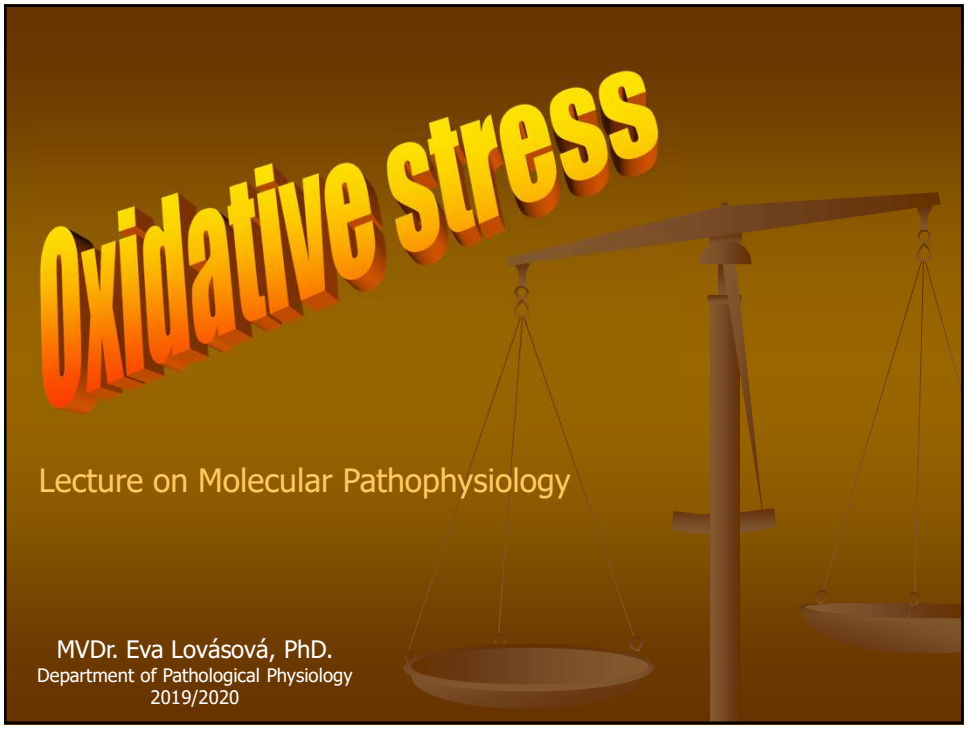
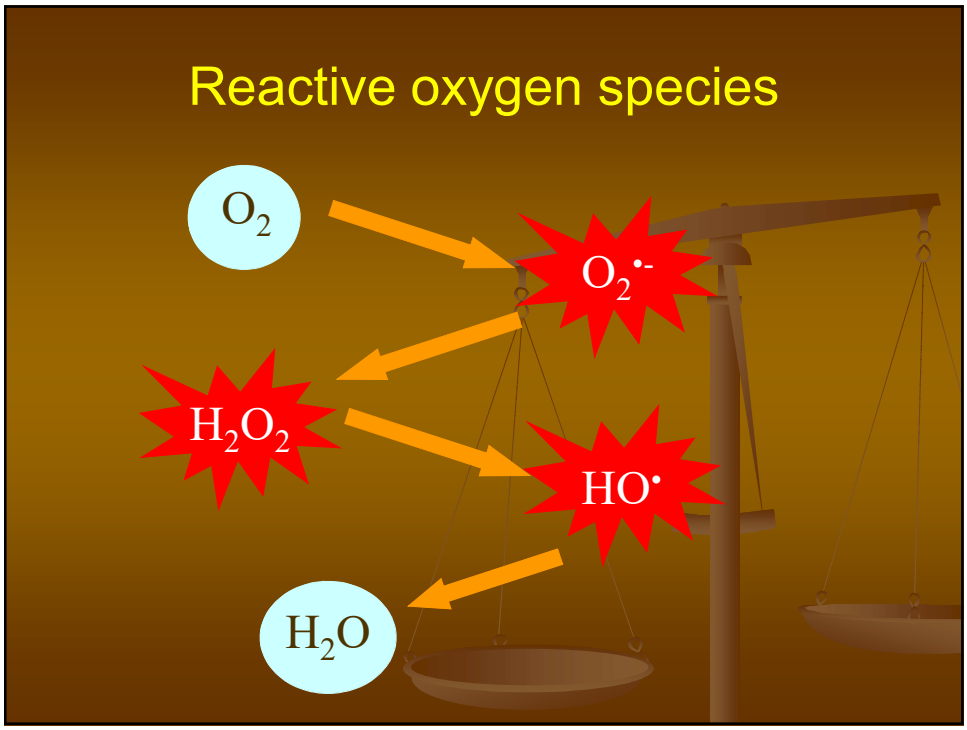
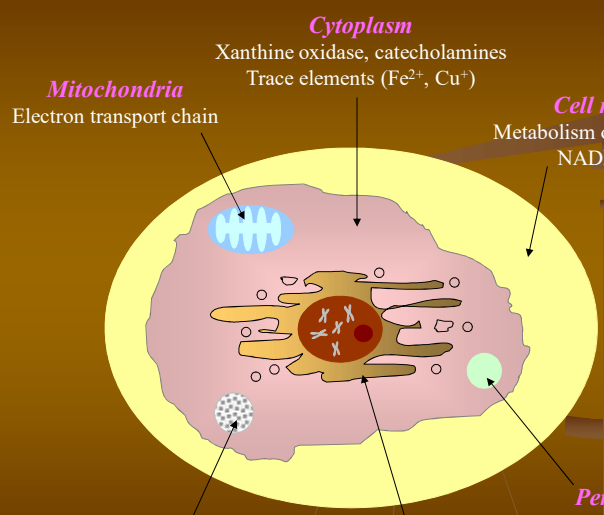

