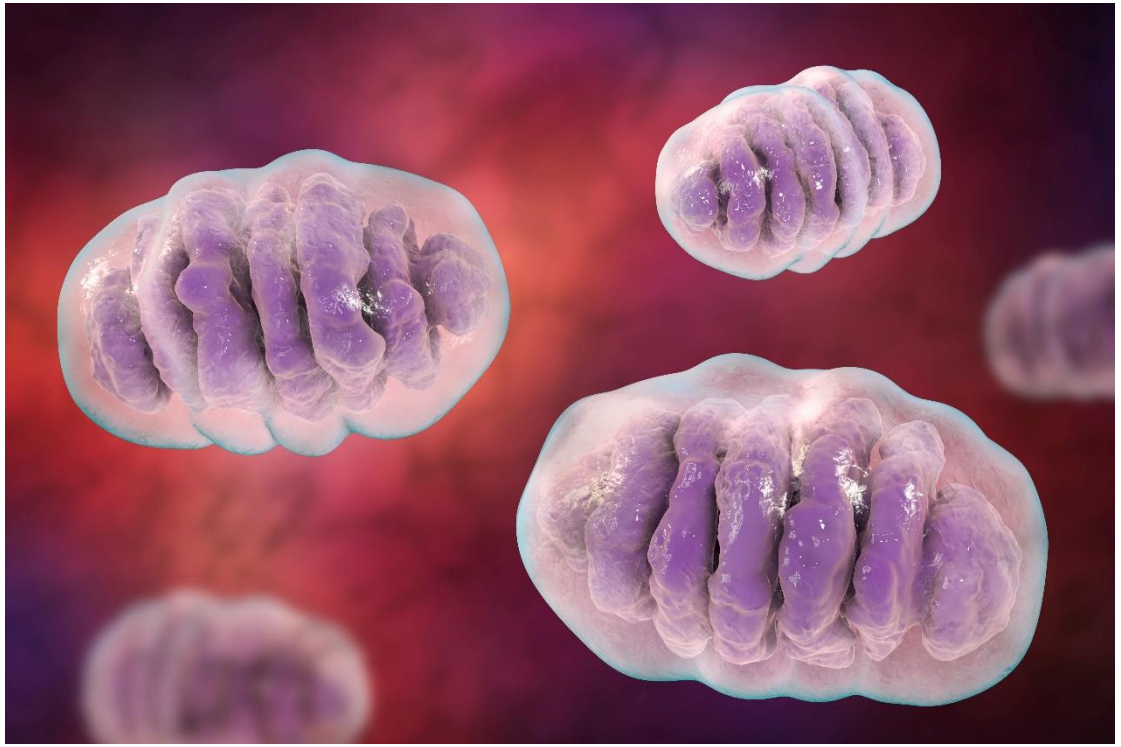
Cancer Metabolics & Epigenetics Update



Dr. Neil McKinney, BSc, ND (non-practicing)

NFH Webinar

Dr. Neil McKinney, BSc, ND

- Clinical practice of integrative medicine for 35+ years – non-practicing.
- Cancer Researcher since 1977.
- Founder of Boucher Institute of Naturopathic Medicine www.binm.org and the B.C. Naturopathic Association www.bcna.bc.ca
- Professor of Naturopathic Oncology (retired).
- Author of textbook *Naturopathic Oncology*

www.vitalvictoria.ca

In Sickness  and in Health

Disadvantage

- Diabetic
- Obese
- Sedentary
- Carnivorous
- Immuno-compromised
- SAD - highly processed food
- Exposed to pollutants, heavy metals

Advantage

- Pesco-vegetarian
- Mediterranean diet
- Low carbohydrate intake
- Whole foods
- Exercise habit
- Non-chaotic lifestyle
- Zest for life
- Clean environment

SAD – Standard American Diet

- Ancestral hunter-gatherer diets, for almost all of human history, provided a sodium to potassium ratio of 1:10.
- Pre-agriculture and early agriculture diets produced a NEAP net alkaline balance of 88 mEq daily.
- The modern western diet creates a **Na to K ratio** > 3:1 - a 30-fold change!
- And with it came a change to a **net acid** residue of 48 mEq daily - a 3 fold change in alkaline to acid!
- Acid forming foods: salt, sugar, sodas, and meat. Alkaline foods: plants.
- Acid reduces oxygen saturation, inducing **hypoxia**.

Acidosis, Hypoxia and Bone, Arnett, Arch. Biochem. Biophys**.** 2010; 503(1): 103-109

pH: Diet Induced Cellular Acidosis, Joe Pizzorno, ND © 2012.

Diet, evolution and aging--the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet, Frassetto, et al., Eur. J. Nutr. 2001 Oct;40(5):200-13.

Cancer and Fetal Cells – Symmetric Mitosis

- Humans start out as an egg and a sperm floating in a low oxygen fallopian tube.
- Undifferentiated biomass accumulates.
- In time the fetus implants and gets a strong blood supply by building a placenta. Oxidative metabolism increases.
- After the first trimester stem cells reproduce differentiated cells by asymmetric mitosis.
- Differentiated cells lost to apoptosis, autophagy, necrosis or trauma, are replaced by stem cells, which also give rise to a replacement stem cell.

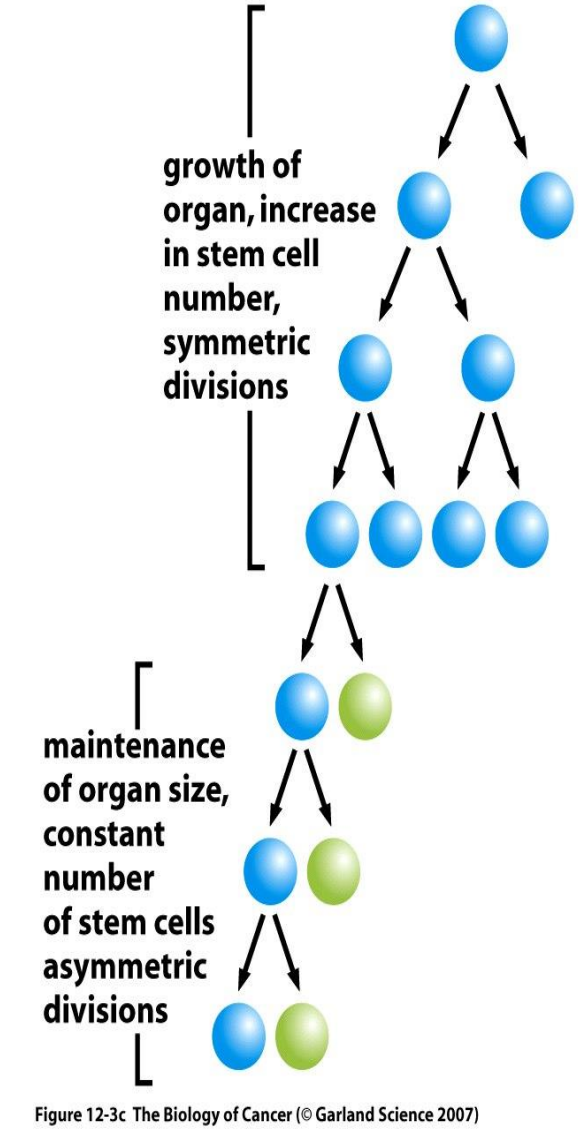
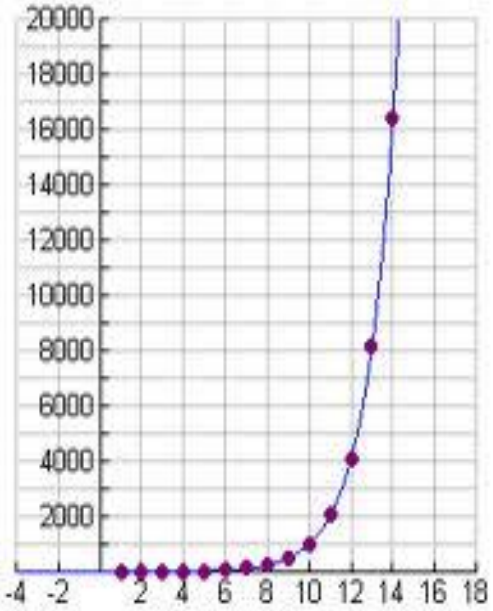
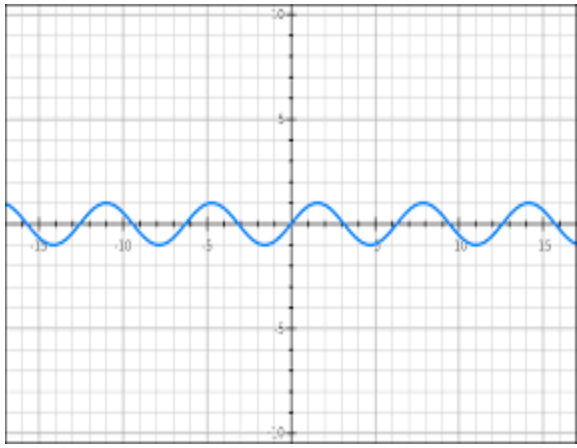
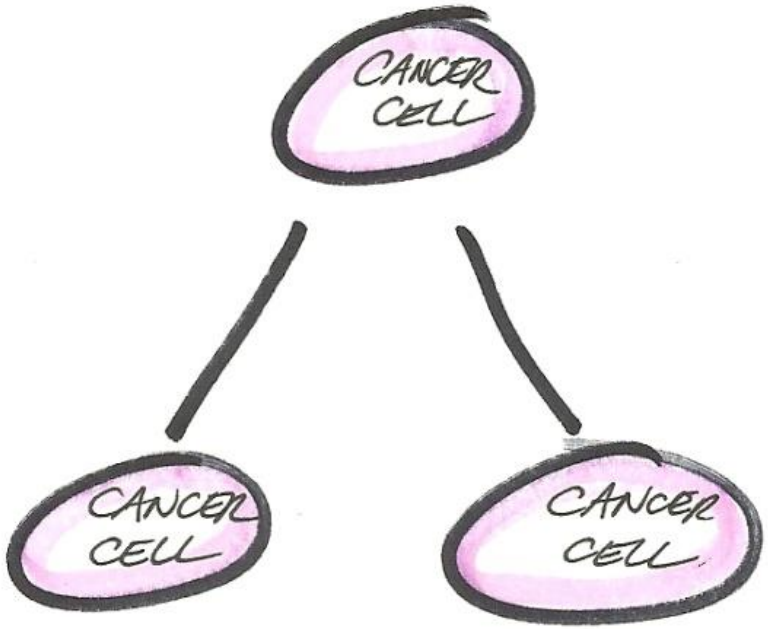


Figure 12-3c The Biology of Cancer (© Garland Science 2007)



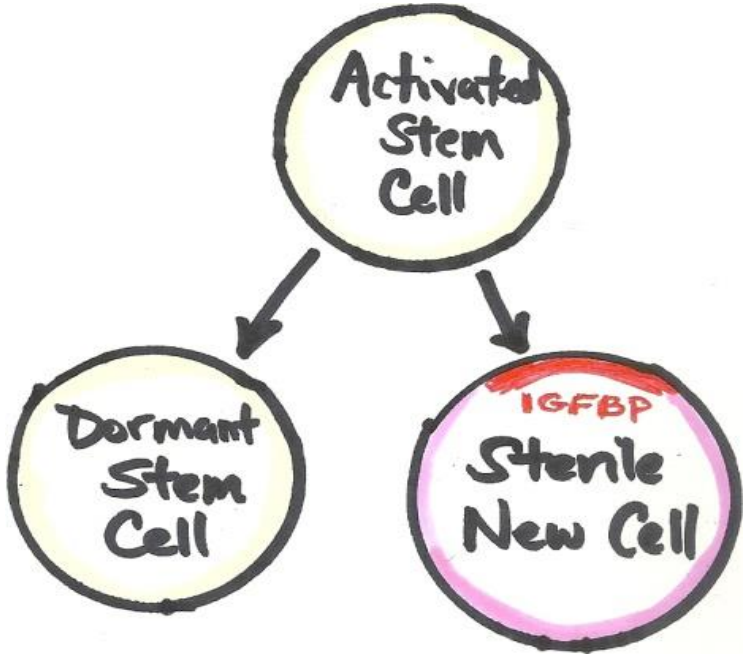


Fetal or Malignant Symmetrical Mitosis



Fetal growth pattern
Exponential growth
Biomass accumulation
Relatively undifferentiated cells
Metabolic and epigenetic controls lost.

Asymmetrical Mitosis of Stem Cells

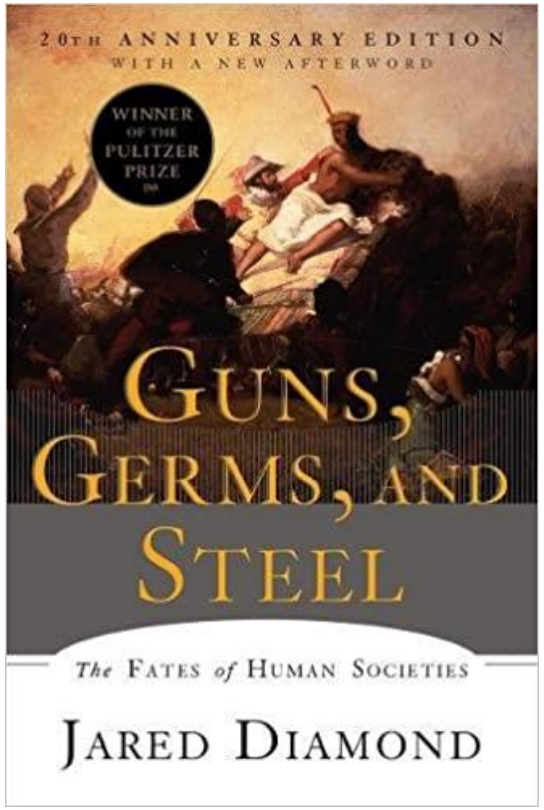


Normal mature growth pattern
Cell numbers stable
Cell replacement only by stem cells
Fully differentiated cells
IGFBP cap prevents division

Insulin, IGF-1 and Diet

- **High sugar and carbohydrate intake** may ramp up insulin, a nutrition pump that some cancers use to drive rapid growth.
- Past a certain threshold of blood insulin, human livers make IGF-1, a potent growth factor and amplifier of hormone receptors. Linked to obesity and disease.
- IGF1 and IGF2 can promote cancer growth through paracrine and autocrine secretion.
- IGF-1 naturally occurs in the **meat and milk** of grazing herd animals – eg. pigs and cows.
- **It is doubled if animals are corn-fed**.

Remember what happened to the last corn-based culture?



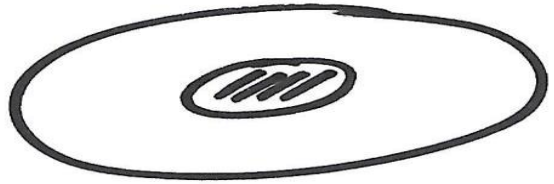
See "*Guns, Germs and Steel*", by Jared Diamond, Pulitzer Prize winner 1998.

