OXIDATIVE STRESS: The effect on mitochondria & energy production

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Disclosures

• None
7 Clinical Systems

Timeline ATMs

Individual Predispositions

Modifiable Lifestyle Factors

The Fundamental Organizing Systems and Core Clinical Imbalances

Assimilation
Digestion, Absorption, Microbiota/GI, Respiration

Defense and Repair
Immune system, Inflammatory processes, Infection and microflora

Energy
Energy regulation, Mitochondrial function

Biotransformation and Elimination
Toxicity, Detoxification, Communication
Endocrine, Neurotransmitters, Immune messengers, Cognition

Transport
Cardiovascular, Lymphatic systems

Structural Integrity
From the subcellular membranes to the musculoskeletal system

Antecedents, Triggers, and Mediators

Mental, Emotional, Spiritual Influences
Genetic Predisposition
Experiences, Attitudes, Beliefs

Modifiable Lifestyle Factors

Sleep & Relaxation
Exercise & Movement
Nutrition
Stress
Relationships
Objectives

• Review mitochondrial structure, energy production and metabolism
• Discuss the pathophysiology of oxidative stress and mitochondrial damage; including dietary factors, ROS, and toxins
• Review ways to support mitochondria with diet, nutrients, and phytochemicals
Mitochondria
Mitochondrial distribution

• Approximately 10 million billion total: ~10% of body weight
• Average of 200 to 2000 per somatic cell
  ~5000 in cardiac cells -- 50% of myocardial cytoplasm -- there is complete turnover of myocardial ATP pool every 10 seconds
  ~800 in hepatocytes
  ~300-400 in neurons (filamentous)
• Mitochondria generate and consume the body’s weight in ATP every day
Mitochondria: powerhouse of the cell

- Mitochondria consume about 90% of the oxygen used by the body for oxidative phosphorylation.
- The oxygen serves as the ultimate electron receptor from the electron transport chain, allowing ATP to be generated.
Mitochondrial anatomy
Healthy cristae (left) vs damaged (right)
Mitochondrial functions

- ATP synthesis
- Buffering Ca^{++} flux (from endoplasmic reticulum & plasma membrane)
- Maintenance of ion gradients (polarized cells)
- Generation of reactive oxygen species (ROS)
- Regulation of cell growth, cell cycle, metabolism
Mitochondrial bioenergetics

• Catabolism of CHO, fats, & amino acids into *carbon skeletons*
• Extraction of energy released via catabolism:
  - Glycolysis
  - Citric acid cycle (Krebs)
  - β-oxidation
  - Oxidative phosphorylation
• 36-38 molecules of ATP per molecule of glucose
Net ATP: 30 to 32 ATP per glucose

Glycolysis: +2 ATP via substrate-level phosphorylation
Citric Acid Cycle: +2 ATP via substrate level phosphorylation (1 per pyruvate)
Electron Transport Chain: +26 to 28 ATP via oxidative phosphorylation
Glycolysis

- Ancient metabolic pathway -- in cytosol of most living organisms

- Glucose (6C): initial electron donor
  - Reduces NAD$^+$ into NADH x 2
  - Generates ATP x 2
    (very rapid but inefficient energy production)
  - Splits into pyruvate x 2

- Pyruvate (3C)
  - Actively transported into matrix for aerobic respiration by mitochondrial pyruvate carrier
  - When mitochondrial metabolism inhibited (anaerobic conditions, etc.), converted into lactate by LDH, which regenerates NAD$^+$
Citric Acid Cycle

- Oxaloacetic Acid
- Malic Acid
- Fumaric Acid
- Succinic Acid

NADH / FADH₂

Electron Transport and Oxidative Phosphorylation

(2) H + ½ O₂ → H₂O

KEY
- Green = cofactor
- Red = inhibitor
Conventional wisdom has been that mitochondria prefer carbohydrates (glucose) as the primary source of energy, however, fatty acids (ketones), and amino acids can also be readily utilized by mitochondria.
Tricarboxylic Acid (Krebs) Cycle

- Final common catabolic pathway for all nutrients (protein, fat, carbohydrates)
- Enzymes located in mt matrix (except for complex II - succinate dehydrogenase)
- Acetyl-CoA oxidized to CO$_2$
- Produces
  - Metabolic byproducts: amino acid precursors
  - NADH, FADH$_2$, GTP
Long chain fatty acids: mitochondrial metabolism