Oxidative stress
Lecture on Molecular Pathophysiology
MVDr. Eva Lovásová, PhD.
Department of Pathological Physiology
2019/2020


Reactive oxygen species
O_2
$O_2^{\bullet -}$
H_2O_2
$HO^{\bullet}$
H_2O

Free radicals	*RS without unpaired electrons*
Reactive oxygen species	
superoxide $O_2^{\cdot -}$	hydrogen peroxide H_2O_2
hydroxyl radical $HO^{\cdot}$	ozone O_3
perhydroxyl radical $HO_2^{\cdot}$	singlet oxygen $^1O_2$
Reactive nitrogen species	
nitric oxide $NO^{\cdot}$	peroxynitrite $OONO^-$
nitric dioxide $NO_2^{\cdot}$	peroxynytous acid HOONO
	alkylperoxynitrite ROON
Reactive organic species	
alkyl radical $R^{\cdot}$	alkylhydroperoxide ROOH
alkoxyl radical $RO^{\cdot}$	alkylperoxynitrite ROONO
peroxyl radical $ROO^{\cdot}$	
thyil radical $RS^{\cdot}$	
Other	
	chlorous acid HOCl

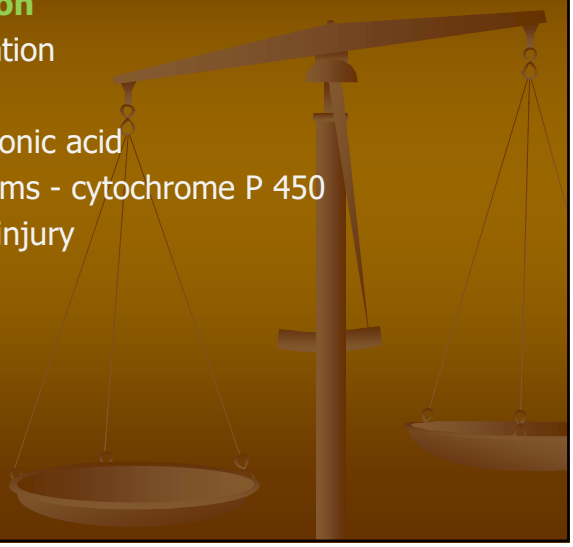
ROS production



Production of ROS

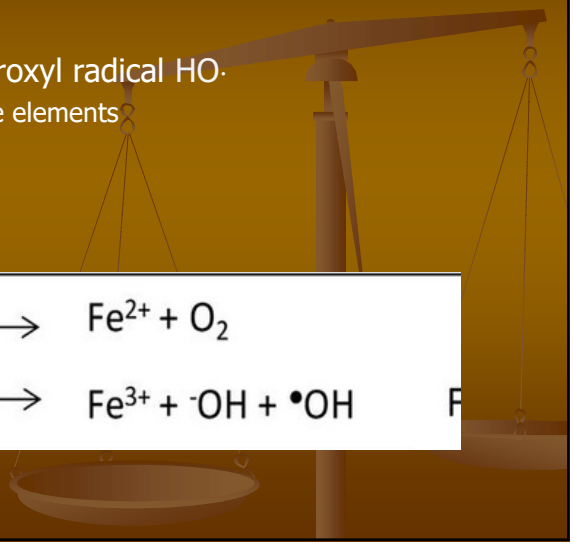
Endogenous production

- Oxidative phosphorylation
- Respiratory burst
- Metabolism of atachidonic acid
- Monooxygenase systems - cytochrome P 450
- Ischemic-reperfusion injury
- Glycation of proteins
- ....



Production of ROS

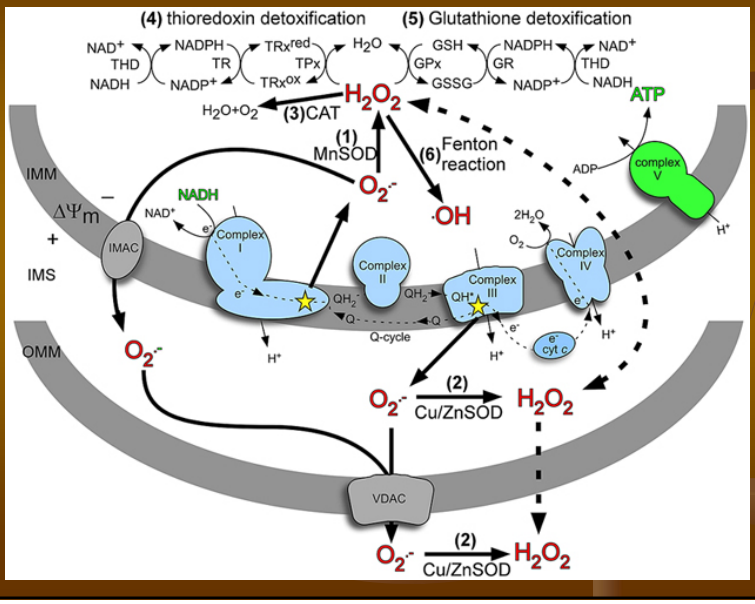
- Fenton reaction
  - Production of hydroxyl radical HO·
    - Catalyzed by trace elements
      - Fe^{2+}
      - Cu^{2+}



