The Mitochondria and Chronic Health Conditions, Part 2

Professor Brigitte Koenig, Magdeburg Molecular Detections
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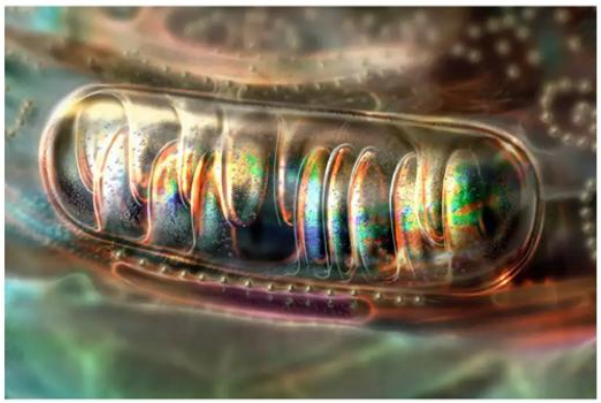
www.aonm.org

This is a follow-on from Part 1 (on the website)



The Mitochondria and Chronic Health Conditions, Part 1

Professor Brigitte Koenig, Magdeburg Molecular Detections
Gilian Crowther MA (Oxon), Dip NT/ND

www.aonm.org

Source: https://aonm.org/mitochondria-webinars/

In Part 2 we will cover some supplementary markers and potential therapeutic support

Supplementary biomarkers:

Mitochondrial mass: Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1α, mitobiogenesis regulator
What to do to increase/decrease mitochondrial mass?
Mitochondrial stress test
Nrf-2, antioxidant regulator
How to quell excess oxidative stress?
Mitochondrial fuel utilisation

Agenda

Supplementary biomarkers:

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Mitochondrial stress test

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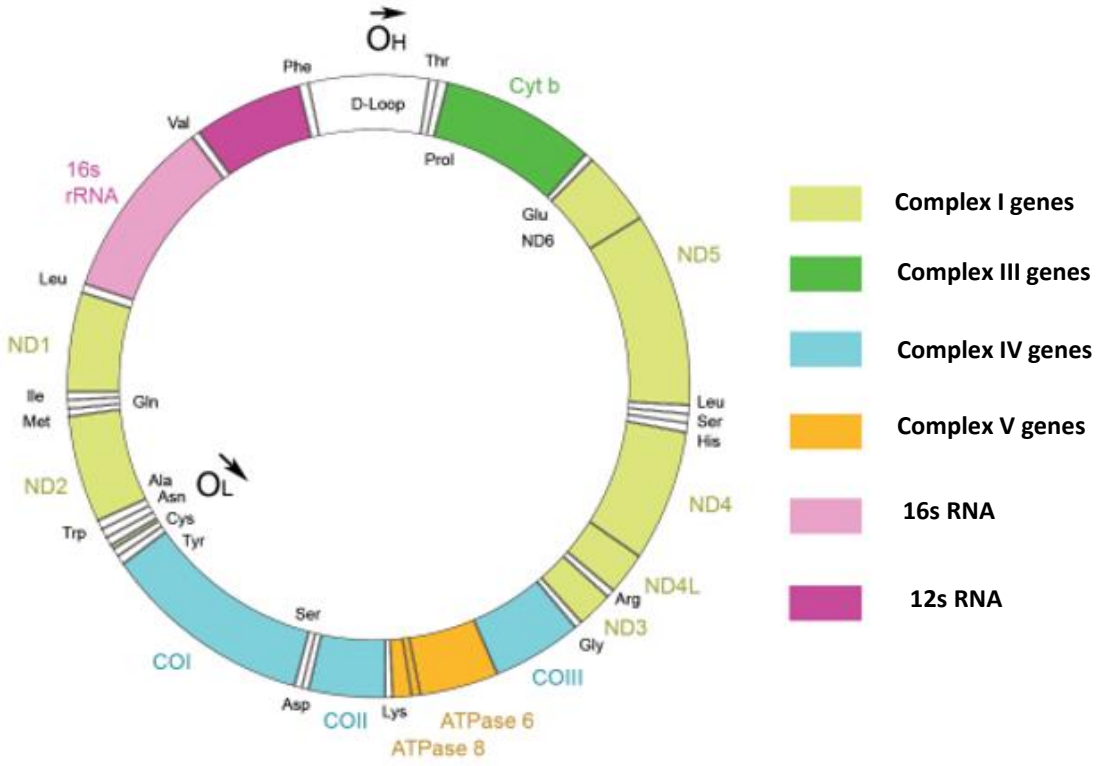
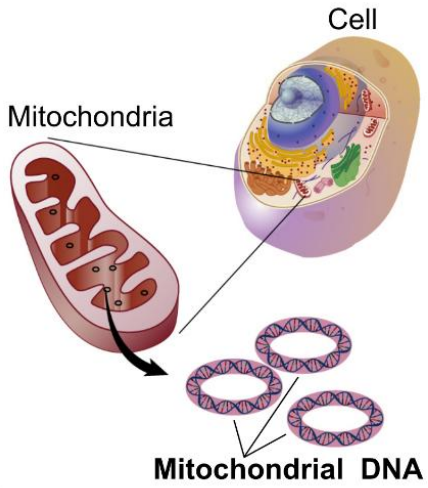
Mitochondrial fuel utilisation

