Basic Mitochondrial Disease

& Supplementation (an Update)

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Mitochondria Background
Mitochondrial Background

• Each cell contains hundreds to thousands of mitochondria
• Are where energy (ATP) is created in each cell
  • Oxidative phosphorylation, pyruvate oxidation, Krebs Cycle (aka TCA cycle, Citric Acid Cycle), and fatty acid oxidation
• Are prokaryotic in nature (very similar to bacteria)
  • Contain their own DNA (mtDNA)
  • Produce some of their own proteins
  • Replicate on their own during cellular division
• Are inherited solely from the mother
  • Ovum contains all fetal mitochondria, sperm does not pass on mitochondria
  • This has been challenged recently, but there is no definitive proof sperm contribute mitochondria or mtDNA to fetal cells
**CYTOSKELETON:** supports organizes and cell shape and plays a role in cell motion. 
- **Microtubule:** tube of protein molecules present in cytoplasm, centrioles, cilia, and flagella.
- **Intermediate filament:** intertwined protein fibers that provide support and strength.
- **Actin filament:** twisted protein fibers that are responsible for cell movement.

**NUCLEUS:** command center of cell 
- **Nucleolus:** site where ribosomes are produced.
- **Nuclear envelope:** double membrane between the nucleus and the cytoplasm.
- **Nuclear pore:** opening embedded with proteins that regulate passage into and out of the nucleus.
- **Ribosomes:** small complexes of RNA and protein that are the sites of protein synthesis.

**Smooth endoplasmic reticulum:** system of internal membranes that aids in the manufacture of carbohydrates and lipids.

**Rough endoplasmic reticulum:** internal membranes studded with ribosomes that carry out protein synthesis.

**Centriole:** complex assembly of microtubules that occurs in pairs.

**Cytoplasm:** semifluid matrix that contains the nucleus and other organelles.

**Mitochondrion:** organelle in which energy is extracted from food during oxidative metabolism.

**Secretory vesicle:** vesicle fusing with the plasma membrane, releasing materials to be secreted from the cell.

**Peroxisome:** vesicle that contains enzymes that carry out particular reactions, such as detoxifying potentially harmful molecules.

**Plasma membrane:** lipid bilayer in which proteins are embedded.

**Lysosome:** vesicle that breaks down macromolecules and digests worn out cell components.

**Golgi complex:** collects, packages, and distributes molecules manufactured in the cell.

For guidance purposes only
Mitochondrial Background

• Damage to the mitochondria cannot be reversed
  • Unhealthy mitochondria will continue to multiply
  • Eventually, as mitochondria are damaged over time, each cell becomes populated with ineffective or non-functioning mitochondria

• Mitochondrial damage can be inherited
  • If mother suffers from a mitochondrial disorder, it will be passed down to children and may be amplified initially or over time

• Everyone has progressive damage to their mitochondria (and cells)
  • Is the reason that we age
  • Is more pronounced in those unable to physiologically cope with that damage
The Krebs Cycle

Krebs Cycle
(Citric Acid Cycle)

Image from:
http://uwstudentweb.uwyo.edu/a/ateeter/krebs_cycle.gif
Electron Transport Chain (ETC)

Image from: http://www.nature.com/nm/journal/v11/n6/images/nm0605-598-F1.jpg

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Electron Transport Chain (ETC)

• Contained in the inner membrane
  • Proton (H+, hydrogen ion) gradient is created in the intermembrane space and flows into the matrix through ATP Synthase

• Complex I – NADH/Co-Q reductase
• Complex II – Succinate/Co-Q reductase
• Complex III – Co-Q/Cytochrome bc1 reductase
• Complex IV – Cytochrome c oxidase
• ATP Synthase (Complex V) – Processes proton gradient and creates ATP
Mitochondrial Damage

SOD = Superoxide dismutase
NO = nitric oxide (free radical)
ONOO⁻ = free radical
·OH = hydroxyl free radical

Images from:
http://www.chinaphar.com/1671-4083/25/figs/977f1.jpg
http://www.nature.com/msb/journal/v3/n1/images/msb4100135-f8.jpg
Mitochondrial Location

• All cells contain mitochondria, but some are more reliant (contain more) than others

• Tissues/sites in decreasing order of vulnerability:
  • Brain, skeletal muscle, heart, kidney and liver
  • Is the reason why most mitochondrial diseases (mtD) are neurodegenerative and neuromuscular
Available Therapies