- Most dietary fatty acids undergo β-oxidation in mitochondria
- High carbohydrate intake impairs β-oxidation, resulting in accumulation of intracellular lipid intermediates and triglycerides, causing insulin resistance
- Fasting, starvation, and low carbohydrate/high fat diets increase hepatic β-oxidation, resulting in ketogenesis
Ketone bodies

- Ketones soluble in water—no protein carriers required
- Plasma levels increase with fasting, high fat/low CHO diets, and uncontrolled diabetes
- Ketones are preferred fuel (vs glucose) for cardiac muscle and renal cortex
- Used in brain (after crossing blood brain barrier) proportionate to concentration in blood, provide energy when glucose availability is limited
Amino acids as fuel sources

- Can be oxidized, degraded into pyruvate, used as citric acid cycle intermediates, or converted into ketone bodies
- Oxidative degradation of AAs produces 10-15% of total metabolic energy
- Act as precursors for gluconeogenesis when glucose supply is low
Amino acid precursors for TCA cycle

- Leucine
- Lysine
- Phenylalanine
- Tryptophan
- Tyrosine
- Arginine
- Glutamine
- Histidine
- Proline
- Isocitrate
- α-Ketoglutarate
- Succinyl-CoA
- succinate
- Phenylalanine
- Tyrosine
- Isoleucine
- Methionine
- Threonine
- Valine
- Acetoacetyl-CoA
- Acetyl-CoA
- Oxaloacetate
- Fumarate
- Malate
- Pyruvate
- Asparagine
- Aspartate
- Alanine
- Cysteine
- Glycine
- Serine
- Tryptophan
**Mitochondrial Matrix**

**FIG.** Mitochondrial Respiratory Chain. Protons ($H^+$) are pumped from the mitochondrial matrix to the intermembrane space through complexes I, III, and IV. Complex V utilizes the proton gradient as a source of energy to produce ATP. Coenzyme $Q_{10}$ transfers electrons from complexes I and II to complex III. Riboflavin is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The amide form of niacin, (nicotinamide) is a precursor for nicotinamide adenine dinucleotide (NAD). Vitamin $K_3$ in combination with vitamin C serve as electron acceptors to bypass a deficiency in complex III. Carnitine function to transfer long chain fatty acids across the mitochondrial membrane.
Introducing: The Electron Transport Chain
Mitochondrial energy production
Stressors
- Aging/Senescence
- Wounding
- Xenobiotics
- Radiation/Light
- Heat & Cold
- Pathogens
- Biotoxins
- Drought
- Heavy Metals
- Air Pollutants
  \( (O_3; SO_2) \)
- Hormones

Oxidative STRESS
- \( O_2^+ \)
- \( H_2O_2 \)
- \( *OH \)

Molecular Damage
- Lipids & Fatty Acids
- Amino Acids
- Proteins
- Nucleic Acids
- Pigments

Cellular Effects
- Membrane Damage
- Loss of Organelle Functions
- Reduction in Metabolic Efficiency
- Reduced Carbon Fixation
- Electrolyte Leakage
- Chromatid Breaks
- Mutations

Cell DEATH
Objectives

• Review mitochondrial structure, function and metabolism
• Discuss the pathophysiology of mitochondrial damage, including dietary factors, ROS, and toxins
• Review ways to support mitochondria with diet, nutrients, and phytochemicals
An individual produces about 1 kg of oxygen radicals per year. The consequence is about 100,000 oxidative attacks on mDNA per cell per day.
Causes of increased mitochondrial ROS

- Caloric excess
- Hyperglycemia (endothelial)
- Inflammatory mediators (TNFα)
- Hypoxia
- Environmental pollutants & toxicants
- Toxic metals (mercury, arsenic)
- Ionizing radiation
**Denham Harman**

• First proposed the idea of “free radicals” in 1956 and postulated that these compounds play a role in aging through cross-linking reactions.
• Free radicals covalently modify lipids, proteins, cellular and mitochondrial DNA.

Free radical theory of aging

- Increased oxidant generation
- Declining defenses and repair
- Accumulation of the end products of oxidative damage
  - Advanced Glycosylated End Products (AGEs)
  - Protein Oxidation (NitroTyrosine)
  - Oxidized LDL, Isoprostane F2, Lipid Peroxides, MDA
  - DNA damage (8-OH dG)
Free radicals, ROS, and RNS…

React with and damage structural and functional components of cells

- Membranes & Receptors
- Enzymes & other proteins
- Cellular DNA & RNA
- Mitochondrial DNA & Membranes
Mitochondria & free radicals

- About 1-2% of oxygen consumed by our mitochondria is converted to superoxide and hydrogen peroxide
- One rat liver mitochondrion produces $\sim 3 \times 10^7$ superoxide radicals per day
- Each liver cell contains $\sim 1000$ mitochondria
NK-kB mediated cellular damage

↑ Oxidative Stress

Activation of NF-KB

Up-regulation of stress and inflammation genes including inducible NOS (iNOS)

↑ RNS, NO, *ONO2⁻

Increased Cellular Damage
How does the body protect itself from ROS?

1. Enzymes
   - Catalase \((Fe)\)
   - Superoxide dismutase-SOD \((Zn, Cu, Mn)\)
   - Glutathione peroxidase \((Se)\) and glutathione reductase

2. Dietary Anti-Oxidants
   - Vitamin C for aqueous compartments
   - Vitamin E for lipid compartments
   - Carotenoids, flavonoids, etc.

3. Endogenous Anti-Oxidant Molecules
   - Glutathione, cysteine, CoQ_{10}, lipoic acid, uric acid, cholesterol.
Understanding oxidative stress

To have a comprehensive understanding of the body’s red-ox potential and level of total oxidative stress, you need to know:

1. What is the antioxidant reserve or total antioxidant capacity?
2. What is the throughput of reactive oxygen species and free radicals?
3. What damage to cellular components is being done?
Mitochondrial function → Generation of ROS → Oxidative damage mtDNA mutations → Mitochondrial dysfunction → Ageing

"The vicious cycle"
What’s the damage?

Oxidative stress from free radicals, ROS, and RNS can damage many cellular components

- Damaged Fats
- Damaged Sugars
- Damaged Proteins
- Damaged DNA
One can evaluate with:

- Damaged Fats $\rightarrow$ Lipid Peroxides, oxidized LDL, Isoprostane F2
- Damaged Sugars $\rightarrow$ HgbA1c, AGEs
- Damaged Proteins $\rightarrow$ 3-Nitrotyrosine
- Damaged DNA $\rightarrow$ 8-OH Deoxyguanosine
Therapies to ↓ lipid peroxides

Consider fat-soluble antioxidants:
- Vitamin E (interrupts rapid propagation of lipid peroxides)
- CoQ10
- Lipoic Acid

For lowering serum lipid peroxides, the combination of Curcumin, cayenne, and garlic is effective

Damaged DNA (8-OHdG)  
8-hydroxy-deoxyguanosine

• When an activated oxygen species reacts with the nucleotide guanosine, 8-hydroxy-deoxyguanosine is created
• 8-OHdG is the most frequent mutagenic lesion in our DNA
• Damage can be triggered by chemical toxicity, inflammation, or radiation
8-OHdG as a marker of oxidative stress

“The biomarker 8-OHdG has been a pivotal marker for measuring the effect of endogenous oxidative damage to DNA and as a factor of initiation and promotion of carcinogenesis.”

Therapies to treat damaged DNA

- Carotene supplementation has been found to decrease DNA oxidation
- Reduce iron overload, if present
- Combination antioxidant support is most effective
- Methylation is critical for DNA synthesis

Effective treatment

- Nutritional Anti-Oxidants (Vit A, C, E)
  - Glutathione, alpha-Lipoic Acid
  - CoEnzyme Q-10 (CoQ-10)
- Plant-based Anti-Oxidants
  - Resveratrol
  - EpiGalloCatechinGallate (EGCG)
  - Many, many, many others
- Proper Methylation Function (B-Vitamins)
- Mineral Co-Factors (Mg, Mn, Fe, Zn)
- Amino Acid Balance and Protein Digestion
- Eat Your Vegetables!
Mitochondrial dysfunction

- Chronic heart failure
- Vascular disease
- Oxidative phosphorylation impairment
- Mitochondrial gene expression alteration
- Abnormalities in FA metabolism
- Inflammatory response impairment
- Enhancement of insulin resistance
- Increase in ROS production
- Fuel and energy homeostasis imbalance
- Insulin resistance, vascular disease

Chronic heart failure, vascular disease, insulin resistance, diabetes, obesity

Insulin resistance, diabetes, obesity

Diabetes, obesity, chronic heart failure

Diabetes, atherosclerosis
Mitochondrial dysfunction & disease

- Metabolic syndrome: insulin resistance, type 2 diabetes, obesity, non-alcoholic fatty liver disease
- Cardiovascular disease (congestive heart failure)
- Cancer
- Neurodegenerative & neuromuscular disorders
- Mood disorders; bipolar disorder
- Chronic fatigue; fibromyalgia
- Multiple chemical sensitivity
- Premature aging
Common mediators of neurodegeneration

- Reactive species and oxidative/nitrative damage – which offending species?
- Mitochondrial dysfunction
- Abnormal protein aggregates
- Inflammation
Common types of neurodegeneration

- Alzheimer’s Disease
  - (a.k.a. Senile Dementia of the Alzheimer’s Type – SDAT)
- Cognitive Impairment
- Memory Loss
- Parkinson’s Disease
- Stroke/ CVA
Damage to lipids, proteins, DNA, & RNA in mild cognitive impairment

“These studies establish oxidative damage as an early event in the pathogenesis of Alzheimer disease that can serve as a therapeutic target to slow the progression or perhaps the onset of the disease.”

Markesbery, W., Arch Neurol. 64(7):954-956; July, 2007
Oxidative stress response

Adaptation Responses

- e.g. Neurotrophic factors, Neurogenesis, DNA repair etc

ROS/RNS

- Oxidation of proteins, lipids and DNA
- Organelle dysfunction

Failure to adapt

- Apoptosis
- Necrosis
- Calcium dysregulation
Metabolic regulation of cognitive dysfunction

- Diabetes aggravates, and energetic challenges attenuate, CNS inflammation.
- Exercise and caloric restriction ameliorate, and diabetes exacerbates, Alzheimer’s disease models.
- Cognitive impairment associated with trauma or ischemia can be modified by caloric intake and exercise.
Regulation of cognitive function

Exercise

Caloric Restriction

Mild Oxidative Stress

Sedentary Lifestyle

Diabetes, Obesity

High cholesterol

Adaptive Cellular Stress Responses

PGC-1α

BDNF

IGFs

HSPs

UCPs

MnSOD

HO-1

Oxidative Stress

Inflammation

Impaired Synaptic Plasticity

Impaired Neurogenesis

Neurodegeneration

Cognitive Impairment

Disease Progression

Adapted from: Stranahan and Mattson, 2011
Is oxidative stress a useful target for brain disorders?

Dual roles of ROS:
- Signaling vs damage → Xenohormesis
- Are ROS merely associated with the disease process or play a causative role?
- Do antioxidant compounds interfere with physiological processes?
- Does redox signaling role interfere with antioxidant efficacy?

Goal of antioxidant therapy in disease states is to normalize elevated ROS levels and decrease oxidative damage
All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and remedy.

Paracelsus (1493–1541)
“What doesn’t kill you, makes you stronger!”

Figure 2. Differential responses to rising oxidative stress.

- Oxidative stress
- Inflammation
- Apoptosis

- Nrf2: mild to moderate
- NFκB: high
- AP-1: extreme

Primed by oxidative stress.
Xenohormesis

Healthy Mitochondrion in Young Cell:
- Relatively few free radicals attack the cell.
- Abundant ATP powers cellular activities.

Damaged Mitochondrion in Distressed Old Cell:
- Free-radical damage increases.
- ATP supply shrinks.

Key Components:
- NUTRIENTS AND OXYGEN
- MATRIX
- INNER MEMBRANE
- MITOCHONDRIAL DNA
- MOLECULAR COMPLEX
- ENERGY-PRODUCING MACHINERY

TIME Arrow Indicates Progression from Healthy to Damaged State.
Ketogenic diet

- Reduces inflammation (NFkB)
- Enhances mitochondrial biogenesis
- Enhances ATP production
- Reduces ROS production
- Reduces apoptosis
- Increases insulin sensitivity
- Increases leptin sensitivity
The ketogenic diet (KD)

• Mimics fasting state – switches metabolism of glucose to metabolism of ketones
• Clinically-used treatment for intractable seizures in children and adolescents
• High fat – low carbohydrate (4:1, fat:non-fat)
• Efficacy appears to be independent of seizure type
• Mechanism of action unknown but attributed to ketone bodies, glycolysis, and mitochondrial metabolism
• Research direction: clinic to bench
Activation of the Nrf-2 adaptive response in the ketogenic diet

Ketogenic Diet → Mild oxidative/electrophilic stress ($H_2O_2$, 4-HNE) → Protein kinase cascade → Keap1, Nrf2 → Keap1 → Keap1 → Nrf2 → Nucleus → Target gene transcription (Gclc, Gclm, Nqo1, Ho-1) → Antioxidant Response → ROS

- 1-3 days
- ~1 week
- ≥3 weeks

Milder and Patel, Epilepsy Res. 2011
The perfect storm (insulin resistance)

- Glucose unable to enter cell
- $\beta$ oxidation is inhibited leading to lipid accumulation in skeletal muscle, liver, & heart
- Gluconeogenesis is inhibited
- Krebs cycle intermediates are depleted
- Only one option remains: break down muscle and replace it with fat
- All these conditions are intracellular energy deficits (obesity, CHF, cachexia, diabetes, fatty liver)
Nrf2, the Oxidant ‘Thermostat’ of the Cell: The ‘Oxidant-stat’

- KEAP1
- Cul5
- Nrf2
- OxStress
- Nrf2 Released to travel to nucleus

Antioxidant Response Elements
ARE: GSH, GST’s, GPx, Catalase and others

Nucleus

GSH
Nrf2 activation

- Oxidative stress
- Caloric restriction
- Curcumin
- Green tea extract
- Pterostilbene
- Sulforaphane
- Garlic (allicin)
- DHA

- Catalase
- Glutathione
- SOD
- GST (Phase II detox)
- Inhibits NF-kB
- Inhibits microglial activation
- Oxidative stress
- Caloric restriction
- Curcumin
- Green tea extract
- Pterostilbene
- Sulforaphane
- Garlic (allicin)
- DHA
Fig. 1. Outline of the Nrf2 regulatory system.
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• Review mitochondrial structure, function and metabolism
• Discuss the pathophysiology of mitochondrial damage, including dietary factors, ROS, and toxins
• Review ways to support mitochondria with diet, nutrients, phytochemicals, and lifestyle
Exercise increases mitochondrial numbers

Moderate intensity exercise 4 months:

- 67% increase in mitochondrial density
- 55% increase in cardiolipin content
- Increase in mitochondrial oxidation enzymes
- All linked to improvement in hemoglobin A1c and fasting plasma glucose

Phytochemicals that support mitochondrial function

- Curcumin (turmeric)
- Sulforaphane (broccoli)
- Berberine
- Quercetin
- Resveratrol (red wine)
- Pterostilbene (purple berries)
- Green tea polyphenols
Nutrients that support mitochondrial function

- Acetyl-L-carnitine: 1500-3000 mg
- Alpha lipoic acid: 300-900 mg
- Coenzyme Q10 (ubiquinone): 50-200 mg
- Magnesium: 100-500 mg
Nutrients that support mitochondrial function

- N-acetylcysteine: 500-3000 mg
- Creatine: 5-15 grams
- Melatonin: 3-20 mg
- Ketogenic & branched chain amino acids
- Nicotinamide riboside: 250-1000 mg
Benefits of enhanced mitochondrial function

- ↓ ROS / Oxidative Stress
- ↑ Metabolic Function
- ↑ Energy Level
- ↑ Exercise Performance
- ↓ Body Fat / ↑ Lean Muscle Mass
- ↓ Age-Related Deterioration
- ↑ Increased Lifespan (?)
- Cancer suppression
Treatment: a TO DO list to support mitochondrial function

- Get adequate nutrition
- Stay cool and hydrate
- Prevent infections
- Exercise (physical & mental)
- Avoid toxins
- Intermittent fasting
- Avoid simple and processed carbs
Treatment: a TO DO list to support mitochondrial function

Supplements:
- CoQ-10
- Omega-3 Fatty Acids
- B-Vitamins (particularly B2 & B3)
- Alpha-Lipoic Acid
- Nrf2 Activators
- Rhodiola
References