Mitochondria have their own DNA

mtDNA:nDNA

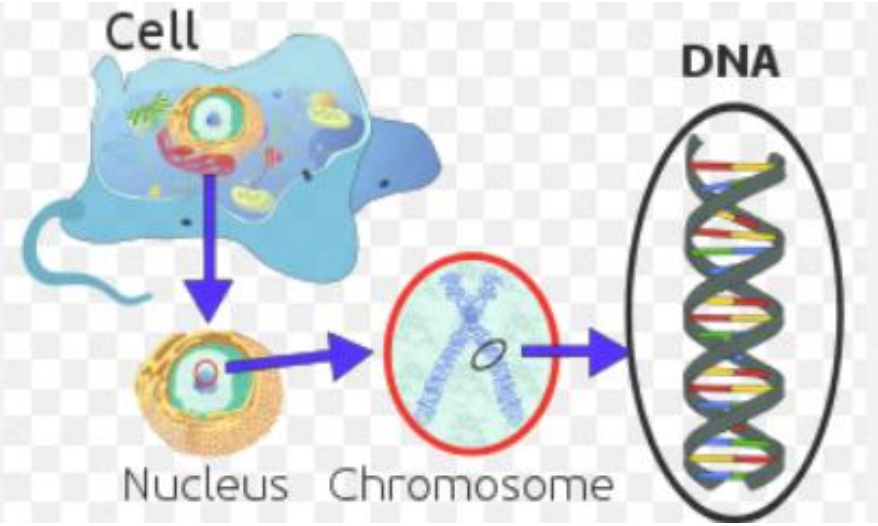


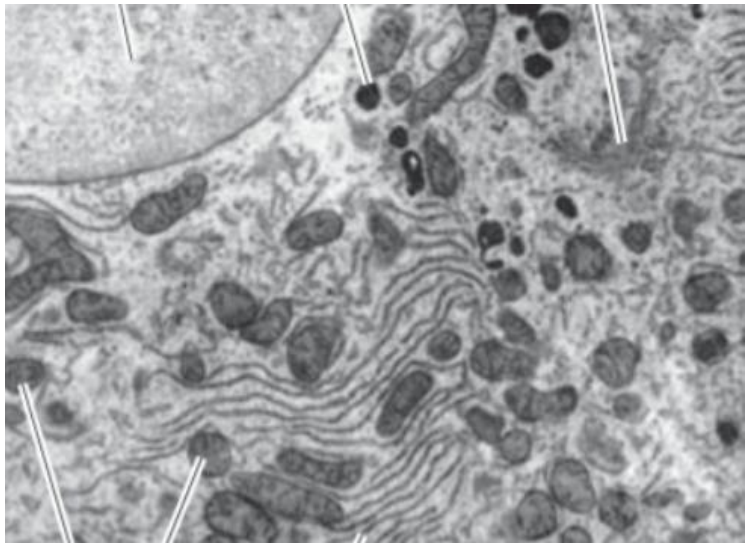


Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König; Hoffmann A, Spengler D. The Mitochondrion as Potential Interface in Early-Life Stress Brain Programming. Front Behav Neurosci. 2018 Dec 6;12:306; https://en.wikipedia.org/wiki/Mitochondrial_DNA: Images free to use under Commons License

It is possible to compare nuclear DNA to sets of mitochondrial DNA per cell: one to many

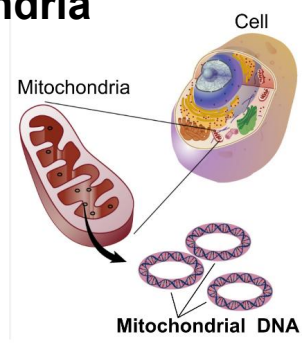
mtDNA:nDNA





The cell nucleus has only one copy of DNA

There are many mitochondria in each cell, each with their own DNA



Source: *https://en.wikipedia.org/wiki/DNA*; https://en.wikipedia.org/wiki/Mitochondrial_DNA: Images free to use under Commons License; http://book.bionumbers.org/how-big-are-mitochondria/

Ratio of mitochondrial DNA to nuclear DNA shows the mitochondrial mass in the cell

mtDNA:nDNA

DNA tests:

Ratio of mitochondrial DNA to nuclear DNA

Example 1:

Orthomolecular and mitochondrial medicine		
DNA		
Test	Result	Reference range
ratio mtDNA:nDNA	202	
Number of mitochondrial DNA copies per 1 copy of nuclear DNA		

