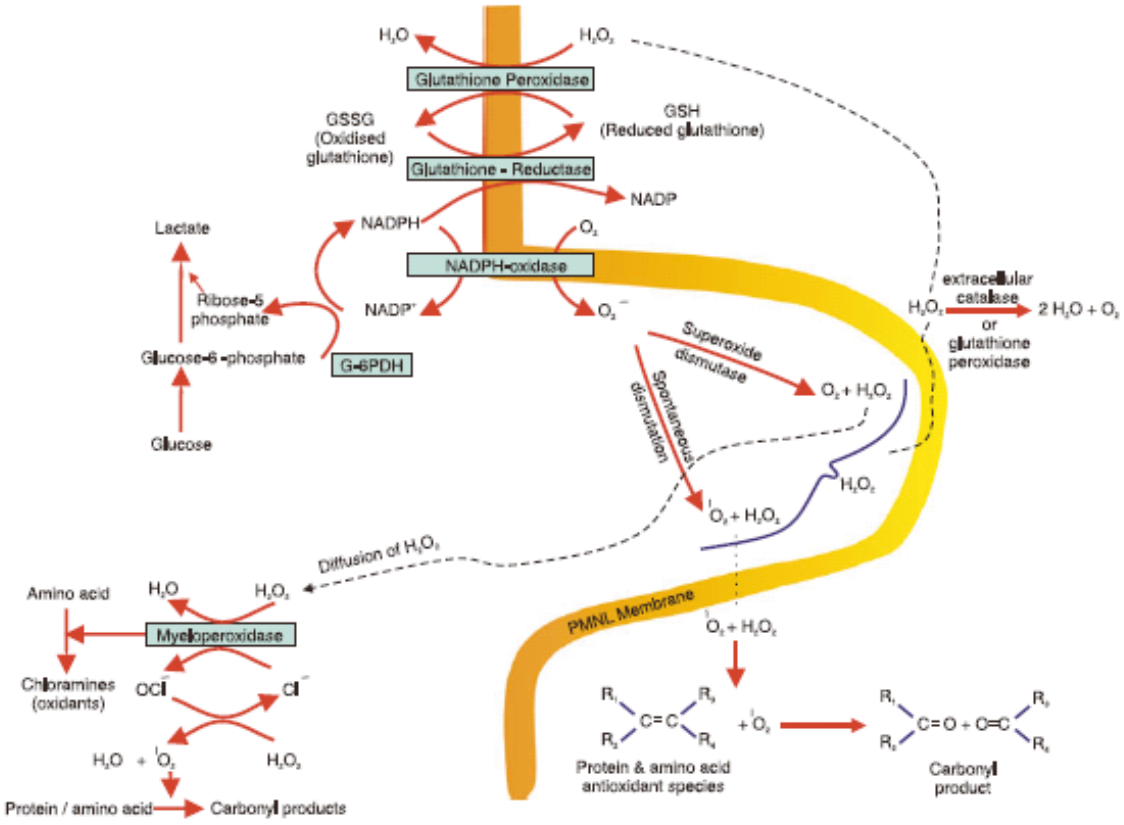
Biomarker	Reference(s)
Osteoarthritis	(5)
Preeclampsia	(5)
Rheumatoid arthritis	(5)
Severe bronchopulmonary dysplasia in neonates	(181)
Synucleinopathies	(5, 132)
Tauopathies	(5)
Cl-Tyr	
ARDS	(5)
Asthma	(5)
Atherosclerosis	(40, 126)
Cardiovascular disease	(125)
Chronic renal failure	(5)
Coronary artery disease	(123, 125)
Cystic fibrosis	(5)
Rheumatoid arthritis	(5)
Di-Tyr	
ARDS	(5)
Atherosclerosis	(14, 28, 40, 182)
Cystic fibrosis	(5)
End-stage renal disease	(5)
Carbonylated proteins	
Aceruloplasminemia	(5)
ARDS	(4, 11)
Acute autoimmune myocarditis	(183)
Acute pancreatitis	(148)
AD	(145)
ALS	(5)
Asthma	(5)
Bronchopulmonary dysplasia	(144)
Cataractogenesis	(143)
Chronic fatigue syndrome	(184)
Chronic hepatitis C	(143)
Chronic kidney disease	(147)
COPD	(60)
Chronic renal failure	(4)
Crohn disease	(143)
Cystic fibrosis	(118)
Diabetes (types 1 and 2)	(4)
Helicobacter pylori infection and inflammation	(143)
Idiopathic pulmonary fibrosis	(185)
Juvenile chronic arthritis	(144, 146)
Lung cancer	(5)
Meningitis	(186)
PD	(143)
Preeclampsia	(144)
Progeria	(11)
Psoriasis	(143)
Rheumatoid arthritis	(143)
Sarcoidosis	(185)
Sepsis	(4)
Systemic amyloidosis	(143)
Uremia	(143)
Werner syndrome	(11)

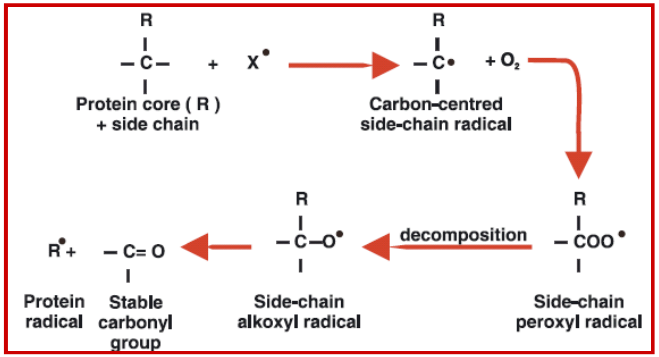
The isoprostanes pathway

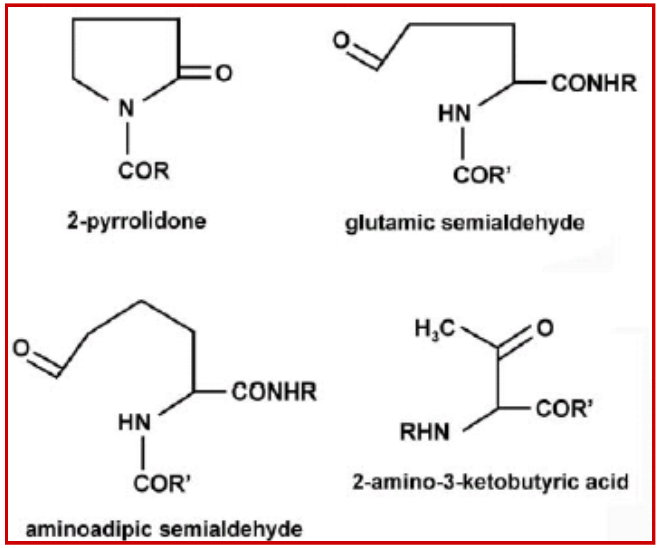


Overview

The carbonylation pathway

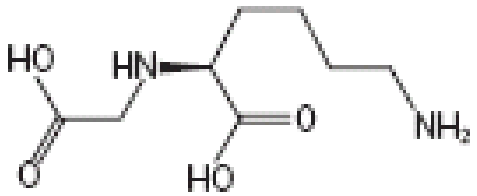




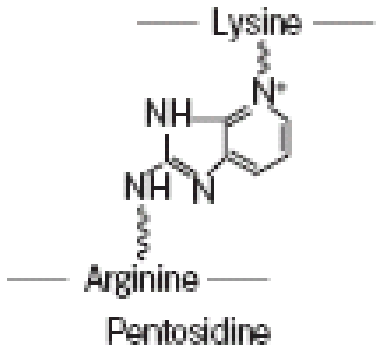


Overview

Other common biomarkers of oxidative stress

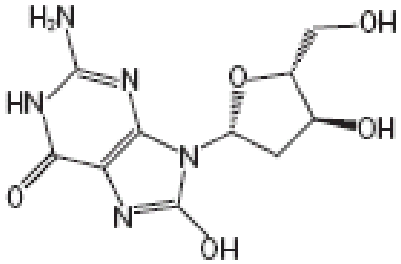


Carboxymethyllysine

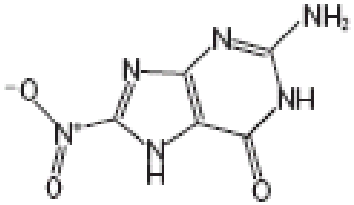


Pentosidine

Advanced glycosilation end-products (AGEs)

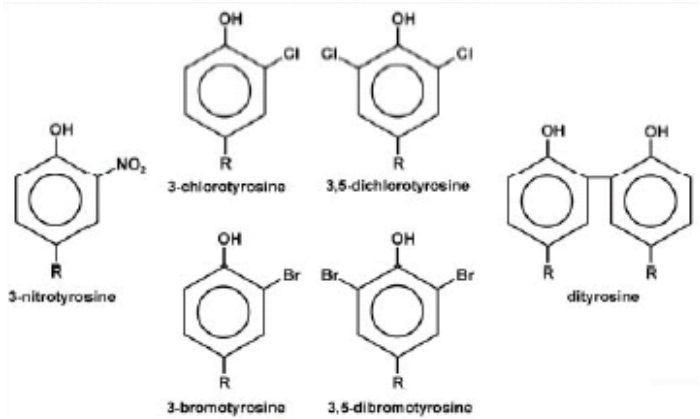


8-hydroxy-2'-deoxyguanosine

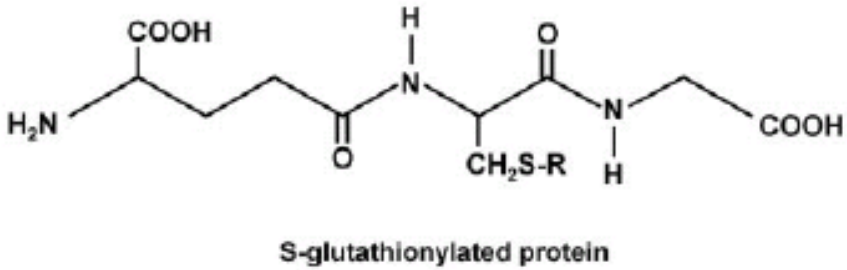


8-nitroguanine

Markers of DNA oxidative damage



Chlorine and nitro-derivatives

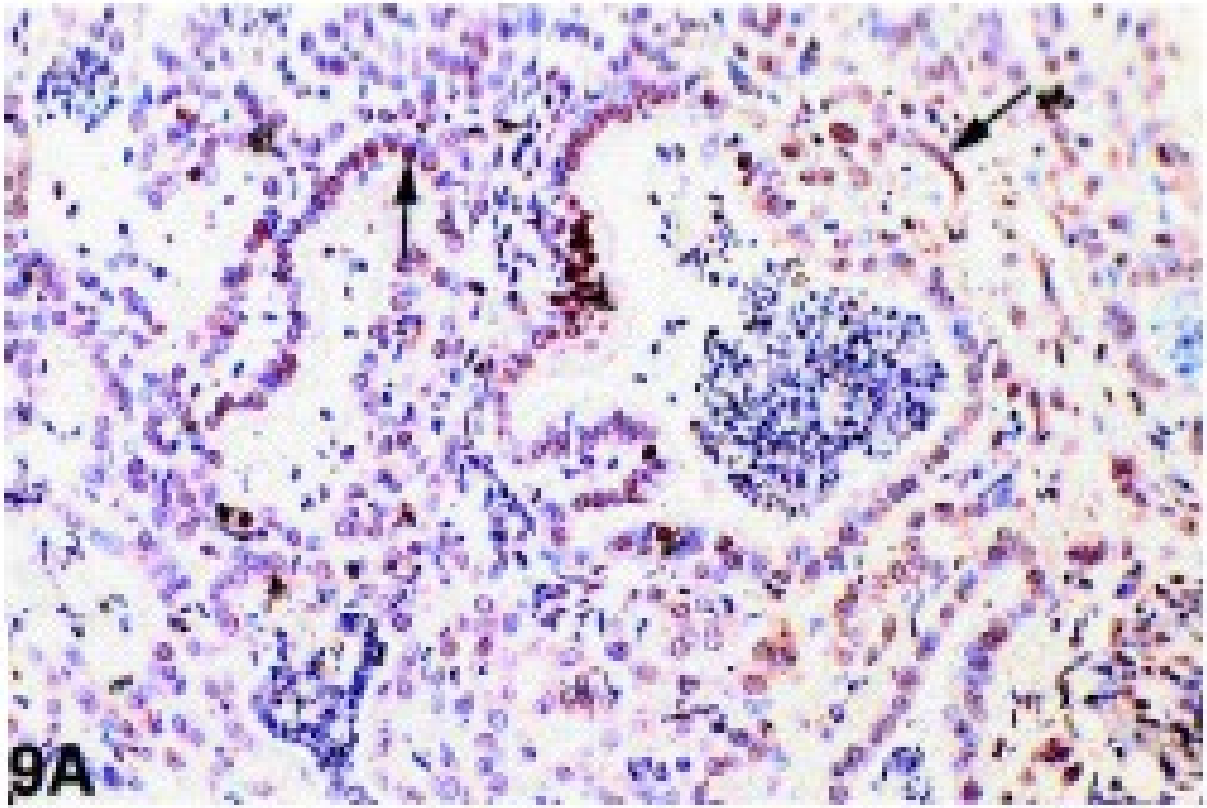


S-glutathionylated protein

Glutathione-adducts

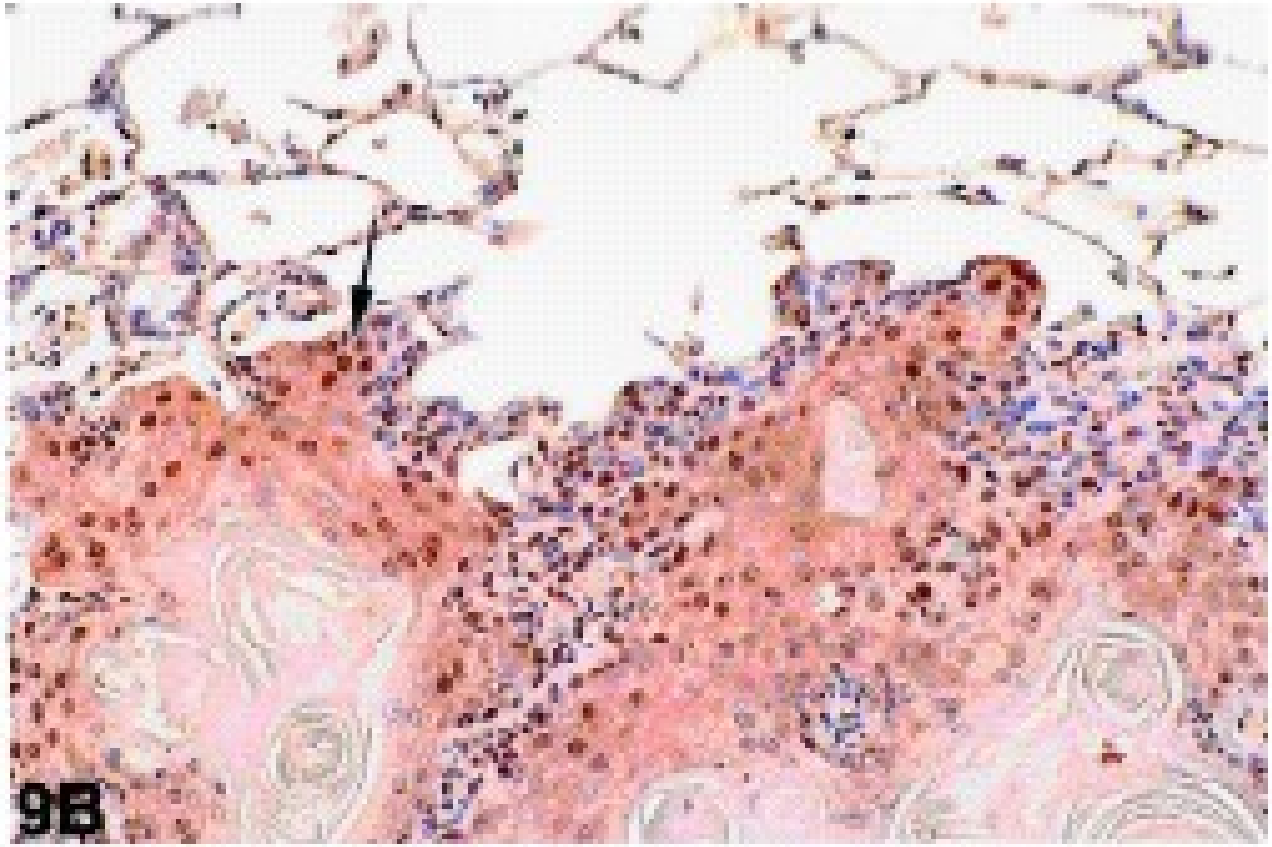
Overview

The challenge of imaging (monoclonal antibodies against 8-OH-dG) (1)



Rat lung slices (atypical dysplasia)

The challenge of imaging (monoclonal antibodies against 8-OH-dG) (2)



Rat lung slices (squammous cystis)

Ozone therapy

ELSEVIER

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OPINION

To What Extent Does Ozone Therapy Need a Real Biochemical Control System? Assessment and Importance of Oxidative Stress

Frank Antonio Hernández

Ozone Research Center Habana, Ciudad de la Habana, Cuba

Received for publication November 7, 2006; accepted March 20, 2007 (ARCMED-D-06-00475).