Nuclear DNA Mutations and Cancer

- Somatic mutation theory postulates mutations in chromosomal DNA result in the cancer cell phenotype – exponential growth by symmetrical mitosis.
- Warren Schaeffer at University of Vermont showed that putting a highly mutated cancer cell nucleus into a normal enucleated cell did not convert that cell to a cancer phenotype.
- However, putting a normal, non-mutated nucleus into an enucleated cancer cell resulted in a fully malignant growth pattern.

Therefore cancer is driven by cytoplasmic factors: Warburg's "grana" = mitochondria!

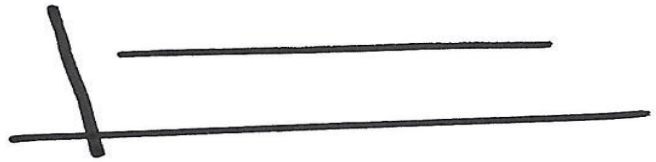
Israel & Schaeffer, *Cytoplasmic Mediation of Malignancy,* In Vitro Cell Dev. Biol. 1988;24 (5): 487-490.



Normal cell,
normal nucleus

↓

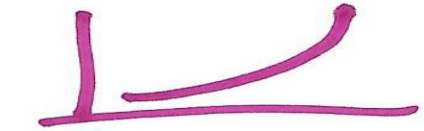
Normal growth

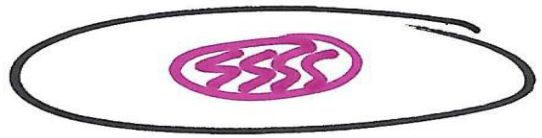


Cancer cell
mutated genome

↓

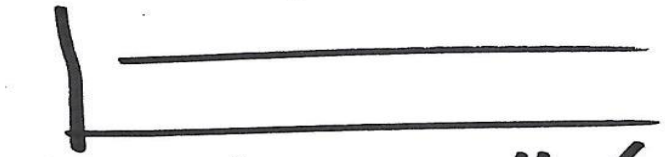
Exponential growth





Normal cell,
Cancer nucleus

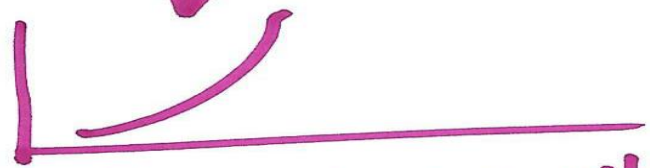
↓



Normal growth!

Cancer cell
Normal genome

↓



Malignant growth!

Metabolic Carcinogenesis

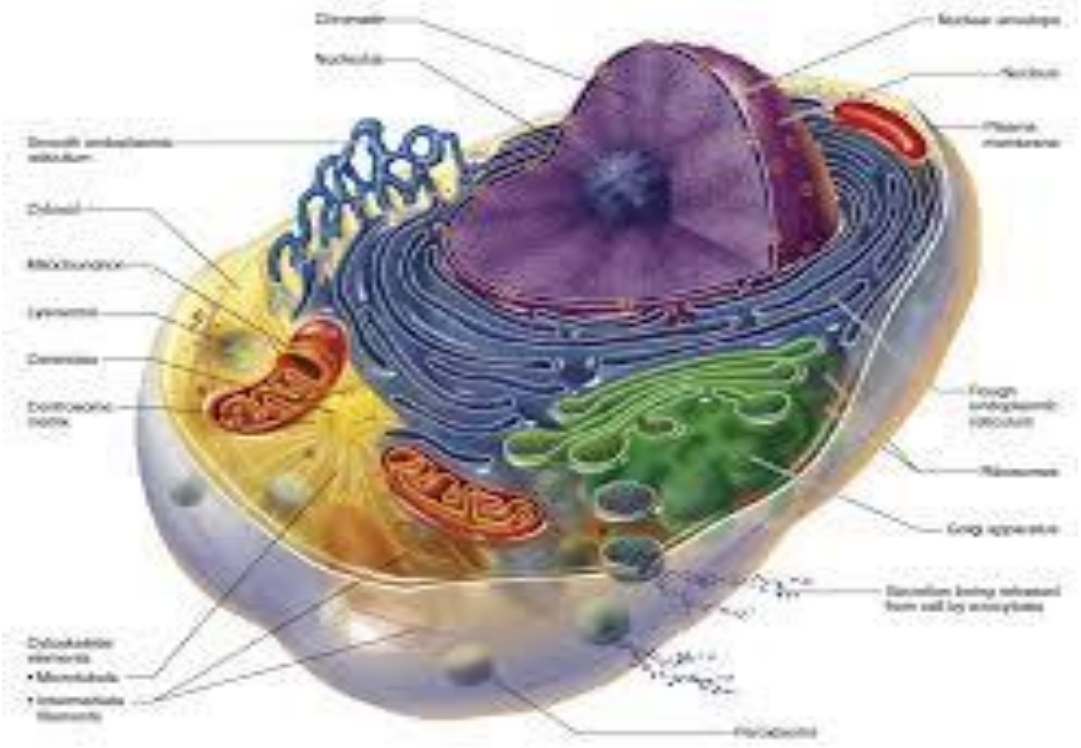
- Warburg showed cancer cells continue anaerobic glycolysis in the presence of oxygen. Fermentation is their metabolic hallmark.
- Pedersen circa 1978 showed **increased fermentation as cancers grow faster and more aggressively, in proportion to loss of mitochondria and structural abnormalities of the remaining mitochondria.**
- Seyfried showed mitochondrial damage alters signals to **transcription factor MYC** controlling about 15% of the genome, triggering carcinogenesis – reversion to a primitive anaerobic metabolic state.
- Retrograde signalling - cytoplasm to nucleus - precedes nuclear mutations!

Cancer As A Metabolic Disease – On the Origin, Management and Prevention of Cancer, Thomas N. Seyfried, PhD, 2012, John Wiley & Sons.

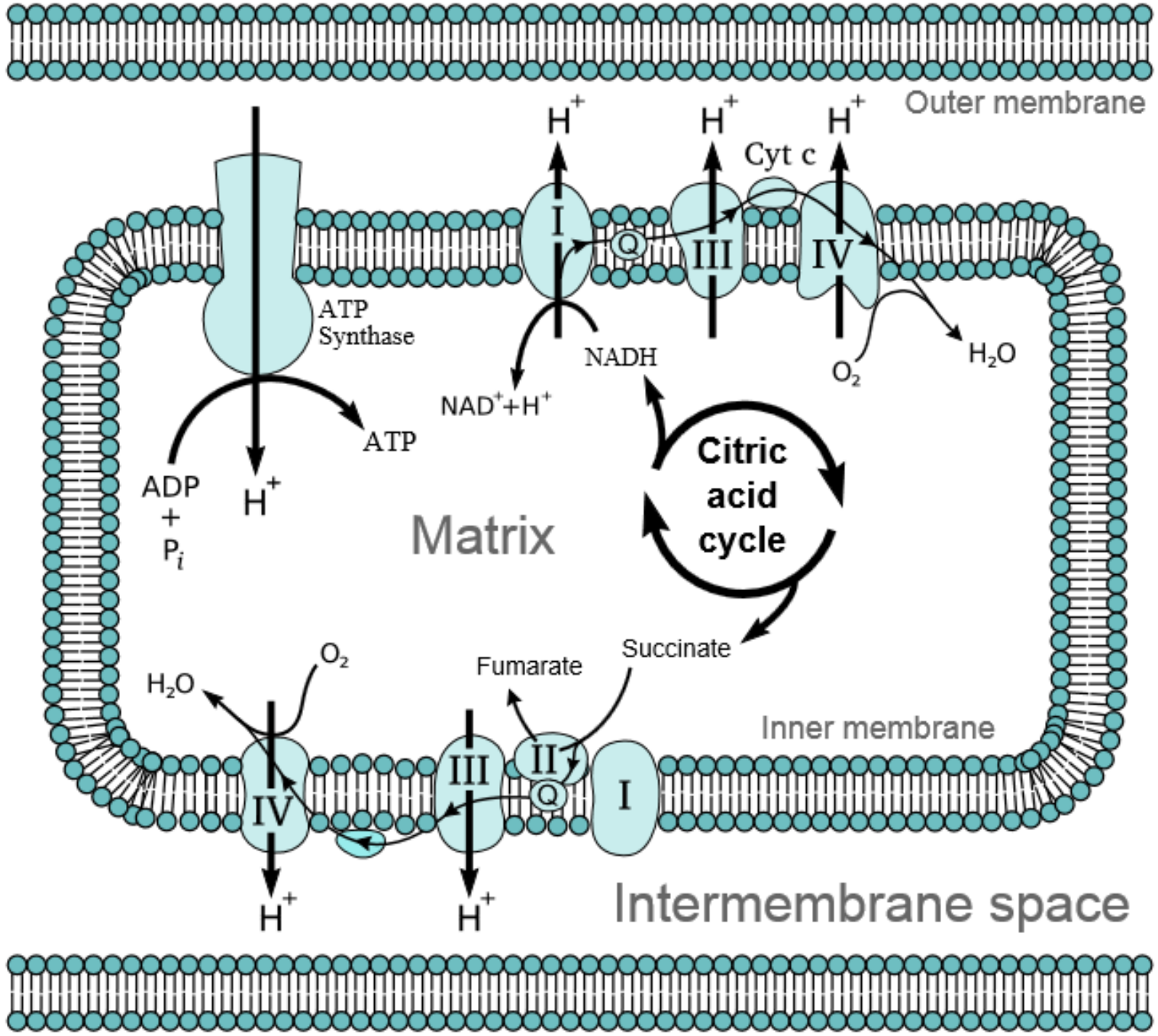
Pedersen, *Tumor Mitochondria and the Bioenergetics of Cancer Cells,* Prog. Exp. Tumor Res. 1978; 22, 190–274. doi: 10.1159/000401202

Mitochondria and Cancer, Wallace, Nature Rev.– Cancer 2012; 12: 685-697.

Mighty Mitochondria from Mommy



- Up to 40 % of a cell's cytoplasm.
- 100,000 in an oocyte.
- 100 in a spermatozoa.
- 10 trillion in an adult.
- 10 % of our body weight.
- Generate our body weight in ATP daily.
- 9 ounces of ATP in circulation at any moment.
- **Mitochondrial fusion with endoplasmic reticulum networks the oxygen and nutrient-sensing pathways**.
- Mitochondrial-ER calcium channels regulate **PDH** activity and **apoptosis**.


Outer membrane
H+
H+
H+
Cyt c
I
Q
III
IV
ATP
Synthase
NADH
NAD+ + H+
O2
H2O
ATP
ADP
+
Pi
H+
Matrix
Citric
acid
cycle
Succinate
Fumarate
O2
H2O
Inner membrane
II
III
IV
Q
I
H+
H+
Intermembrane space

Other Mighty Mito Functions

- Redox modulation.
- Generation of ROS for cellular signal transduction, regulation of nuclear transcription factors.
- Regulation of cytosolic ionic calcium levels.
- Biosynthetic precursors – acetyl-co-A and pyrimidines.
- **Biosynthesis** of anabolic carbon skeletons, lipids, proteins and nucleic acid precursors.
- **Control of apoptosis** – via permeability transition pore for caspases.

Molecular Pathways: Mitochondrial Reprogramming in Tumor Progression and Therapy, Caino & Altieri, Clin. Cancer Res. 2016; 22 (3): 540-545.

Mitochondrial DNA

- **Bacteria-like circular DNA**! A billion years of symbiosis with eukaryote cells.
- 24 genes in mitochondria, 3,000 mito genes in nuclear chromosomal DNA.
- 13 critical OXPHOS genes in mtDNA, some in nuclear chromosomal DNA.
- Mitochondria have their own DNA repair systems. 17X **higher mutation rate** than nuclear DNA.
- High **oxidative stress** from leakage of oxygen and free electrons during oxidative phosphorylation. 10X higher ROS exposure than nuclear DNA. Mitochondrial
- Mitochondrial NADPH is an electron and hydrogen donor in the energy transport chain, and also serves to protect them from degradation by ROS.
- Darlington circa 1948 found **mitochondrial DNA far more chemosensitive** to known “carcinogens” than chromosomal DNA.

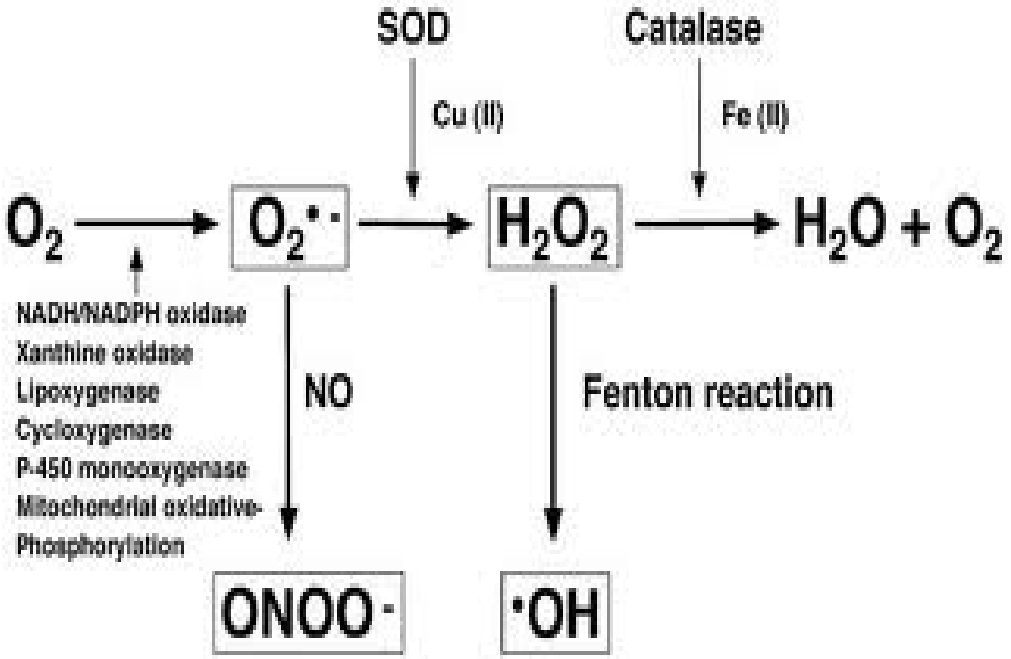
Darlington, *The Plasmagene Theory of the Origin of Cancer,* Br. J. Cancer 1948; 2:118–126. doi: 10.1038/bjc.1948.17