Reference range ratio mtDNA:nDNA						
0 - 100	100 - 150	150 - 200	200 - 250	250 - 300	300 - 500	>500

The ratio of mitochondrial DNA to nuclear DNA is normal, though towards the lower end of the reference range.
Nuclear DNA remains stable at a unit of 1, but mitochondrial DNA will increase proportionally to the number of mitochondria in the cell.
It is important to note though that this does not mean that the mitochondria being detected are healthy/intact.

mtDNA:nDNA – numbers pathologically high/low

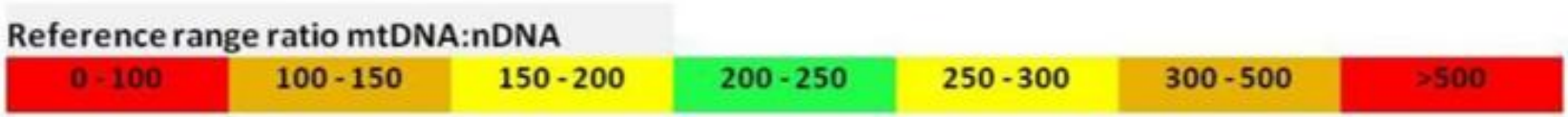
mtDNA:nDNA

Ratio mtDNA:nDNA 82

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Ratio mtDNA:nDNA 1039

Number of mitochondrial DNA copies per 1 copy of nuclear DNA



Too low (top example):

The cell is unable to counteract the lack of energy by increasing the number

Too high (bottom example):

The cell is trying to counteract the lack of energy by increasing the number of mitochondria.

Agenda

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How to quell excess oxidative stress?
Mitochondrial fuel utilisation

If low, useful to first check the key regulator of mitobiogenesis: PGC1-alpha

ELSEVIER

The American Journal of Clinical Nutrition

Volume 93, Issue 4, April 2011, Pages 884S-890S

Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis 1, 2, 3, 4

Fernandez-Marcos Pablo J, Auwerx Johan

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Mechanisms responsible for energy management in the cell and in the whole organism require a complex network of transcription factors and cofactors. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) has emerged as a master regulator of mitochondrial biogenesis and function, thus becoming a crucial metabolic node. We present an overview of the mechanisms by which PGC-1α is regulated, including the transcriptional regulation of PGC-1α expression and the fine-tuning of its final activity via posttranslational modifications.

Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. Am J Clin Nutr. 2011 Apr;93(4):884S-90.

PGC-1-alpha is central for the induction of new mitochondria

PGC-1-alpha

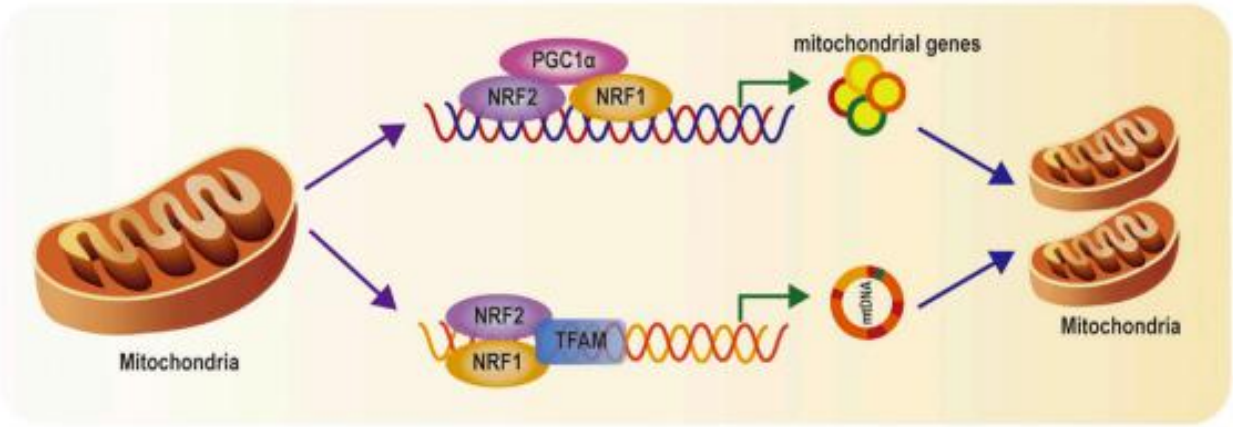

FIGURE 2 | Mitochondrial biogenesis pathways: When PGC-1α is activated, PGC-1a activates NRF1 and NRF2, and subsequently TFAM, which regulate genes involved in subunits of mitochondrial respiratory chain complexes, import of nuclear-encoded mitochondrial proteins, and mtDNA replication and transcription. 1

- PGC-1α regulates mitochondrial biogenesis but also has effects on mitochondrial functions beyond biogenesis.
- Mitochondrial quality control mechanisms, including fission, fusion, and mitophagy, are regulated by PGC-1α.
- PGC-1α-mediated regulation of mitochondrial quality may affect age-related mitochondrial dysfunction and insulin sensitivity. 2

Source: 1. Chen L, Qin Y, Liu B, Gao M, Li A, Li X, Gong G. PGC-1α-Mediated Mitochondrial Quality Control: Molecular Mechanisms and Implications for Heart Failure. Front Cell Dev Biol. 2022 May 27;10:871357; 2. Halling JF, Pilegaard H. PGC-1α-mediated regulation of mitochondrial function and physiological implications. Appl Physiol Nutr Metab. 2020 Sep;45(9):927-936.