Ozone therapy is not officially allowed in many countries, but private medical services are using this therapy worldwide. However, appropriate control systems to assess the benefits and risks of systemic ozone therapy are not always used and in such cases the treatment is based on anecdotal reports. Oxidative stress phenomenon is becoming a highlighted biological process for ozone therapy because it is deeply involved in its mechanism of action. On the contrary, ozone therapy is an efficient regulator of the ox-

Why and how to monitor oxidative stress

Ozone therapy

Ozone therapy is not officially allowed in many countries, but private medical services are using this therapy worldwide. However, appropriate control systems to assess the benefits and risks of systemic ozone therapy are not always used and in such cases the treatment is based on anecdotal reports. Oxidative stress phenomenon is becoming a highlighted biological process for ozone therapy because it is deeply involved in its mechanism of action. On the contrary, ozone therapy is an efficient regulator of the oxidative stress processes. In terms of therapeutic effects, it is convenient to know the metabolic status of the organism to face new oxidative challenges before and during ozone therapy applications. Oxidative stress is also important because it is involved as a cause or effect of many diseases. Since the 1990s, there has been the necessity of developing reliable systems for measuring oxidative stress in humans. In this sense, we have proposed a system for oxidative stress diagnosis that can serve as a control system for systemic ozone therapy applications. The system is based on the blood measurement of eight biomarkers (GSH, GPx, GST, SOD, CAT, DC, SRATB, and HPT) and the interpretation of these values by a computer-developed algorithm yielding four new indices (total antioxidant activity, total prooxidant activity, redox index and grade of oxidative stress). The system shows the patient's redox status and estimation of the oxidative stress level, with this information being relevant regarding implications on dosage and therapeutic effectiveness of ozone therapy.

Key Words: Ozone therapy, Oxidant state, Antioxidant state, Antioxidant enzymes, Oxidative stress.

Why and how to monitor oxidative stress?

Ozone therapy monitoring

Table 3. Grades of oxidative stress and their clinical meaning

Grade	Meaning
0	No oxidative stress
1	Light oxidative stress
2	Moderate oxidative stress
3	Severe oxidative stress
4	Very severe oxidative stress

Table 1. Biochemical indices for a proposed system for measuring the oxidative stress status in humans from a blood sample

Antioxidants	Prooxidants
Reduced glutathione (GSH)	
Glutathione peroxidase (GPx)	Conjugated dienes (CD)
Glutathione S-transferase (GST)	Total hydroperoxides (THP)
Superoxide dismutase (SOD)	Thiobarbituric acid reactive substances (TBARS)
Catalase (CAT)	

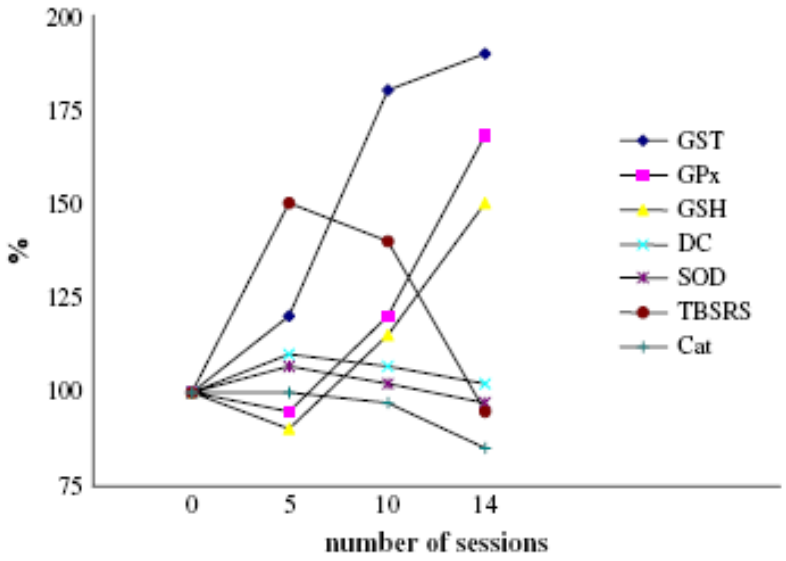


Figure 5. Response of antioxidant and prooxidant parameters during ozone major autohemotherapy application in a cardiopathic patient.

Which test?

Ozone therapy

Table 2. Resulting indices from oxidative stress diagnostic computer program

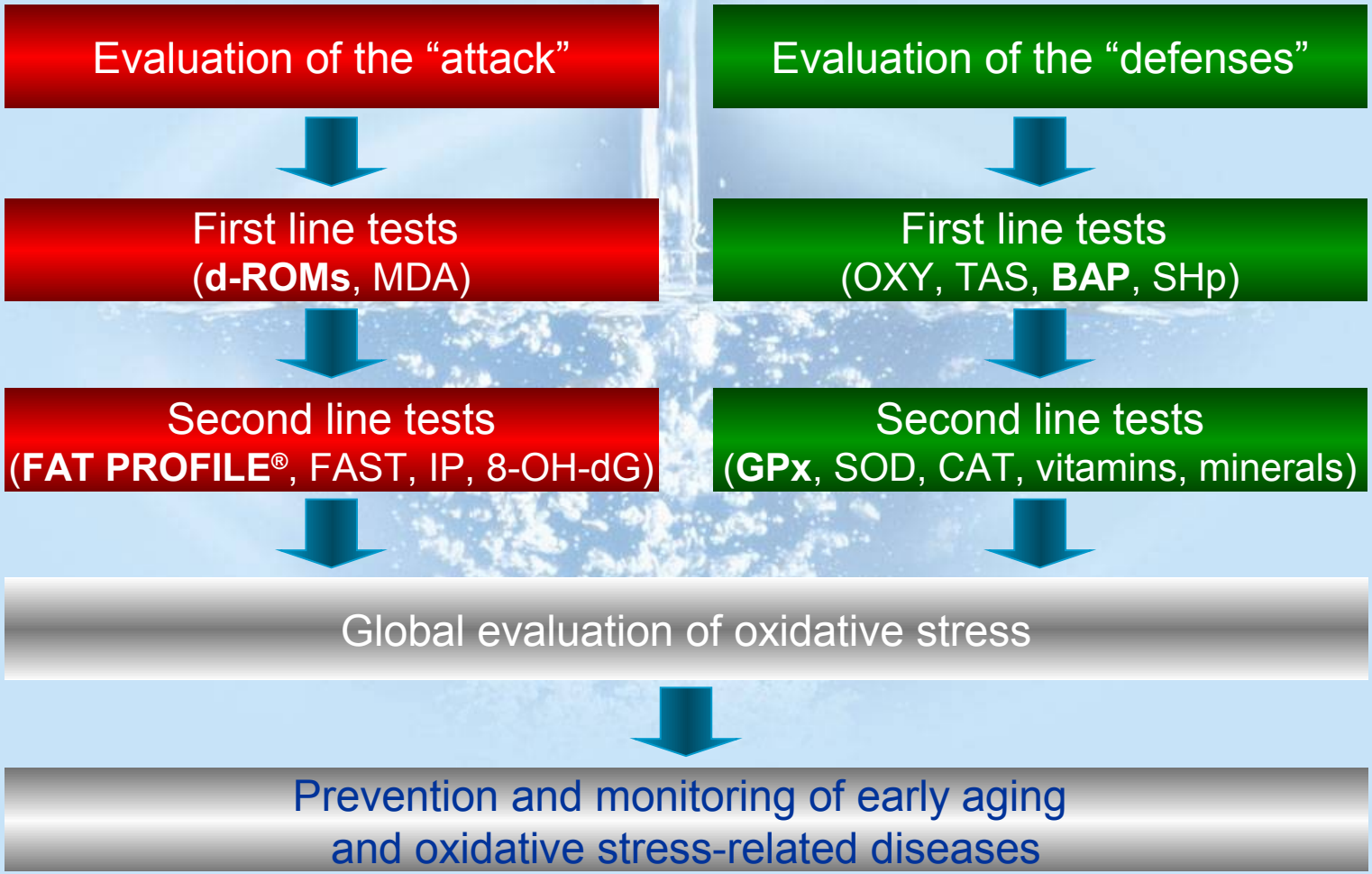
Total antioxidant activity
Total prooxidant activity
Redox index
Grade

Table 4. Oxidative stress diagnosis using the computer program for values obtained with the patient in Figure 5

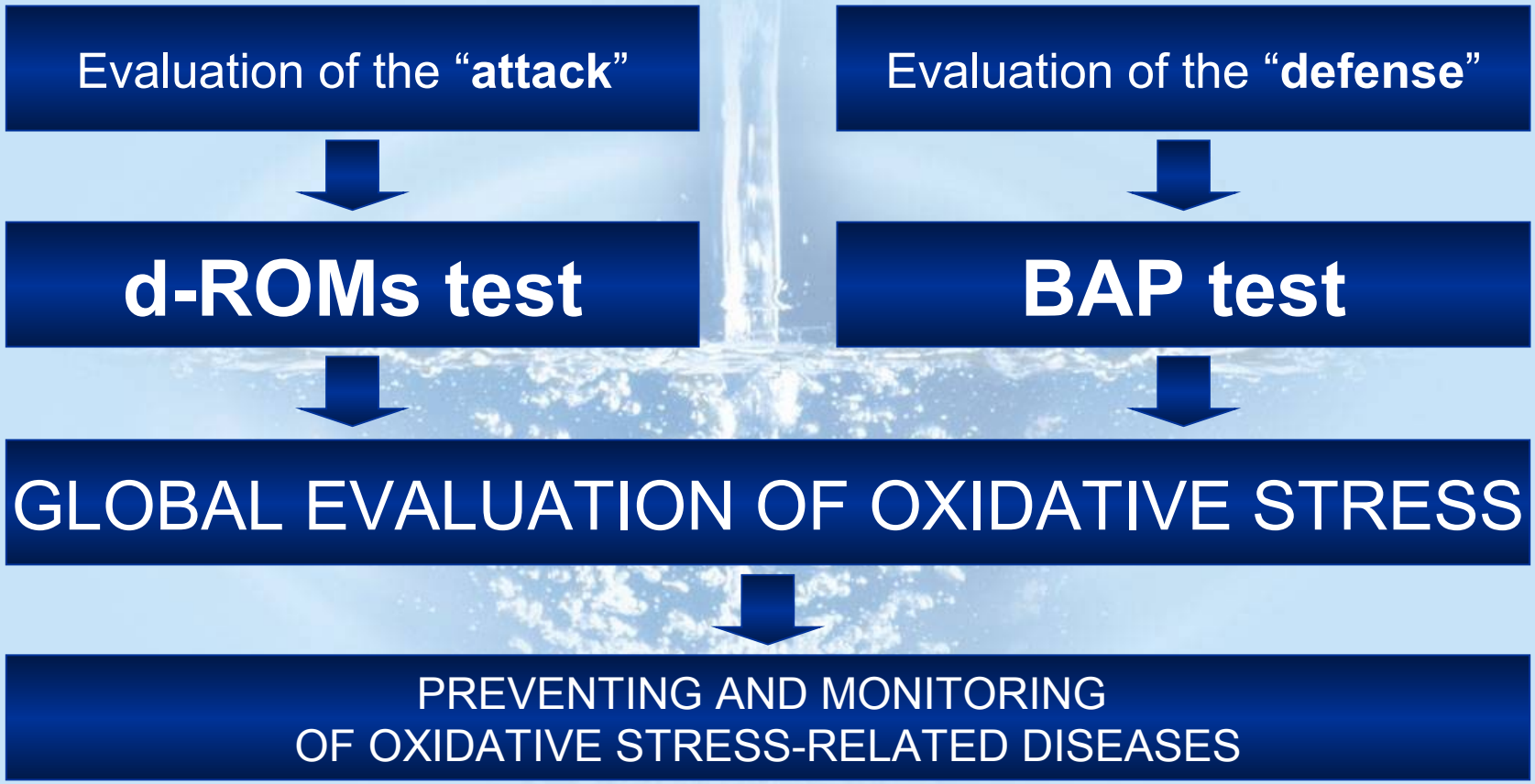
Ozone therapy session number	Total antioxidant activity (units)	Total prooxidant activity (units)	Redox index	Grade of oxidative stress
0	128.38	167.96	0.764	2
5	123.45	178.69	0.691	3
10	138.92	175.21	0.790	2
14	144.28	169.70	0.850	1

How to manage the results?

Evaluating to prevent and to monitor



The Carratelli's panel



Evaluating to prevent and to monitor

d-ROMs test

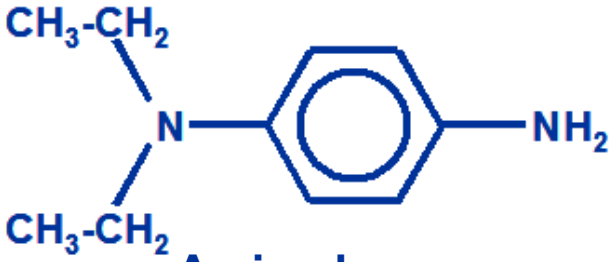
Serum/plasma blood sample

Buffered acidic solution

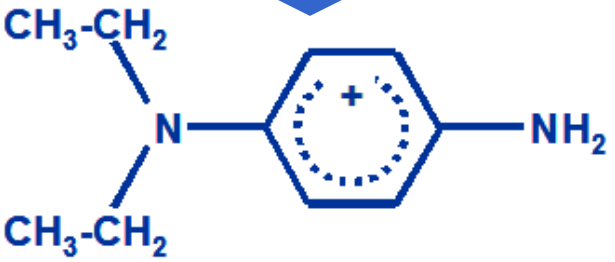
DPPD oxidation

Color increasing

Photometric reading



Amine base
NOT COLORED



Radical cation
COLORED (PINK)

The chemical principle (normal range 250-300 CARR U)

BAP test

Thiocianate + $FeCl_3$

Colored complex

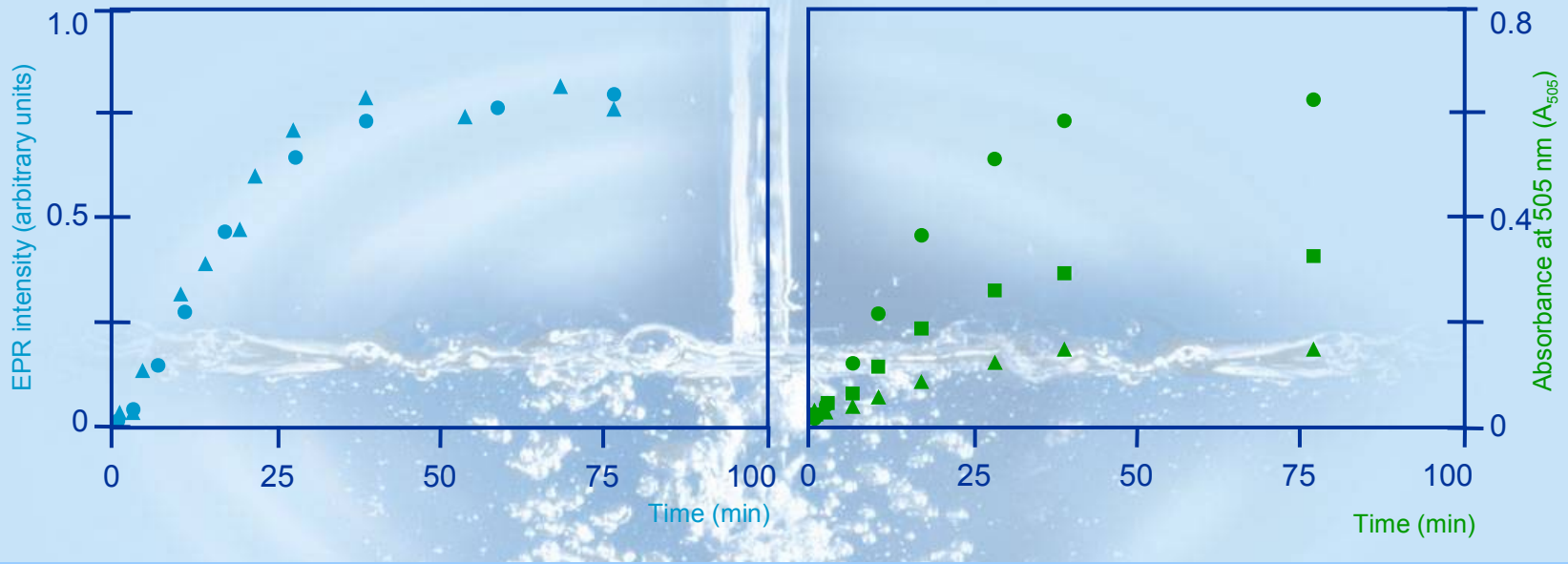
Serum/plasma blood sample

Loss of color

Photometric reading

The chemical principle (optimal value>2200 μmol/L)

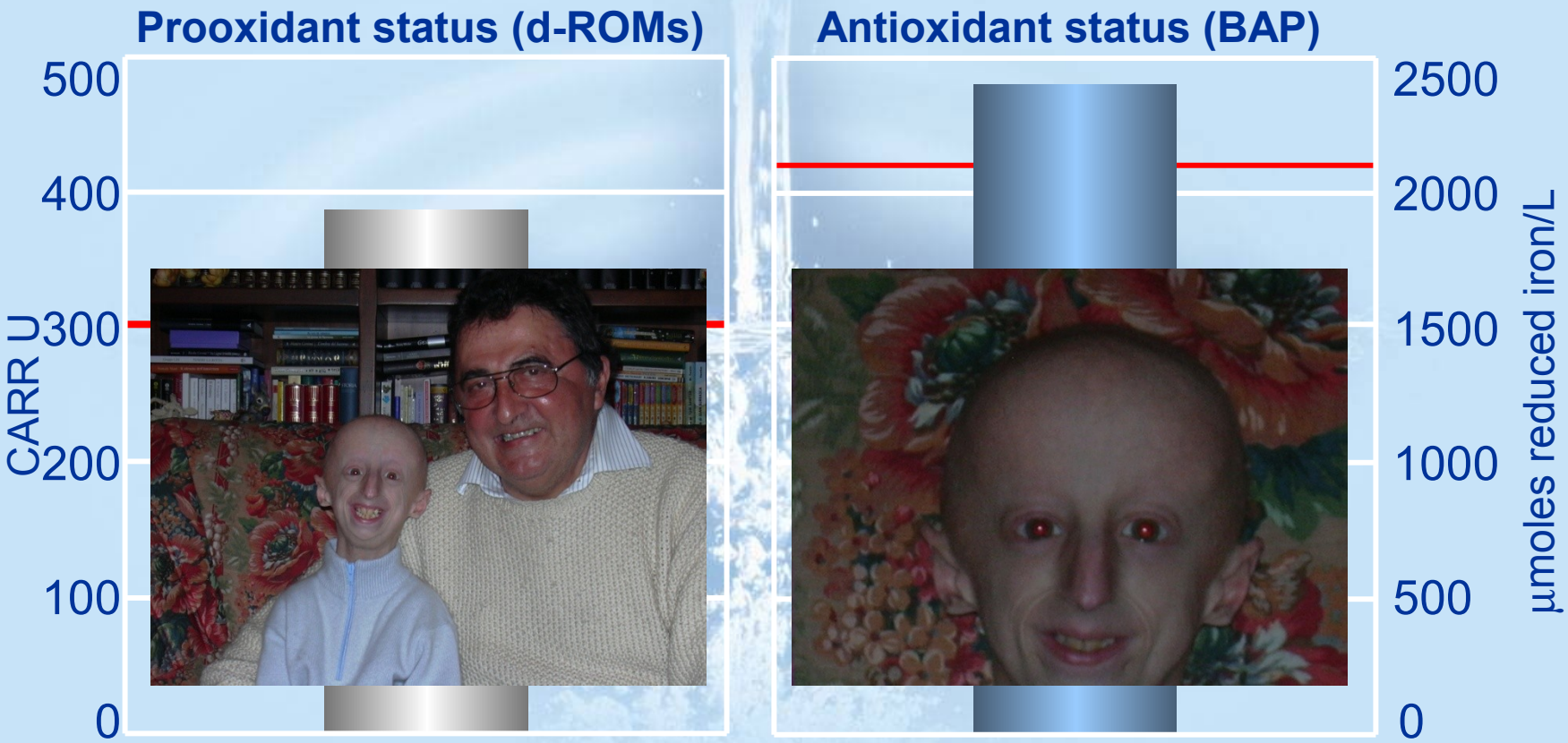
Combined evidence of spectrophotometry and electron spin resonance spectroscopy



