Antioxidant Defence
Ageing
Dietary Antioxidants

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**Ionising radiation:**
Hydroxyl radical originates from ionisation of water:
\[ \text{H}_2\text{O} + \text{hv} \rightarrow \text{H}^- + \text{OH}^- \]

**Reactive oxygen species in the body:**
One-electron reduction of oxygen (mitochondria, NADPH oxidase) forms **superoxide** \( \text{O}_2^- \)
Dismutation of superoxide produces **hydrogen peroxide**:
\[ \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]
Fenton reaction with Fe or Cu converts peroxide to **hydroxyl radical**:
\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^- + \text{Fe}^{3+} \]
Oxidative stress

- Levels of reactive oxygen species are kept within certain limits by mechanisms of antioxidant defence
- Oxidative stress occurs if the oxidant/antioxidant balance shifts to oxidation

Antioxidant defence

- prevention of ROS/RNS formation (regulation of producing enzymes, sequestration of metals)
- scavenging, trapping and quenching of radicals
- reparation systems (phospholipases, proteasome, DNA repair)
Antioxidant in food chemistry

- Reducing agent capable of terminating chain reaction of lipid peroxidation
  …“chain-breaking” …
- E.g.:
  - Butylated hydroxytoluene (BHT)
  - Butylated hydroxyanisol (BHA)
  - Tocopherol (Vitamin E)

Antioxidant defence of human body

- Anatomy of the body limiting tissue oxygen
- Antioxidant enzymes
- Sequestration of redox active metals
- Antioxidant substrates (scavengers)
- Stress response
- (Repair systems)
First organism (anaerobic)

- Develop antioxidant defence?
- Die out?
- Resort to anaerobic condition?

Clump together!
Antioxidant defence I

Regulation of tissue O$_2$

- Inhaled air: 160 mmHg O$_2$
- Lung capillaries: 100 mmHg O$_2$
- Arterial blood: 85 mmHg O$_2$
- Arterioles: 70 mmHg O$_2$
- Capillaries: 50 mmHg O$_2$
- Cells: 1-10 mmHg O$_2$
- Mitochondria: < 0.5 mmHg O$_2$

Fig: Wikipedia

Mitochondria originated from phagocyted/parasitic bacteria ...

Fig: Wikipedia
Antioxidant defence II

Antioxidant enzymes

- Superoxide dismutase:
  \[ \text{O}_2^- + \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]
- Catalase:
  \[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]
- Glutathione peroxidase, peroxiredoxin:
  \[ \text{H}_2\text{O}_2 + 2 \text{R-SH} \rightarrow 2 \text{H}_2\text{O} + \text{RS-SR} \]

Superoxide dismutase (SOD)

- Catalyses dismutation of superoxide:
  \[ \text{O}_2^- + \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]
- Absolutely required for life in oxygen
- SOD1: Cu+Zn (cytosol of eukaryotic cells)
- SOD2:
  - Mn (mitochondrial matrix)
  - Fe (bacteria)
- EC-SOD (SOD3): extracellular, Cu+Zn,
  - MW 135,000; binds to heparane sulfate on the inner surface of blood vessels
Glutathione peroxidases (GPX)

- Reduction of peroxides coupled to oxidation of glutathione:
  \[2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS-SG} + 2 \text{H}_2\text{O}\]
  (glutathione is subsequently regenerated by glutathione reductases)
- Active site contains selenium as selenocysteine
- Cytosolic glutathione peroxidase (GPX1):
  – reduces \(\text{H}_2\text{O}_2\) and \(\text{LOOH}\) after release from phospholipids
- Phospholipide hydroperoxide-GSH-peroxidase (GPX4):
  – reduces \(\text{LOOH}\) even in membranes

Glutathione (GSH/GSSG)