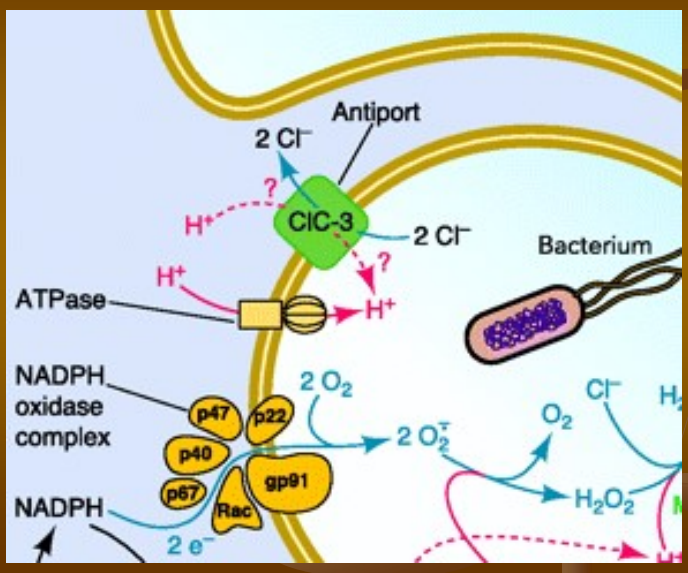
$$Fe^{3+} + O_2^{\bullet -} \longrightarrow Fe^{2+} + O_2$$

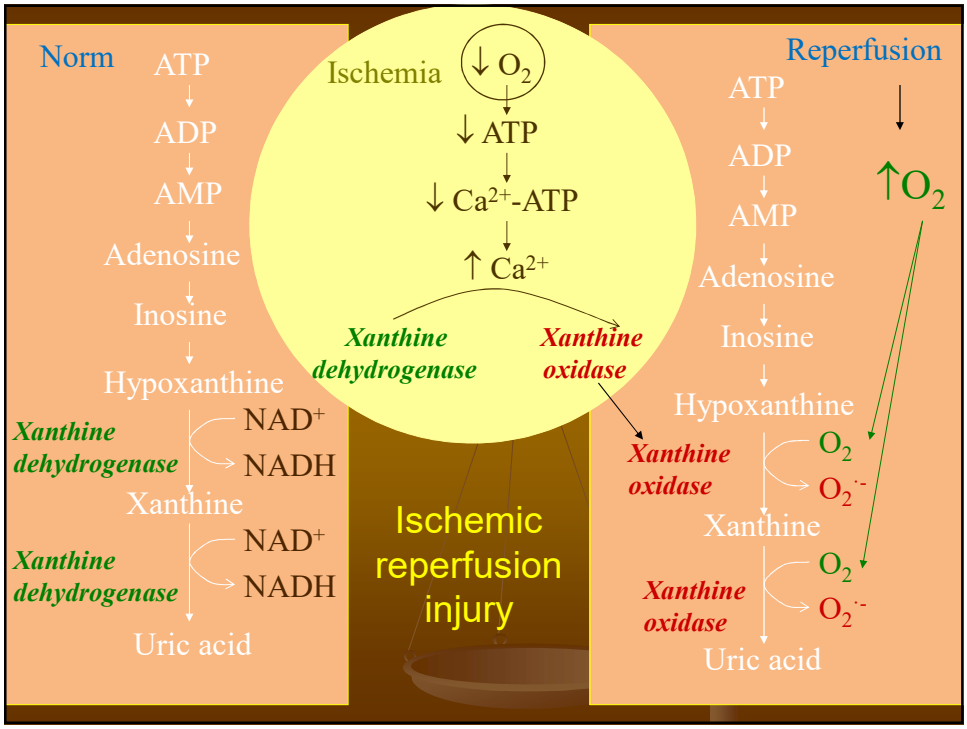
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{-}OH + {}^{\bullet}OH$$

Electron transport chain



Respiratory burst





Production of ROS

External factors

- Smoking
- Radiation – ionizing, gamma, UV
- Ultrasound
- Ozone, nitrogen oxides and other pllutants
- Xenobiotics (heavy metals, adriamycine, primaquin...)

