Mitochondria, Antioxidants and Aging

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Review of the Role of Mitochondria in Aging

I. Mitochondrial Function and Oxidative Stress

II. Mitochondrial Theory of Aging

III. Studies Manipulating Mitochondrial Oxidative Stress in Animal Models-what can we learn?
The diagram illustrates various cellular processes, including:

- **TCA Cycle**
- **Electron Transport Chain**
- **ATP Synthase**
- **Apoptosis**
- **Ca++ Regulation**
- **Fatty Acid Oxidation**
- **ROS Production**
- **ATP Production**

Key components include:

- NADH, FADH
- O₂⁻ → H₂O₂
- cytochrome (cytc)
- H₂O
- ATP synthesis and production

The diagram emphasizes the role of reactive oxygen species (ROS) in these processes.
Sources of Reactive Oxygen Species

Non mitochondrial:

- NADPH Oxidases
- Microsomal cytochrome P-450
- Cyclooxygenases
- Monoamine oxidases
- Peroxisomal β oxidation of fatty acids
- Phagocytes

>90% is mitochondrial

electron transport chain contains several redox centers that may leak electrons to oxygen
Mitochondrial ROS generation

ROS production is at Complex I and Complex III
Mitochondrial Antioxidant Defense:

Enzyme                      Target
MnSOD                        \( \text{O}_2^- \)
CuZnSOD                      \( \text{O}_2^- \)
Gpx1                         \( \text{H}_2\text{O}_2 \)
Gpx4                         lipid peroxides
PrxIII                       \( \text{H}_2\text{O}_2 \)
Trx2

Non-enzymatic:
Ascorbate
Glutathione (GSH)
Tocopherols
## Consequences of mitochondrial oxidative stress

<table>
<thead>
<tr>
<th>Damage to</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td>Membrane peroxidation</td>
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<tr>
<td></td>
<td>Decreased membrane fluidity</td>
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<tr>
<td><strong>DNA</strong></td>
<td>Mutations</td>
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<tr>
<td></td>
<td>Deletions</td>
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<tr>
<td><strong>Proteins</strong></td>
<td>Oxidation of sulfhydryl groups</td>
</tr>
<tr>
<td></td>
<td>Reactions with aldehydes</td>
</tr>
<tr>
<td></td>
<td>Protein aggregation</td>
</tr>
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<td></td>
<td>Protein carbonyls</td>
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</tbody>
</table>

**Markers:**
- F$_2$-Isoprostanes
- 8-iso-PGF$_{2\alpha}$
- oxo8dG
- Sugar
The problem:

Mitochondria are required for energy production

Mitochondria produce potentially harmful reactive oxygen species
What is the link?

mitochondria

AGING
Some Current Theories of Aging:

- Rate of living theory
  - Energy consumption is limiting for longevity

- Evolutionary Theories
  - Aging is a programmed event
    - Mutation accumulation
    - Antagonistic Pleiotropy

- Genomic Instability

- Cell Senescence/Telomere Shortening

- GH/IGF-1 axis

- Free Radical Theory
Free Radical Theory of Aging

“Aging: a theory based on free radical and radiation chemistry”
1956, D. Harman J.Gerontology 11(3):298-300

A single common process, modified by genetics and environmental factors, is responsible for the aging and death of all living things.

“Aging and the degenerative diseases associated with it are attributed basically to the deleterious side attacks of free radicals on cell constituents and on the connective tissues....... The free radicals probably arise largely through reactions involving molecular oxygen catalyzed in the cell by oxidative enzymes and in the connective tissues by traces of metals such as iron, cobalt, and manganese.”
Oxidative Stress Theory of Aging

- Mitochondrial (ETC)
- Extra-mitochondrial (NADPH oxidase)
- Exogenous Sources (chemicals, radiation)

ROS: 
- $O_2^-$
- $H_2O_2$
- $OH^-$
- ONOO$^-$

Antioxidant Defense

Imbalance between pro-oxidants and antioxidants.