(A) Room temperature time profile of the normalised spectral intensity (•) and of A_{505} readings (▲) exhibited by the system DEPPD (3.7×10^{-3} M)/*t*BuOOH (3.9×10^{-5} M)/$FeSO_4$ (2.8×10^{-5} M) at room temperature. (B) Time profile of the A_{505} readinmgs exhibited by the systems DEPPD (3.7×10^{-3} M)/*t*BuOOH (3.9×10^{-5} M)/$FeSO_4$ (2.8×10^{-5} M) (•), DEPPD (3.7×10^{-3} M)/*t*BuOOH (2.0×10^{-5} M)/$FeSO_4$ (2.8×10^{-5} M) (■) and DEPPD (3.7×10^{-3} M)/*t*BuOOH (0.95×10^{-5} M)/$FeSO_4$ (2.8×10^{-5} M) (▲) at room temperature.
tBuOOH: tert-buthylhydroperoxide; DEPPD: **N,N-diethylparaphenylendiamine**

The DEPPD radical responsible for the ESR spectrum is also responsible for the absorption at 505 nm

Genetic disorders with brain impairment.
The Oxidative Balance Progeria Study.



Significantly increased oxidative status in Progeria (*Iorio, 2007*)

The d-ROMs test is a useful test to predict the first atherothrombotic event

Established and Emerging Plasma Biomarkers in the Prediction of First Atherothrombotic Events

Paul M Ridker, MD, MPH; Nancy J. Brown, MD; Douglas E. Vaughan, MD; David G. Harrison, MD; Jawahar L. Mehta, MD, PhD

In the current Adult Treatment Panel guidelines for cardiovascular risk detection,[1] 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).[2] 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.[3] Moreover, the prevalence of traditional risk factors is almost as high in those without disease as in affected individuals.[4]

As our understanding of the pathobiology of atherothrombosis has improved, researchers have attempted to evaluate the activities of these biological processes by measuring markers in plasma or urine (ie, biomarkers). Indeed, a series of candidate biomarkers reflecting inflammation, hemostasis, thrombosis, and oxidative stress have been evaluated as potential clinical tools in an effort to improve risk prediction. To be useful in a clinical setting, the biomarker of interest must be shown in multiple prospective studies to predict future cardiovascular events. Retrospective studies are of limited value because they are prone to bias and cannot exclude the possibility that the particular biomarker is elevated as a result of, rather than a cause of, disease. To be used widely, the proposed biomarker should provide independent information on risk or prognosis beyond that available from global assessment algorithms such as the Framingham Risk Score. The biomarker additionally should be easy to measure in a cost-effective manner in outpatient settings. This typically requires an inexpensive and standardized commercial assay with low variability that does not require specialized plasma collection or assay techniques. Although not a critical issue for risk prediction, the biomarker will have broader acceptance if reduction of the biomarker leads to reduced vascular risk.

Several established and emerging novel biomarkers for vascular risk meet these criteria (Table 1), although few are ready for clinical practice. With the exception of high-sensitivity C-reactive protein (hsCRP), none has demonstrated additive value over and above Framingham risk scoring, and few are supported by commercial assays that achieve appropriate levels of standardization and accuracy for clinical use. Additionally, no clear evidence exists that lowering plasma levels of any of these biomarkers, including hsCRP, lowers vascular risk. However, many of these novel biomarkers provide important insights into the pathophysiology of atherothrombosis and serve as important research tools.

This overview focuses on established and emerging biomarkers in the prediction of atherothrombotic events in apparently healthy individuals and thus includes discussion of markers of inflammation, fibrinolysis, oxidative stress, and altered lipids (Table 1). It is important to recognize that other emerging vascular biomarkers, including brain natriuretic peptide and myeloperoxidase, have shown initial promise in the setting of acute myocardial ischemia[5–8] but have yet to be evaluated in outpatient screening of healthy individuals. Other novel markers emerging in primary prevention include those related to adipocyte function, including adiponectin.[9]

High-Sensitivity C-Reative Protein

Inflammation characterizes all phases of atherothrombosis and provides a critical pathophysiologic link between plaque formation and acute rupture leading to occlusion and infarction.[10] C-reactive protein (CRP) is the best characterized of the currently available inflammatory biomarkers and has emerged as a potential marker for cardiovascular risk.[11] Composed of 5 23-kDa subunits, CRP is a circulating pentraxin that plays a major role in the human innate immune response.[12] Although generally considered to be an acute-phase reactant, CRP is also produced in smooth muscle cells within human coronary arteries and is expressed preferentially in diseased vessels.[13,14] CRP may directly affect expression of adhesion molecules, impact fibrinolysis, and alter

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(Circulation 2004;109[suppl IV]:IV-6–IV-19.)

Circulation is available at http://www.circulationaha.org
DOI: 10.1161/01.CIR.0000133444.17867.56

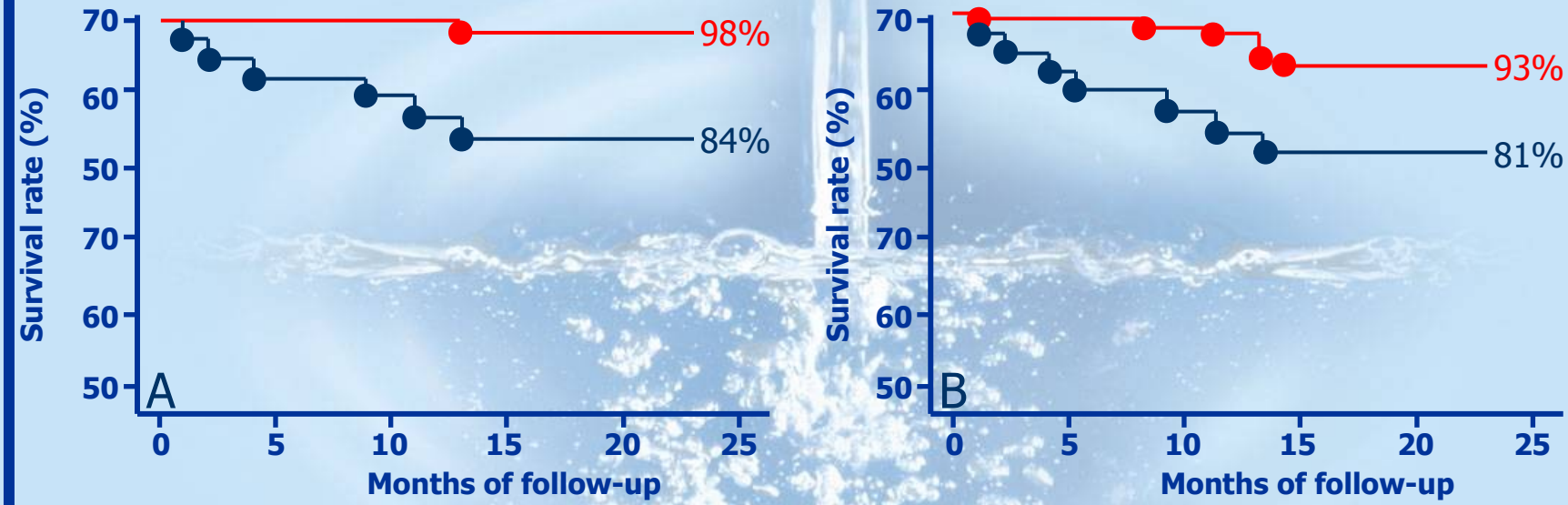
TABLE 2. Proposed Biomarkers of Oxidative Stress

Oxidant Stress Marker	Previous Use	Method of Measurement	Advantages	Disadvantages
F2-isoprostanes[15,16]	↑ 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)[17]	↑ 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)[18,19] antibodies to ox-LDL[20]	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 ex[illegible]
Free oxygen radical monitor (FORM assay or D-ROMs test)[21]	↑ 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)[22,23]	↑ 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[24,25]	↑ 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, proline, 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)[26,27]	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[28]	↑ 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

COPD indicates chronic obstructive pulmonary disease; ELISA, enzyme-linked immunoabsorbent assay; GC, gas chromatography; HPLC, high-pressure liquid chromatography; LDL, low-density lipoprotein; MI, myocardial infarction; TBA, thiobarbituric acid.

Ridke et al. Circulation, 2004

Cardiovascular diseases

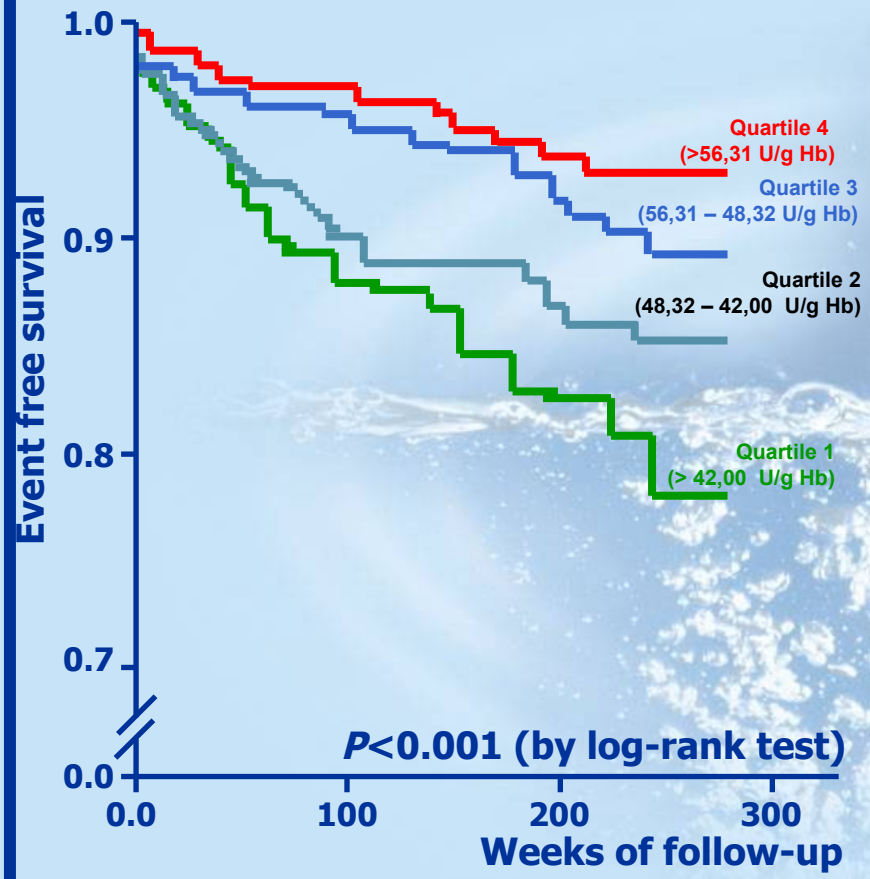


Kaplan–Meier survival curves according to 75th d-ROMs test percentiles, considering cardiac death (A) and total mortality (B) as end points.

Predictive role of d-ROMs test

Vassalle C, Boni C, Di Cecco P, Landi P. *Elevated hydroperoxide levels as a prognostic predictor of mortality in a cohort of patients with cardiovascular disease.* Int J Cardiol. **2005** Nov 14 [Epub ahead of print]

Cardiovascular diseases



Kaplan–Meier curves showing cardiovascular events according to quartile of GPx-1 activity. The numbers of cardiovascular events were 33, 23, 16 and 11 in quartiles 1, 2, 3, and 4, respectively. Glutathione Peroxidase activity 1 is shown in units per gram of haemoglobin.

Predictive role of GPx

Blankenberg S, Rupprecht Hj, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ. *Glutathione Peroxidase 1 activity and cardiovascular events in patients with coronary artery disease.* N Engl J Med. **2003**;349(17):1605 – 1613.

