Oxidative Stress

Fellowship in Anti-Aging Medicine
Module 4

Oxidative Stress

Oxidation of cellular components that damage the cells or tissues.

The damage is caused by an imbalance between the production of free radicals and the antioxidant system, producing a potential state of oxidative cellular lesion.

The oxidative lesion is produced on lipids, carbohydrates, proteins and DNA

Free Radicals (F.R.)

Molecule or fragment of a molecule that contains one or more unpaired electrons in its external orbit; a substance is transformed into a free radical either by gain or loss of an electron

They are highly reactive molecules, with a very short half-life and with the ability to injure cells

Origin of Free Radicals (F.R.)

Exogenous

- Physical Agents : Solar Radiation (UV, Rx)
- Chemical Agents : O_2, ethanol, tobacco smoke, pesticides, some medicine

Endogenous

- Mainly those derived from :
  - Mitochondrial metabolism, ATP (92%)
  - Breakdown of fatty acids in the peroxisomes
  - Enzymes of P450 cytochrome
  - White Blood Cells that attack germs, (phagocytosis) freeing O_2^{-} H_2O_2 ....

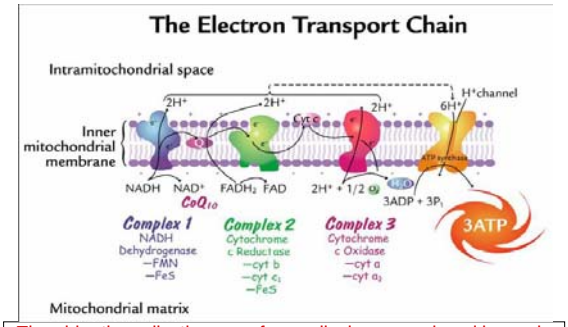
Origin of Free Radicals (F.R.)

ATP Production in the mitochondria

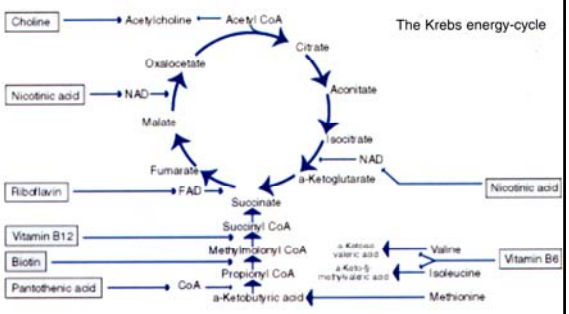
- Krebs cycle and its interaction with the oxidative phosphorylation cycle (matrix mtc)
- Electronic transport chain ETC (interior membrane mtc)

ETC

- Require from Krebs cycle (NADH, $FADH_2$)
- It is the biggest source of production of FR, 2% of O_2 consumed in mitochondria during ETC is converted into O^{2-} => SOD makes O^{2-} into H_2O_2 who spilts it and produces cross-linking in mitochondrial DNA



- The older the cells, the more free radicals are produced by each ATP molecule.



Free Radical Producers of exogenous origin

Atmospheric Pollutions

Ozone, tobacco smoke, car fumes.

Medicines or drugs

Ethanol (increased lipoperoxides)

Cocaine (radical nitroxide norcaine)

Cyclosporin A (decreased GSH)

Paracetamol * (GSH Depletion pool and oxidative stress)

Adriamycine - Phenothiazines.

* Its metabolite N-acetyl-p-benzoquinone imide

Free Radical Producers of exogenous II origin

Oxidative Stress	
Transition Metals	Fe, V, Ni, Al, Cd, Hg, Cr, Co
Chemical Toxins (Xenobiotics)	Paraquat, Diquat, Dieldrin, benzopyrene...
Particles	Asbestos: (associated to Fe) DNA damage (lung cells) Sílicon: (associated to Fe) DNA damage (lung cells)

Drugs & Oxidation

It has been demostrated that ACETAMINOPhEN (Paracetamol) is hepatotoxic.

Its toxic action is exerced through its metabolite: N-acetyl-p-benzoquinone-imine, who causes oxidative stress mainly through the depletion of glutathione.

N-acetyl-cystein is an important source of -SH groups that stimulate the synthesis of glutathione. It is therefore useful to warn the hepatotoxicity of **Paracetamol** during prolonged treatments.

Formation of Free Radicals

Free radicals are continously being produced in the body :

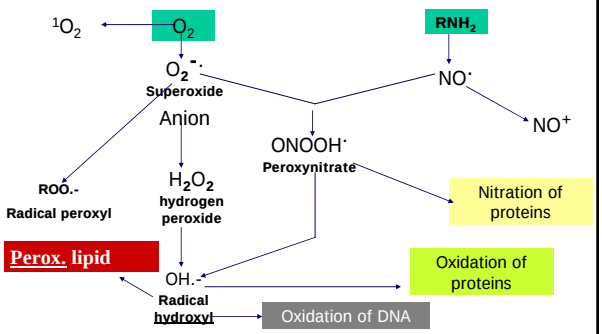
Biochemical redox reactions with oxygen take place in the cellular metabolism

In a controlled inflammatory reaction, phagocyte mediated

As an answer to:

Ionic radiation, ultraviolet rays, environmental contamination, tabacco, excess exercise, ischemia....