Chronic state of oxidative stress
Oxidative Stress Theory of Aging

Mitochondrial (ETC) 

Extra-mitochondrial (NADPH oxidase) 

Exogenous Sources (chemicals, radiation) 

ROS 

O$_2^-$  H$_2$O$_2$  ONOO$^-$  OH$^-$ 

Antioxidant Defense 

DNA 

Protein 

Lipid 

Steady-state accumulation of oxidative damage that increases during aging 

Progressive loss in efficiency of cellular processes
Mitochondria are major source of ROS
Mitochondrial Theory of Aging


Free radicals escaping from the respiratory chain ...would be expected to produce deleterious effects mainly in the mitochondria ...Are these effects mediated in part by mitochondrial DNA functions?
Mitochondria Theory of Aging

Increased Production of ROS

Accumulation of oxidative damage with age

-Damaged mitochondria produce more ROS

-Alterations in ETC function
-mtDNA deletions/mutations

Decreased ATP
Cell Death/Apoptosis
Functional Decline

Aging
Mitochondria Theory of Aging

- Alterations in ETC function
- mtDNA deletions/mutations

Increased Production of ROS

Damaged mitochondria produce more ROS

Accumulation of oxidative damage with age

Decreased ATP
Cell Death/Apoptosis
Functional Decline

Aging
Early studies supported an increase in ROS with age

**Housefly flight muscle**  
(submitochondrial particles)  
Sohal and Sohal (1991)  

**Mouse kidney, heart and brain mitochondria**  
(submitochondrial particles)  
Sohal et al (1994)  
Mech.Ageing Dev. 121-133

**Rat vastus lateralis or soleus muscle**  
(homogenates/isolated mitochondria)  
Bejma and Ji (1999)  
J.Appl.Physiol. 465-470
Other studies show no increase.....

Rat Muscle

Capel et al.

Drew et al
Am.J.Physiol

Rat Heart

Hansford et al
J.Bioenerg.Biomembr., (1997) 89-95

Rat Liver

Lopez-Torres et al.
Increase may be tissue specific?.....

Mouse hindlimb
Skeletal muscle

Mouse heart
Mitochondria Theory of Aging

- Alterations in ETC function
- mtDNA deletions

Increased Production of ROS

Some tissues?
## Aging and ETC function

<table>
<thead>
<tr>
<th>Muscle Tissue</th>
<th>Complex</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
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<tr>
<td>Trounce et al., (1989)</td>
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<tr>
<td><em>Lancet</em> (8639):637-9</td>
<td>Human muscle</td>
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<tr>
<td>Boffoli et al. (1994)</td>
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<tr>
<td><em>BBA</em> 1226:73-82</td>
<td>Human muscle</td>
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<tr>
<td>Sugiyama et al. (1993)</td>
<td></td>
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<tr>
<td><em>Biochem Mol Biol Int</em> 30:937-44</td>
<td>Rat muscle</td>
</tr>
<tr>
<td>Muller-Hocker et al. (1990)</td>
<td></td>
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<tr>
<td><em>J Neurol Sci</em> 100:14-21</td>
<td>Human muscle</td>
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<td>Desai et al. (1996)</td>
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<td><em>Arch Bioc Biophys</em> 333:145-151</td>
<td>B6C3F1 mice</td>
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<td>Muller-Hocker et al. (1996)</td>
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<tr>
<td><em>Mech Aging Dev</em> 86:197-213</td>
<td>monkeys</td>
</tr>
<tr>
<td>Kwong et al. (2000)</td>
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<tr>
<td><em>Arch Bioc Biophys</em> 373: 16-22</td>
<td>C57Bl6 mice</td>
</tr>
</tbody>
</table>
More recent studies in humans, mice

Barrientos 1996
Rasmussne 2003
Chretien 1998,
Kwong and Sohal 2000

No consistent change

Current evidence suggests that there is no consistent overall defect in the ETC with age.
Mitochondria Theory of Aging

