



Basic Mitochondrial Disease

& Supplementation (an Update)

Dr. Ted Toufas, BS, PharmD, RPh

Pharmacist-in-Charge of Compounding Lab

Acton Pharmacy



Acton Pharmacy
563 Massachusetts Ave
Acton, MA 01720
saaddinno@dinnohealth.com
www.actonpharmacy.com
978-263-3901



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Mitochondria Background

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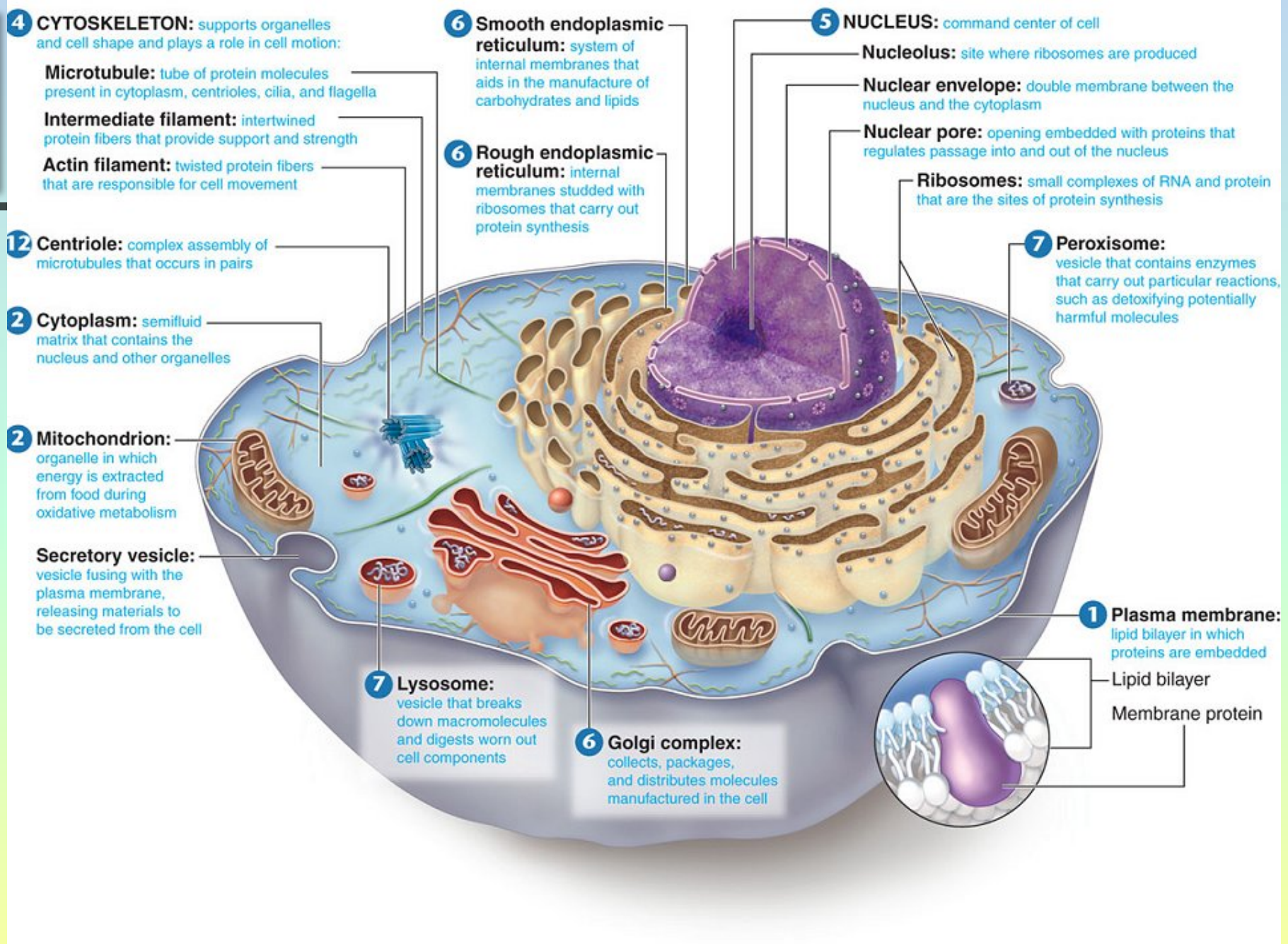
Mitochondrial Background