Reactive Genus



Single Oxygen ($^1O_2$)

Appears when molecular oxygen absorbs a sufficient amount to move one of its two non paired e- to an orbital of superior energy

Strongly electrified molecule

Generated in photosensitivity reactions

Superoxide ($O2^{-\cdot}$)

Is produced in the mitochondria, cytoplasmatic reactions, and by conversion of oxyHb into metaHb.

Very short half-life

Does not spread out far from where it was generated (soon captures the e-)

Able to mutate bacteria, inactivate viruses...

Eliminated by Superoxide Dismutase (SOD)

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

Hydrogen peroxide (H_2O_2)

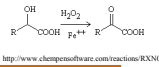
By itself it is not a free radical, however it is with the presence of metal ions giving way to a hydroxyl radical (Fenton reaction).

Is stable

Diffuses far from where it is generated

It is eliminated by Catalase and Glutathione Peroxidase (GPx)

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



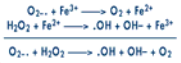
http://www.chempensoftware.com/reactions/RXN067.htm

$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$

Hydroxide (OH.-)

Is one of the most reactive of the chemical species.

It is generated by the Fenton and the Haber-Weiss reaction.



An important cell destruction with the peroxidation of lipids develops, destruction of enzymes, damaged DNA, oxidation of -SH groups

High concentrations are not usually found, however, if they do exist there are no mecanisms of elimination.

Nitric Oxide (NO)

Nitric Oxide (NO)

Clear gas with 11 e- ; the unpaired e- in the atom of the N, or the O.

Functions:
Platelet anti-aggregant
vasodilation
NO = EDRF (Endothelial-derived relaxing factor)

L-Arginine + O2 + NADPH $\xrightarrow[\text{NO-synthesis}]{\text{NOS}}$ NO + L-Citruline

If it reacts with the superoxide anion ($O2^{-\cdot}$), it forms peroxynitrate ($ONOOH^{\cdot}$) (Free Radical)

Nitric Oxide (NO)

Peroxidation of Nitric Oxide

Small amount of Toxicity

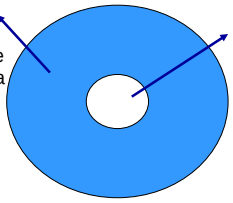
- NO is volatile and does not accumulate.
- Acts as an anti-thrombotic agent, vasodilator,
- As an antimicrobic action,
- Can acts as a destructor of carcinogenic cells, but becomes highly toxic in reaction with a superoxide radical.

Diseases + Peroxinitrate

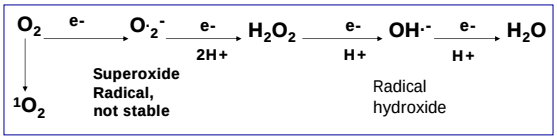
Peroxinitrate is related to:
- renal diseases,
- neurodegenerative processes
- chronic inflammatory diseases
- rheumatoid arthritis.

Oxygen Reduction Canals

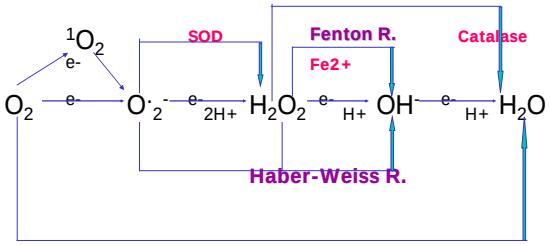
95% Through the oxidase cytochrome system, able to reduce oxygen into water by a tetravalent reduction without producing intermediate components.



5% Univalent reduction of oxygen, generating free radicals - of oxygen-



ELIMINATING OXYGEN



Cytochrome Oxidase

Free Radicals

	Half life (seg)	Antioxidant
HO• Hydroxide Radical	10^{-9}	
RO Alkoxyl Radical	10^{-6}	
ROO• Radical Peroxide	7	alfa-tocopherol
$O_2^{\cdot-}$ Superoxide Anion R.	(enz)	SOD
H_2O_2 Hydrogen Peroxide	(enz)	Catalase/GPx
$^1O_2$ Single Oxygen R.	10^{-5}	beta-carotene Lycopen
NO· Nitric Oxide R.	1^{-10}	SOD

Pro-oxidant Enzymes

NADPH oxidase:

Producer of superoxides in phagocytes and lymphocytes B. It is found in the cytosol and associated with membranes

Nitric Oxide Synthetase: Enzyme of the cytochrome P-450 system

Contains a hemo group and reductase group. In macrophages it is expressed after the exposure to cytokines and produces NO . (independent from Ca). In the brain it is expressed as response to Ca.

Target Points

- Lipids: Lipoperoxidation
- Carbohydrates
- Proteins
- DNA

DNA, Proteins & Carbohydrates

DNA

The damage produced in the mtDNA can cause a mutation during the replication moment, deneutralisation and breaking of the double helix.

Proteins

Breaking of chains and formation of aggregates

Carbohydrates

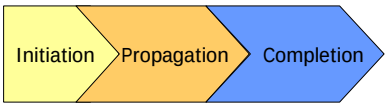
Reactive carboxylic species (RCS) appear and damage the amino groups. Everytime that an HC or a PUFA is attacked the damage expands

Free Radicals produce

- **Protein Oxidation**
  Inactivation of enzymes, which modifies the efficiency of the reactions that are catalised.
- **Lipid peroxidation**
  Malonyldialdehyde : indicator of damaged cells (pigmentation of the skin)
  Oxidation LDL and Atherosclerosis
- **Oxidative Lesion of DNA:**
  Can induce spontaneous mutations that could have a vital role to play in the aging process and carcinogenics.