• Supplementation of nutrients, vitamins and cofactors
• Diet, lifestyle and nutrition
• Exercise, physical therapy
• Speech and cognitive therapies
• Surgery
  • For seizures, organ failures, etc
• Assistive devices
  • For hearing loss, cardiomyopathies
• Genetic therapy/mitochondrial replacement
  • Still highly experimental and speculative
Supplements
Supplementation

• Is a combination of vitamins, cofactors and antioxidants
• Formulas vary from patient to patient based on specific metabolic needs
  • Based on blood work, muscle or tissue biopsy, DNA testing
• May need increased dose/extra supplementation during acute exacerbations of mtD, stress or infection
• Doses may start out the same for all patients initially (depending on what preference a prescriber has) and adjust as lab values come back or patient response to certain supplements
  • No 2 people, despite having the same genetic mutation, will have the same dose since response varies
Supplements

- Most data is anecdotal, not a lot of clinical evidence to support response of mtD to supplementation
  - *Not yet proven that these therapies alter the course of mtD*
- Parikh, Sumit et al “A Modern Approach to the Treatment of Mitochondrial Disease”
  - Reference for supplement information, implementation and dosing
  - Available at [http://www.mitoaction.org](http://www.mitoaction.org)
- Sumit Parkih, et al “Diagnosis and Management of Mitochondrial Disease: A Consensus Statement from the Mitochondrial Medicine Society”
  - Most current reference, tries to tie in clinical research and anecdotal evidence of Mito doctors to generate “consensus statements”
Coenzyme Q-10

- Is normally created in all mammalian mitochondria
- Integral part of the ETC → transfer of electrons from Complex I & II
- Found in all cell and organelle membranes
  - Participates in redox reactions (reduction/oxidation – most common type of biochemical reaction in the body, especially in energy production), acts as an antioxidant and a pro-oxidant
- Involved in apoptosis (programmed cell death), permeability of mitochondrial pores, activates uncoupling proteins (acts as a cellular signal)
- Kinetics
  - Insoluble in water
  - Powder formulations have poor intestinal absorption (max transport in GI tract of 2400mg/day in adults)
  - Improved bioavailability when using nano-particles in suspension
- Is FDA approved for the treatment of mtD


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# Coenzyme Q-10

- **Ubiquinone:** most common, has been available a long time
  - Potency may vary greatly between manufacturers
  - Oil based gels have higher bioavailability vs. suspensions
  - Half-life is 33 hours

- **Ubiquinol:** more recent, 3-5 times better absorbed
  - Half-life of 48 hours

- **Idebenone:** synthetic version, lower molecular weight
  - Proposed to use at lower doses when given SL (sublingually, under the tongue) (0.01-4mg/kg/day SL vs. 100mg/kg/day PO (by mouth))
  - Possible stimulation of nerve growth factor (NGF), serotonin and dopamine
  - May be beneficial in Alzheimer’s, Parkinson’s and Huntington’s Disease, and Friedrich’s ataxia

- All have the side effect of causing wakefulness – take last dose of the day in the afternoon

- Excess that is not utilized by the body gets stored in fat cells and remains around for a long time – potentially harmful, needs to be dosed at high doses by a physician monitoring blood/tissue levels and response.
Riboflavin (Vitamin B2)

• Is a water-soluble vitamin
• Serves as a flavoprotein precursor (utilized by many proteins in the body)
• Is a key building block for Complex I & II, as well as a cofactor in several other enzymatic reactions involving fatty acid oxidation and the Krebs Cycle
• Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) is caused by a gene mutation and is an inborn error of metabolism
  • Riboflavin supplementation alleviates the symptoms and slows the disease’s progression
• Several non-randomized studies have shown riboflavin to be efficacious in treating mtD, specifically Complex I & II disease
• Side effect of anorexia, nausea (at high doses), change in urine color
Levocarnitine (L-Carnitine, Carnitine)

• Critical in β-oxidation of fatty acids (FA)
  • Helps transport long-chain FAs across the mitochondrial inner membrane
  • FAs are then oxidized into Acetyl-CoA and enter the Krebs Cycle
  • β-oxidation is critical in tissues such as: heart, skeletal muscle, and liver