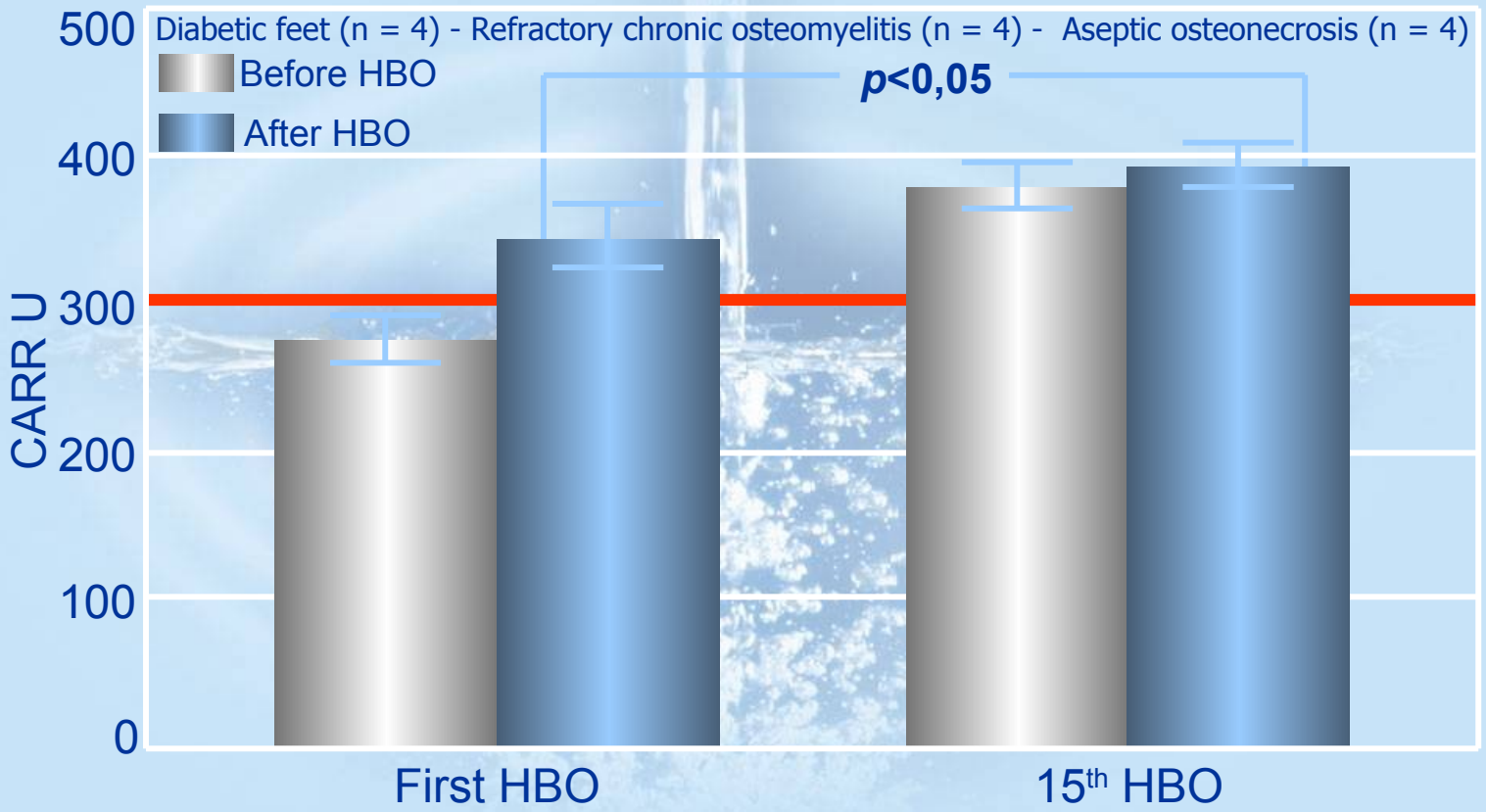
The d-ROMs test

A SIMPLE PROCEDURE FOR SCREENING PURPOSES

THE NEW CARPE DIEM SYSTEM

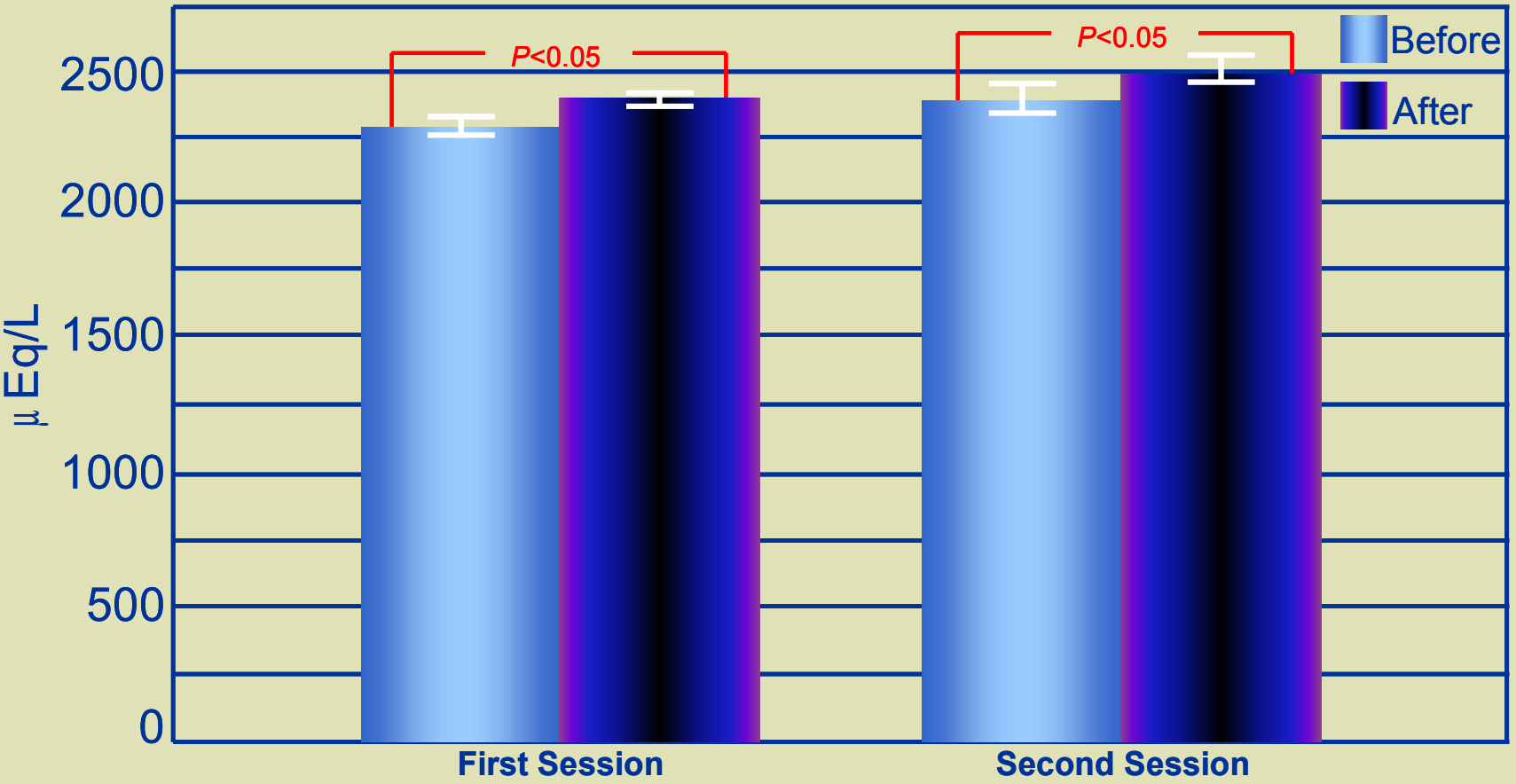
CARRATELLI'S PANEL EVALUATOR

The model of hyperbaric oxygen therapy (HBO). Suitability of d-ROMs test.



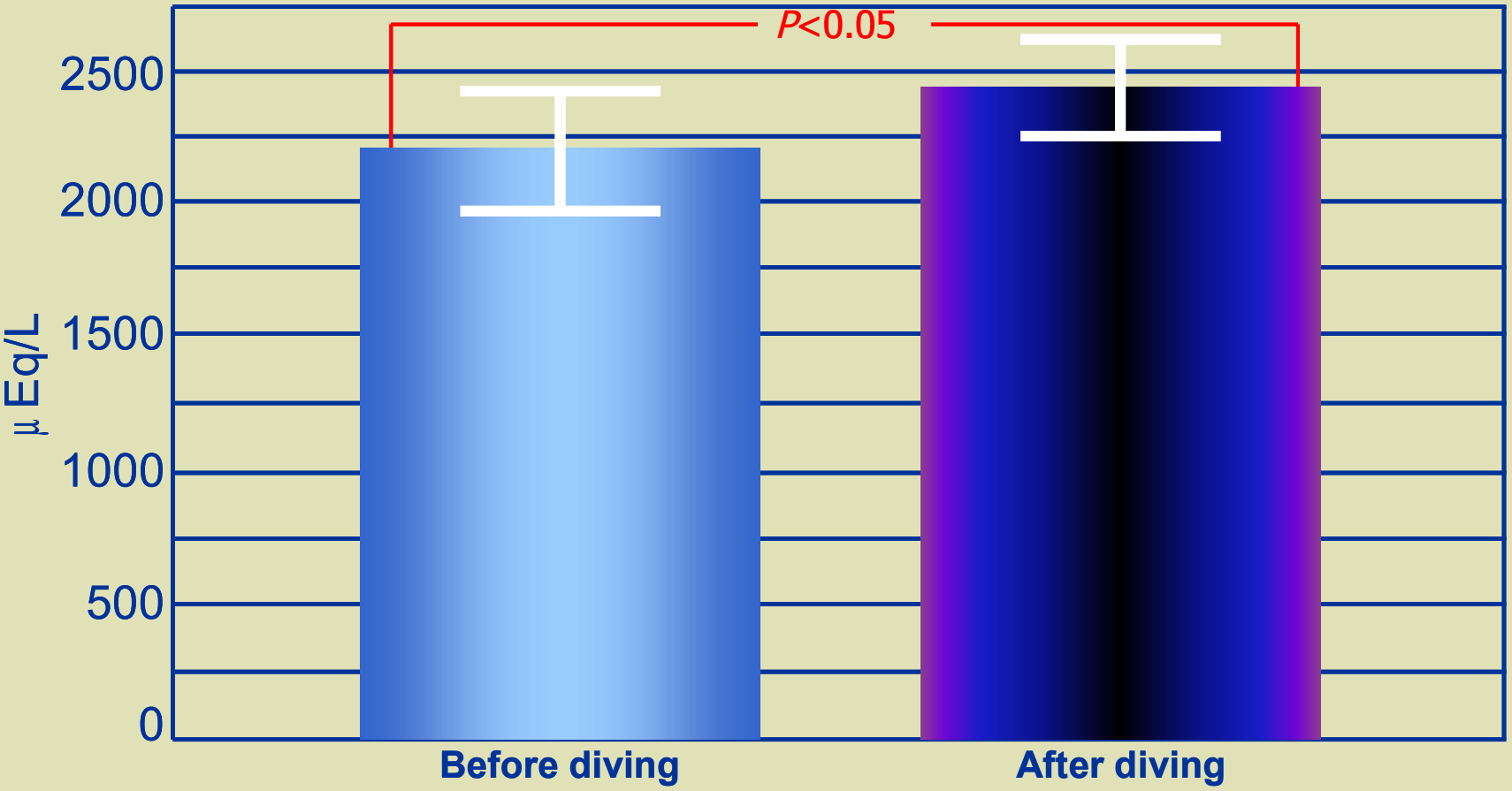
Significant increase of oxidants after repeated cycles of hyperbaric oxygen therapy (*Benedetti, 2004*)

The model of hyperbaric oxygen therapy (HBO). Suitability of BAP test.



Significant increase of BAP test values after two session of hyperbaric oxygen therapy (n=9, means ± SE, one-way repeated-measures ANOVA followed by Dunnett's test) (*modified by Kongoji et al, 2007*).

The model of SCUBA divers. Suitability of BAP test.



Significant increase of BAP test values after diving (n=10, means ± SE, one-way repeated-measures ANOVA followed by Dunnett's test) (*modified by Yamami et al, 2007*).

Ozone therapy

Ozone as an oxidant and its influence on free radical activity and antioxidant levels in the human environment in disease and health

Thomas Marshall-Manifold
Wimbledon Clinic of Natural Medicine, London, UK.

Abstract

A number of free radical species fulfil physiologically important roles within the body, for example, superoxide and nitric oxide function as second messengers. However, free radical levels in the body must be carefully controlled as they are highly reactive and can cause tissue destruction.
Antioxidants help regulate and control the levels of free radicals at the required physiological concentrations. When the production of free radicals and their removal by the antioxidant system becomes unbalanced, tissue damage and disease can occur.
The use of ozone as a therapeutic modality to counteract the disease process in the body would seem to be paradoxical at the least, ozone being an oxidant, and by definition a procurer of free radical activity.
This paper reports the effect of ozone on both free radical and antioxidant levels in the blood of subjects both in disease and health before, during, and after ozone therapy

TheWimbledon's Study

Ozone therapy

Week 1	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	286 U.Carr	216 U.Carr	328 U.Carr
Patient No.2	402 U.Carr	271 U.Carr	378 U.Carr
Patient No.3	396 U.Carr	321 U.Carr	362 U.Carr
Patient No.4	430 U.Carr	311 U.Carr	386 U.Carr

TheWimbledon's Study

Ozone therapy

Week 2 7 days later	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	301 U.Carr	257 U.Carr	289 U.Carr
Patient No.2	379 U.Carr	285 U.Carr	363 U.Carr
Patient No.3	373 U.Carr	302 U.Carr	351 U.Carr
Patient No.4	410 U.Carr	290 U.Carr	311 U.Carr

TheWimbledon's Study

Ozone therapy

Week 3 7 days later	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	291 U.Carr	263 U.Carr	277 U.Carr
Patient No.2	324 U.Carr	300 U.Carr	328 U.Carr
Patient No.3	359 U.Carr	307 U.Carr	363 U.Carr
Patient No.4	367 U.Carr	322 U.Carr	339 U.Carr

TheWimbledon's Study

Ozone therapy

- **Although the trends observed in this limited study suggest the beneficial effect of ozone on the immune system further research on a wider scale especially on the observation of the transient rise in free radical activity 24 hours following ozone application would be welcome.**

- **As there is the possibility, that in patients suffering from autoimmune conditions, free radical activity may not always fall following this observed 24 hour transient rise.**

- **One precaution to eliminate this risk would be to prescribe high antioxidant medication for 24 to 48 hours following ozone treatment.**

TheWimbledon's Study

Ozone therapy

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Full Length Research Paper

The protective effect of plasma antioxidants during ozone autohemotherapy

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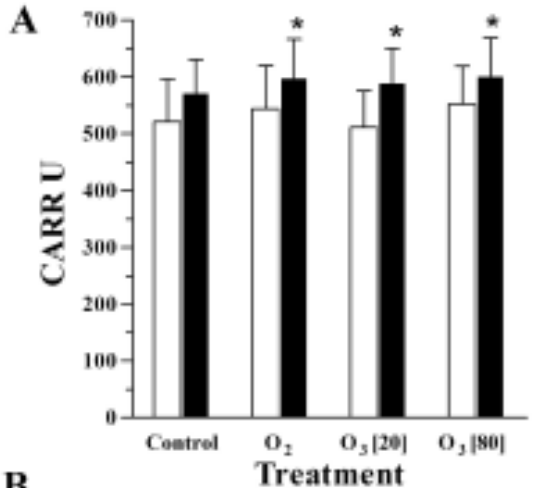
Accepted 5 November, 2007

Ozone (O_3) therapy forms part of a group of complementary and alternative medical therapies and is gaining more and more interest worldwide. There is, however, some concern regarding O_3-toxicity and uncertainty about the effectiveness of O_3-therapy. In this study we investigated the possible protective effects of the plasma antioxidant defense system during O_3-AHT. Venous blood from six apparently healthy human donors was collected. In one part of the study a precise volume of blood was mixed with an equal volume of O_2/O_3 gas mixture containing 20 or 80 µg/ml O_3 for 20 min. In the other part, the plasma was washed out, the cells resuspended in a buffered phosphate solution and treated with same concentrations of O_3. Control samples was not treated or treated with O_2. Ozone-AHT caused increased plasma hydroperoxide levels and glutathione ratio. Antioxidant enzyme (catalase, glutathione reductase, glutathione peroxidase) activity of peripheral blood mononuclear cells (PBMC) decreased, whereas superoxide dismutase levels increased slightly. Plasma antioxidant capacity decreased. These effects were more evident in the absence of plasma antioxidants. Therefore the damaging effects of O_3 were quenched by the antioxidants present in plasma.

The protective effects of plasma antioxidants

Ozone therapy



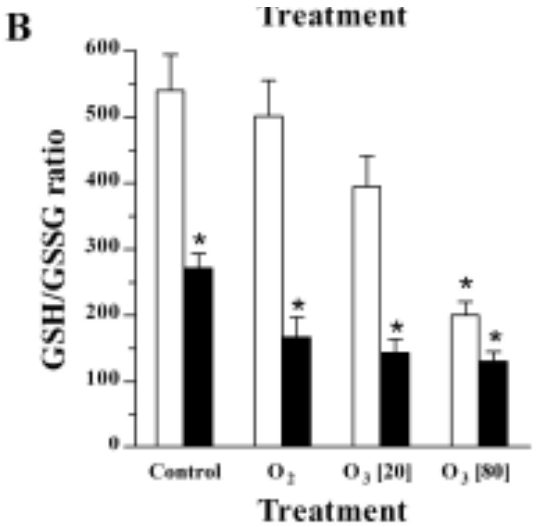


Figure 1. Characterization of the oxidative stress status after treatment. A) Levels of hydroperoxides measured in the two intervention groups Results are given as mean ± 1SEM (n=5)CARR U for whole blood (open bars) and the buffered cells (black bars) at baseline and after exposure to oxygen and 20 and 80 µg/ml ozone.

B) The GSH/GSSG ratio measured in the two intervention groups. Results are given as the mean ± 1SEM (n=6) GSH/GSSG ratio for whole blood and buffered cells at baseline and after exposure to oxygen and 20 and 80 µg/ml ozone. $p<0.05$ with respect to control (*) of the whole blood group (Bonferroni test).

The protective effects of plasma antioxidants

Ozone therapy

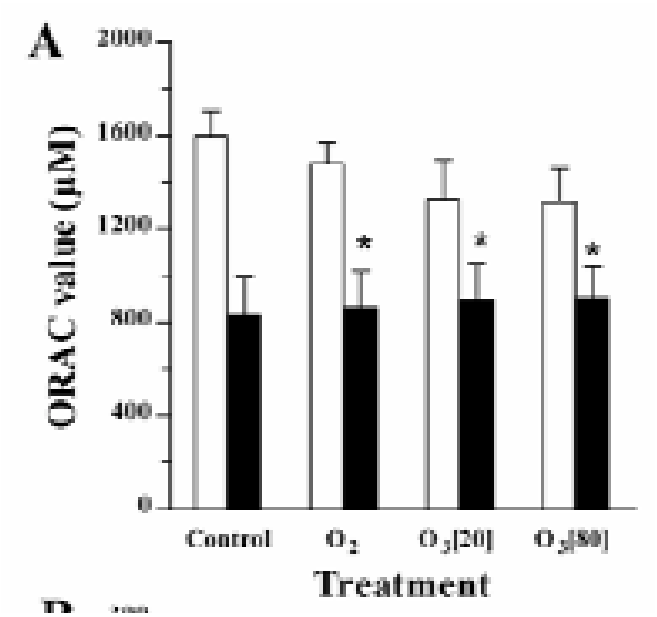
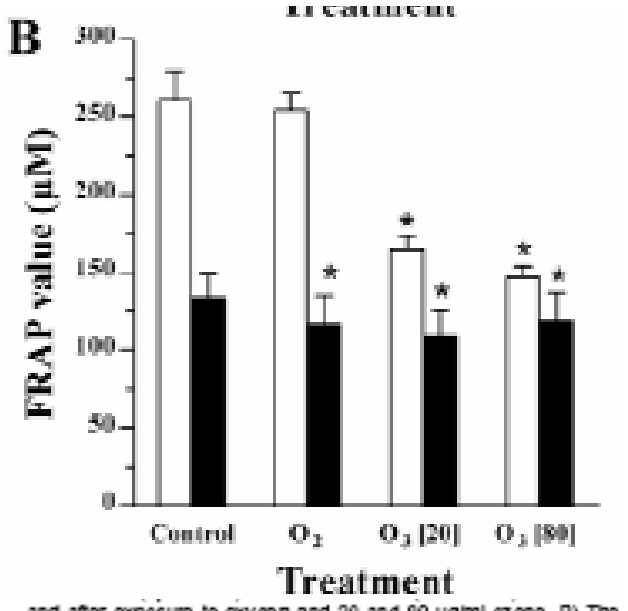


Figure 2. Characterization of the antioxidant status after treatment. A) The ORAC values measured in the two intervention groups. Results are given as the mean ± 1SEM (n=6) ORAC value (µM) for whole blood (open bars) and buffered cells (black bars) at baseline and after exposure to oxygen and 20 and 80 µg/ml ozone. B) The



and after exposure to oxygen and 20 and 80 µg/ml ozone. B) The FRAP values measured in the two intervention groups. Results are given as the mean ± 1SEM (n=6) FRAP value (µM) for whole blood and buffered cells at baseline and after exposure to oxygen and 20 and 80 µg/ml ozone. $p<0.05$ with respect to control (*) of the whole blood group (Bonferroni test). The ORAC and FRAP was measured in plasma for the whole blood and cell homogenate for the buffered cells