- 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



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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



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The Krebs Cycle

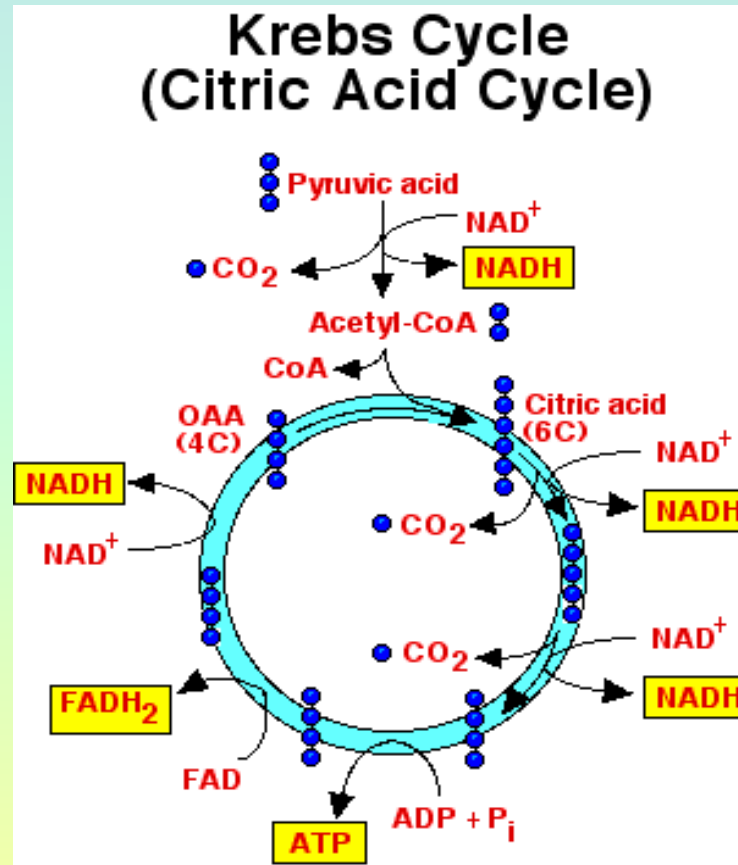


Image from:
http://uwstudentweb.uwyo.edu/a/ateeter/krebs_cycle.gif

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Electron Transport Chain (ETC)