• May help prevent CoA depletion and remove excess acyl compounds which could be toxic

• Some is endogenously produced and the majority comes from diet
  • mtD is not due to lack of transport or synthesis of carnitine, rather patients with ETC defects tend to have a lower free carnitine level (possibly may reflect partial β-oxidation impairment)

• Side effect of body, urine and fecal odor changes – a more “fishy” smell

Image from:

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Carnitine’s Role


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Folinic Acid (Leucovorin)

- Is the reduced form of Folic Acid (Vitamin B9)
- Is a cofactor in multiple metabolic reactions (donates a methyl group)
- Some case reports state that mtD may lead to cerebral folate deficiency
  - Mechanism behind this is unclear
- Deficiency mostly seen in Kearns-Sayre Syndrome
- Side effect of Folic Acid (and therefore potentially Folinic Acid) is itchiness
L-Arginine

- Arginine use in the body is beneficial for some patients to increase nitric oxide (NO⁻) production
  - Is a precursor
  - Utilized to vasodilate in cardiovascular/cerebrovascular disease
  - Not always beneficial in all patients
- May also act as a free-radical scavenger in endothelial cells
- Is not dependent on tissue saturation for effectiveness
- Stroke-like symptoms are present in many mtD syndromes (ex: MELAS)
  - Citrulline can be used as well – more expensive
- Oral and IV administration of L-Arginine does show improvement in clinical symptoms, severity, and frequency of stroke-like episodes

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Image from: [http://www.chemsynthesis.com/molimg/1/big/40/40365.gif](http://www.chemsynthesis.com/molimg/1/big/40/40365.gif)
N-Acetylcysteine (NAC)

- Is a medication that is used for several functions:
  - Inhaled for cystic fibrosis to loosen mucus in lungs
  - Injected for Acetaminophen (Tylenol®) overdoses
  - Supplement for kidney disease to help damaged tissue
- In mitochondrial/metabolic therapy it is used in 2 main functions:
  - To act as an anti-oxidant on its own in the body
  - To act as a replenishing agent for glutathione, the body’s natural (and very powerful) anti-oxidant
- NAC is a modified cysteine molecule, which is a part of glutathione

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Image from:
https://upload.wikimedia.org/wikipedia/commons/3/3a/(R)-N-Acetylcysteine_Structural_Formulae.png
N-Acetylcysteine (NAC)

• How it works
  • It helps reduce oxidated glutathione back to its reduced state
    • Interpretation: when glutathione does its job protecting the body, it becomes oxidized because it “scavenged” damaging free radicals. This makes it non-functional. NAC brings it back to its normal state, allowing glutathione to continue functioning.

• Many people have issues making glutathione, or they cannot make glutathione fast enough to keep up with the free radicals produced by the body
  • Adding NAC helps keep more glutathione “active” in the body
NAC vs. Glutathione

• While it seems to make sense that replenishing glutathione would be ideal, it's very hard to do
  • If administered orally, the body will digest it into amino acids and absorb it as individual components, destroying its function
  • Topical administration works well with getting the medication into the body, but can be expensive, not very well covered by insurances and the body needs a constant supply of the medication since it is always using glutathione (especially in energy-dependent tissues)
  • IV administration is ideal since it makes it into the blood and distributes well in the body & is a constant administration. However, this is expensive, and very cumbersome to patients
NAC vs. Glutathione

• NAC is cheaper and can be administered orally
  • Comes in powder or capsules
    • Can be swallowed as capsules or opened & mixed with foods/beverages
  • Liquid formulations are unstable, exception is the injectable medication, but it is sealed & under pressure with preservatives
  • Smell is pretty powerful – very sulfur-heavy smell (rotten eggs)
    • May have a hard time administering to children due to smell, which affects taste

• Whatever amount of NAC that is not utilized in the body immediately after taking the medication will remain around for a while, either waiting to be used for glutathione or as an antioxidant on its own
  • After a while, it is eliminated by the body, and higher doses are fairly well tolerated

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Other Vitamins and Redox Agents