Lipid Peroxidation

The double bounds of polyunsaturated lipids (PUFA) are the sites mainly oxidized



The process auto perpetuates and the final result will be the fragmentation of the lipid into hydroperoxides and cytotoxic aldehydes.

Lipid Peroxidation

PUFA

Free Radicals

Conjugated diene Radical

O_2

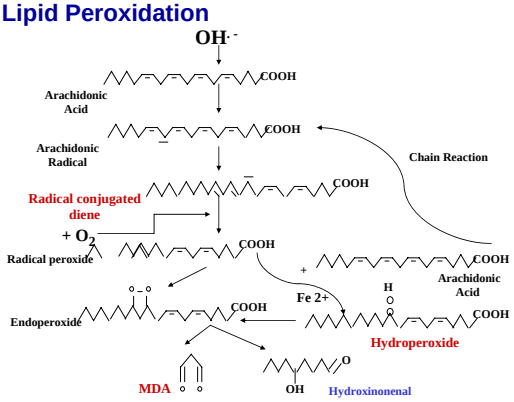
Lipid Hydroxides

Radicals Alcoxyl RO•
- Alcanes
- MDA
- Hydroxinonenal

Radicals Peroxyl ROO•
- F 2 Isoprostanes
- Mono/dehydroxyperoxides in fatty amino acids

Posterior Peroxidation

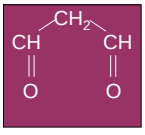
Lipid Peroxidation



ORIGIN of MDA

Lipid peroxidation "in vivo" circulates in the plasma

Freed during the synthesis of the prostaglandin enzyme



MDA

Generated by breaking peroxides "in vitro"

Eliminated by Renal function

PROTEIN OXIDATION : CARBONYL GROUPS

They are chemical compounds -C=O that are formed as a result of the oxidation of proteins (so their function is inactivated).

They are also formed as a result of a sugar reacting with –NH2 (aa) of the proteins. (Maillard Reaction) which happens spontaneously to an Amadori product, from which group C=O can react with other proteins resulting in "Cross-linking".

However, carbonyls can be formed in other situations such as involved with lipids, or DNA, therefore they can cause "cross-linking"; protein-protein & protein-DNA, or protein-lipid, which will also be harmful in the body.

CARBONYL GROUPS

•The molecules that are affected can be: collagen, elastine, enzymes, immunoproteins. Those that facilitate this "cross-linking" are the carbonyl groups that act as "glue" sticking the two proteins together. This can form large agregates (AGEs, or glycotoxins) who can interreact with the free radicals and cause cellular lesions.

•The formation of AGEs accelerates during hyperglycaemic stages such as Diabetes, and in this way can be aided by metals such as Cu & Fe.

AGEs

•Once formed, the AGEs inhibit the cellular transport processes, they stimulate the cells to produce free radicals (such as Superoxide & nitric oxide) and activate pro-inflammatory cytokines such as TNF-a & IL-6.

•Also, some AGEs are immunogenic (causing auto-immune processes) or mutations.

Signs of AGEs

1.- It can be found in cartilage and endothelium, it can be analytically shown through hydrolysis and chromatographical determination of Pentosidine and/or furosine.

2.- Its presence in urine and plasma has been demonstrated in diabetic patients.

3.- Its formation originates from excess glucose.

4.- Can act as indicators in blood serum
Fructosamine serum
Hb1c in blood

Pathologies associated with AGEs

- Diabetic Retinopathy
- Fixation of oxidated LDL (artherosclerosis)
- Fixation in glomerulus (glomerulosclerosis)
- Fixation to immunoglobulins (careful immunitary system)

PROTEIN OXIDATION : AOPP

Advanced Oxidation Protein Products
The levels of AOPP in plasma correlate with those of Dityrosine and AGE-Pentosidine, as index of oxidative protein lesion.

They also correlate with the levels of Neopterine, indicator of inflamatory processes by monocytes.

Do not correlate with TBARS or MDA who are indicators of lipid oxidation.

Free Rad Biol Med (2003)
The J of Inmunology (1998)
Nephrol Dial Transplant (1999)
Kidney International (1996)

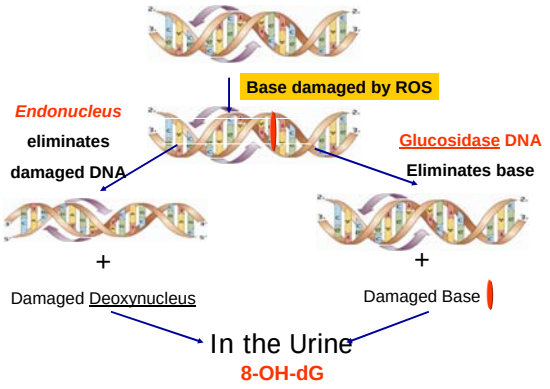
Can one act against the formation of AGEs ?

Aminoguanidine reacts with intermediaries dicarbonyl and blocks the reactionnary sequence of AGEs formation.

During Experiments on diabetic Rats :
Delays microvascular injuries in the retina
Delays microvascular injuries in the glomerulos
Delays neuropathy periferic aparition.
In Humans:
2001. Multicentric study (USA & EU) Faze III

OTHER: Carnosine, Metformine, Acarbose...