Macromolecules damaged by ROS

Lipids

- Lipid peroxidation
- Mostly affected - double bonds of unsaturad fatty acids
- Production of reactive metabolites (peroxids, aldehydes - MDA)

Consequences

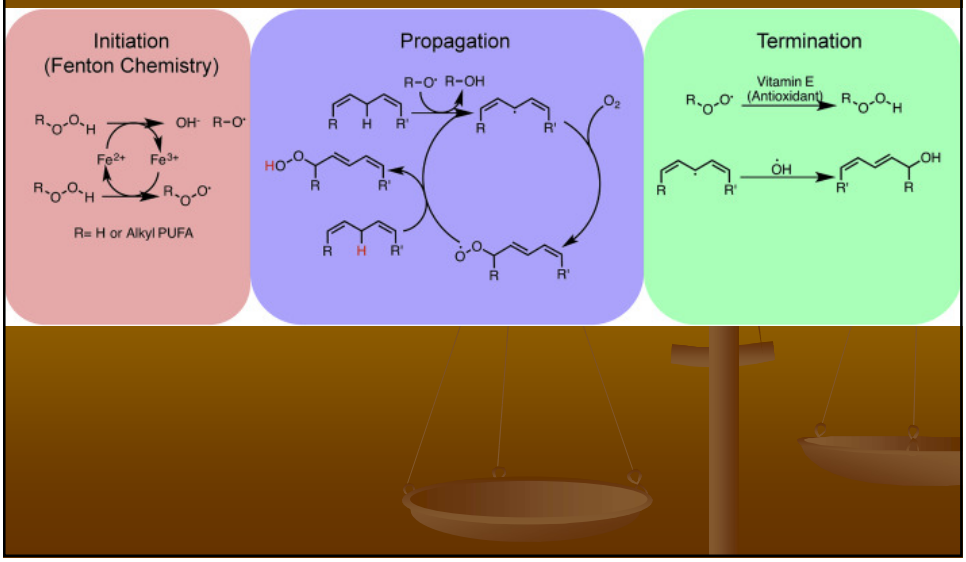
- Change of mambrane fluidity
- Changed transport mechanisms
- Changed activity of enzymes bound on membranes
- ...

Lipid peroxidation

Stages

1. **Initiation**
   - Initiators - reactive oxygen species (ROS) - $OH^{\cdot}$, $HOO^{\cdot}$
   - Production of fatty acid radical
2. **Propagation**
   - The fatty acid radical is unstable molecule - reacts with molecular oxygen - creating a peroxyl-fatty acid radical
   - Peroxyl-fatty acid radical is also an unstable - reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide
   - This cycle continues, as the new fatty acid radical reacts in the same way
3. **Termination**
   - The radical reaction stops when two radicals react and produce a non-radical species.
   - Molecules that terminate by neutralizing free radicals – antioxidants – e.g. vitamin E.
   - The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE)

Lipid peroxidation



Macromolecules damaged by ROS

Proteins

- Mostly affected - SH groups
- Crosslinks between protein chains

Consequences

- Changes in enzyme activity
- Changes in transport and ion concentration

Macromolecules damaged by ROS

DNA

Consequences

- Mutations
- Inhibition of proteosynthesis

Three lines of antioxidant defence

Prevention

- Binding of ions
  - Transferrin, ceruloplasmin – binding of ions – prevention of Fenton reaction
- Integrity of structures
  - Interity of membranes

Active defence

- Enzymes
- Not enzymatic antioxidats

Reparation

- Proteolysis of damaged proteins
- Lipolysis of damaged lipids
- DNA reparation

Enzymatic antioxidants

Superoxide dismutase (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide..

$$2O_2.^- + 2H^+ \rightarrow O_2 + H_2O_2$$

Catalase (peroxisomes) reacts with the hydrogen peroxide to catalyze the formation of water and oxygen.

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

Enzymatic antioxidants

Glutathione peroxidase (GPX) reduces hydrogen peroxide or organic hydroperoxides on water and oxidizes tripeptide glutathione (GSH).

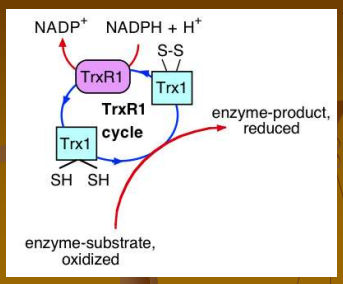
$$ROOH + 2\ GSH \rightarrow ROH + GSSG + H_2O$$
$$H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$$

Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form (GSH).

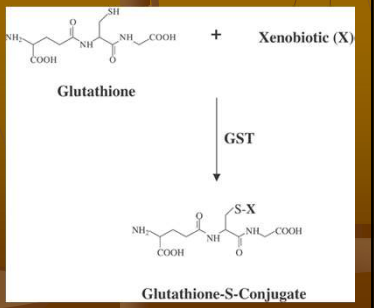
$$NADPH+H^+ + GSSG \rightarrow NADP^+ + 2GSH$$

Enzymatic antioxidants

Thioredoxins (TRX) – proteins that act as antioxidants by facilitating the reduction of other proteins by cysteine thion-disulfide exchange.