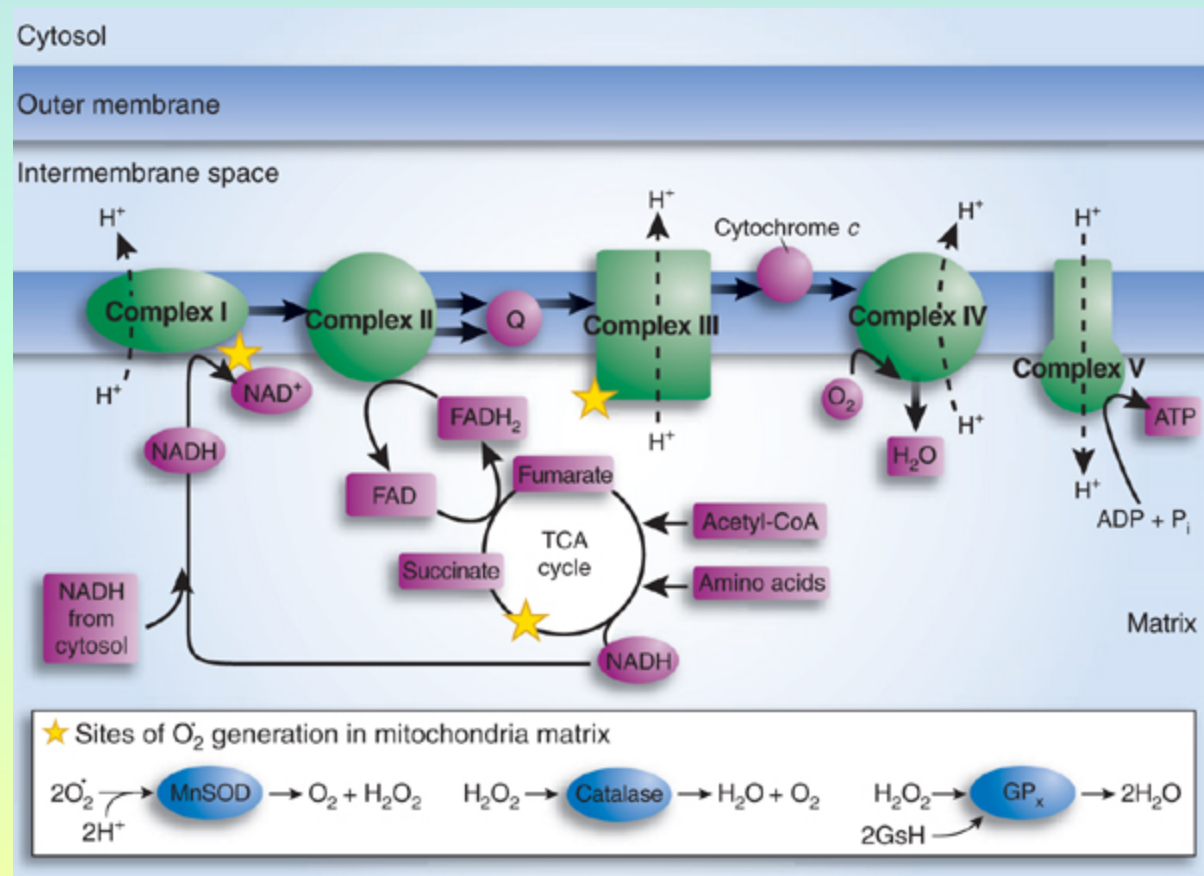


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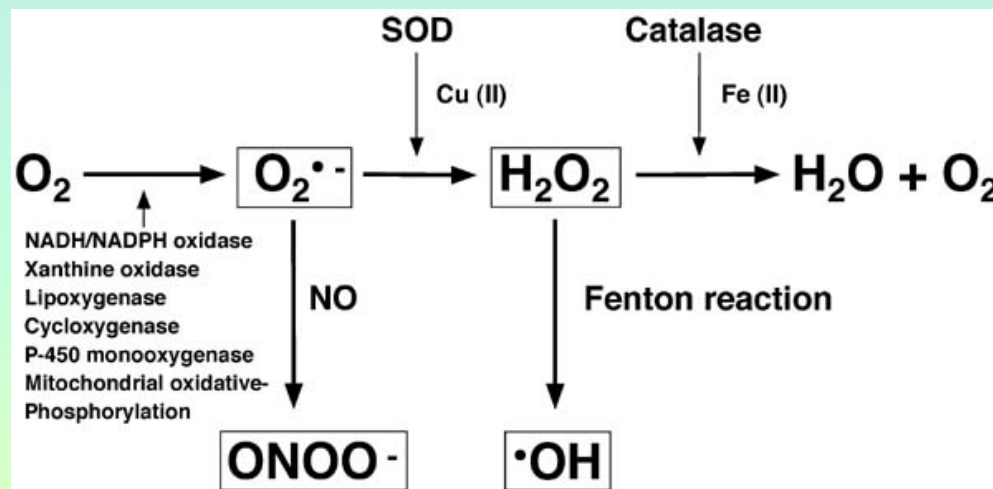
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Electron Transport Chain (ETC)