DNA Damage



Oxidative Stress and its relationship with inflammation

Inflammation:

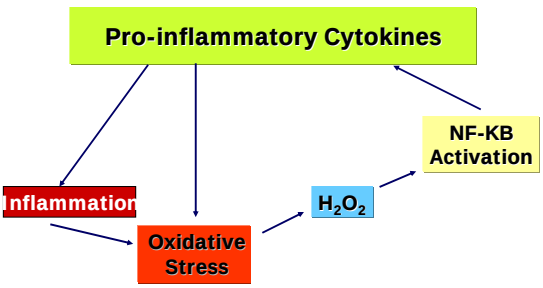
Arachidonic Acid → Free Radical Oxidation

- F2-Isoprostanes **vasoconstriction**
- OH^- **Oedema**
- $PGE_2 + OH^-$ **Pain**

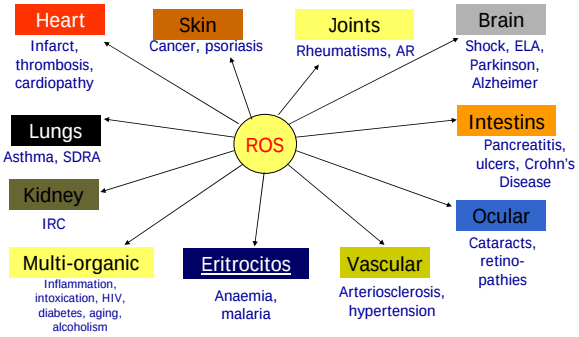
Oxidation by cyclo-oxygenase

Oxidative Stress - Inflammation

"Feed-Back" spiral between Oxidative Stress - Inflammation:



Associated Pathologies



Anti-oxidant defense strategies

1.- Prevention:
- First line of defense is the protection against its formation

2.- Interception
- Once the oxidants are formed, stop them so that they cannot exert its activity = De-activation

Free Radical → Non Radical Product

3.- Repair
- Repair the damage once produced:

DNA / Membranes / Proteins

Anti-oxidant defense strategies

1.- Prevention:

Protection against its formation

Examples:

Cytochrome Oxidase: Does not produce Free Radicals or superoxides

Melanin of the skin: Protects agaisnt UV radiations

Metal fixating proteins : Transferrin, Ferritin, Ceruloplasmine

Enzymatic Reactions in cascade: Glutathione-S-Transferase

Carotenoids: Fixate single oxigen $^1O_2$

Anti-oxidant defense strategies

2.- Interception

- Non enzymatic systems : Anti-oxidants mecanisms in the ample sense
  - Deactivation of a Free Radicals being transformed into a non-Radical molecule they have a half-life < 1 hour.
  - Transfer the Free Radicals into a medium where it has less harmful effects
- In general transfer Free Radicals from the lipid stage to watery stages.
  - For example:
    - •From membranes to cytosol
    - •From lipoproteins to the watery stages of the plasma where its regeneration with anti-oxidants will be easier.

Anti-oxidant defense strategies

Membranes:

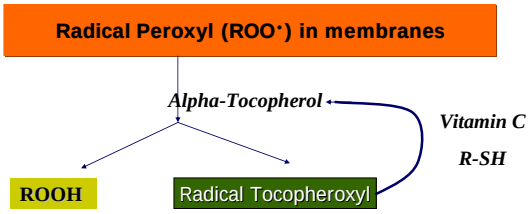
α- Tocopherol → Radical Tocopherol

Ascorbate/Thiols

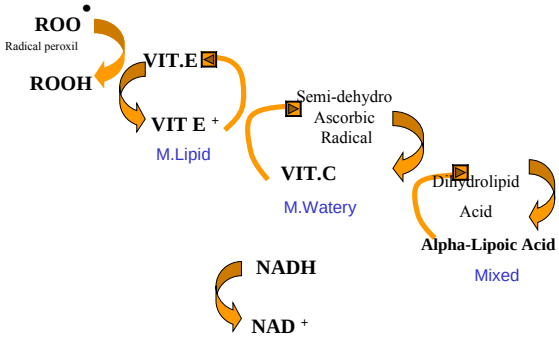
Enzymatic Systems :

- **Superoxide Dismutase**
- **Catalase**
- **Glutathione Peroxidase**
- **Others**

Alpha-Tocopherol Acts:



Redox Cycle of major anti-oxidants

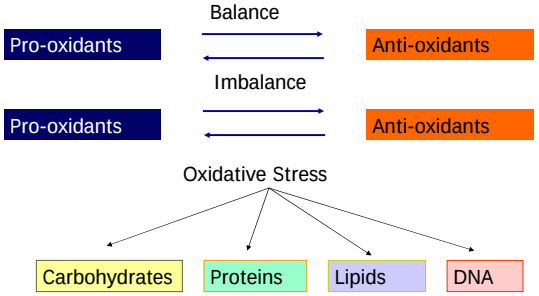


Anti-oxidant defense strategies

3.- Repair

- **Repair damaged molecules by :**
  - Proteins, lipids, membranes, DNA
- **Complete reactions from which it is difficult to pharmacologically react**

Evaluation of aging

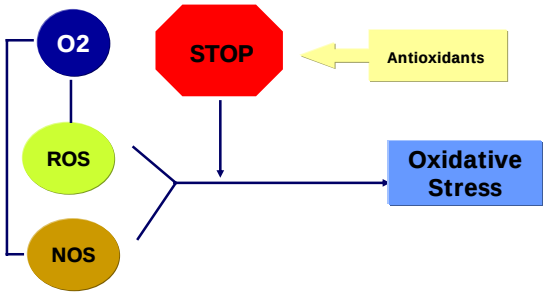


Anti-oxidant Mechanisms

Anti-oxidants

Substances that are present in low concentrations, compared to those of the oxidable substrate, which reduce or prevent its oxidation