- tripeptide, in every cell 1-10 mM
- keeps ICT reduced
- substrate for GPX, etc.
- also non-enzymatic reactions with ROS and mixed disulfides with proteins ... products of GSH oxidation are toxic for cell
- in oxidative stress the cell exports GSSG out
Catalase

• Tetramer, every subunit contains heme with Fe
• Dismutation of hydrogen peroxide:
  \[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]
• Red blood cells, peroxisomes
  • Also peroxidase activity:
    \[ \text{H}_2\text{O}_2 + \text{ROOH} \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{O}_2 \]
    (in comparison to GPX less significant)

**Oxidation of very long chain fatty acids in peroxisomes:**

\[
\begin{align*}
\text{H}_2\text{O}_2 & \xrightarrow{\text{Catalase}} \text{H}_2\text{O} + \frac{1}{2}\text{O}_2 \\
\text{O}_2 & \xrightarrow{\text{Acyl CoA dehydrogenase (ox, FAD)}} \text{Acyl CoA dehydrogenase (red, FADH}_2) \\
\text{Acyl CoA dehydrogenase (ox, FAD)} & \xrightarrow{\text{Further oxidation}} \text{Acyl CoA dehydrogenase (red, FADH}_2)
\end{align*}
\]
Glutathione peroxidase

H$_2$O$_2$

H$_2$O

Glu–Cys–Gly

Oxidised glutathione (GS-SG)

Superoxide dismutase

O$_2$–

Glutathione peroxidase

Glu–Cys–Gly

Reduced glutathione (GSH)

Glutathione reductase

NADPH+H$^+$

NADP$^+$

NAD$^+$

NADH+H$^+$

Transhydrogenase

Pentose cycle

ATP

**PeroxiRedoxin/Thioredoxin**

- Recently discovered antioxidant system, more important for removal of hydrogen peroxide than GPX
Antioxidant defence III

**Sequestration of metals**

- Redox-active transition metals (Fe, Cu) accept/donate one electron easily
  - ... alleviation of spin restriction of dioxygen
  - ... metals are in active centers of all oxygen handling-enzymes
- But, the same properties of Fe, Cu are deleterious if uncontrolled
  - the Fenton oxidant:
    \[
    \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^\cdot + \text{Fe}^{3+}
    \]

oxidative damage to biomolecules
Antioxidant defence III

Sequestration of metals

- Iron/copper handling proteins:
  - **transferrin**: binds 2 atoms Fe\(^{3+}\) (transport)
  - **lactoferrin**: analogous to transferrin, but no Fe release (... only sequestration), leukocytes
  - **ferritin**: H and L subunits, H is ferroxidase, Fe storage (up to 4500 atoms Fe\(^{3+}\))
  - **haptoglobin**: binds hemoglobin in circulation
  - **hemopexin**: binds heme in circulation
  - **ceruloplasmin**: contains Cu, function: ferroxidase (export Fe from the cells)
  - **albumin**: transport of Cu

<table>
<thead>
<tr>
<th>ICT</th>
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<tbody>
<tr>
<td>Superoxide</td>
<td>Superoxide</td>
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<tr>
<td>Peroxide</td>
<td>Peroxide</td>
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<tr>
<td>Fe/Cu</td>
<td>Fe/Cu</td>
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<tr>
<td>Superoxide dismutase</td>
<td>Antioxidant enzymes &amp; glutathione levels very low</td>
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<tr>
<td>Glutathione peroxidase</td>
<td>Tocopherol</td>
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<tr>
<td>Catalase</td>
<td>Ascorbate</td>
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<tr>
<td>Glutathione</td>
<td>Carotenoids, uric acid, albumin, glucose, bilirubin...</td>
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<tr>
<td>Tocopherol</td>
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<tr>
<td>Ascorbate</td>
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<tr>
<td>Labile iron pool (LIP) present</td>
<td>Sequestration of iron and copper:</td>
</tr>
<tr>
<td></td>
<td>- transferrin, lactoferrin</td>
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<td></td>
<td>- hemopexin</td>
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<td></td>
<td>- haptoglobin</td>
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<td></td>
<td>- ceruloplasmin (ferroxidase)</td>
</tr>
<tr>
<td></td>
<td>- Cu bound to albumin</td>
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Antioxidant defence IV
Low-molecular-weight antioxidant substrates