The test for PGC-1-alpha measures its relative expression

PGC-1-alpha

RNA profile

Test	Unit	Result
PGC-1-alpha	Relative expression (to GAPDH)	Not detectable

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

Interpretation: "Basic values of the peripheral blood leucocytes"

PGC-1-alpha expression is undetectable. This indicates absent new mitochondrial formation at the present time.

If this is the case, and mtDNA:nDNA is low as well, then it is often very helpful to take initiatives to increase PGC-1-alpha

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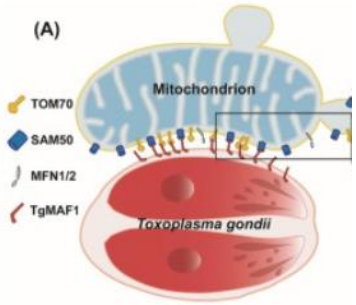
Mitochondrial fuel utilisation

Please do first consider what may be keeping the mitochondrial numbers down – it‘s not just “add“, but also “remove“

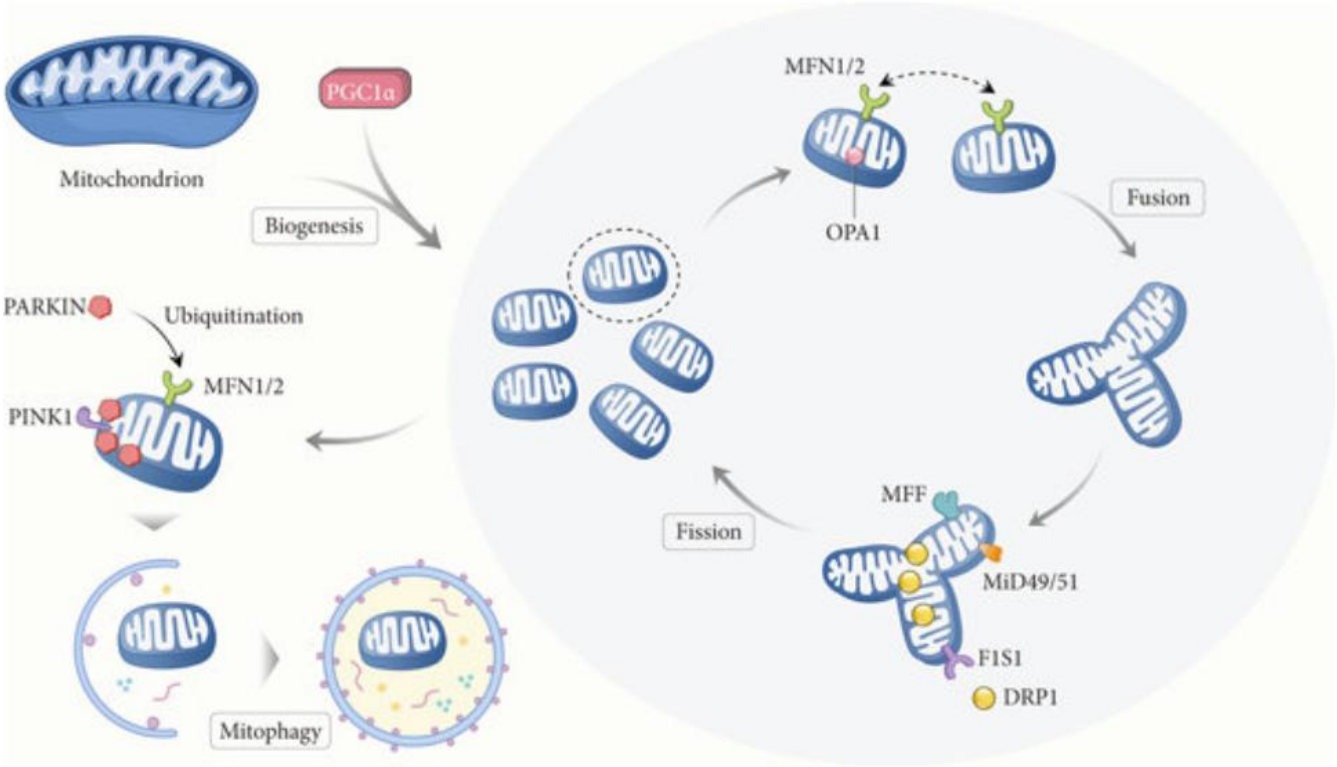
- Viral/bacterial infections: These increase non-mitochondrial respiration because the cell uses oxygen to try to kill the pathogens rather than for energy[1]; pathogens deplete the host’s mitochondrial anti-viral defences to keep themselves alive[2]; Borrelia steal ATP from their host[3]
- Biotoxins/mycotoxins: Ochratoxin A uncouples the mitochondria and inhibits Complex 2[4]
- Some parasites: E.g. Toxoplasma can tether and disable mitochondria[5]
- Heavy metals: Aluminium induces permeability (MPT)[6], Hg induces mito dysfunction[7], Arsenic increases mitochondrial ROS formation, lipid peroxidation and mitochondrial membrane potential collapse[8]