Function of Anti-oxidants



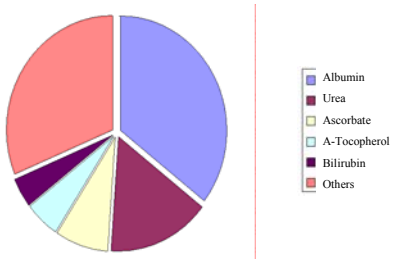
Relative anti-oxidant capacity of different plasmatic metabolites

Albumin	0.69
Ascorbic Acid	1.00
Bilirubin	1.50
Cystine	0.28
Methionine	0.00
alpha-Tocopherol	0.90
Uric Acid	1.00
Urea	0.00
Tyrosine	0.38

Trolox equivalents

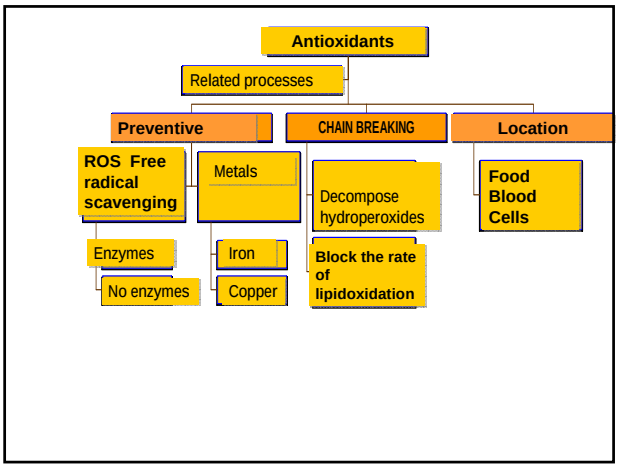
Total anti-oxidant power

Measures the Global state of the anti-oxidant capacity
Relative contribution to PART from different groups of substances



Effects of anti-oxidants

- Breakdown hydroperoxides (ROOH)*
  To convert them into alcohols (ROH).
- Reduce the local concentration of oxygen
- Prevent the initiation process of lipid peroxidation by capturing ROS.
- Fix iron or copper.
- Block the lipoperoxidation chain.



Antioxidants: Chain Breaking

- Breakdown hydroperoxides
  - **Gpx**
- Block lipoid peroxidation chain:
  - Vitamin E
  - ß-carotene
  - Ubiquinone/ Coenzyme Q10
  - Dehydrolipoatrophic

Enzymatic Anti-oxidants : Superoxide dismutase

- Discovered by Mc Cord & Fridovich in 1969.
- $O^{-}_{2} + O^{-}_{2} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$
- **Cu-Zn-SOD**: cytosolic and extra cellular
- **Mn-SOD**: mitochondria

Enzymatic Anti-oxidants : Glutathione Peroxidase

Location

Cytoplasmic Location (liver, renal cortex, adrenal glands, lymphatic nodes)

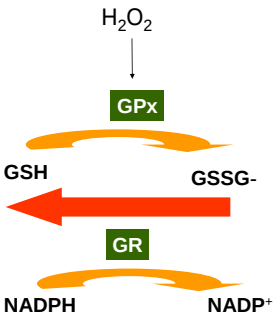
$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2\,H_2O$

GSH: Glutathione reduced
GSSG: Glutathione oxidated

Selenium

Dependent selenium enzyme.

Enzymatic Anti-oxidants : Glutathione Reductase

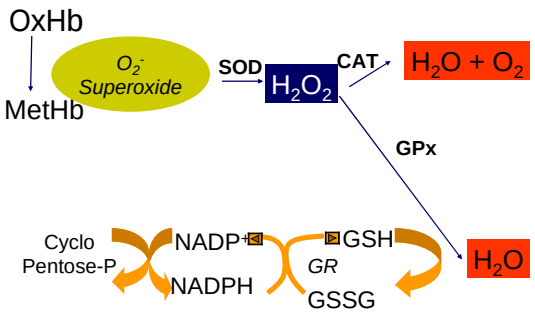


Enzymatic Anti-oxidants : Catalase

Found in peroxisomes,
mainly of the liver and erythrocytes

$2H_2O_2 \xrightarrow{Catalase} 2\,H_2O + O_2$

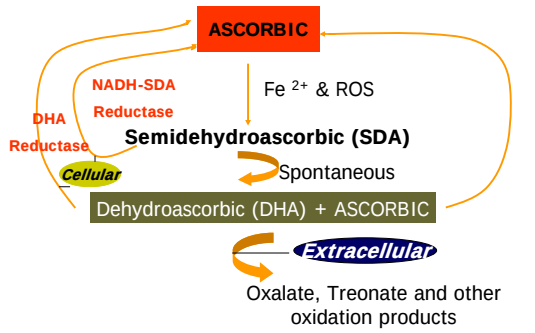
Enzymatic Interactions :