- **THIOLS:**
  - Glutathione
  - Thioredoxin

- **OTHER ENDOGENOUS METABOLITES:**
  - Bilirubin
  - Uric acid
  - Lipoic acid

- **DIETARY:**
  - Ascorbate (Vitamin C)
  - α-Tocopherol (Vitamin E)
  - Carotenoids
  - Plant phenols

Tocopherols (Vitamin E)

- group of 8 isomers, α-tocopherol most effective
- antioxidant of membranes (lipophilic)
- “chain-breaking” ... terminates the chain reaction of lipid peroxidation
Ascorbate (Vitamin C)

- Redox-active saccharide
- In most animals synthesized from glucuronic acid
- Vitamin for humans, other primates, bats and guinea pigs
- Deficit causes scurvy (scorbut)

Ascorbate in the body:
- Main function is pro-oxidant: cofactor of hydroxylases
  - Hydroxylation of Pro and Lys in collagen synthesis
  - Synthesis of noradrenaline from dopamine
  - Synthesis of carnitine (… role in oxidation of fat)
  - Activation of hypothalamic peptidic hormones by amidation (CRH, GRH, oxytocin, vasopressin, substance P)
- Reductant for iron: promotes its intestinal absorption
- Potentially dangerous pro-oxidant if iron sequestration impaired (hemochromatosis) (?)
- Daily need 70-100 mg, high doses p.o. excreted by urine (renal threshold cca 200 mg/24 hours)
membrane compartment:

- LH
- Tocopherol
- LOO·
- LOOH
- L·
- Tocopheryl radical

hydrophilic compartment:

- Semidehydro-ascorbate
- Dehydroascorbate
- Ascorbate
- GSH
- GSSG
- Dehydroascorbate reductase

Activated neutrophiles accumulate dehydroascorbate (DHA)

- GLUT1
- Glutaredoxin
- GSH
- GSSG

DHA

Protects membrane of the neutrophile from its own ROS ...
• **Selenium:**
  – Trace element (daily need 55 µg), possibility of deficiency as well as intoxication
  – Component of several antioxidant enzymes (glutathione peroxidase, thioredoxin reductase) and also e.g. 5‘-dejodase (T4→T3)

• **Carotenoids:**
  – β-carotene (provitamin A) is precursor for synthesis of:
    • Retinal … vision
    • Retinoic acid …regulator of gene expression, cellular growth and differentiation
  – Antioxidants in the skin and in the eye

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**Plant (poly)phenols**

• Thousands of substances (quercetin, resveratrol, curcumin, catechins…)
• Fruits, vegetables, tea, red wine, soy sauce, coffee, chocolate, herbs, spices…
• Excellent antioxidants (reductants) in vitro
• In vivo more complex:
  – Absorption in digestive tract?
  – Conversion to other derivatives?
  – Other specific biological effects?

Fig.: http://www.justaboutskin.com
Antioxidant defence V

**Stress response**

Oxidation or nitrosylation of sensor -SH

Transcription factors (NFκB, Nrf-2…):
activation, nuclear translocation

Induction of gene expression:
• chaperones (heat shock proteins)
• antioxidant enzymes
• metallothionein
• hemoxygenase 1

...→ more resistant to further oxidative stress

Apoptosis as the ultimate antioxidant defence?
**Free radicals in pathogenesis of human diseases**

- **Cause of disease, e.g.:**
  - cancerogenesis due to exposition to ionising radiation
  - retinopathy of the newborn (fibroplasia retrolentalis)

- **Contribute to pathogenesis, e.g.:**
  - atherosclerosis
  - diabetes mellitus
  - hypertension
  - some kinds of cancer
  - brain trauma/hemorrhage
  - ischemia/reperfusion injury of heart and other organs
  - Parkinson disease
  - Alzheimer disease
  - ageing

- **Merely an epiphenomenon** (general consequence of tissue damage)

**What is ageing**

- Looks similar in various animals, but proceeds with variable speed … must be universal process

- Stochastic process, unlike e.g. embryogenesis is not directly programmed by genome (but genes do play a role !)