Glutathione-S-transferase (GST) – group of isoenzymes (α, δ, κ, μ, ω, θ, τ, ζ, mikrosomal) – conjugation of glutathion with xenobiotics



Macromolecular endogennous antioxidants

- Transferrin
- Ferritin
- Ceruloplasmin
- Haptoglobin
- Hemopexin
- Albumin

bind ions – prevention of ROS production

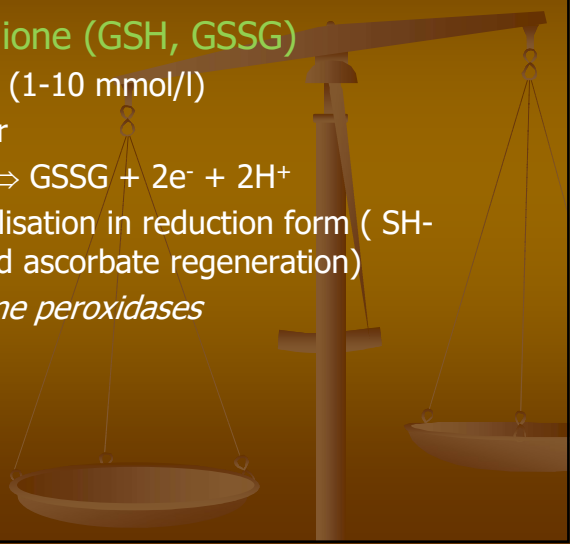
Low-molecule endogennous antioxidats

Glutathione (GSH, GSSG)

- in all mammalian cells (1-10 mmol/l)
- important redox buffer

$$2\ GSH \Leftrightarrow GSSG + 2e^- + 2H^+$$

- ROS elimination, stabilisation in reduction form (SH-groups, tocopheryl and ascorbate regeneration)
- substrate of *glutathione peroxidases*



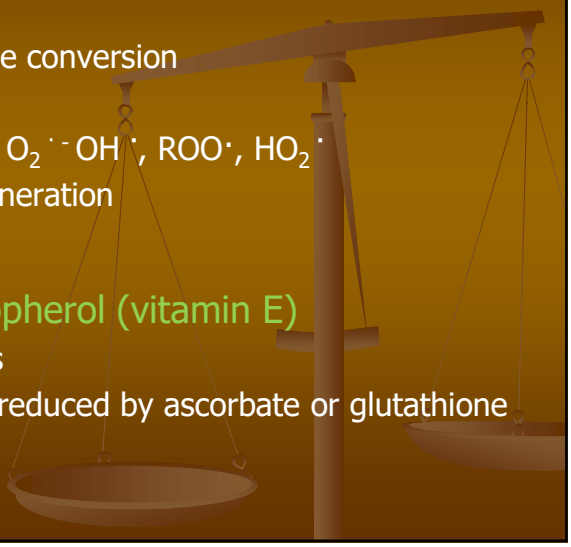
Low-molecular endogennous antioxidats

Ascorbate (vitamin C)

- collagen synthesis
- dopamine to epinephrine conversion
- Fe absorption
- antioxidant = reduction $O_2^{\cdot -}$ $OH^{\cdot}$, $ROO^{\cdot}$, $HO_2^{\cdot}$
- tocopheryl radical regeneration

Alfa-tocopherol (vitamin E)

- localised in membranes
- tocopheroxyl radical is reduced by ascorbate or glutathione



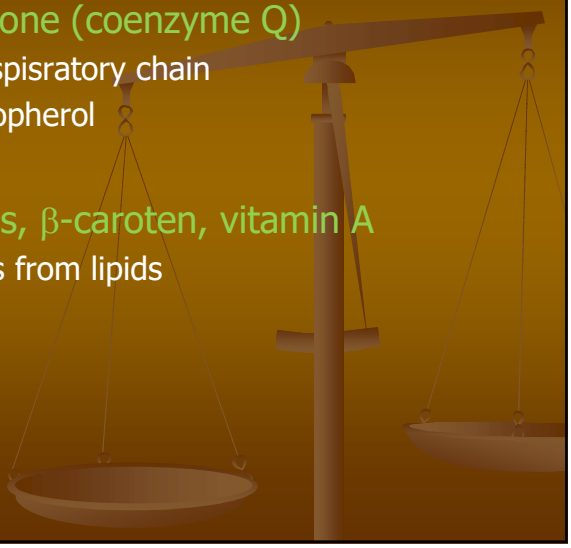
Low-molecular endogennous antioxidats

Ubiquinone (coenzyme Q)