The protective effects of plasma antioxidants

Ozone therapy

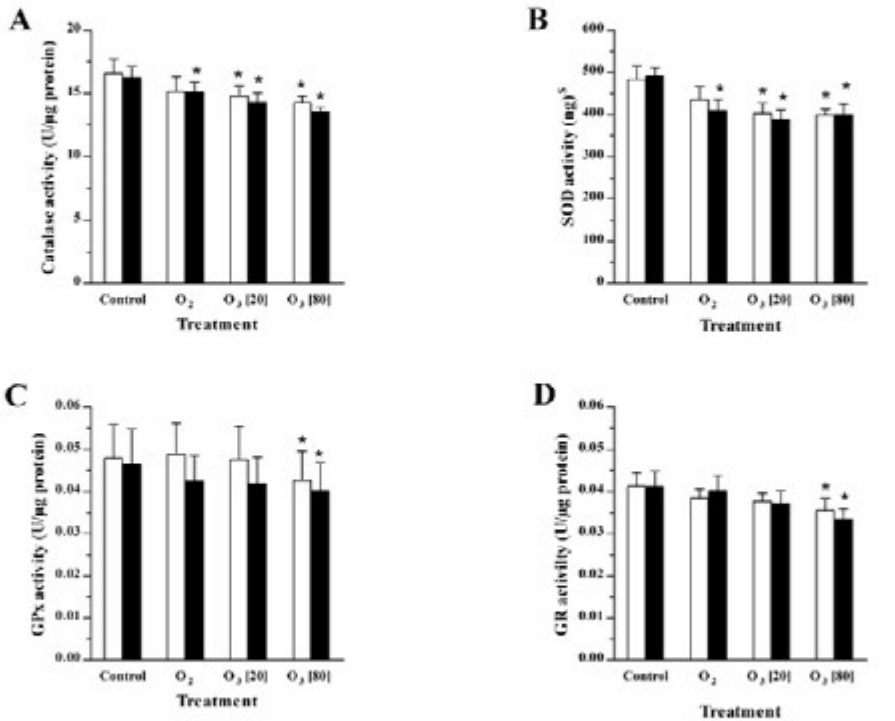
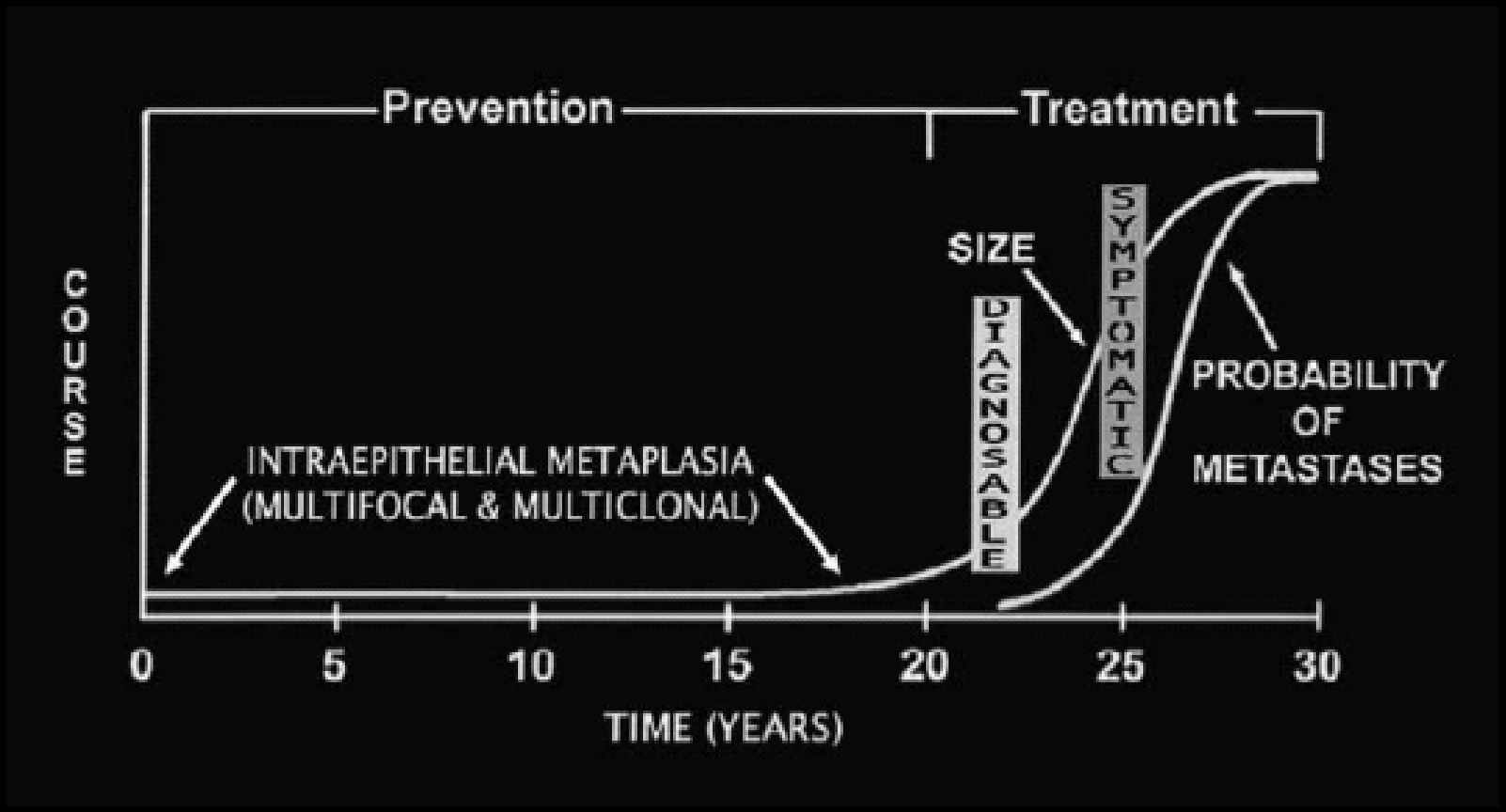


Figure 3. The levels of antioxidant enzymes in PBMC after treatment. A) Catalase activity (U/µg protein) B) SOD activity (ng) C) GPx activity (U/µg protein) and D) GR activity (U/µg protein) for whole blood (open bars) and buffered cells (black bars) at baseline and after exposure to oxygen and 20 and 80 µg/ml ozone. Results are given as the mean ± SEM (n=6). $p<0.05$ with respect to control (*) of the whole blood group (Bonferroni test). [5]ng = the amount of protein that results in 50% inhibition of 6-HD auto-oxidation.

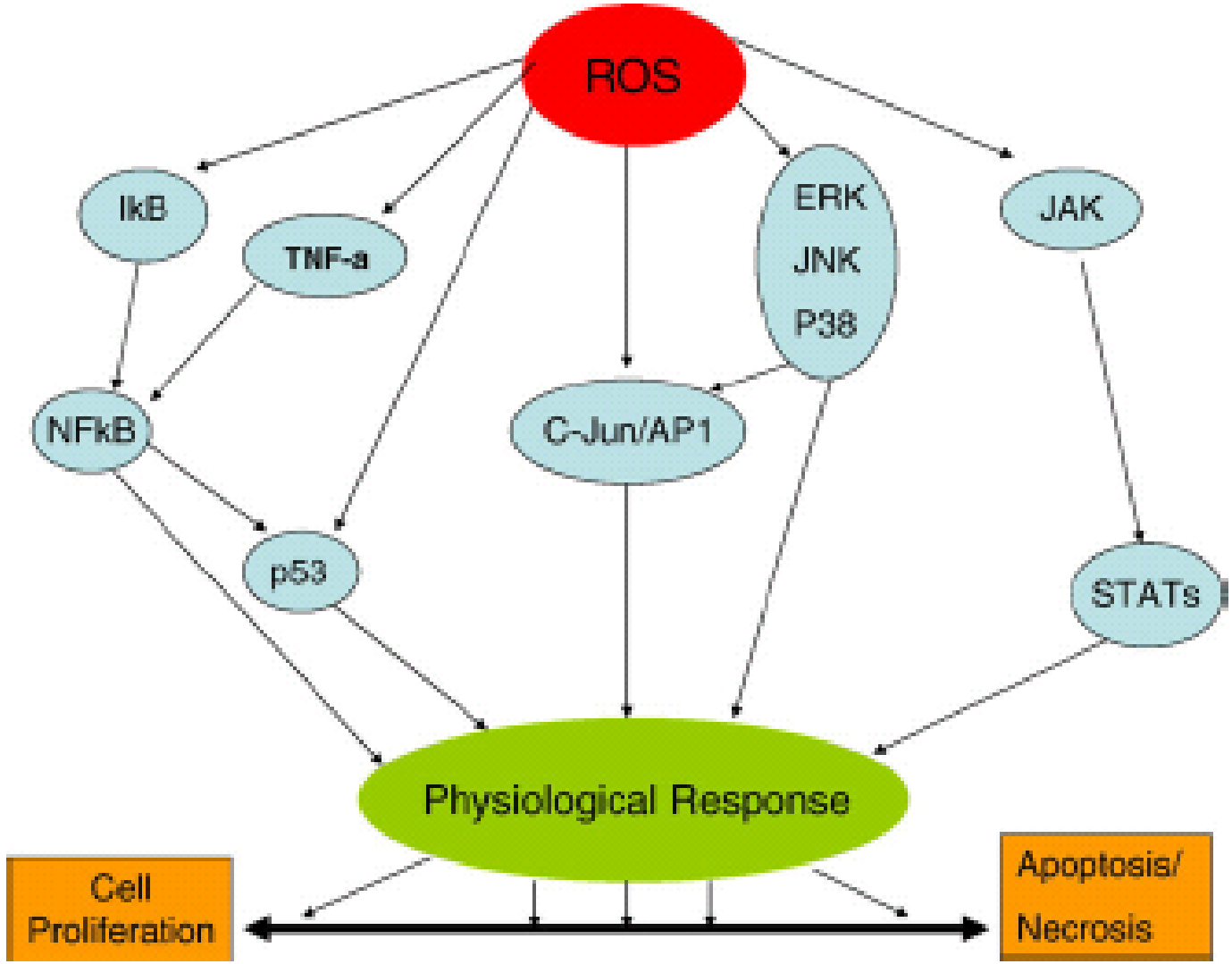
The protective effects of plasma antioxidants

Epithelial cancers



A multi-step process (*Go et al.*)

Cellular response to oxidation



The paradox of oxidant chemical species (1)

Reactive Oxygen Species: A Breath of Life or Death?

John P. Fruehauf and Frank L. Meyskens, Jr.

Abstract New insights into cancer cell – specific biological pathways are urgently needed to promote development of rationally targeted therapeutics. Reactive oxygen species (ROS) and their role in cancer cell response to growth factor signaling and hypoxia are emerging as verdant areas of exploration on the road to discovering cancer's Achilles heel. One of the distinguishing and near-universal hallmarks of cancer growth is hypoxia. Unregulated cellular proliferation leads to formation of cellular masses that extend beyond the resting vasculature, resulting in oxygen and nutrient deprivation. The resulting hypoxia triggers a number of critical adaptations that enable cancer cell survival, including apoptosis suppression, altered glucose metabolism, and an angiogenic phenotype. Ironically, recent investigations suggest that oxygen depletion stimulates mitochondria to elaborate increased ROS, with subsequent activation of signaling pathways, such as hypoxia inducible factor 1α, that promote cancer cell survival and tumor growth. Because mitochondria are key organelles involved in chemotherapy-induced apoptosis induction, the relationship between mitochondria, ROS signaling, and activation of survival pathways under hypoxic conditions has been the subject of increased study. Insights into mechanisms involved in ROS signaling may offer novel avenues to facilitate discovery of cancer-specific therapies. Preclinical and clinical evaluation of agents that modify ROS signaling in cancer offers a novel avenue for intervention. This review will cover recent work in ROS-mediated signaling in cancer cells and its potential as a target for developmental therapeutics.

Cancer chemoprevention by ROS?

Free Radical Biology & Medicine 45 (2008) 97–110

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Review Article

Cancer chemoprevention: A *radical* perspective

Numsen Hail Jr. [a,*], Marcela Cortes [a], Edgar N. Drake [b], Julian E. Spallholz [c]

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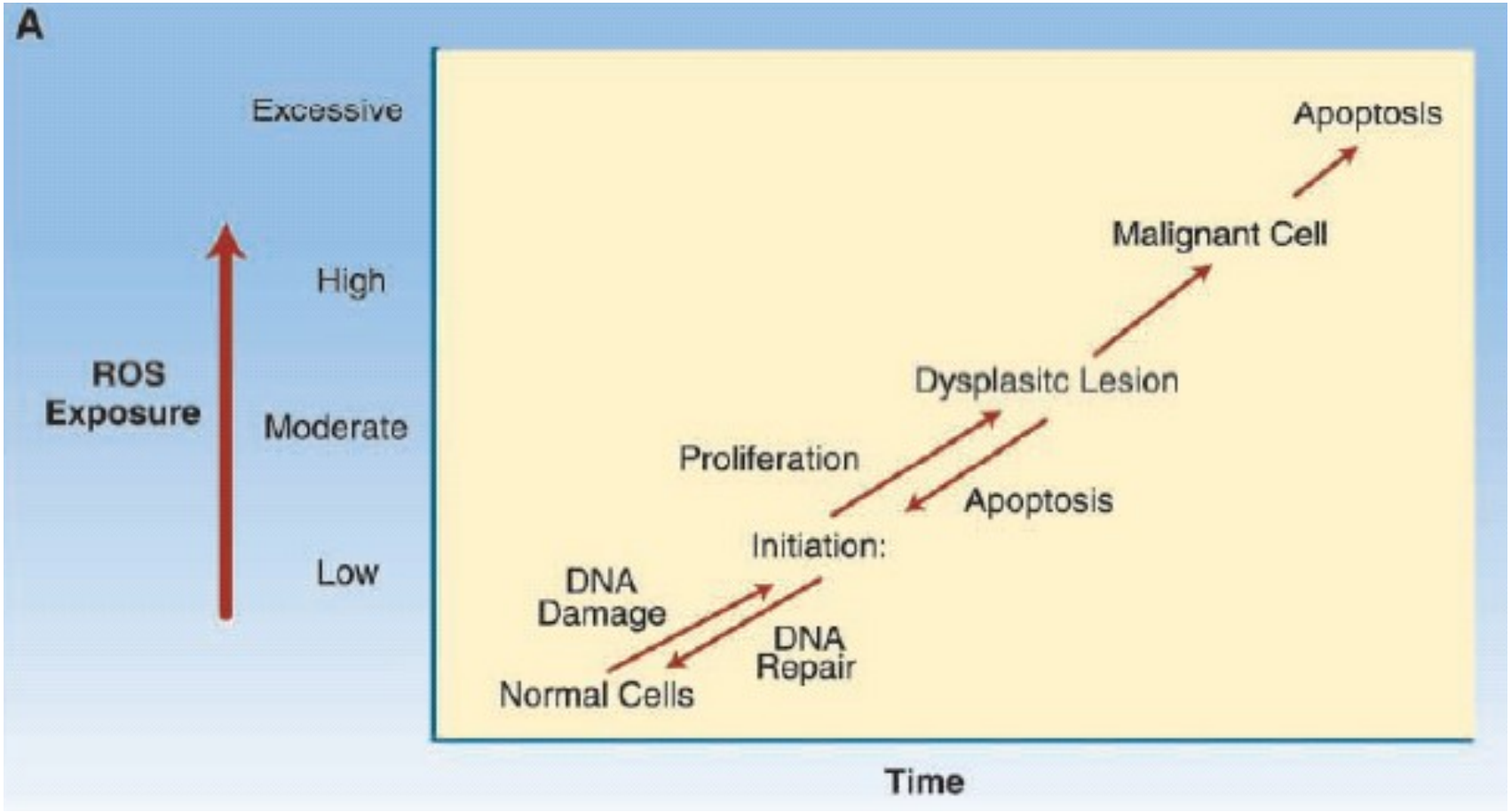
Review

ABSTRACT

Cancer chemopreventive agents block the transformation of normal cells and/or suppress the promotion of premalignant cells to malignant cells. Certain agents may achieve these objectives by modulating xenobiotic biotransformation, protecting cellular elements from oxidative damage, or promoting a more differentiated phenotype in target cells. Conversely, various cancer chemopreventive agents can encourage apoptosis in premalignant and malignant cells in vivo and/or in vitro, which is conceivably another anticancer mechanism. Furthermore, it is evident that many of these apoptogenic agents function as prooxidants in vitro. The constitutive intracellular redox environment dictates a cell's response to an agent that alters this environment. Thus, it is highly probable that normal cells, through adaption, could acquire resistance to transformation via exposure to a chemopreventive agent that promotes oxidative stress or disrupts the normal redox tone of these cells. In contrast, transformed cells, which typically endure an oxidizing intracellular environment, would ultimately succumb to apoptosis due to an uncontrollable production of reactive oxygen species caused by the same agent. Here, we provide evidence to support the hypothesis that reactive oxygen species and cellular redox tone are exploitable targets in cancer chemoprevention via the stimulation of cytoprotection in normal cells and/or the induction of apoptosis in transformed cells.