- Pesticides, herbicides: e.g. glyphosate: blocks Shikimate pathway (bacterial energy generation),[9] it also chelates minerals, including copper …
- Chemical contaminants: e.g. Lindane[10]
- Household chemicals
- EMFs
- Medications: Block various ETC complexes
- Microbiome
- Spike protein

Source: 1. Naviaux RK. Metabolic features of the Cell Danger Response. Mitochondrion. 2014 May;16:7-17; 2. https://www.nature.com/ articles/s12276-021-00602-1; 3. https://pubmed.ncbi.nlm.nih.gov/22710875/; 4. https://pubmed.ncbi.nlm.nih.gov/5441684/; 5. https://www.sciencedirect.com/science/article/ pii/S1471492222000320; 6. https://link.springer.com/article/10.1007/s007750000144; 7. https://link.springer.com/chapter/10.1007/978-3-319-03777-6_1; 8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3813354/; 9. https://roundupprisks.com/2016/04/09/glyphosate-and-mitochondrial-dysfunction/; 10. https://europepmc.org/article/MED/6205709

Mitochondrial biogenesis: the growth of new mitochondria and division of pre-existing mitochondria



Mitochondrial dynamics. Mitochondrial dynamics includes the process of mitochondrial biogenesis, fusion, fission, and mitophagy and determine the proper abundance and function of mitochondria. (1) PGC-1α regulates mitobiogenesis and initiates mtDNA transcription through its downstream factors, such as NRF1/2. (2) Mitofusion 1/2(MFN1/2) on the outer mitochondrial membrane and optic atrophy 1 (OPA1) on the inner mitochondrial membrane regulates mitochondrial fusion. (3) DRP1 serves to constrict mitochondrion physically and uses FIS1 as mitochondrial targets to form the fission complex, and MFF and MiD49/51 also participate in mitochondrial fission. (4) Canonical regulation of mitophagy includes the PINK1/PARKIN pathway; PINK1 recruits PARKIN on mitochondrion where PARKIN ubiquitinates downstream proteins and initiates the form of mitophagy.

Source: Ouyang M, Wang M, Yu B. Aberrant Mitochondrial Dynamics: An Emerging Pathogenic Driver of Abdominal Aortic Aneurysm. Cardiovasc Ther. 2021 Jun 16;2021:6615400; Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. Essays Biochem. 2010;47:69-84.

Weight regulation and mitobiogenesis

REVIEW ARTICLE | Originally Published 30 March 2007 | Check for updates

Defective Mitochondrial Biogenesis: A Hallmark of the High Cardiovascular Risk in the Metabolic Syndrome?

Enzo Nisoli, Emilio Clementi, Michele O. Carruba, and Salvador Moncada | AUTHOR INFO & AFFILIATIONS

Circulation Research • Volume 100, Number 6 • https://doi.org/10.1161/01.RES.0000259591.97107.6c

17,588 / 183 PDF/EPUB

Abstract

Mitochondrial Biogenesis Is Impaired in Obesity

Obese subjects are deficient in energy in the form of ATP.[91,92] Inadequate energy supply in the body results in increased appetite. Although the mechanism of such signaling is not yet known, there is evidence that it exists.[93–95] Treatment of rats with the metabolic inhibitors 2,5-anhydro-d-mannitol or/and methyl palmoxirate reduces the ATP levels in liver cells. Decreasing ATP concentrations in this way has been shown to lead to changes in the eating habits of the animals, including the frequency of eating, the amount of food consumed at a single eating session, and the total amount of food eaten. These experiments indicate that there is an integrated metabolic control of food intake, with liver ATP levels acting as a major sensor of energy status in the body. In obese people, the levels of hepatic ATP are decreased. Moreover, a decreased exercise capacity, together with high fatigability, has been linked to the low ATP levels in skeletal muscle of obese subjects.[96] In this context, genetically heterogeneous rats can be separated into 2 groups according to their aerobic treadmill-running capacity, with low-capacity runners showing higher visceral adiposity, blood pressure, insulin-resistance, plasma triglycerides, and free fatty acids as compared with the high-capacity runners.[97] Intriguingly, mitochondrial dysfunction with a defective oxidative metabolism and a low mitochondrial gene expression were evident in low-capacity runners,[97] suggesting that visceral obesity and related disorders are linked to defective mitochondrial biogenesis and oxidative metabolism with a decreased ATP production. Recent studies in obese rodents have supported this hypothesis, not only at the molecular and biochemical but also at the morphological level.[98,99] In our experiments in 3 models of obesity, we found that eNOS expression was markedly reduced in WAT, BAT, and soleus muscle of *ob*/*ob* mice, *fa*/*fa* rats, and high-fat diet–induced obese mice (DIO mice).[100] Moreover, mtDNA, respiratory protein (such as COX IV and Cyt c) levels, PGC-1α, NRF-1, and mtDNA transcription factor A (Tfam) gene expression were decreased in parallel, as