- 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

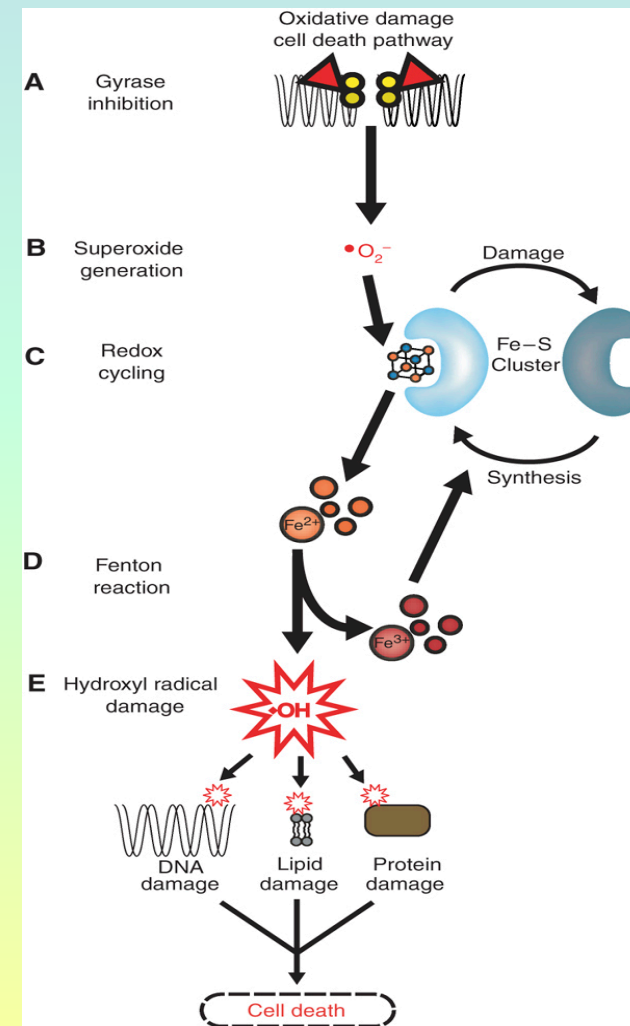


Mitochondrial Damage



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



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Supplements

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Supplementation



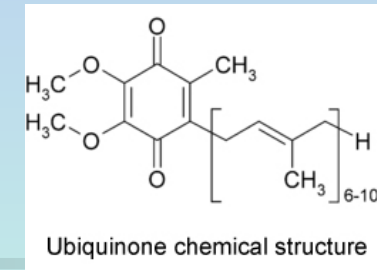
- Is a combination of vitamins, cofactors and antioxidants
- Formulas vary from patient to patient based on specific metabolic needs
 - Based on blood work, muscle or tissue biopsy, DNA testing
- May need increased dose/extra supplementation during acute exacerbations of mtD, stress or infection
- Doses 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>
- 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

Image from:
[https://www.truerenu.com/tr/Images/Ingr_Coenzyme-Q10-\(CoQ10\).jpg](https://www.truerenu.com/tr/Images/Ingr_Coenzyme-Q10-(CoQ10).jpg)

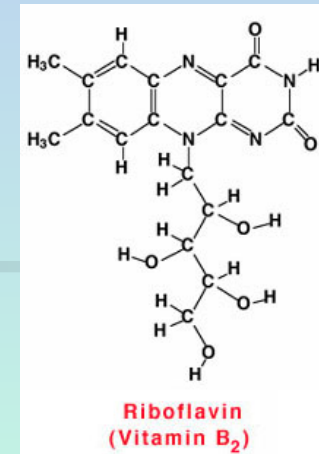
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Coenzyme Q-10



- 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

Image from:
<http://www.chemistry.wustl.edu/~courses/genchem/LabTutorials/Vitamins/images/Riboflavin.jpg>

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

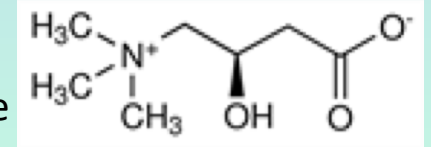


Image from:
<http://www.medicinescomplete.com/mc/martindale/2009/images/c541-15-1.gif>