The paradox of oxidant chemical species (2)



From toxic to therapeutic effect depending on the dose

Chemopreventive –induced oxidative stress may enhance cell elimination

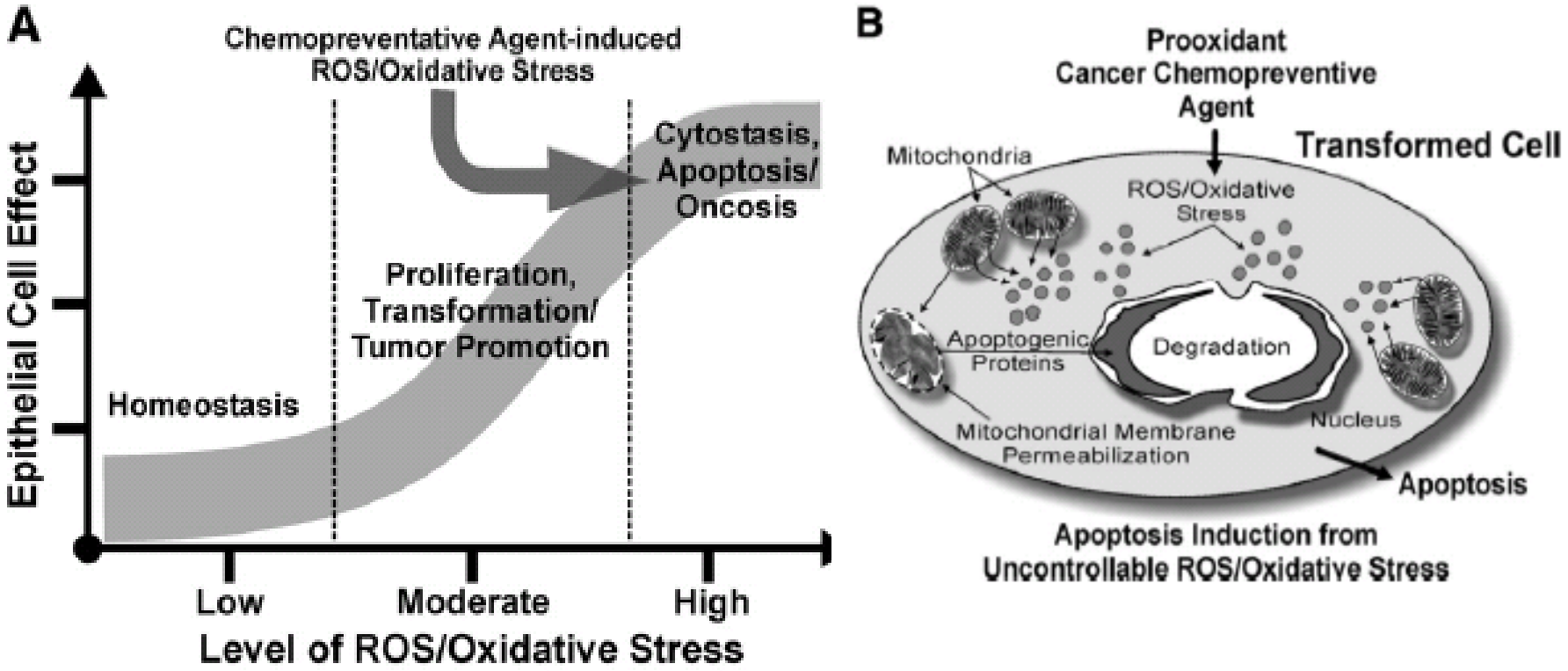
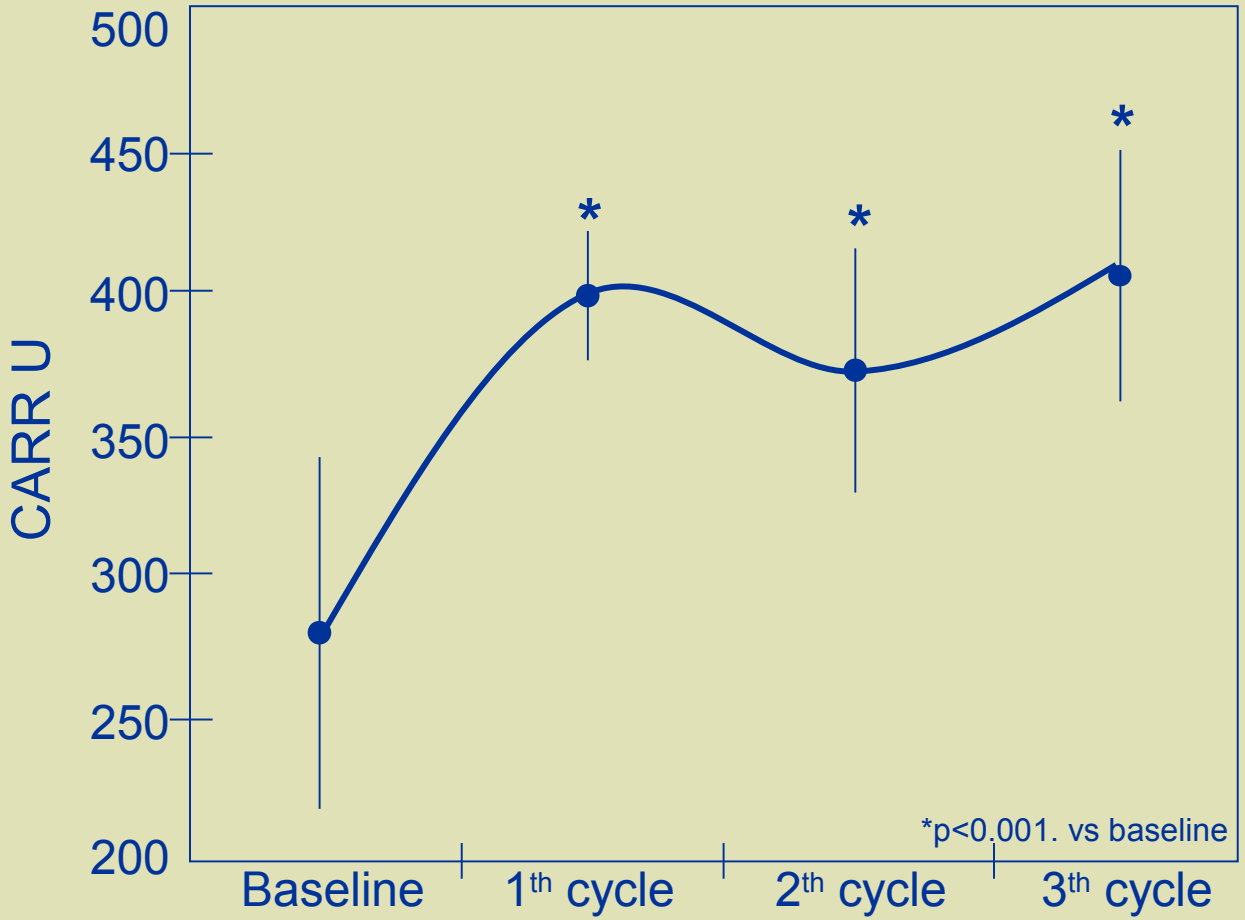


Fig. 3. Exploiting the redox tone of transformed cells encourages cell elimination. (A) Transformed cells in the promotion stage of tumorigenesis are inherently obligated to vie with enhanced oxidative stress. Consequently, their oxidizing intracellular environment should make these cells more likely to succumb to the effects of a prooxidant agent that further promotes and escalates cellular ROS production. Chemopreventive agents that enhance ROS and/or oxidative stress in transformed cells, above the threshold required for anomalous proliferation, could potentially drive these cells to elimination via apoptosis or oncosis. (B) Uncontrollable chemopreventive agent-induced ROS production in transformed cells could inflict widespread damage to numerous cellular components including DNA, protein, and membranes. In addition to ROS and oxidative stress, apoptogenic mitochondrial proteins like apoptosis-inducing factor, cytochrome *c*, and endonuclease G released via MMP could also contribute to cellular degradation. Please refer to the text for additional details.

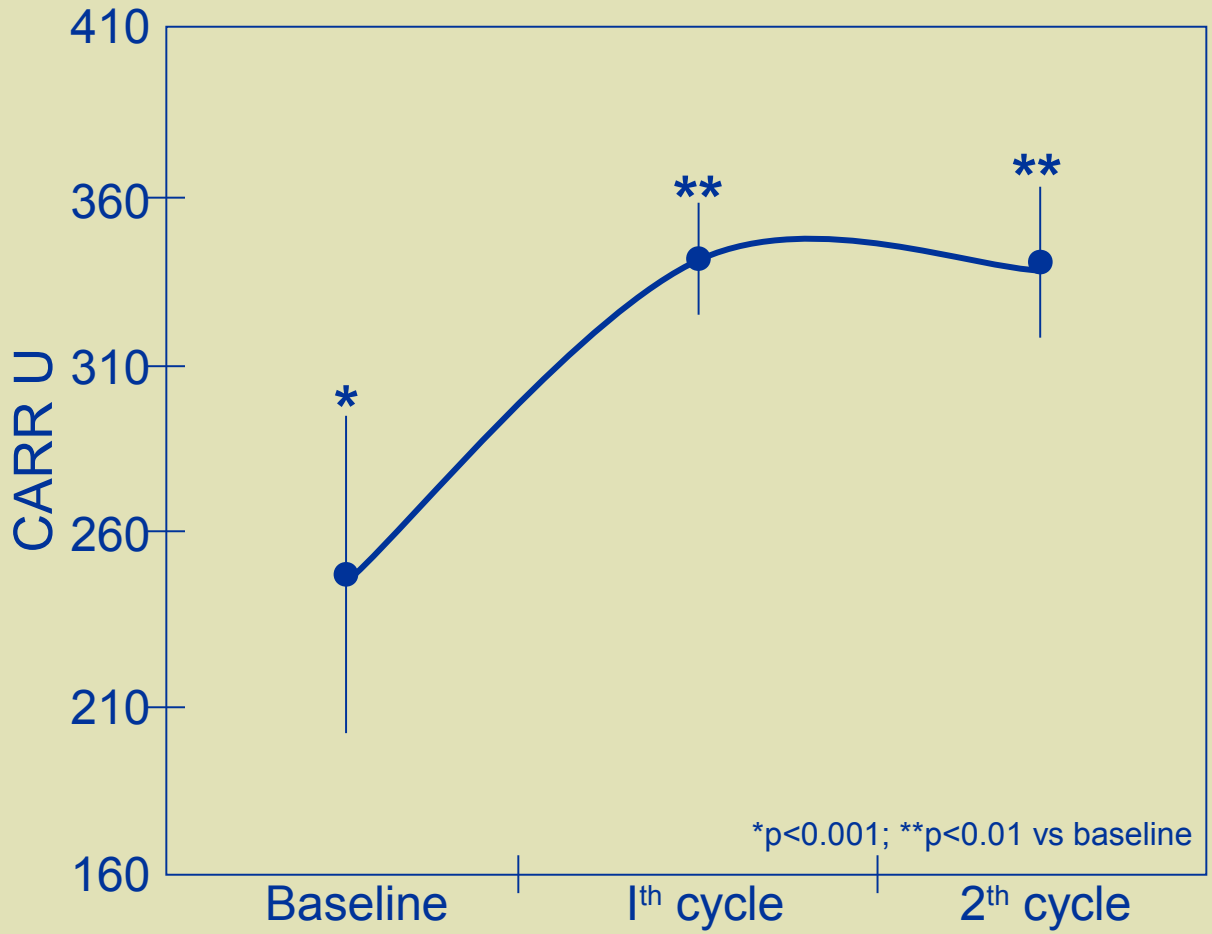
Increased levels of oxidative stress after chemotherapy



La Torre F, Orlando A, Silipigni A, Giacobello T, Pergolizzi S, Aragona M Minerva Medica. 1996. 86: 1-4.

Usefulness of d-ROMs test

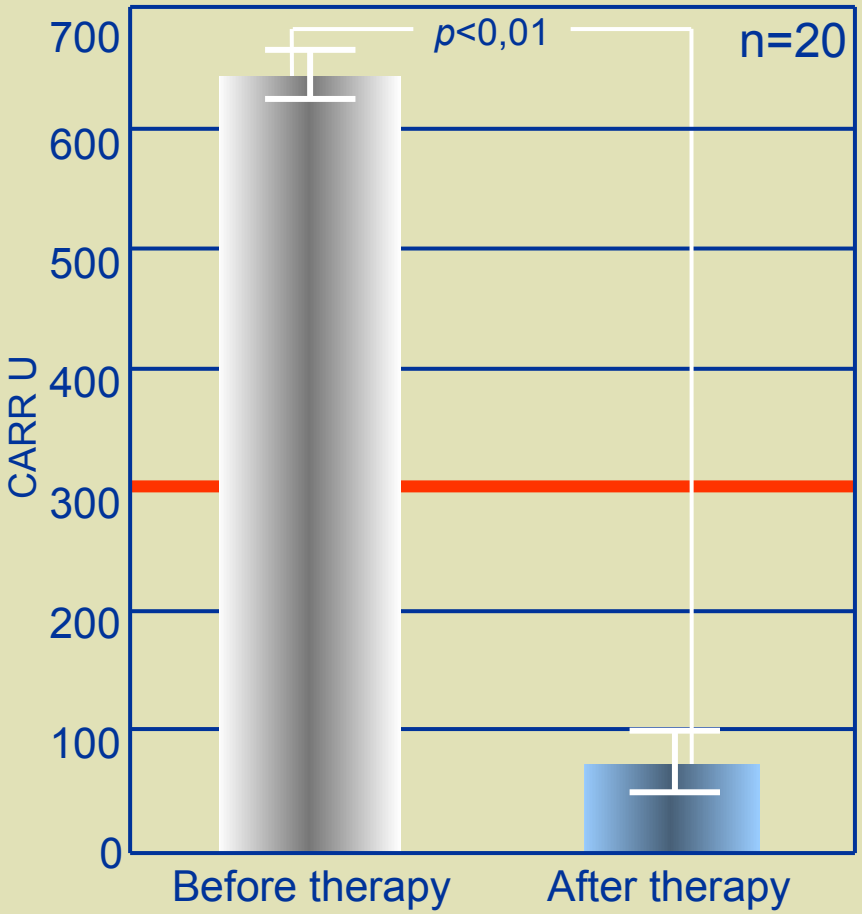
Oxidative stress levels increase during antineoplastic radiotherapy



La Torre F, Orlando A, Silipigni A, Giacobello T, Pergolizzi S, Aragona M Minerva Medica. 1996. 86: 1-4.

d-ROMs test is useful to monitor oxidative stress in radio-treated neoplastic patients

Corticotherapy drammatically reduces free radical levels in patients with leukemia/lymphomas



d-ROMs test is useful to monitor immunosuppressive therapy in oncology

Dionisio C, Pozzi D, Lisi A, Santoro N, Settimo G Report of C.N.R.

Antioxidant formulas and cancer

Free Radical Research, 2003 Vol.. 37 (2), pp. 213–223

Taylor & Francis
Healthsciences

The Impact of Different Antioxidant Agents alone or in Combination on Reactive Oxygen Species, Antioxidant Enzymes and Cytokines in a Series of Advanced Cancer Patients at Different Sites: Correlation with Disease Progression

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Results from a controlled trial

Oxidative stress and cancer

TABLE IV Assessment of blood levels of ROS, GPx activity, serum proinflammatory cytokines and IL-2 of 56 cancer patients and 20 controls

	Controls	Patients	*p* Value
ROS (Carr U)	172 ± 32.2	403.4 ± 78.1	0.000
GPx (U/l)	10813 ± 2134.7	6770.6 ± 2355.2	0.000
IL-6 (pg/ml)	1 ± 2.5	29.1 ± 20.5	0.000
TNFα (pg/ml)	19 ± 6.7	41.0 ± 27.0	0.000
IL-1β (pg/ml)	11.5 ± 5.6	20.0 ± 13.3	0.007
IL-2 (pg/ml)	37.2 ± 23	18.4 ± 12.9	0.000

Results are expressed as mean ± standard deviation. Significance was calculated by Student's *t*-test for comparison to controls.

Significant differences between controls and patients

Oxidative stress and cancer

TABLE VI Assessment of blood levels of ROS and GPx activity in cancer patients at baseline and after antioxidant treatment with single agents (first phase of the study)

		ROS			GPx		
	Number of patients	Baseline	After	*p* Value	Baseline	After	*p* Value
Arm 1	6	445.2 ± 99.8	347.3 ± 98.3	0.051	6033.8 ± 1040.9	8146.8 ± 2741.1	0.082
Arm 2	5	330.8 ± 83.4	257.8 ± 46.2	0.018	6412.4 ± 1340.7	9823.6 ± 2363.9	0.005
Arm 3	7	440.6 ± 87.0	396.3 ± 68.0	0.007	6625.0 ± 1797.2	9160.6 ± 1277.6	0.006
Arm 4	6	434.8 ± 64.4	382.4 ± 57.7	0.050	6765.0 ± 2288.7	8511.6 ± 2295.6	0.052
Arm 5	4	401 ± 63.4	348.3 ± 56.0	0.047	9009.8 ± 4689.2	10414 ± 3034.2	0.340

Arm 1: Alpha lipoic acid 200 mg/day orally. Arm 2: *N*-acetilcysteine 1800 mg/day i.v or carboxycysteine-lysine salt oral solution 2.7 g/day. Arm 3: Amifostine 375 mg/day i.v. Arm 4: Reduced glutathione 600 mg/day i.v. Arm 5: Vitamin A 30,000 IU + Vitamin E 70 mg + Vitamin C 500 mg/day orally. All treatments were administered during 10 days continuously. Significance between values at baseline and after antioxidant treatment was calculated by paired Student's *t*-test. Comparison of the relactive effectiveness of the different antioxidant treatments: Arm 2 vs. 1: ROS $p = 0.615$; GPx $p = 0.508$, Arm 2 vs. 3: ROS $p = 0.192$; GPx $p = 0.348$, Arm 2 vs. 4: ROS $p = 0.303$; GPx $p = 0.153$, Arm 2 vs 5: ROS $p = 0.458$; GPx $p = 0.164$. Arm 2, which showed the highest mean difference between baseline and after-treatment values, was selected as the reference arm.

Significant improvement of oxidative stress balance (d-ROMs⇓ and ⇑ GPx) after single supplements

Oxidative stress and cancer

TABLE VII Assessment of blood levels of ROS and GPx activity in cancer patients at baseline and after antioxidant treatment with combination of different agents (second phase of the study)

		ROS			GPx		
	Number of patients	Baseline	After	*p* Value	Baseline	After	*p* Value
Arm 1	12	394.8 ± 61.8	345.1 ± 50.7	0.008	7641.7 ± 2548.9	10614.4 ± 2064.1	0.223
Arm 2	5	379.2 ± 57.3	350.4 ± 41.9	0.094	4239.4 ± 821.8	7220.8 ± 1126.2	0.010
Arm 3	4	412.5 ± 67.7	361.5 ± 42.8	0.140	7503.0 ± 1783.7	9850.3 ± 2373.6	0.027
Arm 4	3	348.7 ± 77.3	258.0 ± 59.0	0.014	6314.0 ± 859.0	10755.7 ± 1569.5	0.027
Arm 5	4	414.3 ± 112.1	349.5 ± 106.5	0.013	6775.3 ± 3256.9	9711.5 ± 3456.4	0.087

Arm 1: Alpha lipoic acid 200 mg/day + carboxycysteine lysine salt sachets 2.7 g/day. Arm 2: Alpha lipoic acid 200 mg/day + Amifostine 375 mg/day i.v. Arm 3: Carboxycysteine lysine salt sachets 2.7 g/day orally + Amifostine 375 mg/day i.v. Arm 4: Reduced glutathione 600 mg/day i.v. + Amifostine 375 mg/day i.v. Arm 5: Alpha lipoic acid 200 mg/day + reduced glutathione 600 mg/day i.v. All treatments were administered for 10 days continuously. Significance between values at baseline and after antioxidant treatment was calculated by paired Student's *t*-test. Comparison of the relactive effectiveness of the different antioxidant treatments: Arm 4 vs. 1: ROS $p = 0.224$; GPx $p = 0.268$, Arm 4 vs. 2: ROS $p = 0.108$; GPx $p = 0.206$, Arm 4 vs. 3: ROS $p = 0.265$; GPx $p = 0.278$, Arm 4 vs. 5: ROS $p = 0.191$; GPx $p = 0.367$. Arm 4, which showed the highest mean difference between baseline and after treatment values, was selected as the reference arm.