- Thiamine (Vitamin B1): critical in carbohydrate metabolism
  - Also used in nucleic acid (DNA base) synthesis indirectly
- Ascorbic Acid (Vitamin C): antioxidant that helps replenish Vitamin E
- Tocopheryl (Vitamin E): antioxidant in the cellular and organelle membranes
- Alpha-Lipoic Acid: antioxidant for cell and mitochondria
- Pyridoxine (Vitamin B6): helps with neuropathy, component of neurotransmitter synthesis
- Niacin (Vitamin B3): deficiency leads to slow metabolism, intolerance to cold
- Cyanocobalamin (Vitamin B12): RBC (red blood cells) growth and proliferation
Creatine Monohydrate

- Is present in all cells – either made by the body or from diet
- Undergoes a reaction with ATP to phosphocreatine in the mitochondria
  - Source of energy during anaerobic metabolism (when exercising and muscles lack oxygen)
- Acts as an intracellular buffer for ATP and an energy shuttle for high-energy phosphates from mitochondria to cytoplasm
- Found in tissues with high energy demands
- Reduced in muscle tissues of patients who have mitochondrial myopathies
- Small studies have shown an increase in high-intensity, isometric, anaerobic and aerobic power
  - No effect on body composition, 2-minute walk, or activity of daily living (ADL) scores

Image from: [http://www.rice.edu/~jenky/images/creatine_reviewPCT01.JPG](http://www.rice.edu/~jenky/images/creatine_reviewPCT01.JPG)
Side Effects

• All of the vitamins cause GI upset
  • This is due to the fact that the GI tract is trying to dissolve a massive amount of concentrated nutrients to absorb them and floods the GI tract with water
  • Leads to nausea, diarrhea and gas
• Steps to mitigate this are:
  • Take with food & water
  • When starting the medication, if it is an issue, start with 1/4th or 1/2 of the daily dose for the first week, then increase it over the next couple of weeks
• The body adjusts to this side effect over a 7-10 day period as it gets used to the daily dose
  • Do not get discouraged and stop the medication!
  • Talk to you prescriber or pharmacist and go over how you feel with them
Toxicity/Monitoring

- Overall, very safe and if mistakenly given to wrong child or in overdose, and will only result in GI distress

- Chronic use of certain vitamins/supplements may lead to toxicity
  - CoQ-10: possible pro-oxidant effects and pro-signaling pathways triggered
  - Creatine: may elevate SCr (serum creatinine) and crystallize in kidney – is a problem with renal impairment
  - Levocarnitine: possible buildup of toxic metabolites in renal impairment, possible cardiac rhythm disturbances
  - B-complex: neuropathies
  - Vitamin E: possible adverse cardiac risks if >400IU/day over an extended period of time

- Drug interactions are mild to non-existent
  - Possible erythromycin, warfarin interactions
  - Space from any osmotic laxatives (PEG 3350) and bulk-forming laxatives by 1 hour prior, 2 hours after to maximize absorption.
  - Space appropriately from Cholestyramine
Diet, Lifestyle and Nutrition

• Avoiding mitochondrial toxins
  • Certain medications may exacerbate mtD symptoms or are directly toxic to mitochondria (ETC interference, increase in ROS, impaired protein transport, inhibit mtDNA replication, or some combination)
  • Valproic acid: inhibits FA oxidation, Krebs Cycle and ETC; carnitine depletion
  • Anti-retrovirals (HIV medications mostly): impairment of mtDNA replication; lactic acidosis; carnitine depletion; lipodystrophy
  • Statins: CoQ-10 depletion
  • ASA: inhibition and uncoupling of ETC
  • Aminoglycosides, platinum chemo agents: impaired mtDNA translation
  • APAP: oxidative stress (creates ROS compound call NAPQI)
  • Metformin: inhibition of ETC, enhanced glycolysis
  • Beta-blockers: oxidative stress
  • Steroids: unknown

Medication Expectations

• This is not something that will work overnight!
  • Allow 1-2 months of taking the medication before making an assessment if this works for you or not
  • Follow up with prescriber for additional blood work or testing
• Keep a diary
  • Start a diary 1-2 weeks before therapy – write how you feel or how who you are caring for feels/behaves
    • Track fatigue, pain, thought processes, behavior, etc.
    • Track morning, noon and night (if possible)
  • Continue while on the medication and review every few weeks or a month to see how you are doing
• If you decide the medications are not worth the cost based on your diary, stop the medications and continue the diary
  • See how you feel after stopping, you can always restart the medication

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Formulations & Safety
Formulations

• Depending on the pharmacy, there are a myriad of ways to make the compound:
  • Suspension
  • Powder
  • Capsule
  • Effervescent packets
  • Gummies
Formulations