NON ENZYMATIC Antioxidants

WATER SOLUBLE	EFFECT	Plasma Conc. (μM)
Ascorbic	Scavenger of O_2^-, $^1O_2$	30-150
SH Groups, (Glutathione, albumin)	Scavenger of O_2^-, $^1O_2$	1-2
Uric Acid	Scavenger of OH^-, $^1O_2$ Chelation of Fe^2, Fe^3 y Fe^4	160-450
Bilirubin		5-20
Flavonoids		
LIPOSOLUBLES		
α-tocopherol		15-40
γ-tocopherol		3-5
α-carotene		0.05-0.1
β-carotene		0.3-0.6

Ascorbic as a pro-oxidant



Laboratory Profiles

Oxidative Stress Profile

Evaluation of redox equilibrium

Markers of oxidation

Lipids

Proteins

AGEs

DNA

Pro-oxidant Metals

Interception Mechanisms of ROS

Oxidative Stress Profile

1. Markers of lipid oxidation

TBARS (MDA):	Final product of oxidation
Conjugated Dienes :	First phase marker of LDL oxidation
ox LDL	Final phase marker of LDL oxidation correlation with oxLDL in platelets...?
Anti-oxLDL	Correlation titers with oxidation level...?

Oxidative Stress Profile

❷ Markers of lipid oxidation

Hydroperoxides	Oxidation indicator transfered to aqueous phases
F2-Isoprostanes	Marker of the intermediate products of lipid oxidation with metabolic activity.
PUFA (ω_3, ω_6):	Complement of evaluation of the lipid profile.

Inconveniencies of the determination of oxLDL in serum

The oxLDL are in reality various lipid species.

From the extraction of blood and coagulation process, centrifugation, separation of the serum, etc, O_2 from the air oxidates the LDL molecule and these do not diferentiate from oxLDL "in vivo"

The most used method in clinics is by means of **conjugated dienes** first phase of the oxidation process of the LDL- in serum.

Oxidative Stress Profile

Oxidant metals and their indications

- **Iron**
- **Copper**
- **TiBC** → Saturated Transferrin
- **AIBC** → Non-saturated Transferrin
- **Ferritin** → Iron Stock

Oxidative Stress Profile

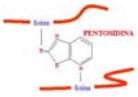
Protein oxidation markers

AOPP: Advanced Oxidation Protein Products
Carbon Groups (=CO)

DNA oxidation Markers

8- OH- deoxy-guanosine (8OHdG)

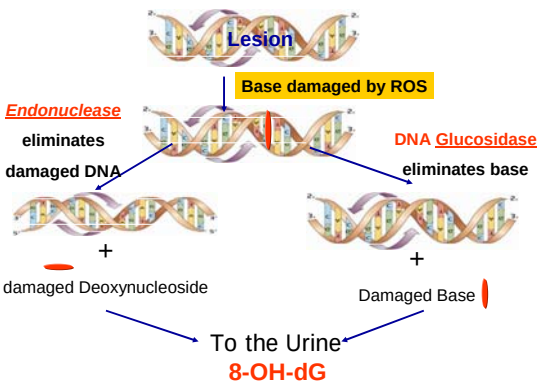
Carbohydrate oxidation /glycation markers



Direct: Pentose, Furosinea

Indirect: Hb1c, Fructosemia

DNA Lesion



Oxidative Stress Profile

ROS InterceptionMechanisms

1.- Anti-oxidant Enzymes

- **Catalase**
- **Superoxide Dismutase (Mn y Cu-Zn) (SOD)**
- **Glutathione Peroxidase (GPx): dependent Se**
- **Glutathione Reductase**

Oxidative Stress Profile

ROS Interception Mechanisms

2.- Protein fixating pro-oxidant metals

- Ferritin
- Transferrin
- Ceruloplasmin
- Lactoferrin
- Cytochrome Oxidase
- Hemoglobin/ Myoglobin

Oxidative Stress Profile

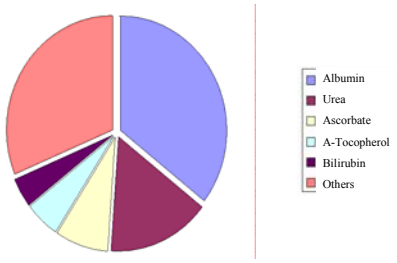
ROS Interception Mechanisms

3.- Neutralisers of Free Radicals (Anti-oxidant)

- Vitamins: A, E, C
- Carotenoids (Carotenes, Lycopene, Lutein...)
- Flavonoids (Quercetin, Rutin, Catequina...)
- Bilirubin
- Uric Acid
- -SH Groups (Total Thiols, GSH...)

Total anti-oxidant power

Measures the Global state of the anti-oxidant capacity
Relative contribution to PART from different groups of substances



Oxidative Stress Profile

ROS Interception Mechanisms

4.- Metals that are co-factors of enzymes

- Manganese (Mn)
- Zinc (Zn)
- Selenium (Se)
- Copper (Cu)

Vitamins, Minerals, Anti-oxidants

Optimal Ranges
Versus
Normal Ranges

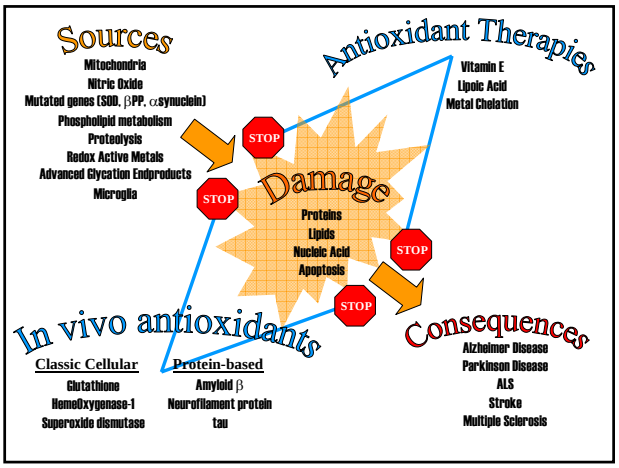
Many Ref.Ranges are not Optimal!!!!!!!!