- Alterations in ETC function (?)
- mtDNA mutations/deletions

Increased Production of ROS
Some tissues?
MtDNA

- Double-stranded, circular molecule

- ~16549 bp

- 2 to 10 copies in each mitochondrion
  > 1,000 in each cell

MtDNA encodes for 37 genes:
  22 tRNA
  13 mitochondrial peptides (ETC subunits)
  16S and 26S rRNA

(Remaining > 67 ETC subunits are nuclear encoded)
mtDNA is more sensitive to oxidative damage than nuclear DNA

Estimated at 1/8000 bases for mtDNA vs 1/130,000 for nDNA
(Richter et al 1988 PNAS)

- high metabolism
- proximity to generation of ROS
- lack of histones
- repair systems less efficient
Tissues that turn-over more slowly (skeletal muscle, heart) have more mtDNA deletions than more rapidly dividing tissues (liver).

Deletions increase with age in muscle heart and brain (identified using PCR).

Common deletion mtDNA4977 increases by a factor of 10,000 in muscle during a normal human lifespan reaching 0.1% of total muscle mtDNA by 84 yrs.

Evidence indicates focal accumulation of deletions in some tissues.
Mitochondrial Theory of Aging

Increased Production of ROS

Some tissues?

Accumulation of oxidative damage with age (mitochondrial and non-mitochondrial)

Alterations in ETC function (?)

mtDNA mutations/deletions

YES

Damaged mitochondria produce more ROS
Age related changes in mitochondrial DNA oxidative damage

Liver Nuclear DNA

Liver Mitochondrial DNA

Van Remmen et al., Physiol. Genomics, 2003
Plasma Free Isoprostanes

Carbonyl Groups in Liver Protein

Jack Roberts, Vanderbilt University

Asish Chaudhuri, San Antonio
Mitochondrial Theory of Aging

- Increased Production of ROS
  - Some tissues?

- Accumulation of oxidative damage with age (mitochondrial and non-mitochondrial)
  - YES

- Alterations in ETC function (?)
  - mtDNA mutations/deletions
  - YES

- Damaged mitochondria produce more ROS

- Decreased ATP
- Cell Death
- Functional Decline
- Aging

Yes
Effects of Manipulating Mitochondrial Oxidative Stress in Animal Models
Altered expression of MnSOD in *Drosophila*


Induced overexpression of mitochondrial Mn-SOD extends the life span of adult *Drosophila melanogaster*.

- Mean life span increased by an average of 16%
- Another study finds no increase in lifespan in MnSOD Tg flies (Orr et al., 2003 JBC)
Increased Expression of Mitochondrial Uncoupling Protein 2

Targeted expression of the human uncoupling protein 2 (hUCP2) to adult neurons extends life span in the fly.

Alterations in Mitochondrial Function and Oxidative Stress

- decrease in ROS production
- decrease in oxidative damage
- increased resistance to paraquat

Increase in lifespan

Fridell et al., Cell Metab. 2005 1(2):145-52
Increased expression of Mitochondrial genes

MnSOD

UCP2

Effect on Lifespan

↑

↑

Seem to be consistent with the theory.....
What can we learn about mitochondrial theory of aging from mouse models?

- MnSOD-mitochondrial antioxidant
- mtDNA polymerase mutant mice
- Mitochondrial targeting of catalase
MnSod (Sod2⁺⁻) Knockout mice

- Mitochondrial antioxidant enzyme (O₂⁻ + O₂⁻ → H₂O₂)
- Homozygous mutant is lethal (survival is less than 2 weeks)
- 50% decrease in MnSOD activity in all tissues studied
- No compensation by other major antioxidant enzymes

(Li et al. (1995) Nature Genetics.)
Sod2+/− Knockout Mice

- Nucleus
- nDNA
- PHGPx
- H2O
- PH
- PHGPx
- H2O
- PH
- Peroxisomes
- H2O2
- H2O
- O2
- Catalase
- β-Oxidation pathway
- MnSOD
- CuZnSOD
- I II III IV V
- PrxIII
- Trx2
- Trx1
- 2H2O
- GPx
- GSSG
- 2GSH
- ATP
- CuZnSOD
- H2O2
- H2O
Mitochondrial Theory of Aging

Alterations in ETC function
mtDNA mutations/deletions

Increased Production of ROS

\( \uparrow \uparrow \) Accumulation of oxidative damage with age

Sod2\(^{+/-}\) mice
(Reduced Antioxidant Protection)

Decreased ATP
Cell Death
Functional Decline

Increased Aging
MnSOD Heterozygous Knockout Mice

Oxidative damage to specific mitochondrial proteins
aconitase
NADH oxidoreductase

Skeletal Muscle

Aconitase Activity (munits/mg protein)

CoQ Reductase Activity (mmoles/min/mg protein)

*
MnSOD Heterozygous Knockout Mice

Altered Mitochondrial Function
/Decreased activities of ETCs

Liver

![Bar graph showing RCR (respiratory control ratio) for different liver samples labeled I, II, and III, with statistical significance indicated by asterisks.](image-url)
MnSOD Heterozygous Knockout Mice

Increased oxidative damage to DNA

Nuclear DNA

![Graph showing increased oxidative damage to DNA in different tissues and ages](Image)
Mitochondrial Theory of Aging

Alterations in ETC function
mtDNA mutations/deletions

Increased Production of ROS

Sod2+/− mice
(Reduced Antioxidant Protection)

Accumulation of oxidative damage with age

Aging
MnSOD Heterozygous Knockout Mice

**Graph: Survival Analysis**

- **Sod2+/+ (n = 69)**
- **Sod2+/- (n = 70)**

**Observations:**
- No change in survival
- Increase in Cancer
Premature ageing in mice expressing defective mitochondrial DNA polymerase

Homozygous knock-in mice for a proof reading deficient PolgA-mtDNA polymerase

- 3-5 fold increase in point mutations and deletions in mtDNA
- Normal appearance until ~6 months (25 weeks)
  - Weight loss, kyphosis, alopecia, decreased fat, osteoporosis
- Decreased ETC activity and ATP production in heart
mtDNA mutations -- Shortened lifespan

- Median lifespan, ~48 weeks, or 336 days
- All died by 61 weeks, or ~430 days

PolgA-mtDNA polymerase mutants

Mitochondrial DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging

- Targeted mutation of PolgA Mitochondrial DNA polymerase
- Residue substitution in the exonuclease domain that impairs proof reading ability

- Increased mutations in mtDNA

- No increase in oxidative damage

- Apoptotic markers were increased during aging [increased caspase 3 activity]
Phenotype shows age-associated characteristics

- Indistinguishable in young age
- Phenotype evident at ~9 months

- Hair loss, graying, and kyphosis.
- Thymic involution
- Testicular atrophy associated with the depletion of spermatogonia
- Loss of bone mass
- Loss of intestinal crypts
- Decrease in circulating red blood cells
- Weight loss
- Hearing loss
- Loss of muscle mass

Kujoth et al Science 259, 2005
• Maximal survival, 460 days.
• Median survival, 416 days
• Wildtype, both are > 850 days

Are mtDNA mutations linked to apoptosis and aging?

Mitochondrial DNA polymerase mutant

Kujoth et al Science 259, 2005
Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria

Increased expression in heart muscle and brain

Increased over 50x in heart mitochondria

Science, 2005- Schriner et al.
Both median and maximum lifespan were increased in MCAT animals.
- Cardiac pathology and cataract development were delayed
- H₂O₂ production was reduced
- Oxidative damage was reduced (aconitase activity, 8-OHdG)
- The development of mitochondrial deletions was reduced.
- 50% reduction in MnSOD
- Increased mtDNA mutations
- Mitochondrial targeting of catalase
Do mitochondria play a key role in aging??

Evidence to date would suggest that mitochondria and oxidative stress in general certainly play a role in age related disease, but a direct link between mitochondria and aging has not been definitively established.