• Depending on the dose of supplements per day, some formulations are preferred over others due to the high volume of supplements
  • Capsules – you may be taking 2-10 capsules a day
  • Powder – a calibrated scoop twice a day
  • Suspension – 5-10mL twice a day (sometimes less)
  • Gummies – usually harder to formulate since a MAXIMUM of 300mg of medication can be added into 1 gummy, typical formulations may require up to 30 gummies a day to meet daily dose
  • Effervescent packets – may take up to 5 packets a day but can taste better than other formulations
Formulations

• All formulations should have the daily dose divided into at least twice a day administration, and can be up to 4 times a day if necessary
  • More frequent dosing leads to better GI absorption of medications since the GI tract does not become overwhelmed
  • Decrease risk of GI upset
  • Helps patients who have absorption issues in GI tract
  • Decrease compliance of medication because need to remember frequent dosing and trying to get a child to take a bad-tasting medication more often may not work well
Considerations

• Make sure to check where you get your supplements from
  • Not all OTC brands are created the same
  • Use brand-name supplements like: Metagenics, Epic4Health, Solgar, RainbowLight, Pure Encapsulation etc.
    • They have a track record of safety and quality because they voluntarily test what they make
  • **NO** over-the-counter supplement is regulated by the FDA, which means that they **DO NOT** need to have what they claim on the label in the medication – if you get CoQ10 600mg capsules, you may get more or less of the medication
    • Contact the manufacturer and ask them what they do for quality control
Considerations

• Make sure to check where you get your compounds from
  • Pharmacies source ingredients from FDA approved wholesalers which are required to maintain a certain degree of testing and standards
    • Pharmacies get information on the powders they order that has a sample analysis for contamination and purity, if it fails, the powder is rejected
  • Compounds are **NOT** FDA-approved, but ingredients should be sourced from FDA-approved facilities
    • Speak with the pharmacy you plan to use and see what their quality standards are – do they send for testing? Who do they source from?
    • If they are PCAB (Pharmacy Compounding Accreditation Board) Certified, most likely they are following good compounding practices
Cost

- Using a trusted source for over-the-counter medications may be more expensive than not, but it is worth the peace of mind knowing you are paying for something that has quality behind it.
- Compounding is even more expensive since there are more rigorous testing procedures utilized at the wholesaler & pharmacy level to make the medication.
- Pharmacies usually work with insurance companies to cover medications, though more and more insurances are requiring prior authorizations (PAs) for compounds or not covering them.
  - PAs are additional paperwork generated by the pharmacy/doctor which have to be filled out by the doctor, faxed to the insurance, insurance has to review them and then make a decision to approve/deny paying for the medication.
  - PAs can take anywhere from 2 days to 2 months for processing, depending on the doctor’s office and the insurance company.


Additional Info
Mitochondrial Background - Genetics

- Some genes for mitochondrial proteins are contained in the nucleus of the cell
  - Allows for proteins to be synthesized in the cytosol (main cell body), then delivered to the mitochondria
  - These genes are inherited from mother and father
- The rest of the genes are contained in the mitochondria
  - Responsible for subunits of Complex I, Complex III, Complex IV and ATP Synthase
- Each mitochondrion contains 2-10 copies of its DNA
  - Are constantly subject to reactive oxygen species (ROS) due to oxidative phosphorylation (main part of energy cycle) byproducts – leads to mtDNA damage
  - Compared to nuclear DNA, there is inadequate repair mechanisms for the mtDNA
  - As copies of mtDNA are damaged, more and more proteins are made inaccurately, which shifts the mitochondrion to an unhealthy state until it is no longer functional (called the threshold effect)
Mitochondrial Structure

- **Outer membrane (phospholipid bilayer)**
  - Contains: transport proteins, lipid conversion proteins

- **Intermembrane space**
  - Contains: enzymes that use ATP to phosphorylate other nucleotides

- **Inner membrane (phospholipid bilayer)**
  - Contains: pores, electron transport chain (ETC), ATP Synthase, transport proteins

- **Matrix**
  - Contains: mtDNA, enzymes for the Krebs Cycle, ribosomes, mtDNA enzymes, tRNA

Images from:
- http://supplementalscience.files.wordpress.com/2009/03/mitochondrion.jpg

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