Significant improvement of oxidative stress balance (d-ROMs⇓ and ⇑ GPx) after multiple supplements

Oxidative stress and cancer

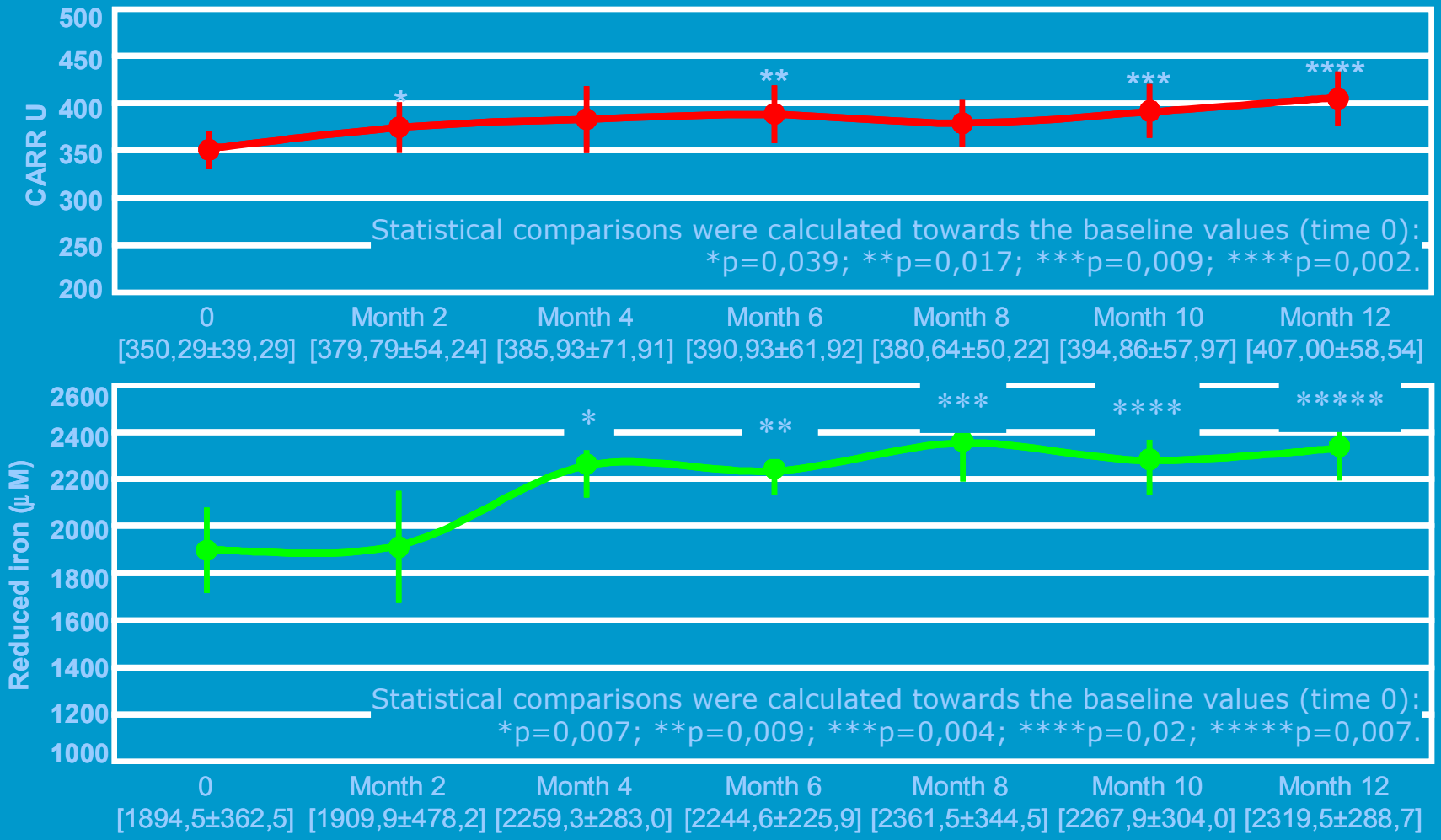
TABLE IX Comparison between baseline levels of ROS, GPx activity, serum proinflammatory cytokines and IL-2 of ECOG PS 0–1 with ECOG PS 0–2 patients

	Patients ECOG PS 0–1	Patients ECOG PS 2–3	*p* Value
ROS (Carr U)	386.6 ± 73.6	436.2 ± 78.0	0.023
GPx (U/l)	7108.4 ± 2605.1	6112.8 ± 1638.3	0.136
IL-6 (pg/ml)	21.6 ± 10.3	42.3 ± 26.9	0.000
TNFα (pg/ml)	38.6 ± 26.2	45.1 ± 28.6	0.398
IL-1β (pg/ml)	18.5 ± 11.4	22.6 ± 16.0	0.273
IL-2 (pg/ml)	19.6 ± 14.3	16.4 ± 10.1	0.389

Results are expressed as mean ± standard deviation. Significance was calculated by Student's *t*-test.

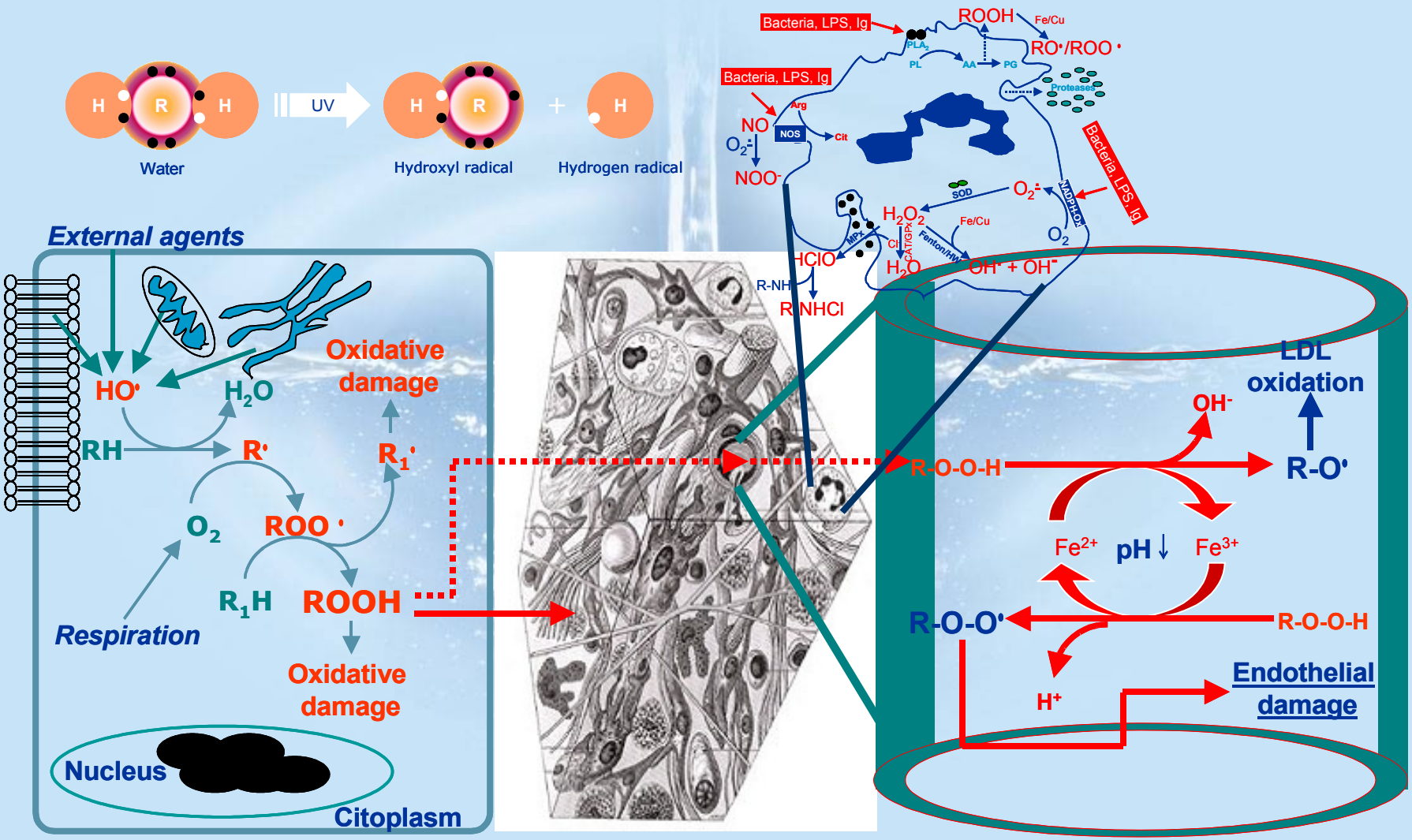
Significant improvement of clinical outcomes after antioxidant treatments

Oncology. *The SERUM MILK PROTEIN Intervention Study on female breast cancer.*

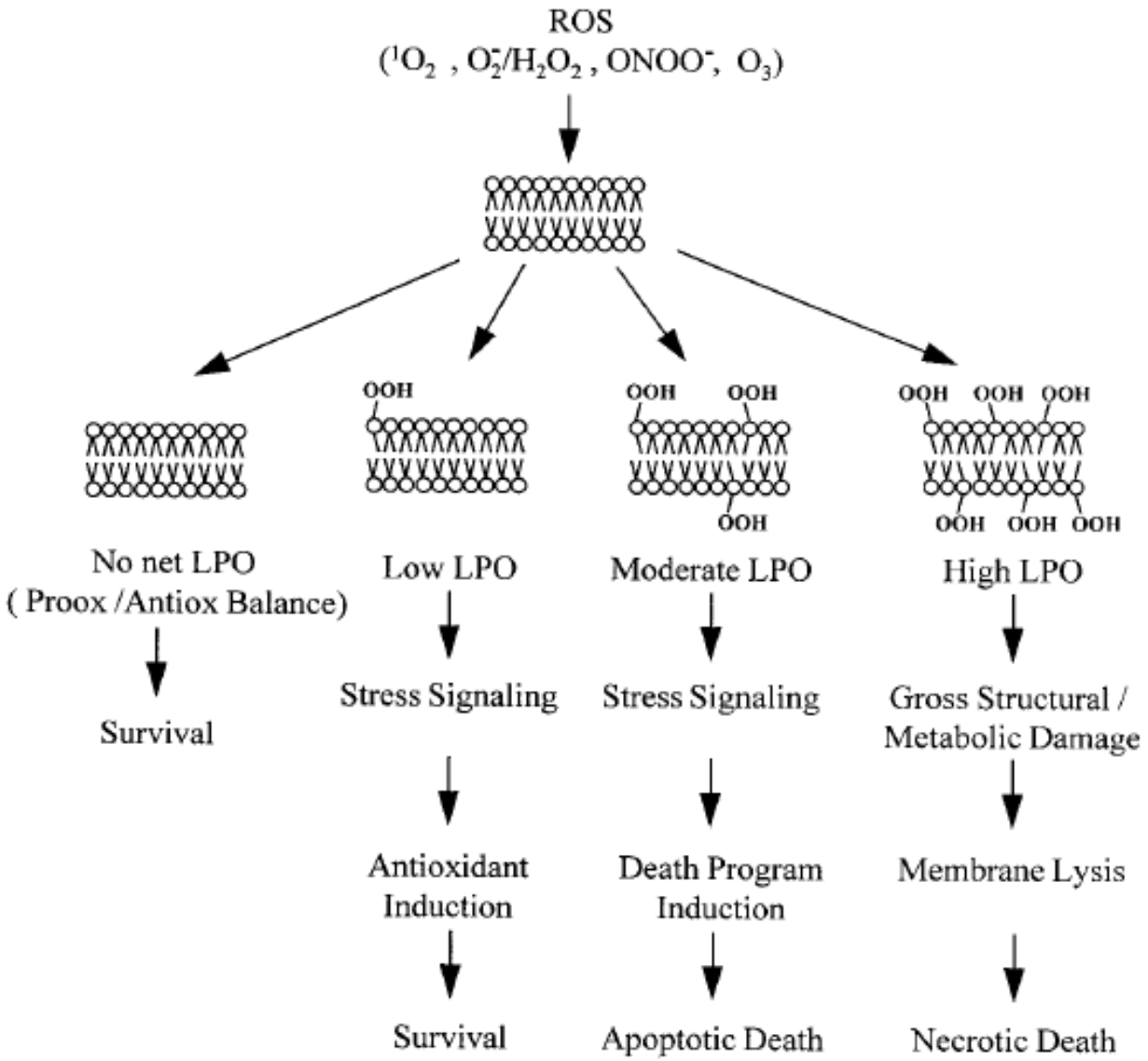


Significant increase of biological antioxidant potential after 12-months supplementation (*Landoni, 2009*)