Source: Nisoli E, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? Circ Res. 2007 Mar 30;100(6):795-806.

Exercise effectively mediates mitochondrial biogenesis

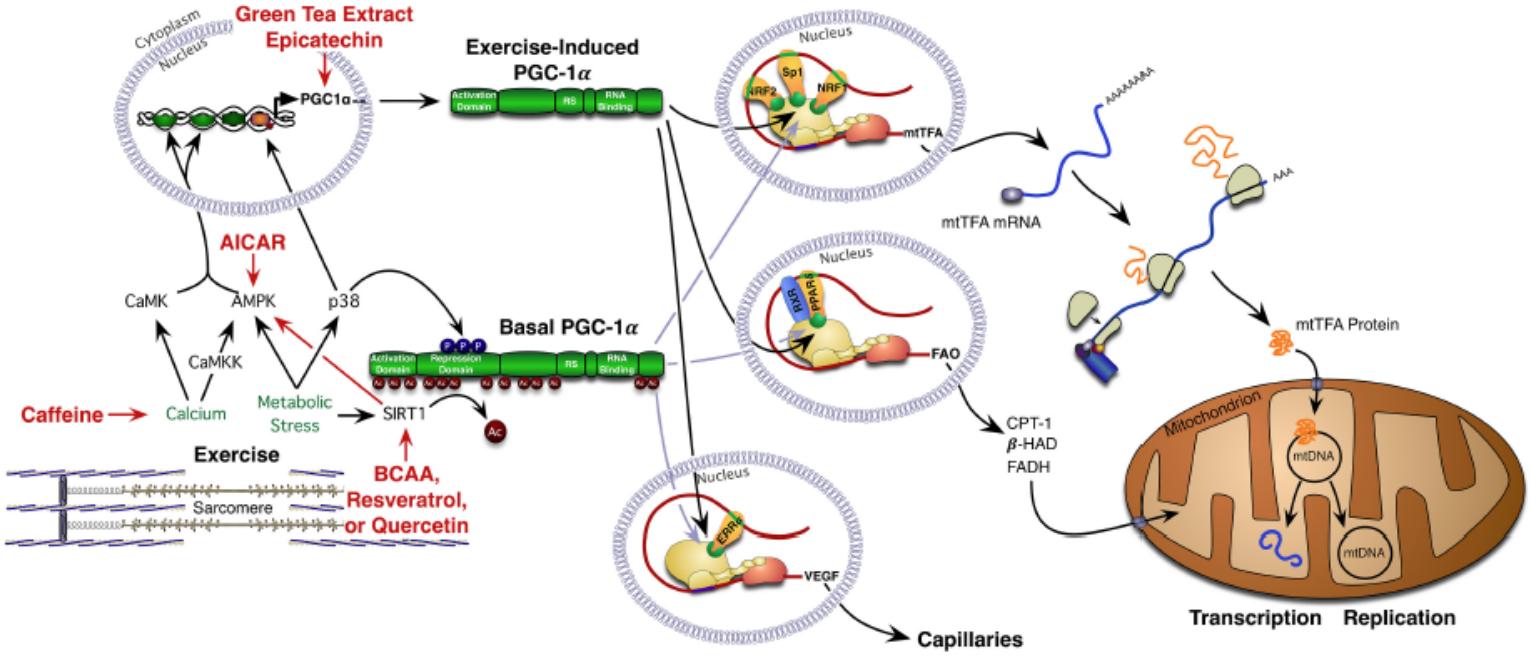


FIGURE 1 | Exercise-mediated mitochondrial biogenesis: exercise triggers mitochondrial biogenesis in skeletal muscle via the activation of numerous signaling pathways that ultimately converge on the transcriptional co-activator PGC-1α. Once activated, PGC-1α translocates to the nucleus to activate numerous transcription factors and nuclear receptors. Bioactive compounds have the capacity to enhance exercise-mediated mitochondrial biogenesis through contraction-dependent signaling cascades. AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, 5′ AMP-activated protein kinase; βHAD, beta-hydroxyacyl-CoA dehydrogenase; BCAA, branch chain amino acids; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CaMKK, Ca(2+)/calmodulin-dependent protein kinase kinase; CPT-1, carnitine palmitoyltransferase I; ERRα, estrogen-related receptor alpha; FADH, flavin adenine dinucleotide; FAO, fatty acid oxidation; mRNA, messenger ribonucleic acid; mtDNA, mitochondrial deoxyribonucleic acid; mtTFA, mitochondrial transcription factor A; NRF1, nuclear respiratory factor-1; NRF2, nuclear respiratory factor-2; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARδ, peroxisome proliferator-activated receptor delta; RS, RS domain; RXR, retinoid X receptor; SIRT1, sirtuin-1; Sp1, specificity protein 1 transcription factor; VEGF, vascular endothelial growth factor.

Source: Craig DM, Ashcroft SP, Belew MY, Stocks B, Currell K, Baar K, Philp A. Utilizing small nutrient compounds as enhancers of exercise-induced mitochondrial biogenesis. Front Physiol. 2015 Oct 27;6:296.

Resveratrol is known to stimulate mitochondrial biogenesis by activating Sirtuin 1 (SIRT1) and PGC-1a

Randomised controlled trial

Randomized Controlled Trial > Am J Clin Nutr. 2016 Jul;104(1):215-27.
doi: 10.3945/ajcn.115.122937. Epub 2016 May 18.

Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial

Jasper Most [1], Silvie Timmers [1], Ines Warnke [2], Johan We Jocken [1], Mark van Boekschoten [3], Philip de Groot [3], Igor Bendik [2], Patrick Schrauwen [1], Gijs H Goossens [1], Ellen E Blaak [4]

Affiliations + expand

PMID: 27194304 DOI: 10.3945/ajcn.115.122937
Free article

Abstract

Background: The obese insulin-resistant state is characterized by impairments in lipid metabolism. We previously showed that 3-d supplementation of combined epigallocatechin-3-gallate and resveratrol (EGCG+RES) increased energy expenditure and improved the capacity to switch from fat toward carbohydrate oxidation with a high-fat mixed meal (HFMM) test in men.

Objective: The present study aimed to investigate the longer-term effect of EGCG+RES supplementation on metabolic profile, mitochondrial capacity, fat oxidation, lipolysis, and tissue-specific insulin sensitivity.

Design: In this randomized double-blind study, 38 overweight and obese subjects [18 men; aged 38 ± 2 y; body mass index (kg/m(2)): 29.7 ± 0.5] received either EGCG+RES (282 and 80 mg/d, respectively) or placebo for 12 wk. Before and after the intervention, oxidative capacity and gene expression were assessed in skeletal muscle. Fasting and postprandial (HFMM) lipid metabolism was assessed by using indirect calorimetry, blood sampling, and microdialysis. Tissue-specific insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp with [6,6-(2)H2]-glucose infusion.

Results: EGCG+RES supplementation did not affect the fasting plasma metabolic profile. Although whole-body fat mass was not affected, visceral adipose tissue mass tended to decrease after the

Cohort: 38 overweight and obese subjects

"Twelve weeks of EGCG+RES supplementation increased mitochondrial capacity and stimulated fat oxidation compared with placebo"

Source: Most J, Timmers S, Warnke I, Jocken JW, van Boekschoten M, de Groot P, Bendik I, Schrauwen P, Goossens GH, Blaak EE. Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial. Am J Clin Nutr. 2016 Jul;104(1):215-27; https://www.nutrition-evidence.com/article/27194304?term=27194304

Peer-reviewed scientific journal evidence of Resveratrol as a PGC-1a activator

Resveratrol (Japanese Knotweed)

Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G, Pearson K, de Cabo R, Pacher P, Zhang C, Ungvari Z. Resveratrol induces mitochondrial biogenesis in endothelial cells. Am J Physiol Heart Circ Physiol. 2009 Jul;297(1):H13-20; https://pubmed.ncbi.nlm.nih.gov/19429820/

Uriho, A., Tang, X., Le, G. *et al.* Effects of resveratrol on mitochondrial biogenesis and physiological diseases. *ADV TRADIT MED (ADTM)* **21,** 1–14 (2021); https://link.springer.com/article/10.1007/s13596-020-00492-0#citeas

Ungvari Z, Sonntag WE, de Cabo R, Baur JA, Csiszar A. Mitochondrial protection by resveratrol. Exerc Sport Sci Rev. 2011 Jul;39(3):128-32; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123408/

Zhou J, Yang Z, Shen R, Zhong W, Zheng H, Chen Z, Tang J, Zhu J. Resveratrol Improves Mitochondrial Biogenesis Function and Activates PGC-1α Pathway in a Preclinical Model of Early Brain Injury Following Subarachnoid Hemorrhage. Front Mol Biosci. 2021 Apr 22;8:620683; https://www.frontiersin.org/articles/10.3389/fmolb.2021.620683/full

Pyrroloquinoline Quinone (PQQ) is able to stimulate mitobiogenesis

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 1, pp. 142–152, January 1, 2010

Pyrroloquinoline Quinone Stimulates Mitochondrial Biogenesis through cAMP Response Element-binding Protein Phosphorylation and Increased PGC-1α Expression*[S]

Received for publication, June 5, 2009, and in revised form, October 14, 2009 Published, JBC Papers in Press, October 27, 2009, DOI 10.1074/jbc.M109.030130

Winyoo Chowanadisai[‡], Kathryn A. Bauerly[‡], Eskouhie Tchaparian[§], Alice Wong[¶], Gino A. Cortopassi[‖], and Robert B. Rucker[‡1]

From the [‡]Department of Nutrition, University of California, Davis, 95616, [§]Amgen, Inc., South San Francisco, 94080, and the Departments of [¶]Anatomy, Physiology, and Cell Biology and [‖]Molecular Biosciences, Veterinary Medicine, University of California, Davis, California 95616

Bioactive compounds reported to stimulate mitochondrial biogenesis are linked to many health benefits such increased longevity, improved energy utilization, and protection from reactive oxygen species. Previously studies have shown that mice and rats fed diets lacking in pyrroloquinoline quinone (PQQ) have reduced mitochondrial content. Therefore, we hypothesized that PQQ can induce mitochondrial biogenesis in mouse hepatocytes. Exposure of mouse Hepa1–6 cells to 10–30 μM PQQ for 24–48 h resulted in increased citrate synthase and cytochrome *c* oxidase activity, Mitotracker staining, mitochondrial DNA content, and cellular oxygen respiration. The induction of this process occurred through the activation of cAMP response element-binding protein (CREB) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a pathway known to regulate mitochondrial biogenesis. PQQ exposure stimulated phosphorylation of CREB at serine 133, activated the promoter of PGC-1α, and increased PGC-1α mRNA and protein expression. PQQ did not stimulate mitochondrial biogenesis after small interfering RNA-mediated reduction in either PGC-1α or CREB expression. Consistent with activation of the PGC-1α pathway, PQQ increased nuclear respiratory factor activation (NRF-1 and NRF-2) and Tfam, TFB1M, and TFB2M mRNA expression. Moreover, PQQ protected cells from mitochondrial inhibition by rotenone, 3-nitropropionic acid, antimycin A, and sodium azide. The ability of PQQ to stimulate mitochondrial biogenesis accounts in part for action of this compound and suggests that PQQ may be beneficial in diseases associated with mitochondrial dysfunction.

Bioactive compounds, such as pyrroloquinoline quinone (PQQ),[2] resveratrol, genistein, hydroxy-tyrosol, and quercetin have been reported to improve mitochondrial respiratory control or stimulate mitochondrial biogenesis (1–5), which is potentially important to a number of health-related issues ranging from increased longevity, improved energy utilization, and protection from reactive oxygen species (6–8). Furthermore, mitochondrial DNA depletion and mutations are associated with cardiomyopathy, lactic acidosis, developmental delay, failure to thrive, and impaired neurological function (9). The response to most biofactors is observed after pharmacological intervention or dietary supplementation, although often neargram amounts per kg of diet, or millimolar quantities, are needed for such responses *in vivo*. PQQ stimulates mitochondriogenesis with the addition of only milligram quantities of PQQ per kg of diet, or micromolar concentrations, *in vivo*. For example, PQQ deprivation depresses mitochondrial function, which is reversed when as little as 200–300 μg of PQQ/kg of diet are added (1, 10). PQQ also remains detectable in tissues when there is no or little dietary exposure (11), which has not been observed for other dietary polyphenolic compounds known to promote mitochondriogenesis.

Recently, PQQ produced by rhizobacterium has been identified as an important plant growth factor (12) and is a possible source of PQQ in plant-derived food. In this regard, the ubiquitous presence of PQQ in a broad range of plants leads to a relatively constant exposure in animal diets. More importantly, levels of PQQ from dietary intake from plants are sufficient to maintain the concentration of PQQ typical of tissues (13). From a chemical perspective, assays that measure redox cycling indicate that PQQ is also 100–1000 times more efficient than other quinones and enediols, such as ascorbic acid (14). PQQ can undergo thousands of reductive or oxidative cycles without degradation or polymerization (14).

Peroxisome proliferator-activated receptor-γ coactivator-1

"We show that PQQ influences PGC-1 activity, which is a major mechanism for the regulation of mitochondrial biogenesis."

Source: Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. J Biol Chem. 2010 Jan 1;285(1):142-52.

Nicotinamide riboside, niacin and quercetin also stimulate PGC-1a ...

Nicotinamide riboside

Gong B, Pan Y, Vempati P, Zhao W, Knable L, Ho L, