Lifestyle Factors, Mitochondrial Dysfunction, & Chronic Disease
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Robert Rountree, MD
Boulder, CO

Disclosure:
Dr. Rountree is a paid consultant and member of the speakers’ bureaus for Thorne Research, Genova Diagnostics, and Albion Laboratories

Objectives
• Review mitochondrial structure, function and metabolism
• Discuss the pathophysiology of mitochondrial damage, including dietary factors, ROS, and toxins
• Discuss the links between mitochondrial damage and disease
• Review ways to support mitochondria with diet, nutrients, and phytochemicals
On the origin of mitosing cells.
Lynn Sagan (Margulis)

Endosymbiosis:
• ~1.5 to 2.3 billion yrs ago, aerobic proteobacteria (Rickettsiales) engulfed by nucleated protozoans, subsequently enslaved to form mitochondria
• These evolutionary changes corresponded with increasing O$_2$ in the environment – hence the energetic advantage of harnessing high-efficiency oxidative phosphorylation
Functional Mitochondrial Structure

• Outer phospholipid bilayer: semipermeable VDACs (porins)
• Intermembrane space: low pH, Ca++, ATP, proteins (e.g., cytochrome c)
• Inner membrane: impermeable bilayer (transport proteins; transition pore)
• Cristae: large folded cardiolipin-rich membrane, ETC protein complexes, ATP synthase
• Matrix: mtDNA, proteins, glutathione, Ca++, nitric oxide, NAD+/NADH (10:1 ratio)

Healthy cristae (left) vs damaged (right)
Mitochondrial Dynamics—Mitochondrial Fission & Fusion in Human Diseases
NEJM, 2013, Vol 369: 2236-2251

- Heterogeneous morphology: mito (thread) + chondros (grain)
- Highly mobile organelles - transverse along actin cytoskeleton (microtubules)
- Continually fuse, divide, and regenerate to fine tune fundamental cellular processes

Mitochondrial Dynamics—Mitochondrial Fission & Fusion in Human Diseases
NEJM, 2013, Vol 369: 2236-2251

- Fission/fusion involved in cell-cycle progression, apoptosis, mitophagy, O$_2$ sensing – highly coordinated with cellular physiology
- Fusion allows mitochondria to restore damaged essential components
- Fission allows for quality control by eliminating damage components

Mitochondrial Dynamics—Mitochondrial Fission & Fusion in Human Diseases
NEJM, 2013, Vol 369: 2236-2251

- Disorders of mitochondrial dynamics emerging as major mechanisms of disease – cancer, cardiovascular disease, endocrine disorders, neurodegeneration
- Most triggered by changes in the cellular milieu (eg oxidative stress) rather than monogenic mutations
Mitochondrial Dynamics

Mitochondrial Genomics

- mt DNA = 37 genes
  - (13 proteins, 2 rRNA, 22 tRNA)
- found in circular double-stranded molecules:
  - heavy strand (28 genes)
  - light strands (9)
- 2-10 circles of mtDNA per mitochondria
- Complexed with proteins but not protected by histones = highly susceptible to oxidative damage
Mitochondrial Genomics

- Mitochondria cannot be created *de novo*: mtDNA must be inherited directly from mother (sperm mitochondria are degraded and destroyed)
- Ovum contains 100,000 to 1 million mtDNA molecules
- This can be used to trace lineage of families, including human evolution back to Africa ("mitochondrial Eve")

Mitochondrial Distribution

- Approximately 10 million billion total: ∼10% of body weight
- Mitochondria generate and consume the body’s weight in ATP every day
- Average of 200 to 2000 per somatic cell
  - ∼5000 in cardiac cells (myocardial ATP pool turns over every 10 sec)
  - ∼800 in hepatocytes
  - ∼300-400 in neurons (filamentous)

Mitochondrial Functions

- ATP synthesis
- Buffering Ca**+** flux (from endoplasmic reticulum & plasma membrane)
- Maintenance of ion gradients (polarized cells)
- Generation of endogenous ROS
Mitochondrial Functions

- Cell signaling (esp. immune)
- Regulation of cell growth, cell cycle, metabolism
- Biosynthetic pathways
- Oxidative deamination of monoamines (MAO)

Mitochondrial Bioenergetics: In a Nutshell

- Conversion of biochemical energy into ATP
- Requires catabolism of CHO, fats, & amino acids into carbon skeletons
- Extraction of energy released from catabolism via
  - Glycolysis
  - Citric acid cycle (Krebs)
  - β-oxidation
  - Oxidative phosphorylation

Mitochondrial Bioenergetics

- Relies on transfer of electrons by reduction of NAD⁺ to NADH which is then oxidized and reduced again
- ATP production by electron transport chain coupled to controlled dissipation of proton electrochemical gradient
- Oxygen is ultimate electron acceptor – creating H₂O, CO₂, & ATP
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 (3C) x 2

Glycolysis

• Pyruvate (3C)
• Actively transported into matrix for aerobic respiration by mitochondrial pyruvate carrier (MPC)
• When mitochondrial metabolism inhibited (anaerobic conditions, etc), converted into lactate by LDH, which regenerates NAD+
• MPC is inhibited by thiazolidinediones – preventing utilization of pyruvate
Selected Metabolic Pathways in Mitochondria


2 NADH

Acetyl-CoA

• Primary substrate for TCA cycle - essential to balance between CHO & fat metabolism
• Produced in mitochondrial matrix from coenzyme A combined with acetyl group (2C) from
  • Pyruvate decarboxylation
  • Fatty acids (beta oxidation)
  • Ketone bodies
  • Amino acids

Acetyl-CoA
Tricarboxylic Acid (Krebs) Cycle

- Final common catabolic pathway for all nutrients (protein, fat, carbohydrates)
- Enzymes located in matrix (except for succinate dehydrogenase—complex II)
- Acetyl-CoA oxidized to CO2
- Produces
  - Metabolic byproducts: amino acid precursors
  - NADH, FADH2, GTP

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

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
Long Chain Fatty Acids: Mitochondrial Metabolism

• β-oxidation in matrix
  • Multistep enzymatic process
  • Removes 2-carbon fragments to make multiple acetyl-CoA subunits,
  • Which can be oxidized by the citric acid cycle, or
  • Converted into ketone bodies (in liver)
• Most dietary fatty acids undergo β-oxidation in mitochondria
• Impaired β-oxidation → accumulation of lipid intermediates → insulin resistance

Ketone Bodies

• β-hydroxybutyrate, acetoacetate, acetone
• Produced in response to hypoglycemia by liver mitochondria (but not utilized there)
• Upregulation of carnitine acyltransferase 1 and β-oxidation increases acetyl-CoA in matrix
• Increased NADH & ATP from β-oxidation inhibits citric acid cycle
• This reroutes acetyl-CoA towards ketogenesis

Ketone Bodies

• Ketones soluble in water—no protein carriers required
• Plasma levels increase with fasting, high fat/low CHO diets, and uncontrolled diabetes
• Preferred fuel (vs glucose) for cardiac muscle & renal cortex
• Used in brain (cross BBB) proportionate to concentration in blood, provide energy when glucose availability is limited
Ketogenic Diets for Neurologic Disorders

- Drug-resistant epilepsy: proven anticonvulsant effects, neuroplasticity
- Alzheimer's disease: overcome impaired glucose metabolism in brain; protects against β-amyloid
- Parkinson's disease: increased mitochondrial efficiency, overcomes complex I defects

Ketogenic Diets for Neurologic Disorders

- Amyotrophic lateral sclerosis (theoretical)
- Traumatic brain injury and stroke (theoretical)
- Brain tumors
- Autism spectrum disorders
- Migraines

Oxidative Metabolism

- Production of reducing equivalents (NADH or FADH2) from glucose & fatty acids
- Electrons are transferred from NADH & FADH2 to ubiquinone by complexes I & II, to cytochrome c by complex II, and to O2 by complex IV
- Electron transfer coupled to pumping of protons across inner membrane by complexes I, III, IV
- Chemiosmotic H+ gradient spins ATP synthase turbine to produce ATP or dissipate heat through uncoupling proteins (UCPs), esp in brown fat
Mitochondrial Dysfunction & Disease

- Chronic fatigue; fibromyalgia
- Metabolic syndrome: insulin resistance, T2DM, obesity, NAFLD
- Cardiovascular disease (esp CHF)
- Cancer
Mitochondrial Dysfunction & Disease

• Neurodegenerative & neuromuscular disorders
• Mood disorders, eg. bipolar disorder
• Premature aging

5 Major Sources of Mitochondrial Dysfunction:

• Oxidative stress
• Macronutrient overload
• Glucotoxicity (including AGEs)
• Lipotoxicity
• Environmental toxicity

Denham Harman, MD, PhD

• 1956: proposed idea that “free radicals” damage macromolecules: proteins, nuclear and mitochondrial DNA, lipids (membranes and free PUFAs)
• Postulated that this free radical damage plays a major role in aging
• 1972: revised hypothesis: mitochondria determine lifespan
• 1998: died at age 98 (after short illness)
An individual produces about 1 kg of oxygen radicals per year. The consequence is around 100,000 oxidative attacks on mtDNA per cell per day.
Causes of Excessive Mitochondrial ROS

• Dietary factors
• Hyperglycemia (impact on endothelium)
• Inflammatory mediators (TNFα)
• Hypoxia (acute & chronic)

Causes of Excessive Mitochondrial ROS

• Environmental chemical pollutants & toxicants
• Toxic metals & metalloids
  – mercury
  – cadmium
  – arsenic
• Ionizing radiation
Does that mean free radicals are always harmful and antioxidants are always good?

How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis).

Exp Gerontol. 2010 Jun;45(6):410-8

In contrast with Denham Harman’s free radical theory of aging, increased formation of ROS within the mitochondria cause an adaptive response (mitohormesis) that culminates in increased stress resistance and a long-term reduction in oxidative stress.

How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis).

Exp Gerontol. 2010 Jun;45(6):410-8

- ROS are essential signaling molecules which are required to promote health and longevity.
- Abrogation of this mitochondrial ROS signal by antioxidants impairs the lifespan-extending & health-promoting capabilities of reduced calorie uptake, glucose restriction & physical exercise.
"What doesn’t kill you, makes you stronger!"

Figure 2. Differential responses to rising oxidative stress.

Antioxidant Mythology

Myth #1: All Free Radicals Are Bad

Myth #2: The More Antioxidants, the Better

Myth #3: All Antioxidants Act the Same

Mitochondrial Poisons
Two Deadly Mitochondrial Poisons

- Cyanide
- Carbon Monoxide
- First identified as mitochondrial toxins in 1940s
- Block mitochondrial energy production by displacing oxygen from heme (hemoglobin, cytochrome c)

Mitochondrial Toxins: Environmental Chemicals

- MPP+ (MPTP metabolite via MAO) binds to and inhibits complex I, reduces mitochondrial motility—“parkinsonian mimetic”
- Rotenone (“fish killer”): blocks ubiquinone binding to complex I, induces PD in rodents
- Pyridaben: increases oxidative stress by inhibiting complex I; induces PD
- Paraquat: increases ROS from complex I; impairs mitochondrial membrane permeability; induces PD
- Maneb (Mn containing fungicide): complex III inhibitor; induces PD

Mitochondrial Toxins: Environmental Chemicals

- Atrazine: inhibits complexes I & II, impairs dopaminergic signaling
- Organochlorines (eg dioxin, dieldren, Agent Orange): induced ox-stress, disrupt mitochondrial stress signaling – PD, T2DM
- Bisphenol A: inhibits complex II
- Organophosphates: neurotoxins
- 2,4 Dinitrophenol: uncouples OXPHOS – causes weight loss but potentially fatal
Persistent organic pollutants, mitochondrial dysfunction, and metabolic syndrome


- Epidemiologic & experimental studies have associated insulin resistance or T2DM with elevated body burdens of persistent organic pollutants, which can damage mtDNA
- Mitochondrial DNA abnormalities are known to cause pancreatic beta cell damage, insulin resistance, and diabetes mellitus

- “We propose that the mitochondrial paradigm for the etiology of metabolic syndrome will facilitate the prevention and treatment of this major health problem”

Mitochondrial Toxins: Metals

- Mercury
- Lead
- Arsenic
- Iron (overload)
- Manganese (overload)
Mitochondrial Toxins: Pharmaceuticals

- Acetaminophen: irreversibly inhibits β-oxidation
- Aminoglycoside antibiotics
- Anti-retroviral drugs (NTRIs)
- Aspirin: inhibits & uncouples OXPHOS
- Statins

Mitochondrial Toxins: Pharmaceuticals

- Cancer chemotherapy agents (platinum compounds)
- Metformin: complex I inhibitor
- Tamoxifen: inhibits complexes III & IV
- Valproic acid: inhibits complex IV

Simvastatin Impairs Exercise Training Adaptations
J Am Coll Cardiol. 2013 Aug 20;62(8):709-14

- 37 sedentary overweight or obese adults at risk for metabolic syndrome
- Randomized to 12 wks of aerobic exercise plus placebo, vs exercise plus 40 mg simvastatin
- Exercise only: CV fitness increased by 10% with exercise, along with 13% increase in skeletal muscle mitochondrial citrate synthase (from biopsy)
- Exercise plus statin: CV fitness blunted to 1.5% increase, with 4.5% decrease in citrate synthase
Dietary Factors and Mitochondrial Function

Mitochondrial Dysfunction: Dietary Factors

- Caloric excess (numerous studies)

Mitochondrial Dysfunction: Dietary Factors

- Advanced glycation end products (preformed & hyperglycemic): Pathological Significance of Mitochondrial Glycation; International Journal of Cell Biology, 2012, Article ID 843605
Links between metabolism & cancer
Front Oncol 2013, Vol 3(292): 1-28
Genes Dev. 2012 26: 877-890

- Excessive caloric intake increases risk for cancer; caloric restriction is protective
- Metabolism generates ROS, which contribute to oncogenic mutations (reprogramming of genes)
- Cancer cells undergo metabolic reprogramming: increased aerobic glycolysis (Warburg effect) – rates up to 200x higher than in normal tissues (basis for PET scanning)

Obesity, T2DM & Mitochondrial Function: The Energy Paradox
2002 Oct;51(10):2944-50

- Obese individuals tend to tire more easily and have decreased physical endurance than those with low BMIs, in spite of increased food intake

- Skeletal mitochondria in obese individuals with T2DM, are…
  - small
  - have reduced contents and
  - impaired electron transport activity
Mitochondrial Biogenesis

- Involves replication of mtDNA - increases mitochondrial mass based on energy needs of cell
- Generates new, healthy mitochondria and replaces damaged ones (mitophagy)
- Requires coordinated interaction between nuclear & mitochondrial genomes
- Mediated by a hierarchy of nuclear transcription factors (e.g., PPARs), all of which are dependent on PGC-1 proteins, esp. PGC-1α

Wang, et al., Insulin Resistance, Chapter 2, 2012 (InTech)
**PGC-1α (PPARγ co-activator 1α)**

- Master regulator of mitochondrial biogenesis and energy homeostasis
- Predominantly expressed in mitochondrial-rich tissues such as heart, skeletal muscle, brown adipose tissue, and to some extent liver
- Increases metabolic switch toward β-oxidation, OXPHOS, antioxidant protection, and uncoupling proteins (exercise, cold)

**PGC-1α (PPARγ co-activator 1α)**

- Activity decreases with
  - Caloric overload
  - Saturated fats
  - Refined carbohydrates, fructose
  - Inflammatory mediators (eg TNFα)
  - Pro-oxidants
  - Inactivity
  - Aging

**PGC-1α (PPARγ co-activator 1α)**

- Activity rapidly increases after
  - Exercise: heart & skeletal muscle
  - Fasting/starvation: heart & liver
  - Cold exposure: brown adipocytes & muscle
  - Also activated by
    - Thyroid hormone (T3)
    - Adiponectin
Mitochondrial Theory of Insulin Resistance


- Depressed PGC-1α levels, oxidative damage, and/or environmental toxins impair mitochondrial β-oxidation & OXPHOS in skeletal muscle & liver
- Intracellular build up of lipid metabolites (fatty acyl-CoA, ceramide, diacylglycerol) & lipid peroxides cause lipotoxicity, which
- Impairs intracellular insulin signaling pathways

The Perfect Storm (Insulin Resistance)

- Glucose unable to enter cell
- Lipolysis is inhibited: lipid accumulation in skeletal muscle, liver, & heart
- Gluconeogenesis is inhibited
- Krebs cycle intermediates are depleted
- Only one option remains: Break down muscle
- All these conditions are intracellular energy deficits (obesity, CHF, cachexia, diabetes, fatty liver)

PGC1α Activators

- Mitochondrial damage
- Fasting / calorie restriction
- Exercise (skeletal muscle contraction)
- AMP-Kinase (↑AMP/ATP)
- Sirt1 (deacetylase): activated by NAD⁺
- Nitric oxide
- Triiodothyronine (T3)
- Cold temperature

- Packed with mitochondria
- Brown color from heme (cytochromes)
- Uncoupling protein (UCP-1) in inner mitochondrial membrane dissipates H+ from OXPHOS, generating heat instead of ATP
- UCP-1 activated in response to beta adrenergic stimulation: ↑free fatty acids
- PGC-1α highly expressed – induced by cold

Exercise Increases Mitochondrial Numbers

- Moderate intensity exercise x 4 months
- 67% increase in mitochondrial density
- 55% increase in cardiolipin content
- Increase in mitochondrial oxidation enzymes
- All linked to improvement in A1c and fasting plasma glucose

Benefits of Increased Mitochondrial Biogenesis

- ↓ROS / Oxidative Stress
- ↑Metabolic Function
- ↑Energy Level
- ↑Exercise Performance
- ↓Body Fat / ↑Lean Muscle Mass
- ↓Age-Related Deterioration
- ↑Increased Lifespan (?)
- Cancer suppression
Phytochemicals that support mitochondrial function

- Curcumin: 500-1500 mg
- Sulforaphane glucosinolate: 50-200 mg
- Quercetin: 250-500 mg bid
- Resveratrol: 50 mg bid
- Pterostilbene: 50 mg bid
- Green tea polyphenols: 50-100 mg qd
- Berberine: 500 mg bid

Supportive Nutrients for Mitochondrial Function

- Acetyl-L-carnitine: 1500-3000 mg
- Alpha lipoic acid: 300-900 mg
- Coenzyme Q10 (ubiquinone): 50-200 mg
- Magnesium: 100-500 mg
Supportive Nutrients for Mitochondrial Function

Int. J. Mol. Sci. 2014, 15, 20169-20208
Neurobiol Aging. 2013, pii: S0197-4580(13)00525-3

• N-acetylcysteine: 500-3000 mg qd
• Nicotinamide riboside: 250-500 mg bid
• Creatine: 5-20 grams qd
• Melatonin: 3-20 mg qhs

Branched chain amino acid supplementation promotes survival and supports cardiac and skeletal mitochondrial biogenesis in middle-aged mice

Cell Metab. 2010 Oct 6;12(4):362-72

• Life expectancy increased by 12%
• Number of mitochondria increased
• Old mitochondria changed to look like young mitochondria
• Decreased oxidative stress
• Antioxidant defense systems turned on
• New genes turned on, similar to caloric restriction

• “In summary, we have provided evidence that an original BCAA mixture increases average life span in male mice.
• This was likely the consequence of increased mitochondrial biogenesis and reduced oxidative stress in cardiac and skeletal muscle via eNOS-mediated mechanism.
• Our study offers a rational for deeply exploring the role of amino acids in prevention and control of age-related disorders in humans.”
NAD+

- Universal energy and signal-carrying molecule
- Actively transported into mitochondrial matrix
- "Metabolic monitor": modulates redox reactions & rate of oxidative phosphorylation
- Declines with aging

NAD+

- Regulates mitochondrial transition pore
- Rate-limiting co-substrate for sirtuins 1 & 3 (deacetylases)
- Upregulates PGC1α, which activates mitochondrial biogenesis

NAD+ Regulation of Sirtuins
Nicotinamide Riboside (NR)

- Vitamin B3 analog, produced endogenously and found in food (milk, beer)
- Extensively researched at Weill Cornell Medical College
- The most efficient dietary precursor to cellular NAD⁺

Nicotinamide Riboside (NR)

- NAMPT: rate limiting enzyme that converts nicotinamide into NMN (salvage pathway)
- NAMPT declines with aging and is inhibited in metabolic organs by high fat diet
- NR bypasses NAMPT -- converted directly into NMN by Nr-kinase pathway
ATP depletion:
Calorie restriction, Cold Temperatures, Exercise

NR is the most efficient NAD+ precursor

Mitochondrial Function

NR treatment significantly enhanced mitochondrial function and mitochondrial biogenesis

Gene regulation (numerous)
Nutraceuticals:
Alpha Lipoic Acid, Amino acids, Berberine, Curcumin, Green tea polyphenols, Nicotinamide Riboside, Pterostilbene, Quercitin, Resveratrol

AMPK
↓
SIRT1
↓
PGC-1alpha
[muscle, brain, liver, brown adipose tissue]
Gene regulation (harmonizes)

Mitochondrial Biogenesis
↑ ATP
↑ Fatty acid oxidation, ↑ Energy, Endurance, Weight loss, Longevity

Supporting Mitochondrial Function
• Adequate nutrition – judicious caloric intake
• Avoid toxins in foods, personal care products, lawn chemicals, home cleaning chemicals
• Eat curry, broccoli, spinach, blueberries
• Drink green tea, red wine or purple grape juice
• Stay cool and well-hydrated
• Challenge brain and body with physical & cognitive exercise
• Minimize emotional stress

“Be honest—how much are you exercising?”