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Carnitine's Role

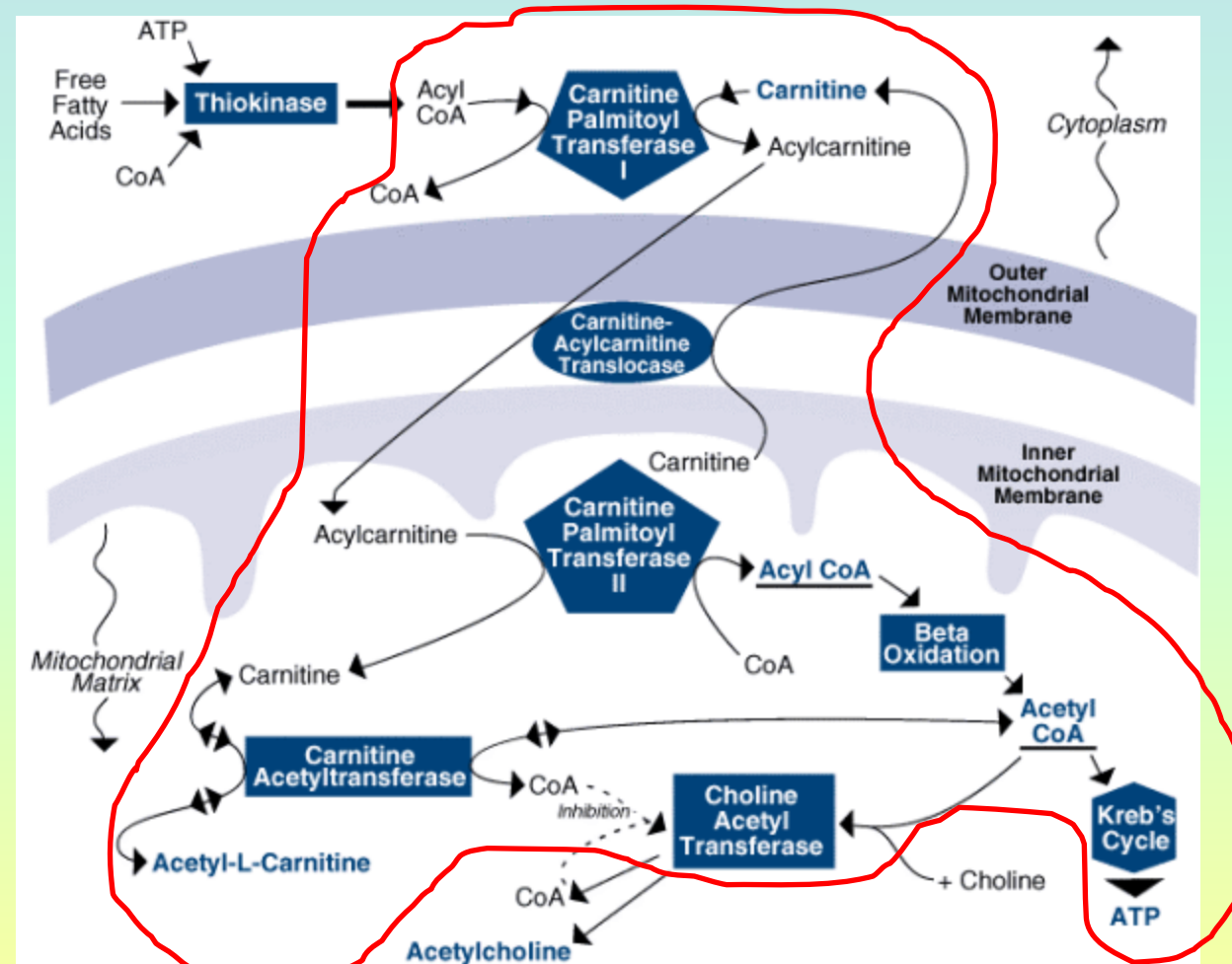
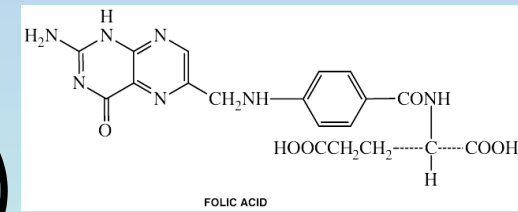


Image from:
http://www.siteground247.com/~bettergy/index.php?option=com_content&view=article&id=155:acetyl-l-carnitina&catid=118:integrazione

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Folinic Acid (Leucovorin)

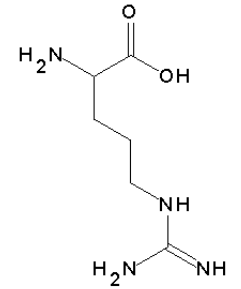


- Is the reduced form of Folic Acid (Vitamin B9)
- Is a cofactor in multiple metabolic reactions (donates a methyl group)
- Some case reports state that mtD may lead to cerebral folate deficiency
 - Mechanism behind this is unclear
- Deficiency mostly seen in Kearns-Sayre Syndrome
- Side effect of Folic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

Image from:
<http://home.caregroup.org/clinical/altmed/interactions/Images/Nutrients/folicaci.gif>