Basic cell mechanisms of oxidative tissue damage

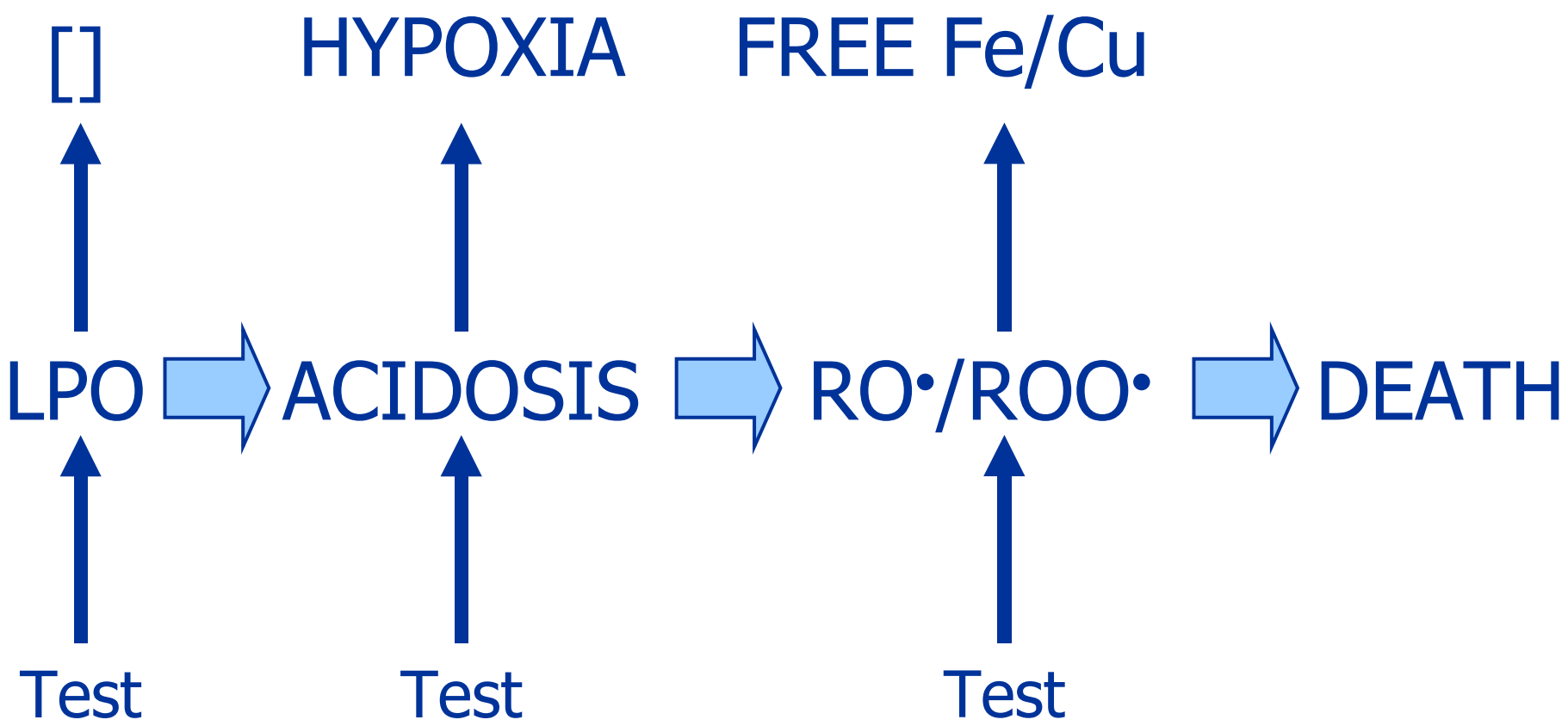


The role of lipoperoxides (LPO) in cell physiology modulation



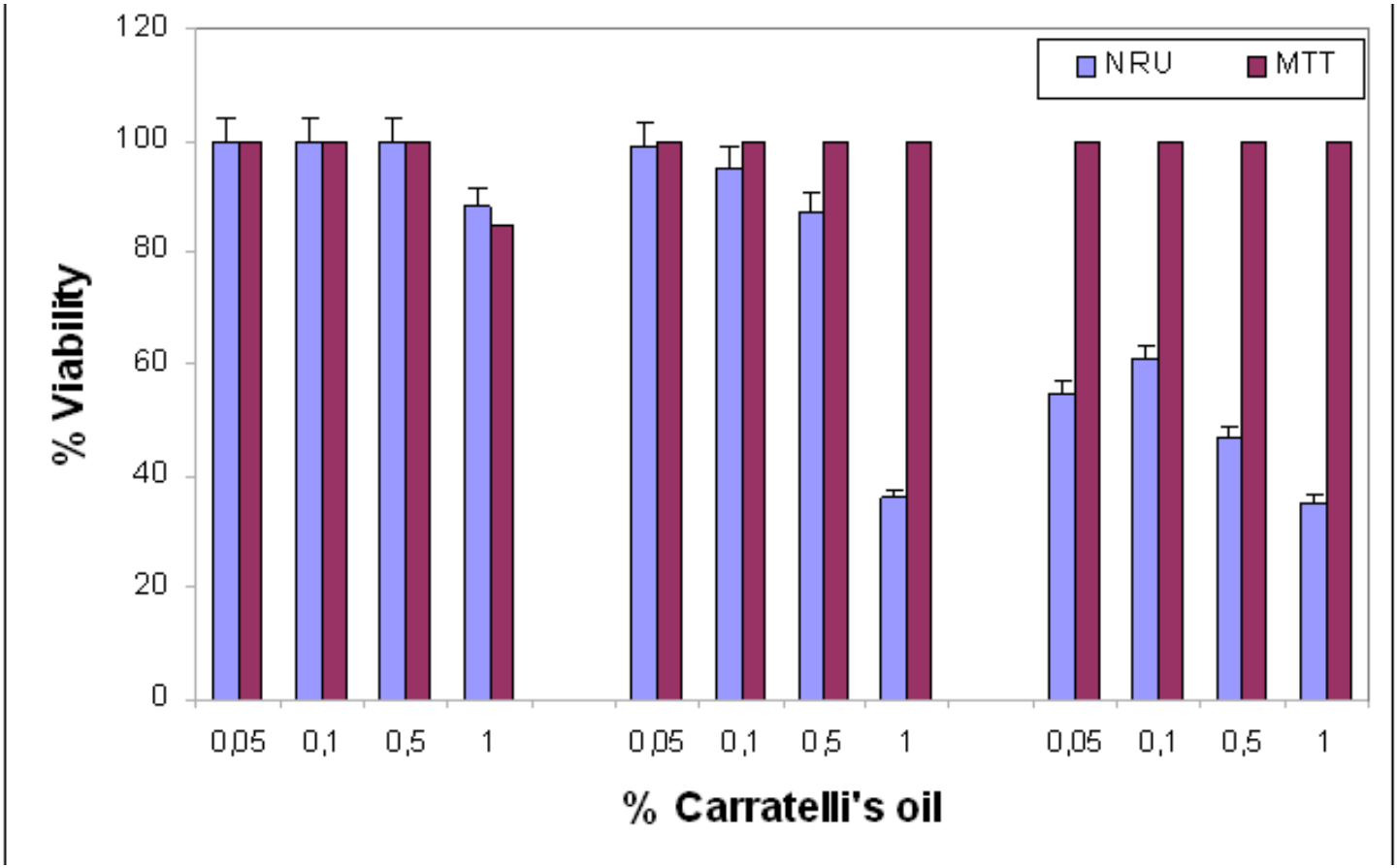
Girotti, 1998.

OXIDANTS AND CANCER



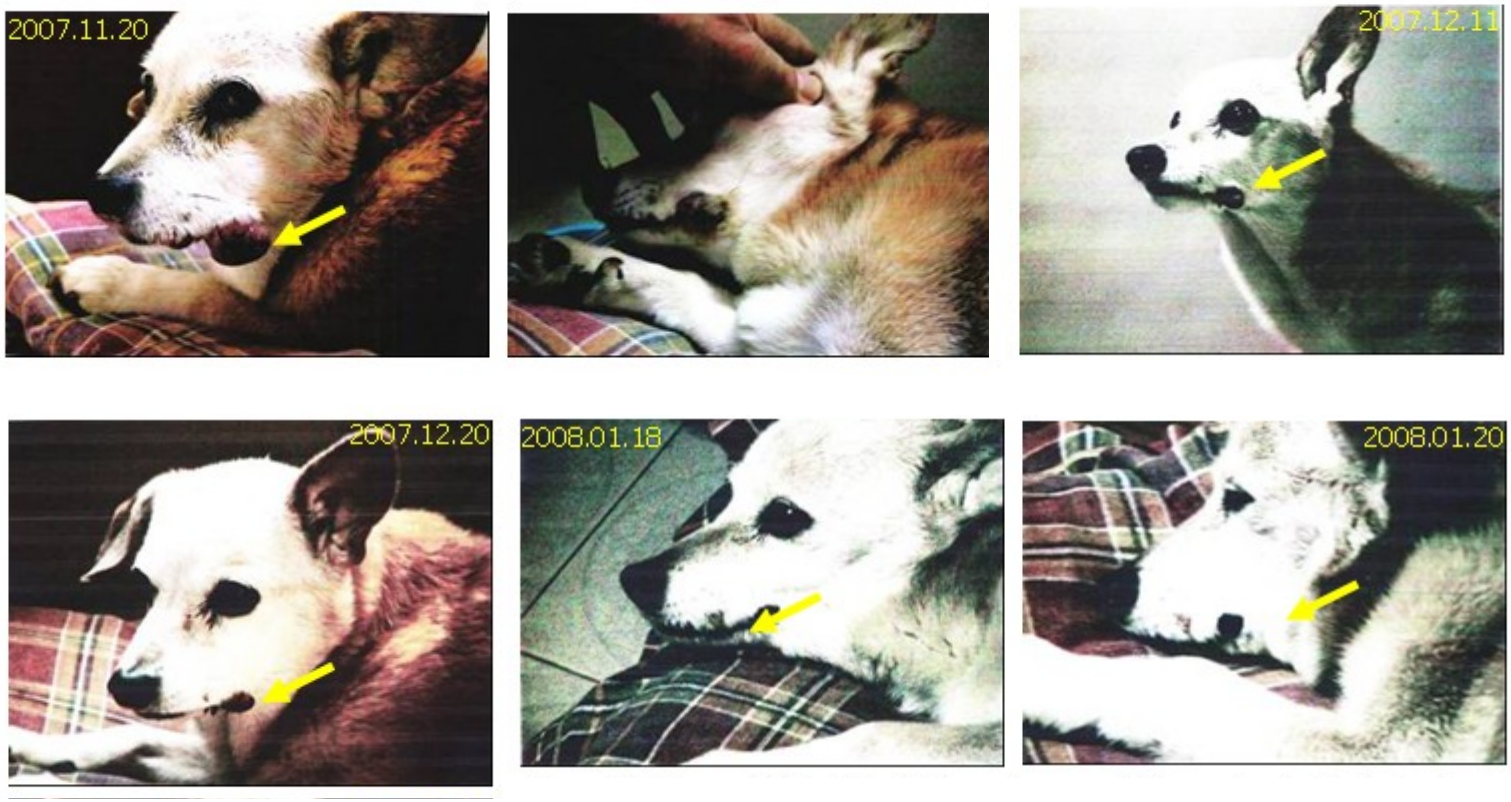
OUR HYPOTHESIS (CARRATELLI AND IORIO)

CARRATELLI AND IORIO HYPOTHESIS



STUDIES *IN VITRO* WITH PEROXIDISED EXTRA-VIRGIN OLIVE OIL

CARRATELLI AND IORIO HYPOTHESIS



STUDIES *IN VIVO* WITH PEROXIDISED EXTRAVIRGIN OLIVE OIL

Potential usefulness of γ-linolenic acid in cance therapy

© Med Sci Monit, 2007; 13(7): RA119-131
PMID: 17599036

www.MEDSCIMONIT.COM
Review Article

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Accepted: 2007.05.10
Published: 2007.07.01

γ-linolenic acid therapy of human glioma-a review of *in vitro*, *in vivo*, and clinical studies

Undurti N. Das

UND Life Sciences, Shaker Heights, OH, U.S.A.

Source of support: Departmental sources

Summary

γ-linolenic acid (GLA) induced apoptosis of tumor cells without harming normal cells. Both cyclo-oxygenase (COX) and lipoxygenase (LO) inhibitors did not inhibit the selective tumoricidal action of GLA in some, but not all, tumor cells suggesting that GLA by itself is active. In contrast, anti-oxidants such as vitamin E blocked the tumoricidal action of GLA. GLA-treated tumor but not normal cells produced a 2–3-fold increase in free radicals and lipid peroxides. GLA decreased the anti-oxidant content of tumor cells, expression of oncogenes *ras*, and *Bcl-2*, enhanced the activity of p53, protected normal cells and tissues from the toxic actions of radiation and anti-cancer drugs, enhanced the cytotoxic action of anti-cancer drugs and reversed tumor cell drug resistance. In the animal glioma model, GLA induced tumor regression and preserved the surrounding normal brain tissue. In three open-label clinical studies, intra-tumoral injection of GLA induced significant reduction of glioma without any significant side effects. The low neurotoxicity of GLA to normal brain neurons and selective activity against tumor cells suggests that it could be an effective anti-glioma molecule.

key words: gamma-linolenic acid • glioma • free radicals • lipid peroxidation • apoptosis • oncogenes • neuronal cells • polyunsaturated fatty acids • anti-cancer drugs

Oxidative stress and cancer: a *radical* approach.

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A radical approach to cancer

Undurti N. Das

EFA Sciences LLC, Norwood, MA, U.S.A.

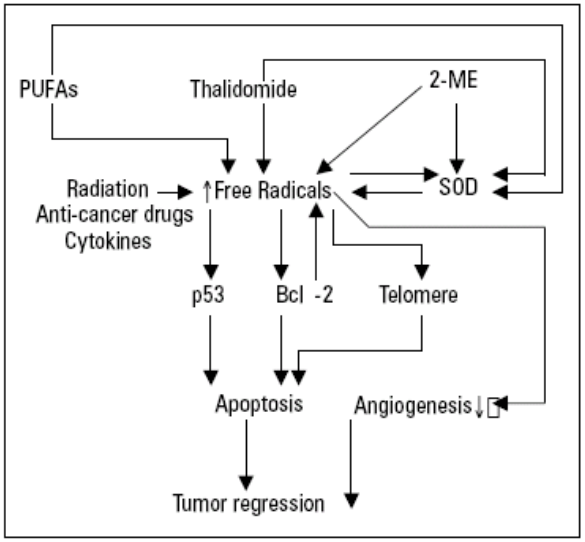
RA

Summary

Reactive oxygen species are known to be potentially dangerous, but are also needed for signal-transduction pathways. Tumor cells have relatively low amounts of superoxide dismutase (SOD), which quenches superoxide anion ($O_2^{-\bullet}$), and as a result of a higher level of aerobic metabolism, higher concentrations of $O_2^{-\bullet}$, compared to normal cells. But this may not be true of all tumor cells. Some tumor cells have relatively higher amounts of vitamin E, a potent anti-oxidant, and a higher level of anaerobic metabolism, resulting in a balance that is tilted more towards higher anti-oxidant capacity. In both instances of higher aerobic and anaerobic metabolism methods designed to augment free radical generation in tumor cells can cause their death. It is suggested that free radicals and lipid peroxides suppress the expression c Bcl-2, activate caspases and shorten telomere, and thus inducing apoptosis of tumor cells Ionizing radiation, anthracyclines, bleomycin and cytokines produce free radicals and thu are useful as anti-cancer agents. But they also produce many side-effects. 2-methoxyoestradic and polyunsaturated fatty acids (PUFAs) inhibit SODs and cause an increase of $O_2^{-\bullet}$ in tumo cells leading to their death. In addition, PUFAs (especially gamma-linolenic acid), 2-methoxy oestradiol and thalidomide may possess anti-angiogenic activity. This suggests that free radi cals can suppress angiogenesis. Limited clinical studies done with gamma-linolenic aci showed that it can regress human brain gliomas without any significant side-effects. Thus PUFAs, thalidomide and 2-methoxyoestradiol or their derivatives may offer a new radica approach to the treatment of cancer.

key words: free radicals • 2-mehtoxyestradiol • polyunsaturated • fatty acids • superoxide dismutase • tumor cells

Full-text PDF: http://www.MedSciMonit.com/pub/vol_8/no_4/2474.pdf



Peroxidative cancer: a possible approach to cancer therapy.

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 33, pp. 23643–23651, August 18, 2006

Intracellular Dissemination of Peroxidative Stress

*INTERNALIZATION, TRANSPORT, AND LETHAL TARGETING OF A CHOLESTEROL HYDROPEROXIDE SPECIES BY STEROL CARRIER PROTEIN-2-OVEREXPRESSING HEPATOMA CELLS**

Received for publication, January 25, 2006, and in revised form, May 30, 2006 Published, JBC Papers in Press, June 12, 2006, DOI 10.1074/jbc.M600744200

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Pro-oxidant action of high ascorbate concentration

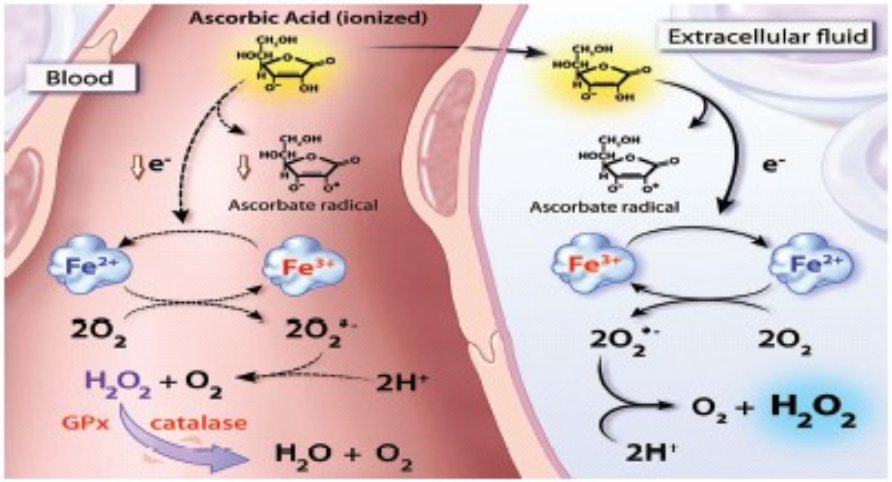

Fig. 1. Proposed mechanism of preferential formation of $Asc^{\bullet-}$ and H_2O_2 in extracellular fluid compared with blood. After oral and parenteral administration, ascorbic acid is proposed to achieve equivalent concentrations in blood (left side) and extracellular fluid (right side). In extracellular fluid, pharmacologic concentrations of ascorbic acid lose one electron and form $Asc^{\bullet-}$. The electron reduces a protein-centered metal: An example reaction is shown as reduction of Fe^{3+} to Fe^{2+}. Fe^{2+} donates an electron to oxygen, forming active oxygen including superoxide ($O_2^{\bullet-}$) with subsequent dismutation to H_2O_2 (17). In blood (left side), it is proposed that these reactions are damped or inhibited (dashed lines). $Asc^{\bullet-}$ appearance will be inhibited by red blood cell membrane-reducing proteins (18) and/or by large plasma proteins that do not distribute to the extracellular space. Any formed H_2O_2 will be immediately destroyed by plasma catalase and red blood cell GSH peroxidase, so that no H_2O_2 will be detectable (14–16). The identities of the metal-centered proteins are unknown.

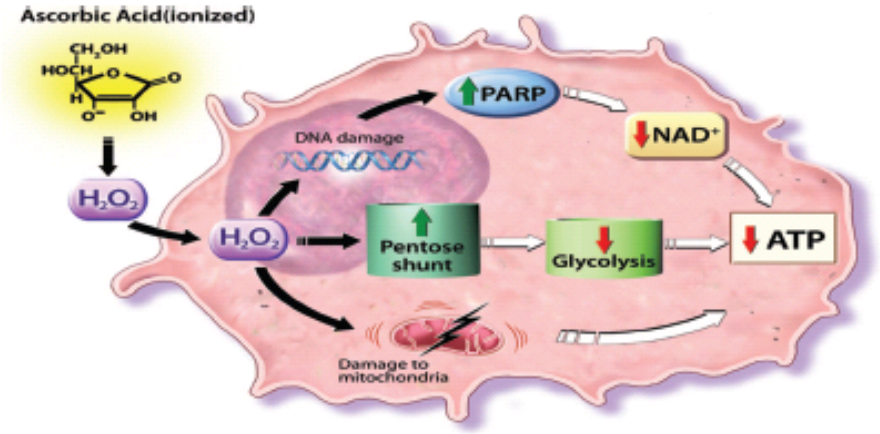

Fig. 5. Pharmacologic ascorbic acid concentrations: mechanisms for selective cell death. Pharmacologic ascorbic acid concentrations produce extracellular H_2O_2, which can diffuse into cells, deplete ATP in sensitive cells, and thereby cause cell death. ATP may be depleted by three mechanisms. (*i*) DNA damage induced by H_2O_2 activates PARP. Activated PARP catabolizes NAD^+, thereby depleting substrate for NADH formation and consequent ATP synthesis. (*ii*) H_2O_2 is catabolized by concurrent oxidation of GSH to GSSG. To reduce GSSG back to GSH, GSH reductase utilizes NADPH, which is provided by the pentose shunt from glucose. Glucose used to reduce $NADP^+$ to NADPH cannot be used for glycolysis or NADH production so that ATP generation is decreased. (*iii*) H_2O_2 may directly damage mitochondria, especially ATP synthase, so that ATP production falls. Some cancer cells rely primarily on glycolysis rather than on oxidative phosphorylation respiration for ATP production (the Warburg effect). Compared with oxidative phosphorylation, ATP generation by glycolysis is inefficient. In glycolysis-dependent cancer cells, decreased glycolysis may lower intracellular ATP. Cancer cells that are glycolysis-dependent may be particularly sensitive to pharmacologic ascorbic acid concentrations, compared with cells that use oxidative phosphorylation. See text for additional details.

INTERNATIONAL CONFERENCE. New horizons for the ozone therapy - *Pontevedra. June 5th, 2009.*

MUCHAS GRACIAS!

www.oxidativestressobservatory.org
www.oxigenlab.com