- Blood levels:

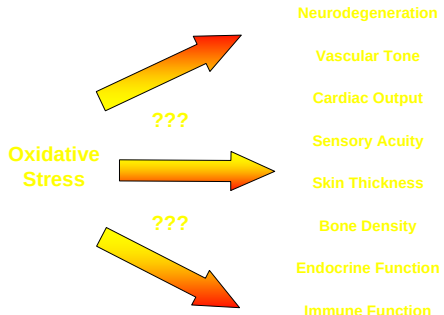
	low risc	Ref.Range?	Opt.Range
-Vit.E µmol/l:	>=30	14-40	30-80
-Vit.C µmol/l:	>=50	20-70	50-150
-Vit.D nmol/l:	>=78	25-100	78-250
-Vit.B6nmol/l:	>=85	12-30	85-180
-Vit.B11µmol/l:	>=30	12-50	30-150
-Vit.B12pmol/l:	>=400	115-850	400-2000
-Selenium pg/l:	>=250	70-250	250- 410
-Homocysµmol/l:	<6-7	5-12	<6-7

Nutrient shortage and DNA damage

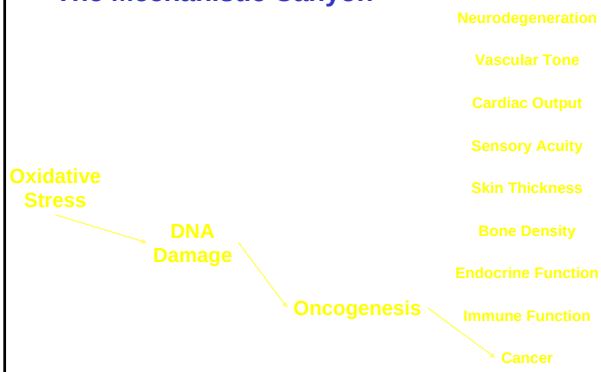
- Vitamin B3 Reduces DNA recovery
- Vitamin B6 Chromosome breakage
- Vitamin B11 Chromosome breakage
- Vitamin B12 Chromosome breakage
- Vitamin C "Irradiation" (DNA oxidation)
- Vitamin E "Irradiation" (DNA oxidation)
- Iron Chromosome breakage/Irradiation
- Zinc Chromosome breakage/Irradiation



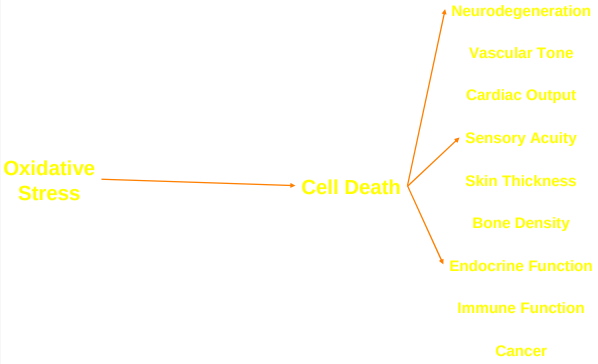
The Mechanistic Abyss



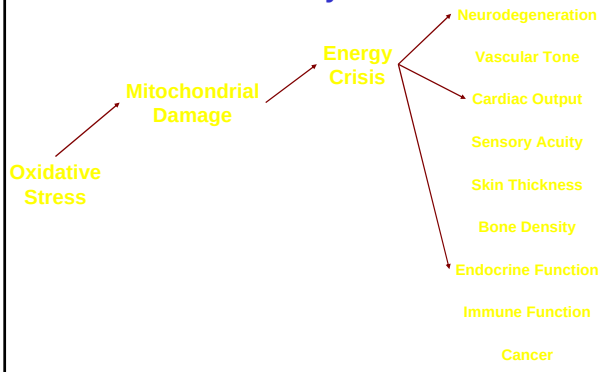
The Mechanistic Canyon



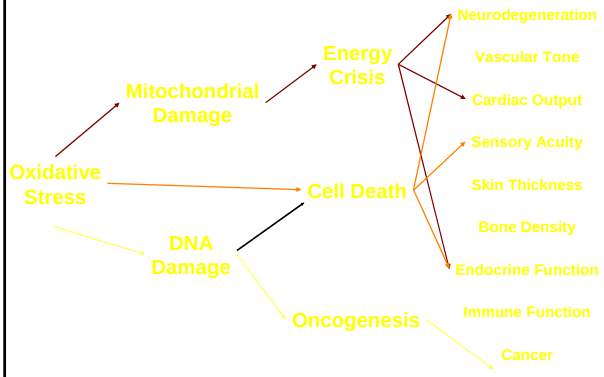
The Mechanistic Canyon



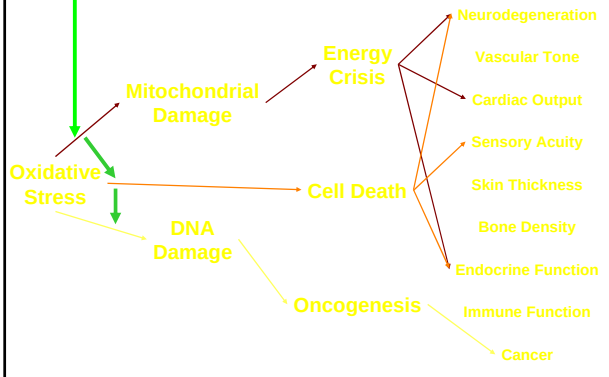
The Mechanistic Canyon



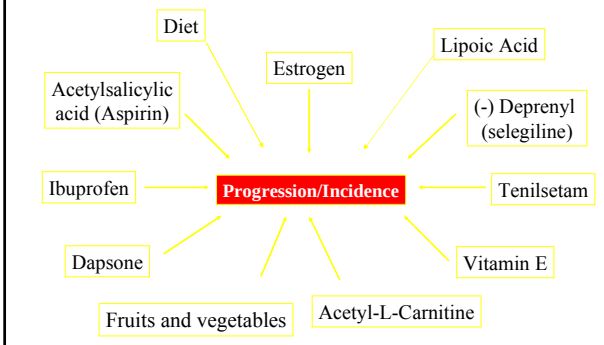
The Mechanistic Canyon



Potential sites of intervention



Antioxidants and anti-inflammatories are protective



Lab Test – Evaluation

Oxidative Stress

- Total Antioxidant Status
- Lipid Peroxides
- Glutathione
- SOD

- Oxidative Profile
  - **DNA: 8-OHdG**
  - **Muscle: Allantoin**
  - **Protein: Carbonyl Proteins**
  - **Membrane: MDA**

- Iron
- Copper
- Heavy metals

Antioxidant Levels

- Vitamin Levels
  - Vitamin A (Retinol), Lycopene, α & β Carotene.
  - Vitamin C (Ascorbic Acid),
  - Vitamin E (α & γ -Tocopherol),
  - CoQ10.
- Amino Acids
  - tryptophan, tyrosine, cysteine and homocysteine
- Minerals
  - Zinc, Selenium
- Other
  - Melatonin
  - E2

Antioxidant Factors Lab report

Preferred Anti-Oxidant Status Assessment Test

Vitamin A				
RETINOLS				
Retinol	1209		800-1400	ug/L
CAROTINOIDS				
Carotenes:				
Beta carotene	754	*H	381-628	ug/L
Alpha carotene	191	*H	67-171	ug/L
Lycopene	280		225-368	ug/L
Xanthophylls:				
Luteine	666	*H	267-400	ug/L
Zeaxanthine	5.0		2-9	ug/L
Crytoxanthine	26.0		2-30	ug/L
Vitamin E				
Alpha Tocopherol	11.0*L		12.9-17.3	mg/L
Gamma Tocopherol	2.3*H		0.75-1.28	mg/L
Vitamin C				
Ascorbic Acid	11.5	*L	14-30	mg/L
CoQ10				
CoQ10	0.46	*L	0.7-1.2	mg/L

Test Protocol

- The Oxidative Stress panel includes measurement of:
  - blood glutathione,
  - lipid peroxides,
  - Glutathione peroxidase (GSH-Px),
  - Superoxide dismutase (SOD) and

Test Protocol
(best & cost effective panel)

- **Oxidative Damage Markers**
  - **DNA: 8-OHdG markers**
  - **Muscle: Allantoin**
  - **Protein: Carbonyl Proteins**
  - **Membrane: MDA**

Preferred Test for Oxidative Damage Assessment

Oxidative Damage Markers
Lab Report

- **Membrane Oxidative Damage**
- Malonydialdehyde (MDA) 64 *H (40-60) ug/L
- **Protein Oxidative Stress**
- Carbonyl Proteins 0.98 *H (0.6-0.9) nm/mg
- **Muscle Mass Oxidative Damage**
- Allantoin 166.6 *H (70-130) *umol/L*
- **DNA Oxidative Stress**
- Urinary 8-OHdG 5.0*H (1.09-1.45) nmol/mmol Cr

Comments & Treatments
on Reports

- **8-OHdG** is an index of genetic oxidative damage (damage to chromosomes/genes), which is a major contributor to the ageing process and related degenerative diseases.
  Smokers excrete 50% more 8-OHdG than non-smokers.
- **Elevated levels are consistent with excess free radical damage of genes.*
  *Higher levels of **water** soluble and **fat** soluble anti-oxidants are indicated. Supplementation with alpha lipoic acid and glutathione also indicated.*

- **Malondialdehyde (MDA),** the end product of lipid peroxidation. This is a test for fat soluble vitamin deficiencies. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in humans. MDA reacts readily with amino groups on proteins and other bio-molecules to form a variety of adducts, including cross-linked products. MDA also forms adducts with DNA bases that are mutagenic and possibly carcinogenic.
- ** Elevated levels are suggestive of the need for supplementation with Vitamin A & E.*

If needed…
Genomics - SNPs and Gene expression

- Enzymes involved in protection from free radical/stress/ detoxification and metabolism
- SOD-1 & 2 & 3
- GSHPx
- GSTM, GSTP, GSTP1
- CYP1A1-1/ CYP1A1-2/ CYP1B1
- CYP17/ CYP19
- COMT
- Estrogen receptor-a
- MTHFR
- PPARg