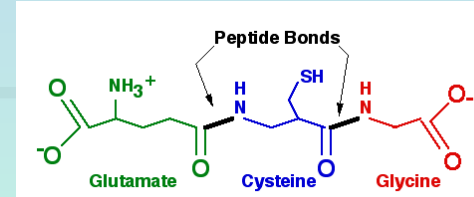
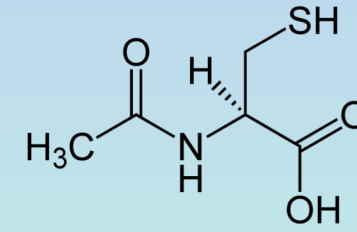
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L-Arginine



- Arginine use in the body is beneficial for some patients to increase nitric oxide (NO \cdot) 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

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

Image from:
[https://upload.wikimedia.org/wikipedia/commons/3/3a/\(R\)-N-Acetylcysteine_Structural_Formulae.png](https://upload.wikimedia.org/wikipedia/commons/3/3a/(R)-N-Acetylcysteine_Structural_Formulae.png)
<http://www.madsci.org/posts/archives/2003-10/1066260582.Bc.1.gif>

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N-Acetylcysteine (NAC)



- How it works
 - It helps reduce oxidated glutathione back to it 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, its 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



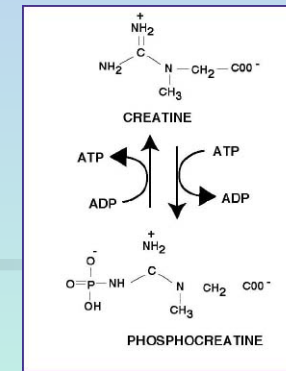
- NAC is cheaper and can be administered orally
 - Comes in powder or capsules
 - Can be swallowed as capsules or opened & mixed with foods/beverages
 - Liquid formulations are unstable, exception is the injectable medication, but it is sealed & under pressure with preservatives
 - Smell is pretty powerful – 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

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

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 your 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
- <http://www.mitoaction.org/files/Mito%20Toxins%20Chart.pdf>

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

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Formulations



- Depending on the pharmacy, there are a myriad of way 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

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Additional Info

Mitochondrial Background - Genetics



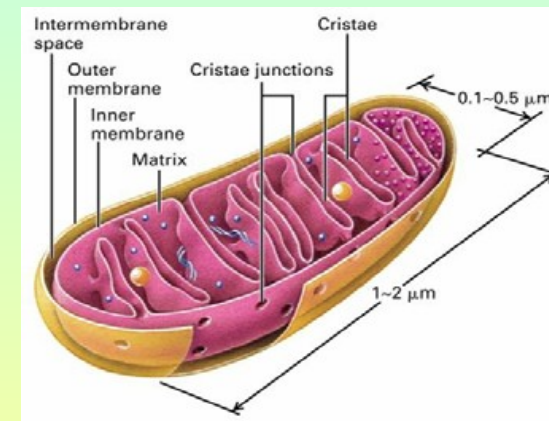
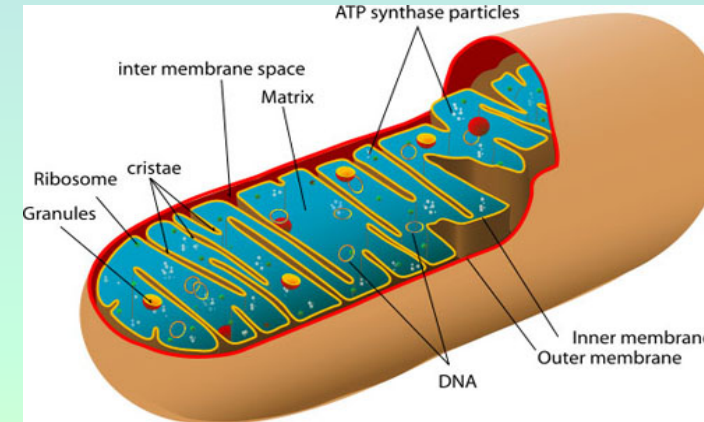
- Some genes for mitochondrial proteins are contained in the nucleus of the cell
 - Allows for proteins to be synthesized in the cytosol (main cell body), then delivered to the mitochondria
 - These genes are inherited from mother *and* father
- The rest of the genes are contained in the mitochondria
 - 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*)



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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://www.eloscense.com/blogoscense/wp-content/uploads/2009/09/MitochondriaSMALL2.jpg>

<http://supplementalscience.files.wordpress.com/2009/03/mitochondrion.jpg>

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