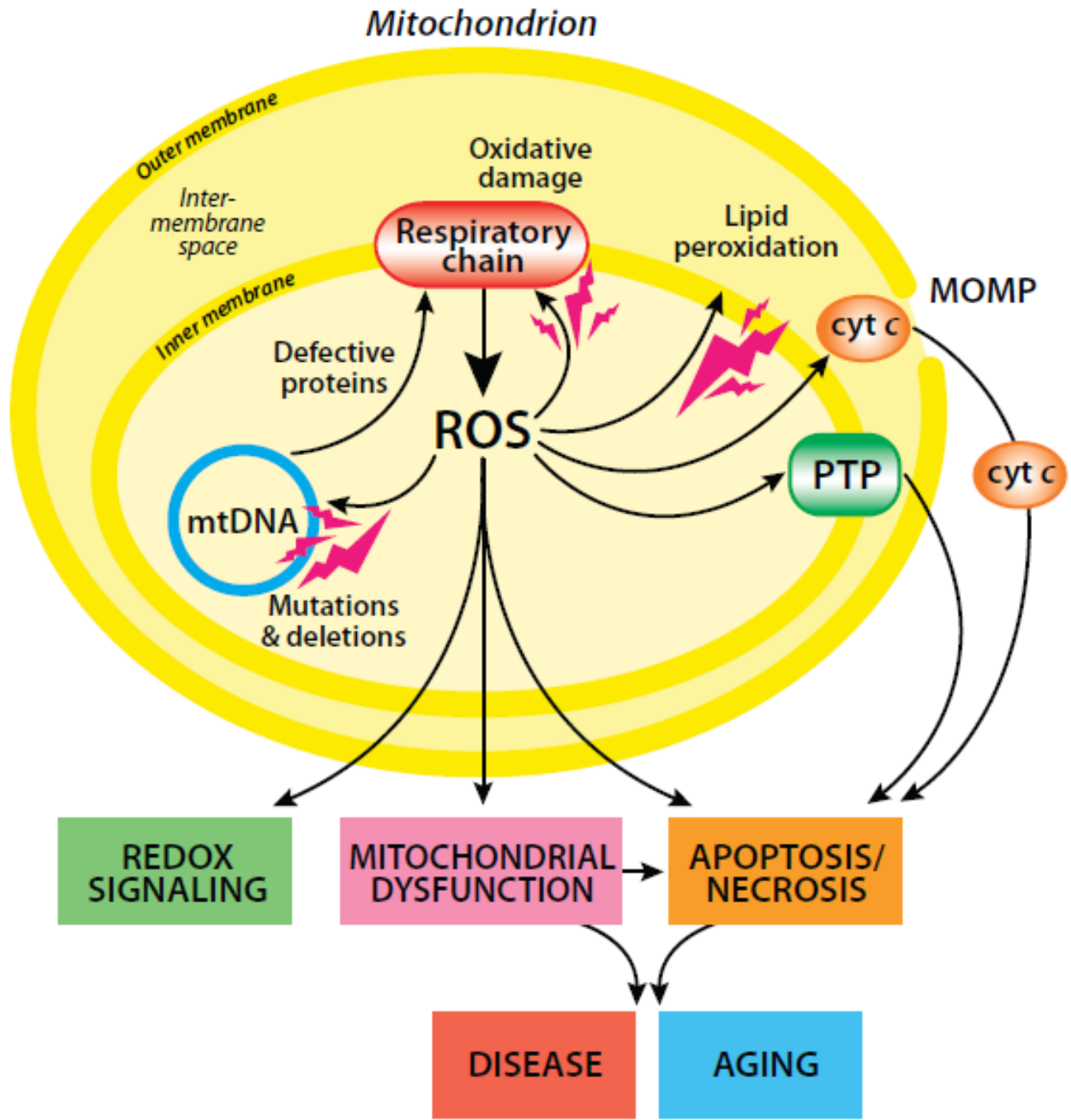
Mitochondria Burn Out

- Mitochondrial DNA and it's repair systems are distinct from and less robust than for parental, nuclear, chromosomal DNA.
- Electrons transport spills 1 to 2% of electrons and 6% of oxygen as ROS (free radicals of oxygen) eg superoxide radical.
- Over-feeding of sugars and carbohydrates are stoking these fires.

Healthy

Age-Diminished




Mitochondrion
Outer membrane
Inter-membrane space
Inner membrane
Oxidative damage
Respiratory chain
Lipid peroxidation
MOMP
cyt c
Defective proteins
ROS
PTP
cyt c
mtDNA
Mutations & deletions
REDOX SIGNALING
MITOCHONDRIAL DYSFUNCTION
APOPTOSIS/ NECROSIS
DISEASE
AGING

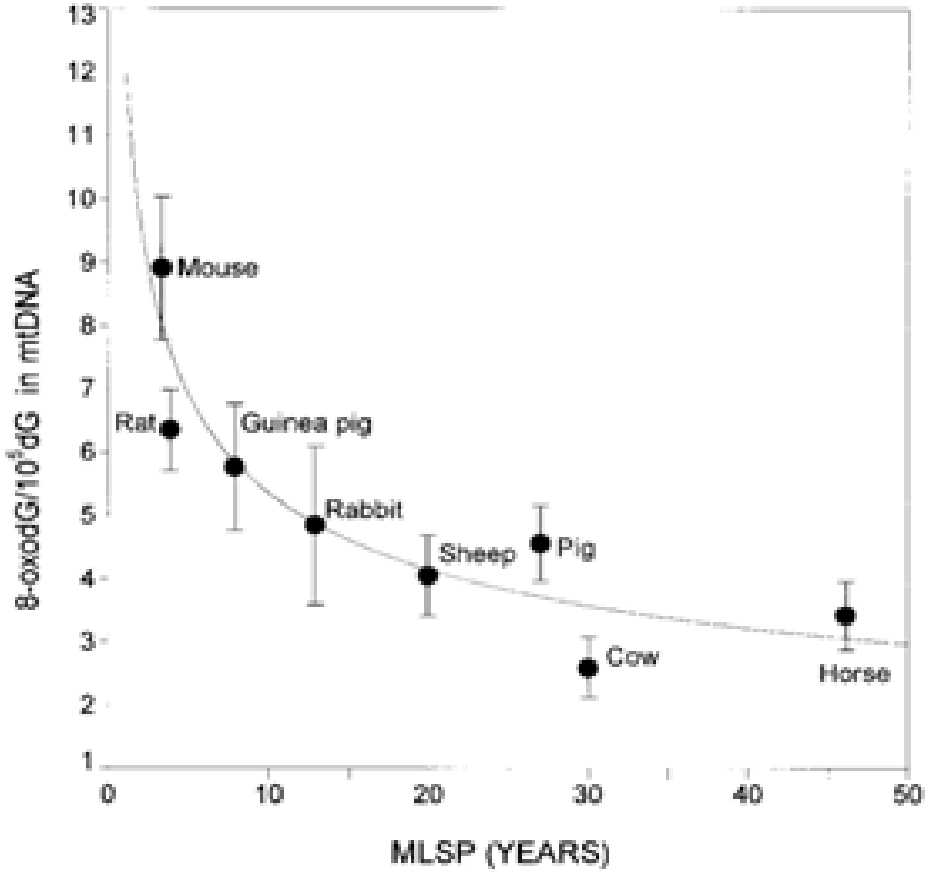
Mitochondrial Poisons

- arachidonic acid.
- alcohol, tobacco, cocaine, methamphetamines,
- AIDS drugs such as AZT; anti-viral nucleoside analogues such as ganciclovir.
- antifungals such as ketoconazole, griseofulvin, cyclosporine; cordyceps (?).
- NSAIDs such as ibuprofen, indomethacin, acetaminophen, aspirin.
- antibiotics such as rifampin, isoniazid, tetracycline, gentamycin, fluoroquinolone, adriamycin.
- cyclophosphamide, amiodarone, valproate, phenytoin, chloroquine, quinidine, clofibrate, fenofibrate, fluoxetine, haloperidol, risperidone, chloroform, amytal, propofol, hydrazine, isoflurane, chlorpromazine, metformin, lidocaine, bupivacaine, capsaicin, cholic acid, L-DOPA.
- cyanide, heavy metals
- pesticides eg heptachlor, chlordane, rotenone, and dioxin.

➢ Pizzorno, *The Toxin Solution –How hidden poisons in the air, water, food and products we use are destroying health-AND WHAT WE CAN DO ABOUT IT*, 2017, HarperCollins.

Mitochondria Damage and Lifespan

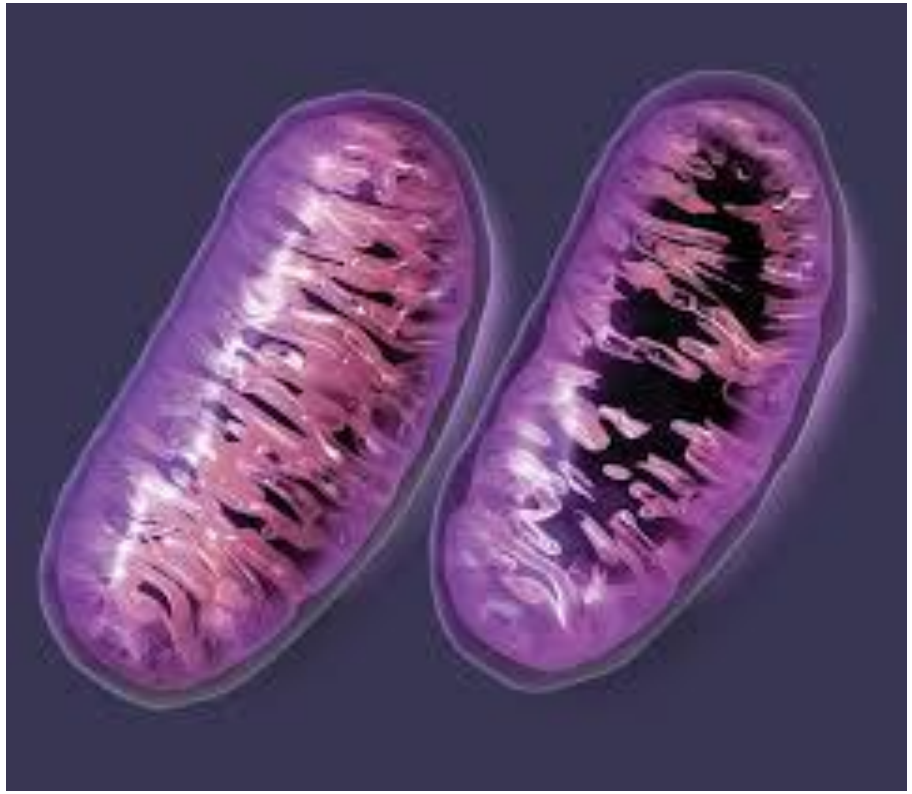
- 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), is a marker of oxidative stress and mitochondrial destruction.
- 8-OHdG > 4 = high toxic load, eg mercury, tobacco, persistent organic pollutants (POPs).
- Inverse relationship between maximum lifespan and 8-oxodG mtDNA – not observed with nuclear DNA.



Oxidative Damage to Mitochondrial DNA is Inversely Related to Maximum Lifespan in the Heart and Brain of Mammals, Barja & Herrero, FASEB J. 2000; 14 (2): 312-318.

Mitochondropathies

- Metabolic syndrome
- Diabetes
- Cardiovascular disease
- Neuro-degenerative disease
- Neuro-behavioural disorders
- Psychiatric disease
- Fatigue syndromes
- Musculo-skeletal disease
- Aging
- Mitochondropathies (MCP) should be considered in any patient with unexplained progressive multisystem disorder. Finsterer 2004



Mitochondrial Initiation of Carcinogenesis

Seyfried has shown damaged mitochondria adapt by

→ activation of the p13/akt network

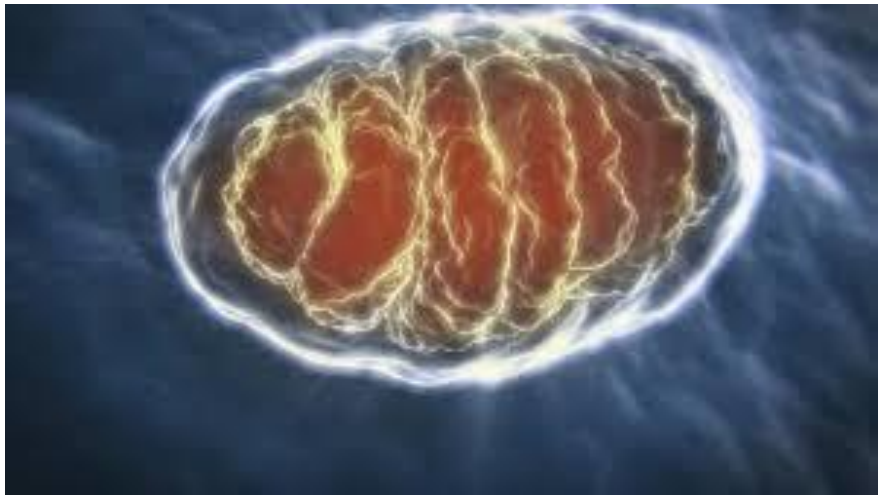
→ retrograde signalling

→ epigenetic changes

→ altered nuclear DNA

→ metabolic reprogramming

→ changes in many cellular processes



Cancer As A Metabolic Disease – On the Origin, Management and Prevention of Cancer, Thomas N. Seyfried, PhD, 2012, John Wiley & Sons.

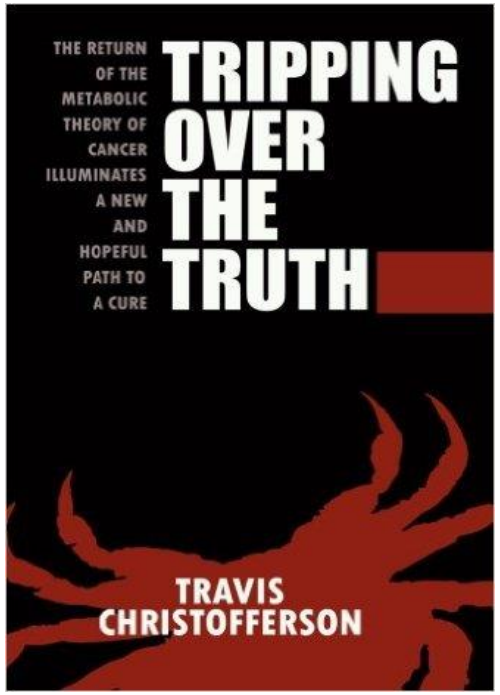
Glycolysis Drives Cancer Growth and Spread

How fast a tumour grows and how aggressive the cancer is directly related to:

- a lower number of mitochondria - Warburg's "*grana*" - as low as 50% of normal.

- loss & damage to mitochondria in cancer cells distorted into dumbbell and cup shapes, missing internal membranes, and with abnormal fats and proteins.

- the increase in glycolytic fermentation of glucose and other fuels for biosynthesis of cell components, as well as energy.



Tripping Over the Truth: The Return of the Metabolic Theory of Cancer Illuminates a New and Hopeful Path to a Cure, Travis Christofferson, 2014; CreateSpace Independent Publishing Platform.