- electron carrier in respisratory chain
- co-operates with tocopherol

Carotenoides, β-caroten, vitamin A

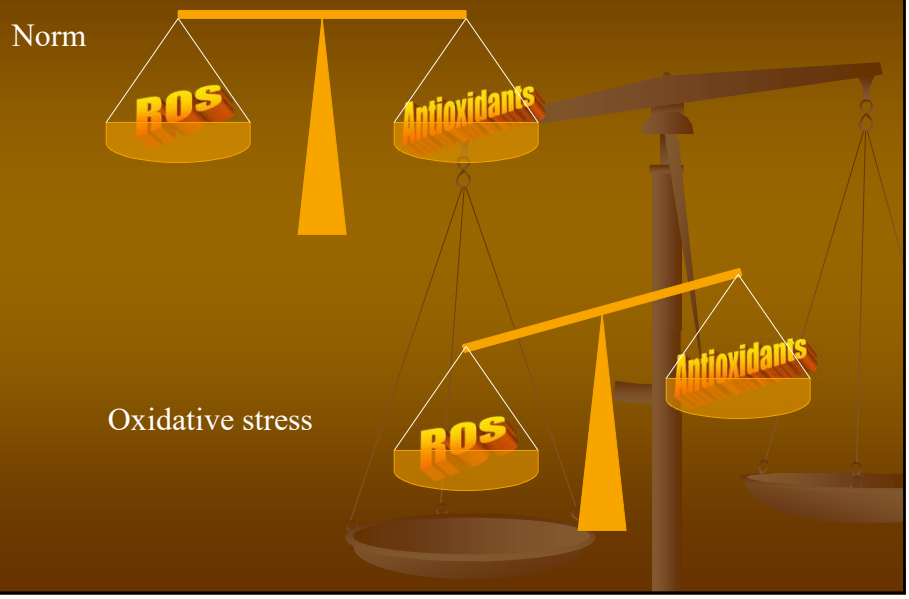
- removing the radicals from lipids



Low-molecular endogennous antioxidats

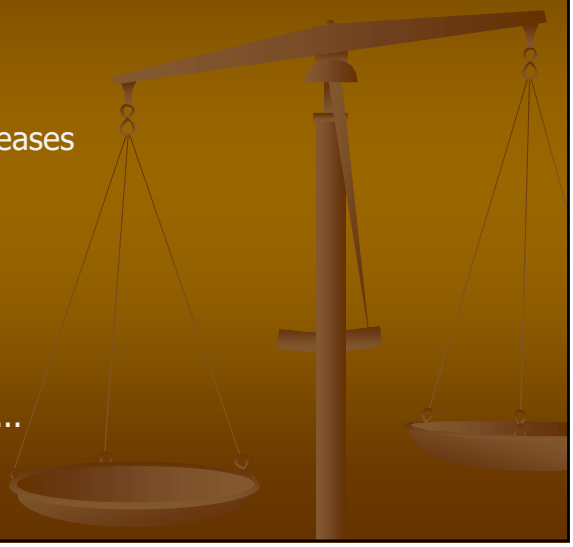
- Lipoic acid
- Uric acid
- Bilirubin
- Flavonoids
- Melatonin

Oxidative stress



Disorders associated with oxidative stress

- Inflammation
  - arthritis
  - pancreatitis
  - autoimmune diseases
- Intoxications
  - smoking
  - pollutants
  - alkohol
  - adriamycine
  - tetrachlormetan...



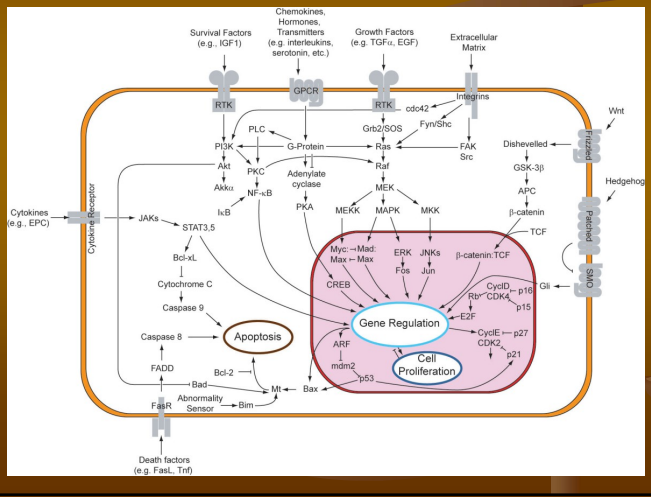
Disorders associated with oxidative stress

- Ischemic-reperfusion injury
  - CHD and MI
  - Brain ischemia
  - transplantations
- Cancers
- Atherosclerosis
  - oxLDL
- Diabetes mellitus
- Hemochromatosis
- Unfertility

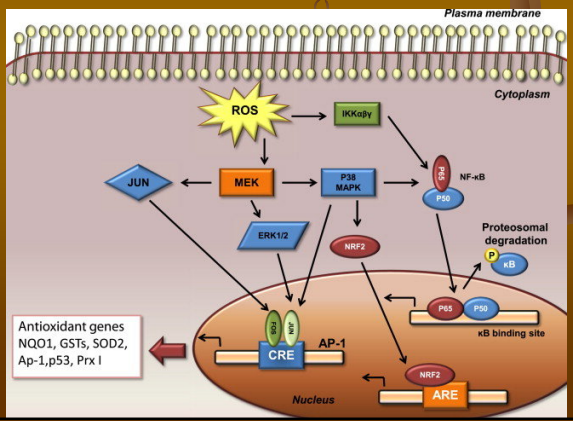
Disorders associated with oxidative stress

- Neurological and psychiatric diseases
  - Amyotrophic lateral sclerosis
  - Parkinson´s disease
  - Alzheimer´s disease
  - Huntington´s disease
  - Schizophrenia
  - Epilepsy
  - Multiple sclerosis
- Ageing
- Sport and muscle work

Physiological role of free radicals in signal transduction



The wide spectrum of proteins (as well as their genes), important in the process of signal transduction has been demonstrated to be targets of direct effect of ROS. These proteins include growth factors receptors, protein kinases, G-proteins and nuclear transcription factors.