- On molecular level: **inability to keep fidelity of biomolecules indefinitely**
  
  „systemic molecular disorder“ (Hayflick)
Mitochondrial genome

Mitochondrial DNA mutates 10 times faster than nuclear DNA
Exposed to oxygen radicals?
Not covered by histones
Repair insufficient?

Vicious circle of mitochondrial oxidative damage

Production of oxygen radicals in mitochondria
Accumulation of mtDNA mutations with age
Respiratory chain deficiency
Heart failure, muscle weakness, diabetes mellitus, dementia, neurodegeneration …
Free-radical/mitochondrial theory of ageing:

- Accumulation of oxidative damage with age (Denham Harman, 1956)
- Later refined to mitochondrial theory
  - …mitochondria are the main source of ROS in the body
- But: Difficult to prove that human mitoDNA is more damaged by ROS, or that significantly accumulates mutations with age
- Model of Kirkwood and Kovald:
  - Certain amount of ROS always escapes mitochondria and damages other cellular structures
  - Prevention of ROS formation and repair systems are never 100% effective
  - Slightly damaged mitochondria produce less energy than the cell would need

How the cells get rid of worn-out proteins and organelles

- Calpains, proteasome (short-lived proteins)
- Autophagy (long-lived proteins, organelles):
  - Macroautophagy (whole organelles)
  - Microautophagy (macromolecules, small organelles)
  - Chaperone-mediated autophagy (KFERQ proteins)

Fig.: http://cpmcnet.columbia.edu/dept/gsas/anatomy/Faculty/Kessin/autophagy.html
Ageing as a catabolic insufficiency

Incomplete digestion in lysosomes, release of Fe from mito, ROS, lipid peroxidation, cross-linking, aggregation and polymeration of oxidised proteins and lipids

↑ LIPOFUSCIN (in lysosomes)
In cytosol defective mito and indigestible protein aggregates

Loss of hydrolases delivered to lipofuscin-loaded lysosomes
Damaged and hypertrophic (giant) mito not degradable

Less ATP, more ROS, damaged mito & lysosomes can initiate apoptosis…

Stress response becomes permanent in ageing

Fig.: http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMtLyso.html

Nick Lane: Oxygen. The Molecule that made the World. Oxford University Press 2002
Antioxidants as elixirs of youth?

- Vitamin E (tocopherol)
- Vitamin C (ascorbate)
- β-carotene
- Selenium

Fig.: http://www.osel.cz

Antioxidant dietary supplements can even be harmful!

- Recent meta-analysis of total mortality in 68 studies on administration of antioxidant supplements (232,606 participants, 385 publications):
  - β-carotene, vitamin A and vitamin E significantly increase mortality
  - Vitamin C and selenium have no effect

(Bjelakovic G et al., JAMA 2007; 297: 842-857)
Why the antioxidants do not help or even harm ???

- High doses are ineffective
- Suppress the beneficial oxidations
  - Inhibition of the stress response
  - Impair defence against infection, cancer, physiologic apoptosis?
- Have other effects in addition to antioxidant
  - tocopherols: anti-inflammatory
  - β-carotene: co-carcinogen (together with smoking or environmental toxins)

Diet rich in fruit and vegetables (optim. 5x 80 g daily) is associated with lower risk of cardiovascular diseases, diabetes and certain kinds of cancer (lung, oropharynx, pancreas, stomach, prostate)

(but we do not know why…)