Phosphatases, Kinases and Hexokinases

- Phosphatases cleave off phosphate groups, often inactivating the protein or enzyme. Phosphatases are turned down in cancer cells. The metabolic machinery is revved up.

- Kinases are signalling proteins that attach a phosphate group to proteins to turn on a function. Cancers turn on the kinases. **PK** & **PDK** create a biochemical bottleneck, shifting metabolism towards anaerobic glycolysis = **fermentation.**

- **Hexokinase II compensates for energy loss** due to damaged and absent mitochondria. Cancer cells alone produce hexokinase II to turn glucose to glucose-6-phosphate, but unlike normal hexokinase I, Hex-II is not slowed by build-up of its product. Without feedback, fhe fermentation throttle is stuck on.

- Hexokinase binds to voltage dependent anion channels VDAC and inhibit the release of cyto chrome c, preventing apoptosis, and this **immortalizes cells.**

Targeting Hexokinase II

- Inhibiting Hexokinase II shifts the cell away from fermentation on which malignant growth depends (Mathupala, 2006; DeWaal, 2018).
- Dr. Davis Lamson, ND and others such as Israel and Schwarz in France put forward several candidates, including hydroxycitrate from *Garcinia cambogia (*Israel, 1988, 2011; Schwarz 2010, 2012,2013, 2014; Guais, 2012). Clinical responses are not robust.
- Lectins from Solomon's seal herb (*Polygonatum spp.*) strongly inhibited Hex-II in pre-clinical studies (Wang, 2011; Zhang, 2017), and GLUT2 (Wang, 2018). When a whole-plant tincture was added to patient protocols the clinical impact was clear. Since that time it has become apparent that the lectins are most abundant in the leaf, explaining why the whole-herb extracts seem to be much more bioactive in humans than the root extracts commonly used for arthritis.

Polygonatum spp.

Solomon's seal herb or *Polygonatum spp.*:

- Induces autophagy in cancer cells.
- Induces apoptosis in cancer cells.
  (Both involve mitochondria-mediated ROS-p38-p53 pathways).
- Blocks Ras-Raf and P13K-Akt pathways.
- Blocks epidermal growth factor receptor EGFR.
- **Inhibits expression of hexokinase II** – a substance unique to cancer cells which drives them into glycolysis or fermentation, and which locks the mitochondrial voltage dependent anion channels (VDAC) through which move ATP, caspases and cytochrome c, thereby blocking apoptosis and immortalizing the cancer cell.
- Clinical outcomes with mitochondrial rescue protocols have been outstanding.

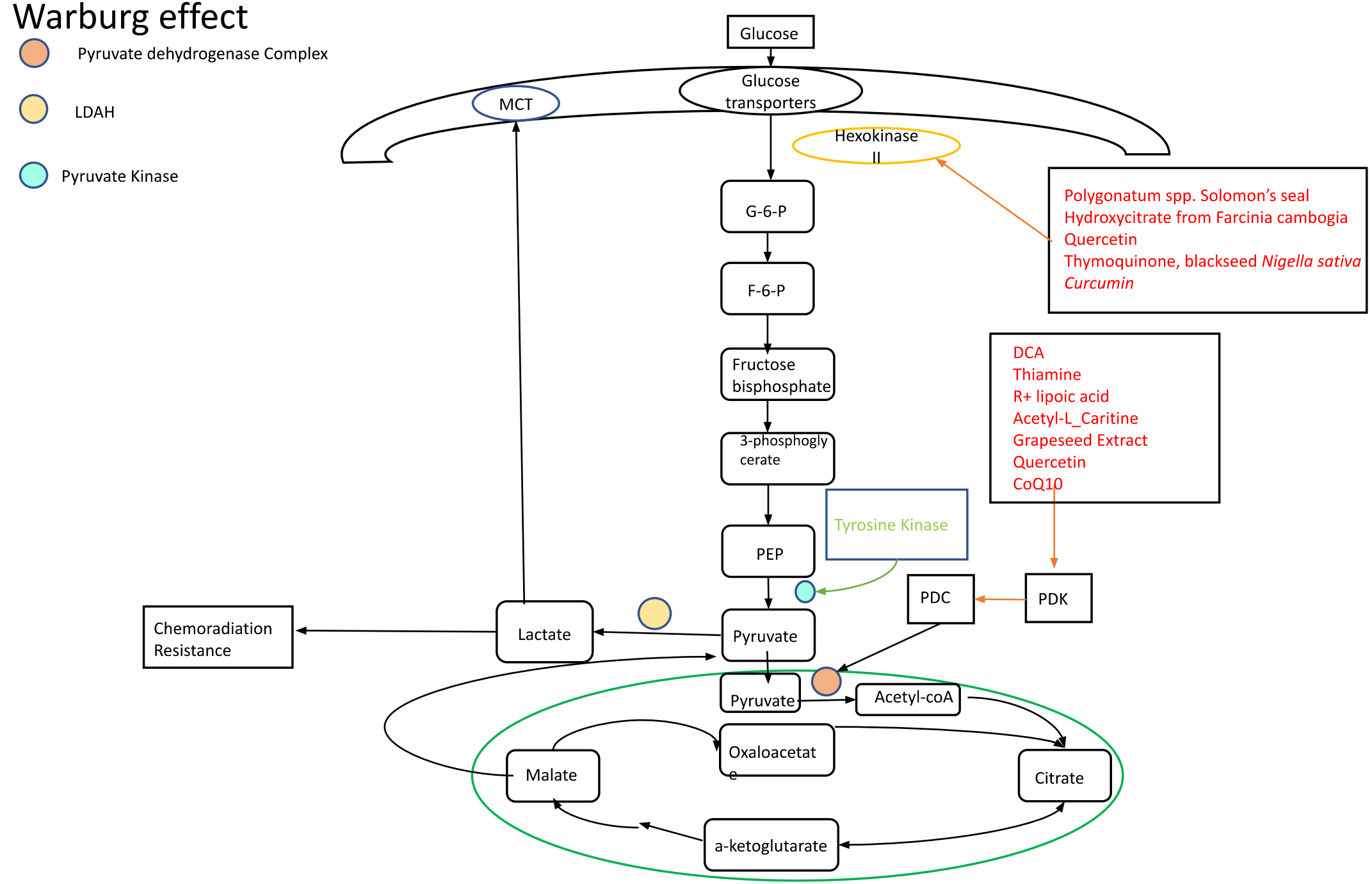
Wang, Yu, Bao & Liu, *Polygonatum cyrtonema Lectin, A Potential Antineoplastic Drug Targeting Programmed Cell Death Pathways*, Biochem. Biophs. Res. Comm. 2011; 406: 497-500.

Zhang, Du, Sun, et al., *Lectin PCL Inhibits the Warburg Effect of PC3 Cells by Combining with EGFR and Inhibiting HK2*, Oncol. Rep. 2017 Jan. 16, DOI:10.3892/or.2017.5367. PMID: 28098871

Best Interventions for Hexokinase II

- *Polygonatum spp*. Solomon's Seal whole herb (minus seeds which are toxic) 5:1 tincture – 30 drops (½ tsp) bid.
- Quercetin 1,000 mg bid - tid, less if in liposomal format, delivering up to 140 mg bid of dihydroquercetin (Graziani, 1977).
- Thymoquinone, from blackseed *Nigella sativa*, 100-200 mg bid.
- Curcumin –standardized, absorption enhanced format, bid.
- Hydroxycitrate from *Garcinia cambogia* 1,000 mg bid.
- Itraconazole is an antifungal drug repurposed as a Hex II inhibitor (Gu, 2016).

Warburg effect



Reversing Cancer's Fermentative Metabolism

- Inspired by the discovery in 2007 of the remarkable ability of Dichloroacetate (DCA) to overcome the PDH and PDK metabolic bottleneck and restore apoptosis in cancer cells, but alarmed at the severe neurotoxicity, I looked for non-toxic alternatives to DCA.
- PubMed revealed a number of candidates, the most promising being alpha lipoic acid and thiamine. Ironically both of these have a long published history of utility to *treat* neuropathy.
- Over time novel combinations have been used and case studies published, and the concept has taken hold of *metabolically* healing cancer cells.

- *A Mitochondria-K+ Channel Axis is Suppressed in Cancer and its Normalization Promotes Apoptosis & Inhibits Cancer Growth,* Bonnet, Archer, Allalunis-Turner, et al., Cancer Cell 2007; 11 (1): 37-51.
- *Cheap, Safe Drug Kills Most Cancers,* Coghlan, New Scientist January 2007.
- *Metabolic Modulation of Glioblastoma with Dichloroacetate*, Michelakis, Sutendra, Dromparis, et al., Science Transl. Med. 2010; 13: 31–34.
- *On the Metabolic Origin of Cancer: Substances That Target Tumour Metabolism*, Israel & Schwartz, Biomed. Res. 2011; 22 (2): 130-164.
- *Mitochondrial Rescue –Turning Cancer Cells Off,* McKinney, Integr. Healthcare Pract. 2011; 4 (4): 78-82.

Mitochondrial Rescue

- **R+ ALA** 300 mg bid – tid
- D-ALA by IV 150 mg biweekly IV-DCA.
- **Thiamine** or benfotiamine - vit. B1 - 100-160 mg bid.
- Acetyl-L-carnitine 1,000 mg bid
- Co-enzyme Q-10 300+ mg
- Glumetza (ER metformin) Rx 500 mg qd-bid.
- Low-dose Naltrexone Rx 4.5 mg hs.
- IV-DCA, thiamine, grapeseed extract, quercetin, berberine, riboflavin-5-phosphate, niacinamide, taurine, D-ribose, Ca, Mg, Zn, Cu, Cr, Fe, proline, HBO2T.
- Acup: CV-4, PC-6, ST-36.

Mitochondria Rescue (Possibly) Heals Cancer? McKinney, Naturopathic Doctor News & Review (NDNR) 2008; 4 (5): 10-11.

Schwartz, Buhler, Icard, et al., *Metabolic Treatment of Cancer: Intermediate Results of a Prospective Case Series,* Anticancer Res. 2014; 34 (2): 973-980.

DCA - Dichloroacetate

- DCA selectively **promotes mitochondria-regulated apoptosis** and inhibits tumour growth in preclinical models by shifting the glucose metabolism in cancer cells from anaerobic to aerobic glycolysis.
- DCA selectively induces phosphatidylserine externalisation but **suppresses caspase 3/7 activation,** interfering with efficacy of cisplatin and doxorubicin.
- DCA does not influence the cytotoxicity of temozolomide, use freely.
- Accumulates in nervous tissue. Neuropathy limits its oral use. Published results with human brain tumours at 6.25mg/kg bid - about 750 mg daily.
- Oral doses of 12.5 mg/kg X 1 month, about 500 mg bid, then tid **-** or double dose, until AEs occur – numbness, tingling, psychosis, paralysis, ataxia.

DCA **inhibits pyruvate dehydrogenase kinase**, triggering an influx of acetyl-CoA into mitochondria. This drives more NADH into complex I.

Superoxides that form are converted into hydrogen peroxide by manganese-super oxide dismutase. The H2O2 inhibits proton (H+) efflux, reducing mitochondrial membrane potential $\Delta\psi m$, the proton-driving force → ATP

This opens the **mitochondrial transition pore** (MTP), inhibiting **calcium** ion entry via voltage-dependent channels. Reduced intra-mitochondrial calcium (Ca++) suppresses a tonic activation of nuclear factor of activated T-lymphocytes (NFAT).

NFAT1 is a nuclear transcription activator, similar in action to activator protein 1 (AP-1) and nuclear factor kappa B (NFκB).

This reduces Kv1.5 expression, increasing potassium ion K+ efflux, reducing inhibition of caspases, and finally **triggering cancer cell apoptosis**.

Managing DCA Neuropathy

- **Thiamine** or B1 as fat soluble **benfotiamine** 100-200 mg bid prevents peripheral neuropathy
- **R+ alpha lipoic acid** 300 mg bid – tid prevents sedation, confusion, hallucinations, memory problems, hand tremor. Can be given IV or nebulized.
- Proton pump inhibitors such as **Pantoprazole** (Pantoloc) 40 mg qd prevents heartburn, nausea, vomiting and indigestion.
- **Methylcobalamin** (B-12) 1000-2000 mcg, acetyl-L-carnitine 1,000 mg tid.

Effect of Thiamine Phosphates on the Activity of Regulatory Enzymes of the Pyruvate Dehydrogenase Complex, Parkhomenko, Chernysh, Cjurilova, et. al., Ukr. Biokhim. Zh. 1987; 59 (5): 49-54.

IV-DCA

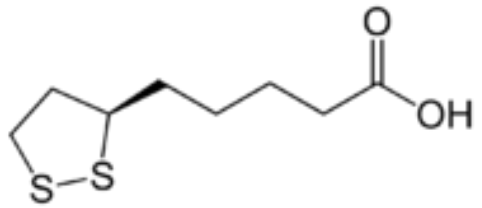
- Lemmo protocol: DCA – 1,000→2,000→3,000 mg
 (ramp up in 3 increments: 4, 8 and 12 mL of 250 mg/mL DCA).
- Saline 100 ml.
- Vitamin C – 2,500mg.
- B-complex – 1 cc.
- B12 – 1 mg.
- B6 – 100 mg.
- B5 – 250 mg.
- B1 – 100 mg.
- Infused over 30-60 min.
- Twice weekly for two weeks, then a week break, repeat.
- Evaluate progress at 10 – 12 infusions.

A Novel Form (IV) of Dichloroacetate Therapy for Patients with Advanced Cancer: A Report of 3 Cases, Khan, Marier, Marsden & Andrews, Altern. Ther. 2014; 20 (Suppl. 2): 21-28.

DCA Synergies

- Give piggy-back with D- ALA 150 mg infusions.
- Metformin is highly synergistic.
- Other synergists are grapeseed extract, curcumin, quercitin, IV-vitamin C, resveratrol, selenium, Temozolamide.
- An interesting new concept is alternating weekly with artemesinin, or IV artesunate, as DCA helps cancer cells recharge with iron.

Alpha Lipoic Acid

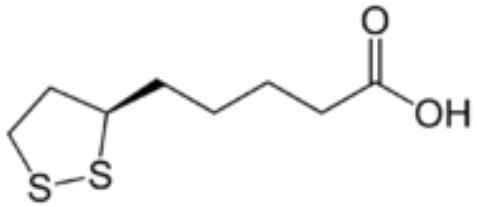


- Antioxidant, hypoglycemic, mitochondrial and epigenetic effects.
- Potent natural PDK activator (cf thiamine – B1), inhibits PDK1 and reprograms cancer cell metabolism, like DCA.
- ALA activates pyruvate dehydrogenase (PDH) by inhibition of pyruvate dehydrogenase kinase (PDK), which results in an increased amount of pyruvate entering the tricarboxylic acid cycle tips the metabolism towards Oxyphos and away from the Warburg effect anaerobic glycolysis on which cancer cells depend (whereby pyruvate can no longer be converted to acetyl-CoA.

R-Lipoic Acid Inhibits Mammalian Pyruvate Dehydrogenase Kinase, Korotchina, Sidhu & Patel, Free Rad. Res. 2004; 38 (10): 1083-1092.

Alpha Lipoic Acid

- Modulates cancer stem cells, chelates heavy metals, increases glutathione & immunoglobulins. Inhibits TGFβ-1, angiogenesis, NFκB.
- Prevents and treats neuropathy.
- May provoke hypoglycemia. May mildly inhibit thyroid function.
- avoid artemesinin (and curcumin?) while on ALA therapy.



Alpha-Lipoic Acid induces Apoptosis in Human Colon Cancer Cells by Increasing Mitochondrial Respiration with a Concomitant O2--Generation,* Wenzel, Nickel & Daniel, Apoptosis 2005; 10 (2): 359-368. PMID 15843897

Curcumin Induces the Mitochondrial Permeability Transition Pore Mediated by Membrane Protein Thiol Oxidation, Morina, Barthelemyc, Zinia, et al., FEBS Letters 2001; 495 (1-2): 131-136

Right or Wrong? R, D, L, or S – ALA?

The natural form is right-handed version, designated by the letter **R**.

When used as a drug, by IV or nebulizing, by convention we use the Latin designation for right which is *dextro*, so it becomes **D**-alpha lipoic acid.

When made synthetically half will be in the left-handed version of the molecule, designated **L**, or the Latin **S** for *sinestre*. The left-handed moiety is not only useless, it may be toxic.

DL or DS racemic mixtures must be dosed at twice the strength as D/R products.

- R-ALA is dosed at 300 mg bid – tid.
- D-ALA by IV is dosed at 150 mg twice weekly or nebulized at 100 mg or more daily.
- Caution: May provoke hypoglycemia. May mildly inhibit thyroid function

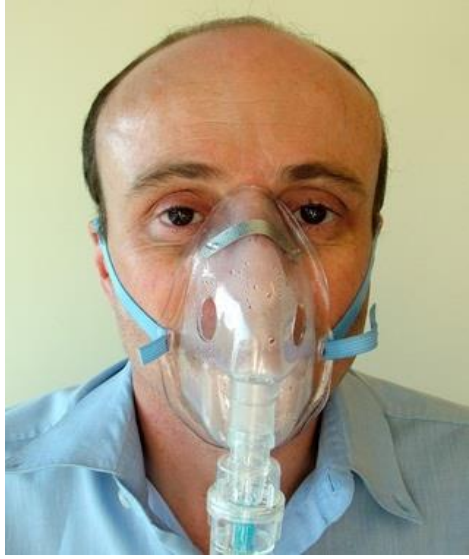
Nibber, *Your Two-faced Lipoic Acid*, Adv. Orthomolec. Res**.** Oct. 2001; 2 (1): 5-28.

IV-D-ALA

- Biweekly D-ALA 150 mg IV drips.
- 10 mL of 15 mg/mL D-ALA in 250 mL saline.
- nothing else in the bag.
- protect from light, with foil.
- run in ≤ 1 gtt/sec.
- takes about 1.5 hours.
- continue oral dosing of R+ALA 300 mg bid.

.

Nebulizing D-ALA



- Injectable grade preservative –free 5 -50 mg/mL D-ALA.
- NOT racemic DL-ALA!
- Keep away from light as much as possible.
- Purchase a nebulizer, or rent from a pharmacy.
- Use a 3 cc syringe to pull out the medicine from a rubber-top multi-dose vial.
- Always wipe the top of the vial with alcohol before putting away in the fridge.
- Put 1 mL medicine in the medicine cup of the nebulizer, which is protected from light by wrapping it with tinfoil or masking tape.
- Dilute in 4 mL sterile saline.
- Over time you can try increasing the dose to 2 to 3 mL of medicine, diluted in 2 to 3 mL saline qs to make 5 mL total.

Nebulizing D- ALA and DCA

- Turn on the pump and through a face mask or breathing tube breath in the medicine as a mist. Breathe normally. After about 10 minutes the medicine well will go dry and you'll hear it sputtering. Turn off the nebulizer pump, and rinse everything off for next time.
- You can do this twice a day at home, it is about as effective as an intravenous drip, and a lot cheaper.
- It is synergistic to add DCA (dichloroacetate) 250 mg/mL with the D-ALA. We start with 1 mL of each medicine, plus 3 mL of saline.
- Later we can go up to 2 mL of each plus 1 mL saline.
- Do not let stand long, as a precipitate can form.

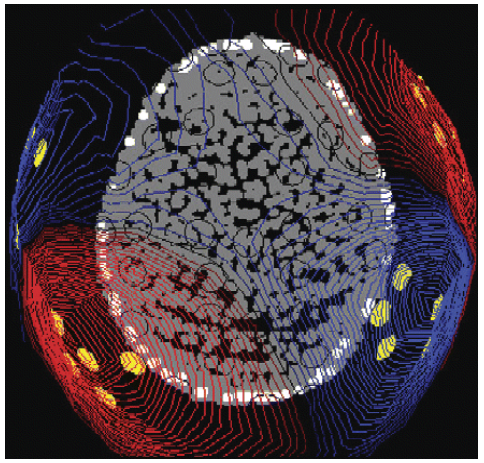
Lipoic Acid Mineral Complex

- LAMC aka Poly-MVA® is a palladium-lipoic acid complex.
- Redox agent, facilitates energy charge transfer, co-factor for oxidation of pyruvate to acetyl co-A.
- Reduces lipid peroxidation and increases glutathione (GSH).
- Oral doses: 10 - 20 mL bid – tid.
- IV – 5 to 40 mL 2X weekly in 100 - 250 mL D5W/NS.
- In stage 4: up to 5 days/week X 4 weeks, then 3X/week X 8 weeks, then 1X/week for 12 weeks.
- Synergistic with thiamine, dichloroacetate.

Acetyl-L-Carnitine

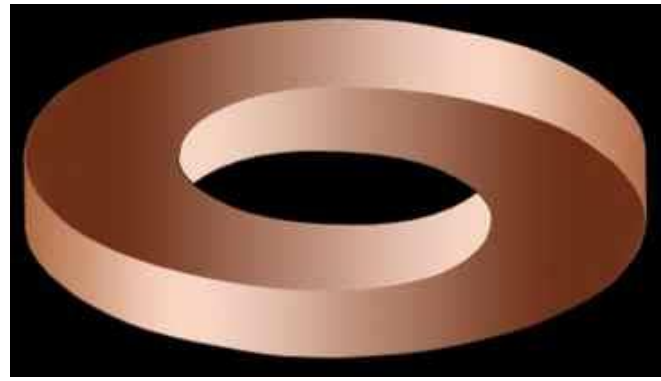
- Fat soluble conditional energy source for mitochondria and brain.
- Acetyl-L-carnitine restores beta-oxidation of fatty acids to acetyl-co-A.
- Outstanding for **chemo brain** – also stroke and head injury.
- Synergistic with R+ Alpha Lipoic Acid for neuropathies and mitochondrial rescue.
- L-carnitine overcomes resistance to EPO blood builders.
- Protects the heart from Herceptin.
- Rx 1,000 mg bid – tid.

Caution: Does not interact well with some anti-epileptic medications.



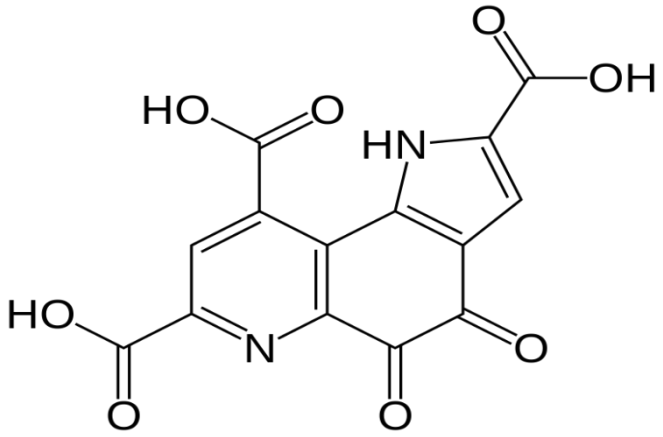
Plotnikov & Zorov, *Pros and Cons of Use of Mitochondria-Targeted Antioxidants,* Antiox. (Basel) 2019 Aug 17;8(8). pii: E316.

Co-enzyme Q-10



- Electron transport molecule in oxidative respiration, essential to ATP production.
- Free radical scavenger. Protects mitochondria from oxidative stress.
- Co-Q10 strongly protects the heart from damage from anthracyclines. Doxorubicin is an antibiotic lethal to healthy cells as well as cancer cells, linked to injury to mitochondria, which have bacteria-like properties.
- Supports recovery from organ failure including heart, liver and kidneys.
- Rx daily 300+ mg of ubiquinone or 100+ mg of ubiquinol.

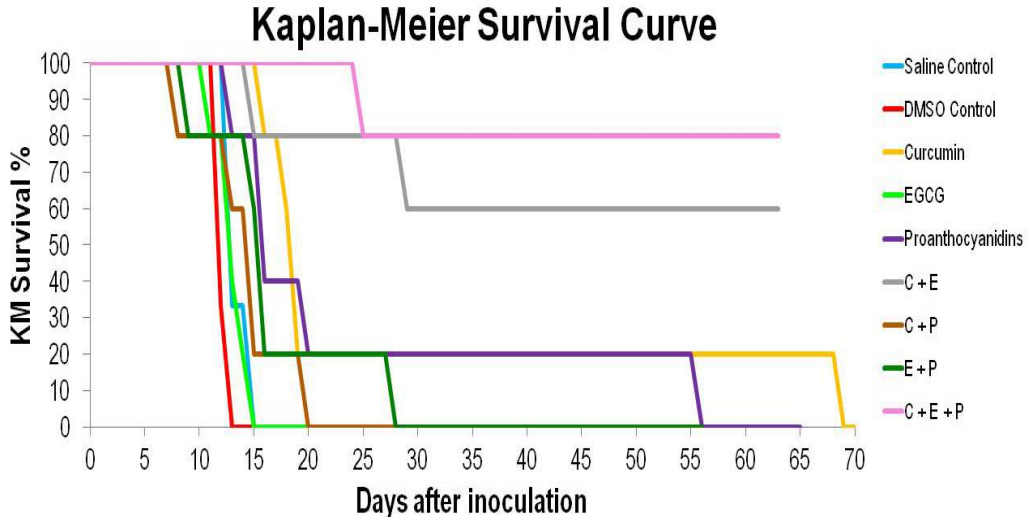
PQQ



- Pyrroloquinoline quinone is a **potent continuous cycling antioxidant**.
- **Activates AMPK** - an enzymatic master regulator of energy metabolism.
- found in parsley, green tea, green peppers, spinach, carrots, kiwi, papaya, and tofu.
- Supplemented at 10-20, up to 40 mg, it is highly effective for memory.
- Protects mitochondria from oxidative stress. Works with CoQ-10 to reduce electron transport ROS damage to mitochondrial membranes.
- **Stimulates mitochondrial biogenesis**.
- Chemo-protective, neuro-protective, stimulates nerve growth factors, blocks intrinsic nitric oxide synthase, protects cells from beta amyloid.

Grapeseed Extract

- Grapeseed extract contains oligomeric proanthocyanidins (OPCs) which are potent antioxidants.
- OPCs are significantly cytotoxic to human breast, lung and gastric adenocarcinomas.
- OPCs regulate cell cycle/apoptosis genes p53, Bcl-2, p21, JNK, Cip1 and c-myc.
- OPCs are anti-angiogenic, reducing VEGF induction by TNFα.
- OPCs inhibit cyclin kinase, NFκB, EGF, MAPK, AP-1, aromatase.



Wake Forest Baptist Health – Cancer Center, North Carolina, 2014

Bagchi, Bagchi, Stohs, et al., *Free Radicals and Grape Seed Pro-anthocyanidin Extract: Importance in Human Health and Disease Prevention,* **Toxicol.** 2000; 148 (2-3): 187-197.

Niacinamide – Vitamin B3

- Niacinamide becomes nicotinamide adenine dinucleotide **NAD+**, a critical regulator of redox and mitochondrial function.
- NAD+/NADH determines function of **sirtuin metabolic sensors & regulators**.
- Mammalian **longevity protein sirtuin** SIRT1-7 utilizes NAD+ to deacetylate proteins in different functional subcellular compartments with a strong convergence on optimizing mitochondrial function.
- NAD+ is needed by **PARP** [poly(ADP-ribose) polymerase] family, protectors against geno-toxic stress.

NAD+ Metabolism, A Therapeutic Target for Age-Related Metabolic Disease, Mouchiroud, Houtkooper & Auwerx, **Crit. Rev. Biochem. Mol. Biol.** 2013 ; 48(4): doi:10.3109 /10409238. 2013.789479.

Quercetin

- Abundant in apples and onions. The most consumed bioflavonoid in the human diet.
- Plants use it to extract nitrogen – less if artificially fertilized, so eat organic.
- Is quercetin mutagenic and *carcinogenic*? Not to human DNA.
- **Inhibits mitochondrial membrane-bound hexokinase**. Hexokinase supports OXPHOS while **hexokinase II** supports glycolysis.
- Blocks lactate export, supports mitochondria.
- Supports clearance of cancer cells by apoptosis, via mitochondrial cytochrome-C release.
- Inhibits Heat Shock Protein 90 from suppressing apoptosis.

Bioflavenoid Regulation of ATPase and Hexokinase Activity in Erlich Ascites Cell Mitochondria, Graziani, Biochim. Biophysica Acta 1977; 460 (2): 364-373.

Spontaneous Mitochondrial Membrane Potential Change During Apoptotic Induction by Quercitin in K562 and K562/adr Cells, Kothan, Dechsupa, Leger, et al., Can. J. Physiol. Pharmacol. 2004, 82 (12): 1084-1090.

Quercetin

- Quercetin can increase or decrease mitochondrial membrane potential Delta Psim ($\Delta\psi m$) depending on concentration, inducing apoptosis (Kellner 2004, Kothan 2004, Yang 2006, Zhang 2005).
- This versatile anti-cancer agent interferes with glycolysis via reduced generation of glycolytic substrates adenosine diphosphate and inorganic phosphate.
- Dose at 1,000 mg bid-tid, or qs to provide 140 mg dihydroquercetin.
- Liposomal forms are clinically robust.

Apoptosis of Murine Melanoma B16-BL6 Cells Induced by Quercetin Targeting Mitochondria, Inhibiting Expression of PKC-alpha and Translocating PKC-delta, Zhang, Chen, Xia & Xu, Cancer Chemother. Pharmacol. 2005; 55 (3): 251-262. PMID: 15538571

The Effect of Flavonoids on Aerobic Glycolysis and Growth of Tumor Cells, Suolina, Buchsbaum & Racker, Cancer Res. 1975; 35 (7): 1865-1872.