Receptors

Growth factor receptors – epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) receptors as well as insulin receptor are receptors with tyrosine kinase activity that have been reported to be affected ROS. Although exact mechanism is unknown, it is documented that EGF receptor can be activated by ROS generation, which may trigger the conformational changes in the receptor.

Protein kinases

Besides receptor tyrosine kinases, non-receptor proteine kinases can be activate ROS. Mitogen-activated protein kinases (MAPKs) are the group of intracellular serine/threonine protein kinases that relay signals from receptor to the nuclear transcription factors via phosphorylation of proteins in signal transduction pathway. There are four known MAPK families: extracellular-regulated kinase (ERK), c-jun-NH_2-terminal kinase (JNK), p38, and big MAPK-1 (BMAPK-1). Recent studies have documented the ROS-mediated regulation of MAPK. Production of superoxide and especially hydrogen peroxide activates ERK1 and ERK2 and also the recently discovered BMAPK-1.

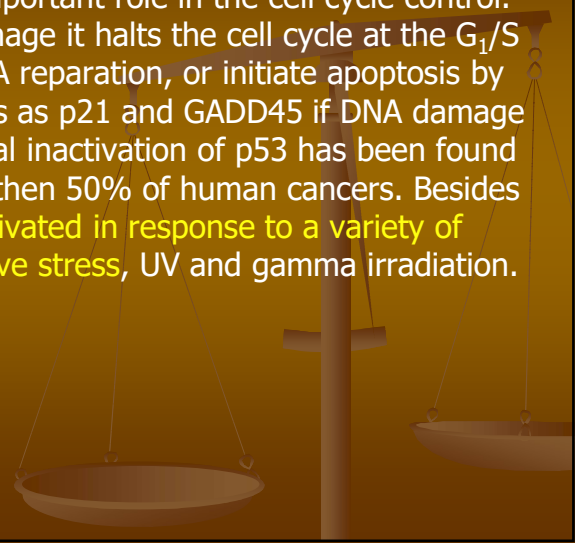
Src is a proto-oncogenic non-receptor tyrosine kinase, member of several signal transduction pathways. This protein plays a role in the regulation of such important processes as growth, differentiation, adhesion and transcription. Its activity can be influenced also by ROS and UV radiation.

G-proteins

Ras gene products are membrane-bound G-proteins (GTPases). They are involved in cellular signal transduction and regulate cell growth, differentiation and apoptosis. Ras gene mutation has been found in about 30 % of all cancers include lung, skin, liver bladder and colon carcinomas. Mutations in two ras genes (H-ras a K-ras) are associated with metal-induced carcinogenesis. These mutations may result in constitutive activation of the signal transduction pathways and constant growth activation. Ras mutations can be induced by oxidative stress and UV radiation.

Nuclear transcription factors

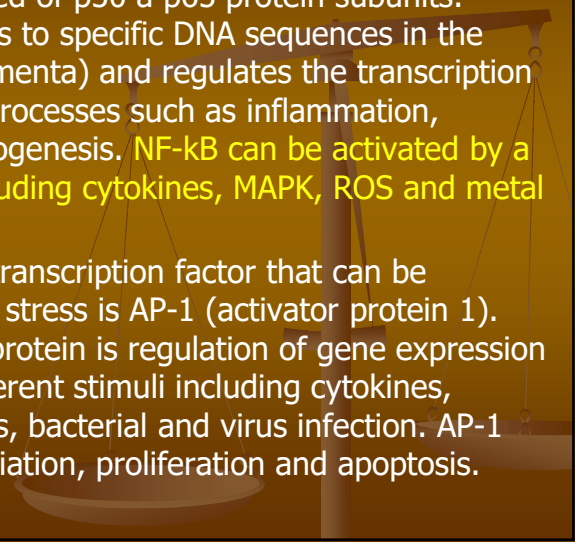
The nuclear transcription factor p53 is a tumor suppressor protein that plays an important role in the cell cycle control. In the case of DNA damage it halts the cell cycle at the G_1/S regulation point for DNA reparation, or initiate apoptosis by activation of such genes as p21 and GADD45 if DNA damage is irreparable. Mutational inactivation of p53 has been found to be involved in more then 50% of human cancers. Besides DNA damage p53 is activated in response to a variety of stimuly, such as oxidative stress, UV and gamma irradiation.



Nuclear transcription factors

Transcription factor NF-kB (nuclear factor kapaB) is a heterodimer composed of p50 a p65 protein subunits. Activated NF-kB binds to specific DNA sequences in the target genes (kB elementa) and regulates the transcription of genes mediating processes such as inflammation, apoptosis and carcinogenesis. NF-kB can be activated by a variety of stimuli including cytokines, MAPK, ROS and metal ions.

The additional nuclear transcription factor that can be affected by oxidative stress is AP-1 (activator protein 1). The function of this protein is regulation of gene expression as an answer on different stimuli including cytokines, growth factors, stress, bacterial and virus infection. AP-1 controls cell differentiation, proliferation and apoptosis.



Protein	Function	Factors
EGFR	receptor	ROS, oxidative stress
VEGFR	receptor	ROS, oxidative stress
PDGFR	receptor	ROS, oxidative stress
IR	receptor	ROS, oxidative stress
MAPK	Protein kinase	ROS, oxidative stress
Src	Protein kinase	ROS, UV radiation
Ras	G protein	ROS, UV radiation
P53	nukleárny transkripčný faktor	ROS, oxidative stress, MAPK, hypoxia
NF-kB	nuklear transcription factor	ROS, oxidative stress, cytokins
AP-1	nuklear transcription factor	ROS, MAPK, cytokins, UV radiation

Oxidative stress and some diseases

Neurodegenerative diseases

Amyotrophic lateral sclerosis

- Amyotrophic lateral sclerosis (ALS) is a group of neurological diseases that mainly involve the neurons responsible for controlling voluntary muscle movement.
- Mutations (over 150 identified, mainly autosomal dominantly inherited) in SOD1 gene have been linked to familial ALS.
- Wild-type SOD1, under conditions of cellular stress, is implicated in a significant fraction of sporadic ALS cases, which represent 90% of ALS patients.
- The exact molecular mechanism (or mechanisms) by which SOD1 mutations cause disease are unknown. It appears to be some sort of toxic gain of function.

Alzheimer's disease

- Alzheimer's disease (AD), as one of the most common neurodegenerative diseases, is characterized by progressive neuronal loss and accumulation of proteins including extracellular amyloid plaques (Aβ) and intracellular tau tangles (neurofibrillary tangles).
- The accumulation of Aβ seems to increase oxidative stress and lead to mitochondrial dysfunction and energy failure even in early stage of AD
- Oxidative stress can also aggravate the production and aggregation of Aβ and promote the phosphorylation of tau protein.

Parkinson's disease

- Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective neuronal loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and decreased DA levels in the nigrostriatal DA pathway in the brain. Although the exact mechanism still remains unclear, oxidative stress has been considered as one of major pathophysiological mechanisms underlying PD.
- Reduced activity in Complex I of the respiratory chain in SNc of patients with PD, may contribute to the generation of excessive ROS and, in turn, induce apoptosis.
- Genetic mutations in proteins including α-synuclein, parkin, and phosphatase and tensin homolog-induced putative kinase (PINK) were linked to the familial forms of PD. Mutations of these genes have been known to affect mitochondrial function and increase oxidative stress.

Down syndrome (DS)

- The enzyme superoxide dismutase (SOD) is a constitutive enzyme coded by a gene located in Chromosome 21 (21q22.1).
- The tissues from patients with trisomy 21 contain 50% more SOD activity.
- It is often suggested that the increased SOD content in the cells of Down's syndrome patients is responsible for many of the symptoms of this disease.
- Increased SOD may be responsible for the increased incidence of Down's syndrome in children of older women. The augmented antioxidant protection resulting from an extra copy of chromosome 21 may, with time, selectively protect human oocytes from apoptosis, increasing their proportion with age, explaining the higher incidence of this disease.
- The clinical signs are connected also to overexpression of other genes also located in chromosome 21 such as the beta amyloid precursor, as well as oncogenes and other Down's syndrome-related genes.

Markers of oxidative stress

The assesment of oxidaive stress markers improve understanding of disease pathogenesis, and help the diagnosis of disease, develop therapeutic strategies, and monitor treatment compatibility, performance, and complications.

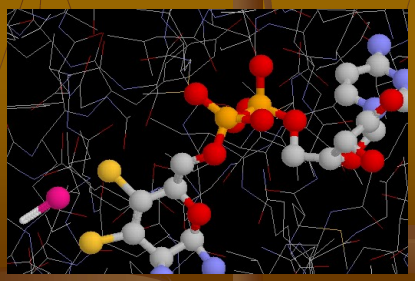
Free radicals

- superoxide $O_2^{\cdot -}$, hydrogen peroxide H_2O_2, hydroxyl radical $HO^{\cdot}$

Methods
- Spin trapping
- Electron paramagnetic resonance
- Pulse radiolysis
- ...

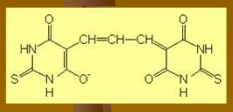
Sources of ROS and RNS

- Xanthine oxidase
- NO synthase



Products of oxidative stress

- Conjugated diens and lipid peroxides
- Malondialdehyde
- 4-hydroxy-2-nonenal
  = Products of lipid peroxidation



- Oxigenated lipoproteins - oxLDL
- Oxidative damage of saccharides

Products of oxidative stress

- Carbonyl groups
- Protein hydroperoxides
  = Products of oxidative damage of proteins
- Oxidative damage of nucleic acids
- Assesment of autoantibodies against modificated biomolecules
- AGEs – advanced glycation end-products

Antioxidants

- Glutathione
- Ascorbate
- Tocopherols, ubiquinol, carotenoids and retinoids
- Superoxide dismutase
- Glutathione peroxidase
- Catalase

Antioxidant capacity

- TAS – Total antioxidant status
- TRAP – total radical antioxidant parameter

THANK YOU
FOR YOUR
ATTENTION