Metformin Hurts Cancer

- Inhibits mTOR signalling
- Inhibits MAPK involved in glutaminolysis.
- Inhibits NFκB
- inhibits VEGF
- Inhibits cyclin D1
- Inhibits ovarian cancer cell growth, proliferation, metastasis and increases survival time.
- Turns off cancer stem cells in breast, ovarian and endometrial cancers.

Metformin

- Biguanide is found in goat rue herb *Galega officinalis*
- ↓ liver gluconeogenesis, ↓ glycogenolysis.
- Hypoglycemic, ↓ insulin, ↓ IGF-1, ↑IGFBP-1.
- Activates AMPK, which inhibits lipogenic enzymes, and inhibits fatty acid release from adipose cells.
- Reduced lipid biosynthesis inhibits cell membrane generation, slowing tumour growth.
- Reduces protein synthesis.
- Rx extended release 500 mg bid, may upset GI tract.

Rena, Hardie & Pearson, *The Mechanisms of Action of Metformin*, Diabetologica 2017; 60 (9): 1577–1585

Berberine

- Berberine significantly increase mRNA expressions of AMPK, PGC1α, UCP2, CPT1α, and Hadhb related to mitochondrial energy metabolism.
- This may be driven by increased expression of Fiaf - fasting-induced adipose factor, a key protein negatively regulated by intestinal biome.
- Berberine increases insulin sensitivity and protein kinase C-dependent up-regulation of insulin receptor expression.
- Reduces insulin resistance without provoking hypoglycemia.
- Davis Lamson, ND cautions that berberine is not completely inter-changeable with metformin.
- It is a cold herb = risk of diarrhea, and may be mildly immuno-suppressive.

Mitochondrial Healing

- Encourage olive oil, berries, grapes, pomegranate, apples, chili peppers, onions, garlic, lemongrass, *Brassica*s and whole grains.
- *Centella asiatica* reduces mitochondrial ROS and prevents dysfunction of lipids, proteins and DNA.
- **glutathione, n-acetyl cysteine**, cysteine, and methionine are controversial in cancer care.
- **Heavy metals** increase mito ROS increasing DNA mutations. **Cadmium** is a potent mitochondrial poison. Walter Crinnion, ND: liver enzymes that detoxify organics function better after toxic metals are removed. Test and chelate, MCP.

Miscellany for Mitochondria

- **Curcumin**
- **Melatonin**
- **Indole-3-carbinol**
- Hesperidin
- Diosmin
- DHEA
- Vit. B2 - Riboflavin
- D-ribose
- Carnosine
- Selenium, magnesium, iodine.
- Resveratrol
- Ellagic acid
- Cinnamic acid
- Lutein & Zeaxanthin
- Lycopene
- *Ginkgo biloba*
- *Coriolus*
- B-complex vitamins,
- Vit. A
- Vit. E - mixed tocopherols

Mitochondrial Biogenesis

- Cellular nitric oxide levels; increased by **grapeseed extract**.

- Activation and/or increased content of the protein **AMPK** - AMP-activated protein kinase, a metabolic master switch. AMPK is activated by exercise, resveratrol, curcumin, quercetin 1,000 mg bid, metformin up to 500 mg bid, berberine 300-500 mg bid, and green tea EGCG 500-700 mg.

- Activation and/or increased content of the "fountain of youth" sirtuin protein *SIRT1,* a NAD-dependent histone deacetylase; SIRT1 is increased by caloric restriction, **resveratrol, quercetin and exercise**.

Mitochondrial Biogenesis

- Mitochondria and endoplasmic reticulum interact to create micro-environments that direct fission.
- PGC-1α transcriptional coactivator of the fusion mediator mitofusin-2 is a key to mitogenesis, and is modulated by **resveratrol** 250 mg bid.
- **PQQ** – pyrroloquinoline quinone stimulates biogenesis.
- inhibit peroxisome proliferator-activated receptor (PPAR) gamma γ coactivator 1α (PGC-1α) with fermented wheat germ extract (FWGE) 9 gm qd –bid, and red wine 1 glass daily.
- Fuelled by high energy substrates such as **ketones** and lactate which can be scavenged from stromal fibroblasts. Ketones can be made by gut bacteria acting on **plant fibre**, as well as a **ketogenic diet**.

Mitochondrial Dynamics – Mitochondrial Fission and Fusion in Human Diseases, Longo, N. Eng. J. Med. 2013; 369 (13): 2236-2251.

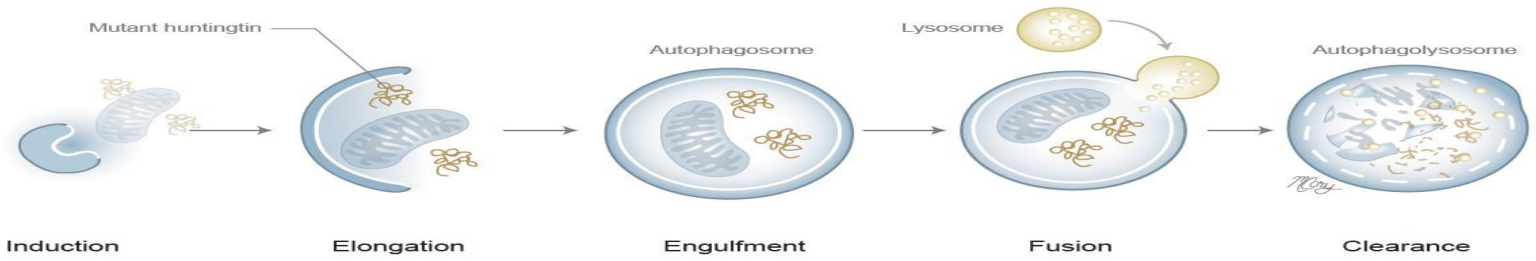
Restore Mitochondria-Mediated Cell Death by Apoptosis

- Block the glycolytic pathway via downregulation of GLUT2 mRNA and proteins with phloretin 1,000 mg bid, or an apple a day.
- Restore gated membrane channels which release caspases by a diet with ample omega 3 marine oils, olive oil, lemongrass, berries, pomegranate, grapes, apples, cabbage family vegetables, chili peppers, onions, garlic, and whole grains.
- Interventions: chemotherapy, radiation, mistletoe, quercetin, curcumin, grapeseed extract OPC's, green tea EGCG, gamma vitamin E, berberine, metformin, R-alpha lipoic acid, feverfew, ginger, betulinic acid, caffeine, genestein, baicalen from *Scutellaria, Bupleurum,* vitamin C, melatonin, ellagic acid, limonenes, indole-3-carbinol, taheebo, reishi, trans-resveratrol, vitamin D3, niclosamide.

Address Underlying Sub-Acute Hypoxia

- Correct sodium-to-potassium ratio, limit salt intake and increase potassium-rich vegetable and fruit intake.
- Aerobic exercise at least twice a week.
- Alkaline diet - reduce salt, sugar and meat intake.
- Sodium bicarbonate – up to ½ tsp bid.

AUTOPHAGY



- Autophagy removes protein accumulations involved in neurodegenerative diseases, and **purges and recycles damaged cell organelles.**
- Autophagy is mediated by membrane phospholipids, and mTOR.
- Dysfunction of autophagy may result in abnormal *mitophagy.*
- Loss of mitochondrial function and oxidative stress are hallmarks of aging.
- Support autophagy with **quercetin, green tea EGCG, vitamin E, curcumin, berberine, sulforaphane, co-enzyme Q-10, resveratrol**.

Yang, Zhang, Nakahira & Gu, *Mitochondrial DNA Mutation, Diseases, and Nutrient-Regulated Mitophagy*, Annu. Rev. Nutr. 2019 Aug 21;39: 201-226.

Fermentation → High Lactate Output

- Cancer cell glycolysis creates a large acid (lactate) load, as noted by Warburg in 1956.
- Lactate can behave as a hypoxia mimetic factor, activating transcription factor HIF-1 in normoxic cancer cells, a key step in angiogenesis (Koukourakis, 2005, 2006), and triggering cancer cells to develop stem cell properties. Lactate reduces tumour antigenicity to dendritic immune cells (Gottfried, 2006), and directly accelerating cellular proliferation (Walenta, 2000, 2004).
- This unique malignant fermentative phenotype is an obvious therapeutic target (McCarty & Whitaker, 2010; Martin, 2012; Pilon-Thomas, 2015; Huber, 2017) but one that has proven elusive. Laypeople as well as doctors have assumed from a cursory view of Warburg's findings that an alkalizing diet and alkaline therapies such as intravenous bicarbonate will address this problem, but clinical results have been marginal (Wenzel, 2004; Martin 2012). Alkalizing increases patient quality of life, but has little to no impact on progression of the disease.

Reverse Malignant Intracellular Alkalosis by Inhibiting Acid (Proton) Efflux Pumps

- Cancer cells are distinctly more **alkaline** on the inside than normal cells of the same type (Hao, 2018), although the environment right around them is highly acidic (Newell, 2003). This effect is due to the dramatic up-regulation of proton efflux pumps, shifting the acid from the cancer cell cytoplasm out into the pericellular spaces.
- We can kill cancer cells by inhibiting proton-coupled monocarboxyate transporters, especially MCT1 and MCT4 (Colen, 2011; Pérez-Escuredo, 2016; Benjamin, 2018; Payen, 2019; Sun, 2020). Inhibit MCT1 and MCT4 with quercetin.
- Conessine from bitter oleander *Holarrhena antidysenterica?*

Sun, Wang, Wang, et al., *Role of Proton-Coupled Monocarboxylate Transporters in Cancer: From Metabolic Crosstalk to Therapeutic Potential*, Front. Cell Dev. Biol. 2020; 8: 651.

Hao, Xu & Li, *Manipulating Extracellular Tumour pH: An Effective Target in Cancer Therapy*, RSC Adv. 2018; 8: 22182.

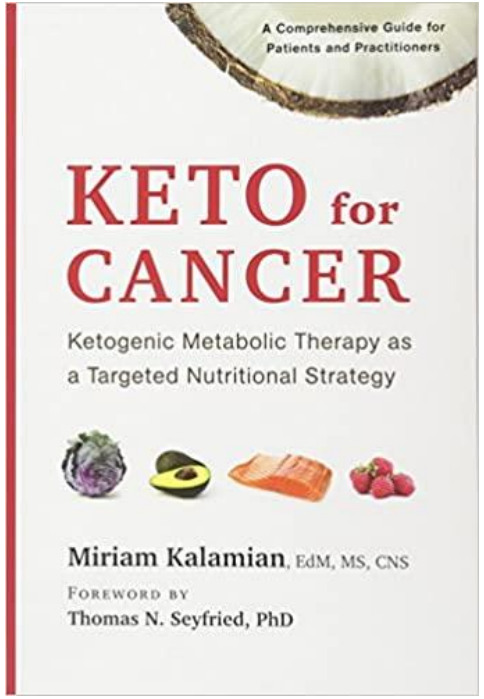
Ketogenic Diet for Cancer

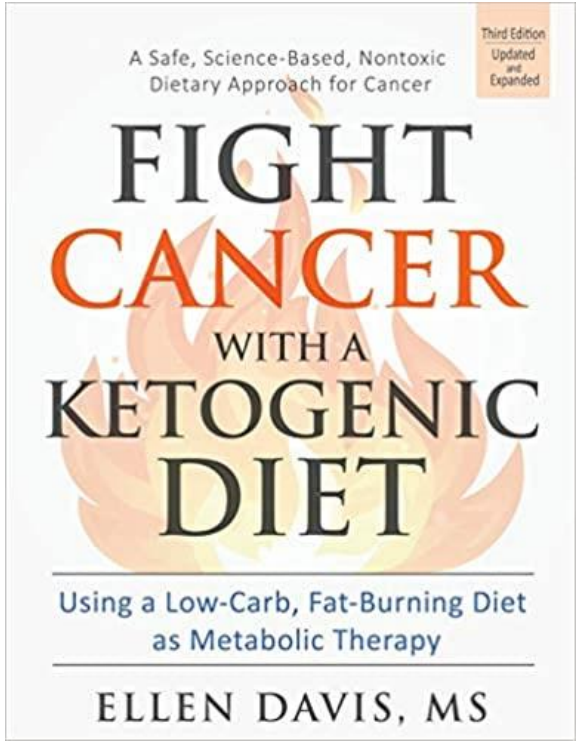
Ketogenic diets are a relatively new concept in cancer care (Seyfried, 2009; Poff, 2015; Katz, 2017; Winters, 2017, Harper 2019; McKinney, 2020).

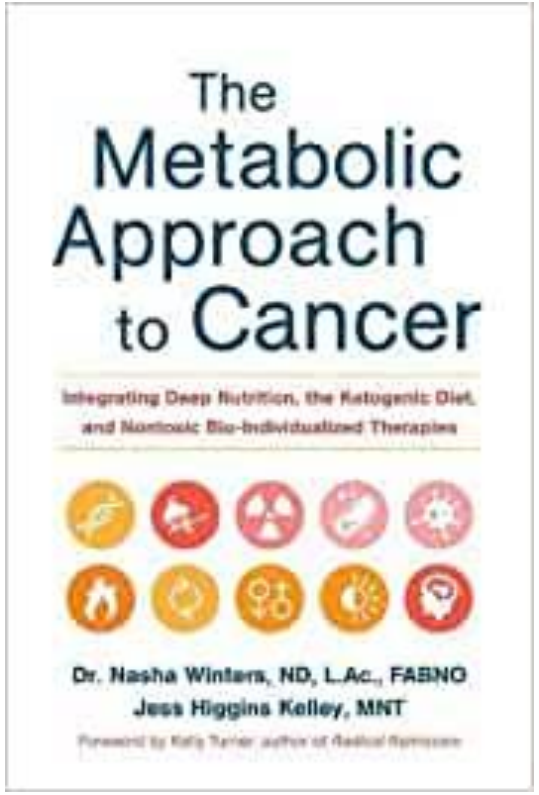
Very high fat, moderate protein and very low carbohydrate ketogenic diets take advantage of the ability of normal cell mitochondria to adapt to ketones as fuel, while cancer cell mitochondria cannot do so ((Longo, 2009; Lee 2011; Davis, 2017; de Cabo. 2019).

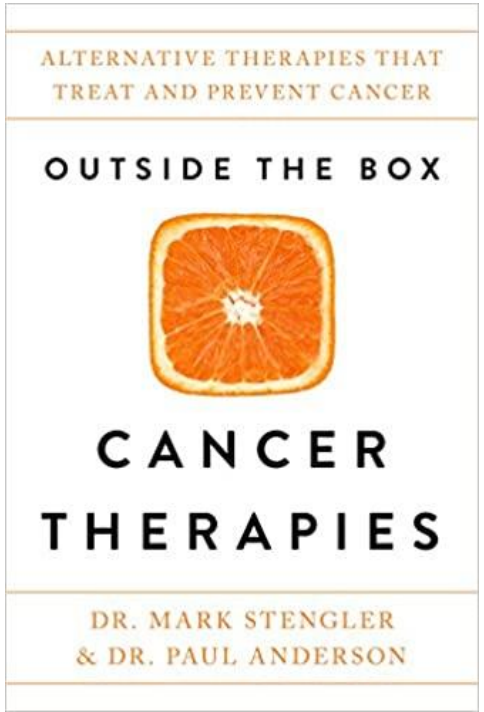
Some possible adjuncts to the ketogenic diet:

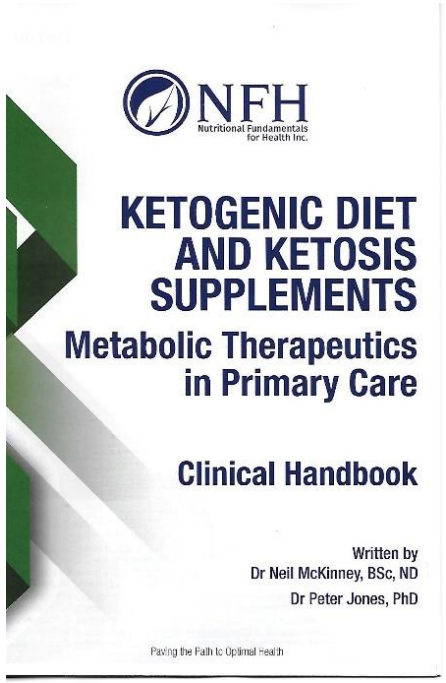
- Supplemental ketones such as β-hydroxybutyrate, acetoacetate or 1,3,butane-diol.
- Metformin.
- Berberine.
- LAMC lipoic acid-palladium complex.
- Hyperbaric oxygen therapy.
- Vitamin A retinol (Anderson, 2018; Stengler, 2018).


A Comprehensive Guide for Patients and Practitioners
KETO for CANCER
Ketogenic Metabolic Therapy as a Targeted Nutritional Strategy
Miriam Kalamian, EdM, MS, CNS
Foreword by
Thomas N. Seyfried, PhD


A Safe, Science-Based, Nontoxic Dietary Approach for Cancer
Third Edition Updated and Expanded
FIGHT CANCER WITH A KETOGENIC DIET
Using a Low-Carb, Fat-Burning Diet as Metabolic Therapy
ELLEN DAVIS, MS


The Metabolic Approach to Cancer
Integrating Deep Nutrition, the Ketogenic Diet, and Nontoxic Bio-Individualized Therapies
Dr. Nasha Winters, ND, L.Ac., FABNO
Jess Higgins Kelley, MNT

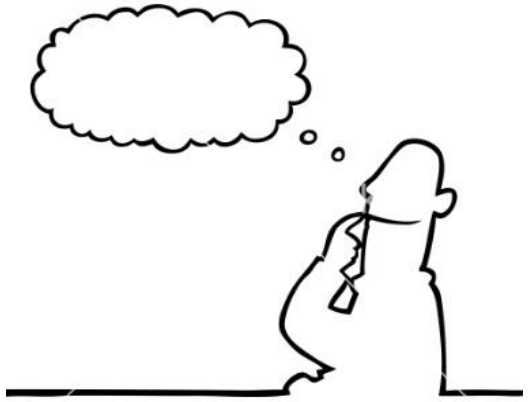

ALTERNATIVE THERAPIES THAT TREAT AND PREVENT CANCER
OUTSIDE THE BOX
CANCER THERAPIES
DR. MARK STENGLER & DR. PAUL ANDERSON


NFH
Nutritional Fundamentals for Health Inc.
KETOGENIC DIET AND KETOSIS SUPPLEMENTS
Metabolic Therapeutics in Primary Care
Clinical Handbook
Written by
Dr Neil McKinney, BSc, ND
Dr Peter Jones, PhD
Paving the Path to Optimal Health

Epigenetics

- Epigenetic switches determine the expression and function of genes without changing the inherited nucleotide sequences. Cells can adapt and reprogram the gene set to optimize survival.

- Environmental signals "select, modify, and regulate gene activity…..Our genes are constantly being remodeled in response to life experiences….Our perceptions of life shape our biology."



Bruce Lipton The Biology of Belief 2015 Hay House

Simple gene set – but complex controls

- The human genome is remarkably simple, but the complexity of the epigenetic modulators is also remarkable.
- Epigenetic controls act through changes in DNA:
    - methylation and hydroxyl-methylation
    - methylation, acetylation, and phosphorylation of histone tails
    - non-coding microRNA
    - chromatin structure
- Inactivating mutations in genes that control the epigenome are common in cancer, impacting DNA methylation patterns, histone modifications and nucleosome positioning and hence, gene expression; can cause point mutations, and disable DNA repair functions.

Paluch, Naqash, Brumberger, et al., *Epigenetics: A Primer for Clinicians*, Blood Rev. 2016

You & Jones, *Cancer Genetics and Epigenetics: Two Sides of the Same Coin?* Cancer Cell 2012; 22: 9–20.

Genes ↔ Metabolism

- Nutrition, behaviour, stress and toxins → nutrient-sensing pathways, adaptive patterns of methylation and hydroxyl-methylation; methylation, acetylation, and phosphorylation of histone tails, non-coding microRNA, chromatin structure, and non-coding RNA → Epimutations.
- These epigenetic changes are reversible are reversible or modifiable by dietary polyphenols such as soy genestein, resveratrol, curcumin, sulforaphane and catechins.
- Nutrients and metabolites such as NAD+, iron, acetyl-coA, ketoglutarate, and S-adenosyl-methionine are necessary for DNA repair enzymes, methylation, histone modifications, microRNA and chromatin rearrangements. **These depend on mitochondrial function and the balance of glycolysis and aerobic metabolism.**
- *"Gene regulation is thus linked to the metabolic status of cells."*

Epigenetic Impact of Dietary Polyphenols in Cancer Chemoprevention; Lifelong Remodeling of Our Epigenomes, W.V. Berghe, Pharmacol. Res. 2012; 65: 565-567.

Epigenetic Targets of Bioactive Dietary Components for Cancer Prevention and Therapy, Meeran, Ahmed, & Tollefsbol, Clin. Epigen. 2010; 1 (3-4): 101-116.

Epigenetics: A Primer for Clinicians, Paluch, Naqash, Brumberger, et al., Blood Rev. 2016

Chromatin Remodeling

- Chromatin states determine activity in many tumour-promoting genes.
- Chromatin remodeling factor Bmi-1 is suppressed and its phosphorylation decreased by green tea polyphenol EGCG.
- Bmi-1 is a member of the polycomb repressive complex 1 which protects DNA integrity.
- Inhibiting Bmi-1 reduces survival of transformed cells such as squamous cell cancers.

Ross, Dwyer, Umar, et al., *Introduction: Diet, Epigenetic Events and Cancer Prevention*, Nutr. Rev. 2008; 66 (Suppl. 1): S1-S6.

Methyl Cycle

Methyl cycle influences:

- Ornithine/arginine cycling.
- Catecholamine reduction.
- Histamine reduction.
- Sulfite reduction.
- Phase 2 primary detox: ammonia, urea.
- Rapidly growing tissues: GI lining, bone marrow, muscle, cancer.
- Low methylation = low AMP!

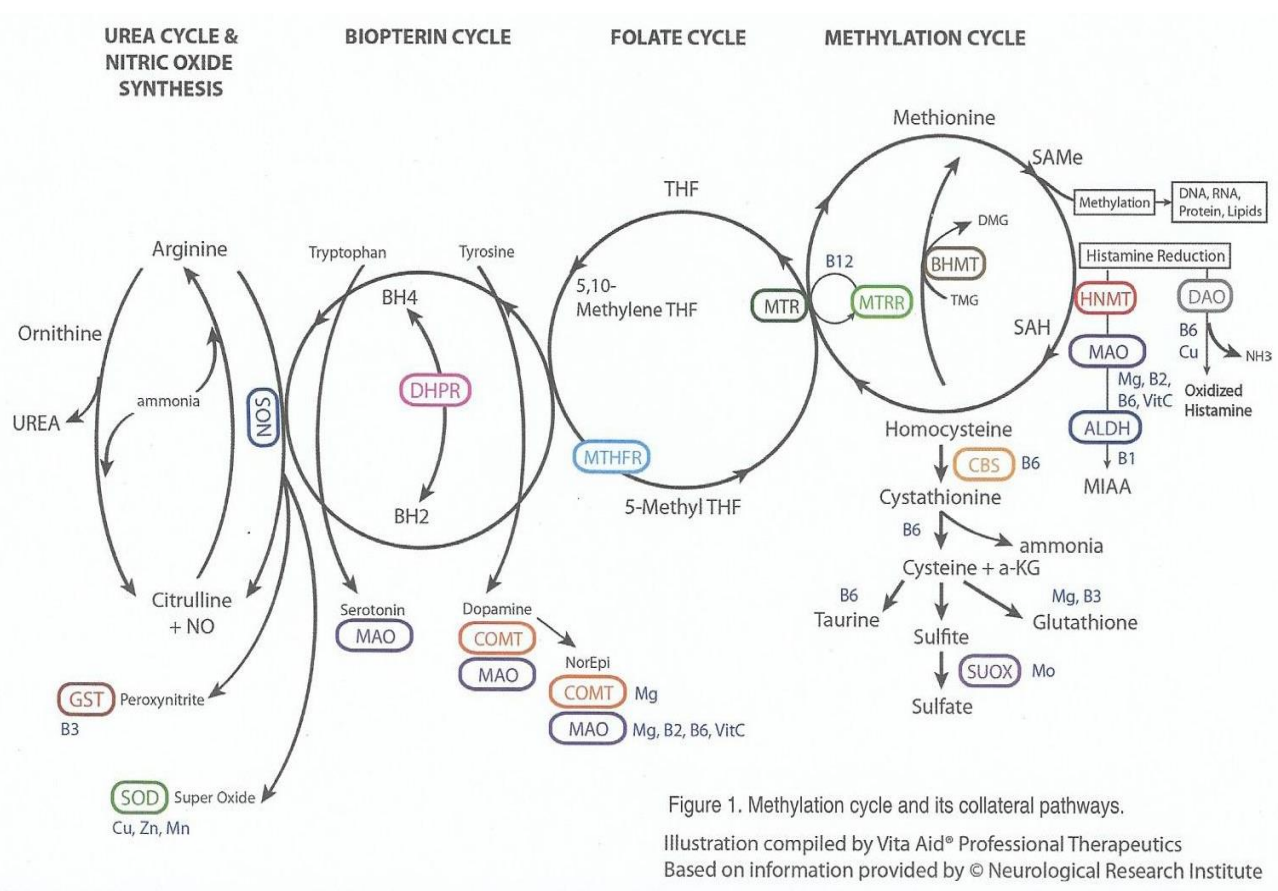


Figure 1. Methylation cycle and its collateral pathways.

Illustration compiled by Vita Aid® Professional Therapeutics
Based on information provided by © Neurological Research Institute

Stem Cells and Aging

- Progressive epigenetic changes explain many aging processes.
- Aged cells have an intact genome, but stem cells are losing pluripotency due to epigenetic alterations.
- Altered mitochondrial substrate use is linked to cardiac disease, with retrograde signalling to epigenetic regulators.
- PPARα coordinates and regulates transcription and ultimately the cellular phenotype.

Lopez-Leon & Goya, *The Emerging View of Aging as a Reversible Epigenetic Process*, Gerontology 2017; doi: 10.1159/000477209.

Warren, Oka, Zablocki & Sadoshima, *Metabolic Reprogramming via PPARα Signaling in Cardiac Hypertrophy and Failure From Metabolomics to Epigenetics*, Amer. J. Physiol. Heart Circ. Physiol. 2017: doi:10.1152/ajpheart.00103.2017.

“Methylation Age”

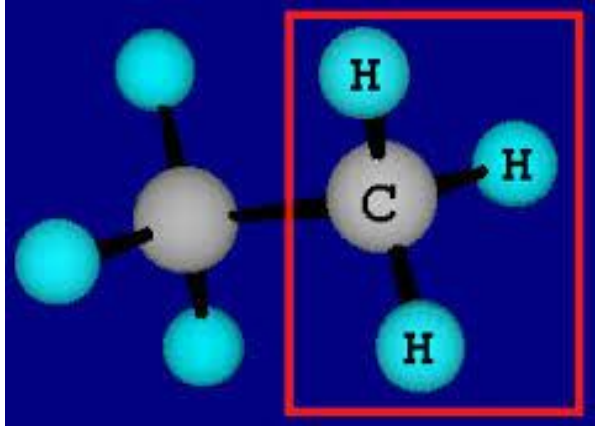
- Methylation impacts aging, and aging impacts methylation. There is an “epigenetic clock”.
- Persons found to have gene methylation more than 5 years over their chronological age have increased risk from all-cause mortality.

Deary, *DNA Methylation Age of Blood Predicts All-Cause Mortality in Later Life*, Gemone Biology 2015, doi 10.1186/s13059-015.0584.6

Agouti Mice (from NWNPC 2012)

- Short lifespan due to cancer, diabetes, obesity – if fed basic Purina Rat Chow.
- Hypo-methylation leads to loss of imprinting of the IGF-1 gene.
- Feeding extra zinc, methionine, folate, choline and B12 alters their genes, and as long as a good diet is given, the offspring are normal for generations!

Methylation in Cancer



- Methylation is a critical epigenetic modulator of oncogenes.
- Methylation resources are used up in detoxification of carcinogens, excess hormones, catecholamines, hormone disruptors, and toxic pollutants ie POPs. Glutathione depletion correlates with methylation arrest.
- Methylation deficit triggers inactivation of phosphatases. The damaged phosphatases localize to the nucleus, triggering dysregulation of cell cycle proteins, and inactivation of controllers of mitosis.
- Reactivation of the fetal growth gene cassette → symmetrical mitosis → undifferentiated and exponential cancer cell growth.

PP2A Methylation

- An epigenetic brake on excess mitosis is methylated phosphatase PP2A.
- Cellular distribution of methylated PPA2 is altered by accumulation of **triglycerides**, creating dysmethylation.
- Poor methylation of PP2A allows the insulin receptor to operate unopposed, triggering mitosis.
- Unmethylated PP2A fails to dephosphorylate M2 **pyruvate kinase** (PK) and **pyruvate dehydrogenase** (PDH). These kinases create a biochemical bottleneck, which creates a new metabolic economy.

Methylation of microRNA and Carcinogenesis

- Methylation of specific microRNAs (MIR1, MIR9, MIR124, MIR137, MIR34B/C) often occurs in an age-dependent manner, as a field defect in some instances, and may be an early event in colitis-associated carcinogenesis.
- Specific mRNA signature patterns could be used to identify patients with ulcerative colitis (UC) patients who are at increased risk for colorectal neoplasia.
- Methylation of all miRNAs was significantly higher in samples from patients with dysplasia or CRC compared to samples from patients without neoplasia.
- Methylation levels of miRNAs in rectal mucosa accurately differentiated patients with CRC from those without.

Toiyama, Okugawa, Tanaka, et al., *A Panel of Methylated MicroRNA Biomarkers for Identifying High-Risk Patients With Ulcerative Colitis-Associated Colorectal Cancer*, Gastroenterology 2017 Aug 25;[EPub Ahead of Print].

Diabetes and Methylation

- Type 2 diabetes is linked to hypermethylation in the promotor region of the insulin gene, reducing gene expression, which correlates with pancreatic islet cell insulin output.
- A key player in this are persistent-organic pollutants (POPs) – pesticides, herbicides, flame retardants, phthalates, etc. Very impactful during gestation.
- These can also hypomethylate tumor suppressor genes and cause chromosomal instability (cancer!).

Pizzorno & Katzinger, *Autoimmune Thyroiditis and Iodine Therapy* J. Restor. Med. 2013; 2 (1): 4-13.

Betaine

- Betaine is found in food such as **whole grains, marine invertebrates/seafood, beets and spinach**: eg Pesco-vegetarian Mediterranean diet.
- Betaine is a methyl donor. Methylation is vital to lipid metabolism and epigenetic controls.
- Key in metabolism of B6, B12,folate, methionine, homocysteine.
- Betaine protects proteins, enzymes and cells from stress.
- Prevents and treats fatty liver disease (steatosis) due to excess intake of sucrose, fructose and fat.
- Betaine and **methylation resources are used up in detoxification** of carcinogens, excess hormones, hormone disruptors, and toxic pollutants. So...***organic* pesco-vegetarian Mediterranean**.

Betaine In Human Nutrition, Craig, Am. J. Clin. Nutr. 2004; 80: 539–549.

Demethylation by Botanicals

- O6-methylguanine-DNA methyltransferase (MGMT) eliminates mutagenic, carcinogenic and cytotoxic lesions from O6-alkylguanines induced by exogenous alkylating agents.
- Neem, ashwagandha, holy basil and oregano increase MGMT microRNA and its demethylation activity, increasing DNA repair, protecting the genome.
- Less potent MGMT effects are seen with gooseberry, spearmint and common basil.

Niture, Rao & Srivenugopal, *Chemopreventative Strategies Targeting the MGMT Repair Protein: Augmented Expression in Human Lymphocytes and Tumor Cells by Ethanolic and Aqueous Extracts of Several Indian Medicinal Plants*, Int. J. Oncol. 2006; 29 (5): 1269-1278.

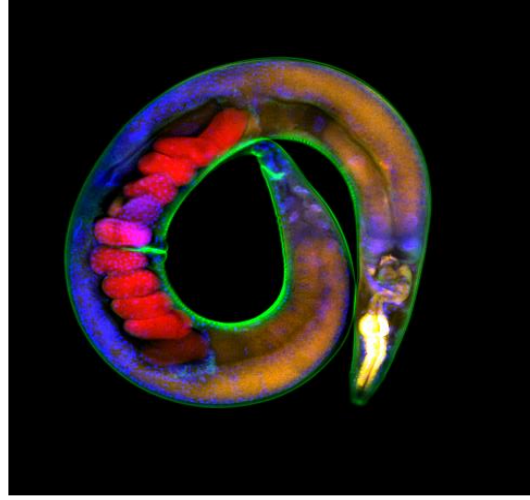
Epigenetics and Extravirgin Olive Oil

- Olive oil acts through the endocannabionoid receptor CB1 to up-regulate CNR1 gene.
- 10 days intake resulted in a 4-fold increase in CB1 activity.
- This stimulation was inversely correlated with DNA methylation at the CNR1 CpG promotor miR23a and miR-301a.
- This may be used prevent or treat colon cancer.

Francesco, Falconi, Germanio, et al., *Extravirgin Olive Oil Up-Regulates CB1 Tumor Suppressor Gene in Human Colon Cancer Cells and In Rat Colon Via Epigenetic Mechanisms*, J. Nutr. Biochem. 2014; 26 (3): 250-258.

Razquin & Martinez-Gonzalez, *A Traditional Mediterranean Diet Effectively Reduces Inflammation and Improves Cardiovascular Health*, Nutrients 2019; 11 (8): pii: E1842.

Leaving a Legacy



- DNA methylation is alterable and durable through generations.
- Non-coding RNA and chromatin proteins in gametes transmit phenotypes to offspring.
- Environmental memories passed on for 14 generations in roundworms.

……

- Nematodes briefly exposed to high temperatures alter the methylation of histones in transgenes for fluorescence.
- These genes remain activated in 14+ generations of progeny kept at lower temperatures than would activate the gene.
- Short-lived worms leave a very long legacy of their environmental challenges.

Environmental Epigenetic Inheritance Through Gametes and Implications for Human Reproduction, Wei, Schatten & Sun, Human Reprod. Update 2015; 21 (2): 194-208.

Klosin, et. Al., *Transgenerational Transmission of Environmental Information in C. elegans.* Science, April 21 2017.

Human Epigenetics and Stress

- Overly sensitive, aversive reactions to stress seem to run in families. The literature abounds with reports of relatives in these populations predisposed to depression, anxiety, and even suicide.
- Some family members present with glucocorticoid levels notched abnormally high, and in curiously deregulated concentrations.
- Behaviorally, they seem to exist at a permanent state of high alert.
- Methylation regulates the HPA axis.
- Transplacental cortisol effects the developing fetal brain, passing down the trait.

Medina, *The Epigenetics of Stress,* Psych. Times 2010; 27: 4.

Perinatal and Childhood Imprints

A critical set of 9 inflammation regulating genes is influenced by neo-natal, peri-natal and childhood circumstances, including:

- Socioeconomic status
- Birth in a stressful period, such as the dry season in the tropics
- Extended parental absences in childhood
- Nutritional stress
- Microbial stress
- Psychosocial exposures

These can result in adult inflammatory diseases such as cardio-vascular events and diabetes, as well as depression, anxiety, bipolar illness, PTSD and resilience. Suicide risk is increased.

McDade, Ryan, Jones, et al., *Social and Physical Environments Early In Development Predict DNA Methylation of Inflammatory Genes In Young Adulthood*, Proc. Nat'l. Acad. Sci. 2017; 114 (29): 7611-7616.

Jiang, Postovit, Cattaneo, et al., *Epigenetic Modifications in Stress response Genes Associated with Childhood Trauma*, Front. Psych. 2019; 10: 808.

Epigenetic Miasms – Taints that Last a Lifetime, or More.

- Epigenetic changes have been found in the lungs of smokers and cord blood of infants prenatally exposed to smoke.
- Studies show an association between famine in Sweden, Germany and China and shortened lifespans and schizophrenia in subsequent generations.
- In mice and humans studies of nutritional deficiencies that lead to disease, there is an indication that epigenetic changes may occur early in life and can be heritable.
- The modern revolution in gene sequencing has revealed many mutations in cancers that control epigenetic factors.

Feinberg, *The Key Role of Epigenetics in Human Disease Prevention and Mitigation*, N. Eng. J. Med. 2018, 378 (14): 1323-1334.

Sins of the Father

- Paternal gene methylation is remodeled during spermatogenesis.
- Lifelong exposure to folate excess or deficiency caused variance in gene methylation.
- Sperm methylation changes can also be seen 10 years after chemo for osteosarcoma.
- Offspring inherited unstable methylation patterns and increased risk of mortality.

Ly, Chan, Aarabi, et al., *Intergenerational impact of paternal lifetime exposures to both folic acid deficiency and supplementation on reproductive outcomes and imprinted gene methylation,* Mol. Human Reprod. 2017.

The Gamete Game

- Pre-diabetic fathers have changes in their sperm cytosine methylation.
- Differentially methylated loci can be transmitted to pancreatic islet cells in progeny. These increase risk of diabetes for at least 2 generations!
- Other epigenetic alterations in gametes, including chromatin changes and non-coding RNA, can transmit phenotypes to offspring.
- Gamete epimutations are seen in fruit flies, roundworms, rats, mice and humans.

Wei, Schatten & Sun, *Environmental Epigenetic Inheritance Through Gametes and Implications for Human Reproduction*, Human Reprod. Update 2015; 21 (2): 194-208.

Epigenetic Taints are Reversible

- Mice exposed to trauma pass along to progeny increased glucocorticoid receptor expression in the hippocampus, altering behavioural responses to stressors relative to controls.
- The GR gene is relatively demethylated in the germline cells of stressed mice. The “sperm methylome” passes to the progeny.
- The adaptive and coping responses to trauma are enhanced, including increased avoidance of stressors.
- This GR alteration can be corrected by providing a very calm and supportive environment for the impacted progeny. Enhanced sensory, motor and cognitive stimulation and socialization reverse the inherited changes.

Gapp, Bahacek, Grossmann, et al., *Potential of Environmental Enrichment to Prevent Transgenerational Effects of Paternal Trauma*, **Neuropsychopharm.** 2016; 41: 2749-2758.

Hug ‘em up!

- Holding and attending to infants improves their resistance to epigenetic aging.
- High contact children had altered methylation in 5 genes, involving functions such as immunity and metabolism.
- They exhibit less stress and fussing. They thrive.
- Four years later the benefit persists.
- Perinatal physical contact and comfort improves their life, lifelong.

Moore, McEwen, Quirt, et al., *Epigenetic correlates of neonatal contact in humans*, Devel. Psychopathol. Pub. online November 22 2017 doi:10.1017/S0954579417001213

Loss of Oncogene Silencing Histones

Shifts in **histone deacetylation** trigger up-regulation of oncogene kinases. Tyrosine kinases are necessary to reprogram mitosis.

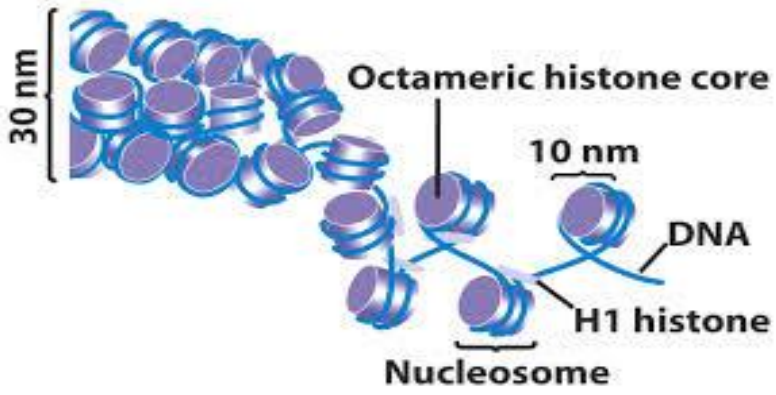
Insulin-tyrosine kinase signaling increases, triggering an influx of glucose, an increase in mitosis, and an inhibition of apoptosis , ie increased cancer cell survival.

Pyruvate kinase and pyruvate dehydrognase kinases favors tumour production of cellular components: lipids for cell membranes, RNA, DNA, ribosomes, etc. This mixed aerobic and anaerobic economy supports rapid cell reproduction.

Israel & Schwartz, *On the Metabolic Origin of Cancer: Substances That Target Tumour Metabolism*, Biomed. Res. 2011; 22 (2): 130-164.

Citrate Replaces Pyruvate

- Cancer cells must find another way to obtain acetyl-Co-A , since pyruvate is no longer generated. Instead, tumours rely on lipolysis and fatty acid β-oxidation from body fat stores.
- This acetyl-Co-A is condensed into citrate.
- Less **ketone bodies and butyrate are produced, de-inhibiting histone protein deacetylation.**
- Remember plant fibre, gut biome and ketogenic diet provide ketones to correct this.



Histone Regulation

Modulating histone protein de/acylation prevents silencing of good tumor suppressor genes, turns off oncogenes, and supports DNA regulation:

- vitamin C.
- cruciferae isothiocyanates – eg sulforaphane downregulates deacetylation enzymes.
- curcumin, green tea EGCG, grape cyanidins.
- garcinol, milk thistle silymarin, parsley apigenin, baicalein, rosemary,
- niacinamide.

Foods that Protect and Repair Genes

- Red bell peppers
- Tomato
- Paprika
- Cinnamon
- Epazote spice
- Endive
- Spinach
- Asparagus
- Tea polyphenols
- Berries
- Cruciferous vegetables
- Broccoli sprouts
- Mustard greens
- Turnip greens
- Basil
- Ginger
- Garlic
- Persimmons
- Grapes
- Eggs
- Duck liver

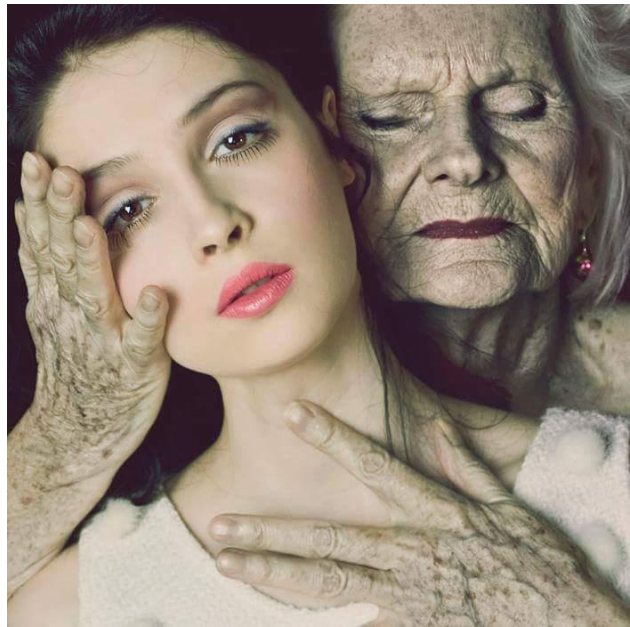
Vitamins and Epigenetics

- Vitamin C potentiates TET's catalytic activity.
- Vitamin C enhancement of DNA histone demethylases regulates epigenetic signatures of stem cells, improving somatic cell reprogramming.
- Vitamin C also acts as a cofactor for Fe+2/αKG-dioxygenases which regulate **stem cell epigenetics**.
- **Vit. A** stimulates expression of ten-eleven translocation demethylases (TET).
- Synergistically **vit. C and A enhance reprogramming** of differentiated cells.

Hore, *Modulating Epigenetic Memory Through Vitamins and TET: Implications for Regenerative Medicine and Cancer Treatment*, Epigenomics 2017; 9 (6): 863-871.

Keys to Healthy Metabolism, Mitochondria, & Epigenetics

- Mediterranean diet – vegetables, seafood, whole grains, olive oil, legumes, seeds, berries. Organic is best.
- Reduce sugar, salt, meat, SAD.
- Quercetin, alpha lipoic acid, thiamine, Co-Q10 or PQQ, proanthocyanidins, curcumin, vit. A, sulforaphane, catechins, omega 3 oils, vit. C, resveratrol.
- Berberine, *Centella asiatica*.
- Exercise.
- A peaceful, secure, supportive environment.
- Cognitive and social stimulation. Hugs. Love.
- Detox from heavy metals, avoid drugs, and de-chemicalize.



www.drneilmckinney.ca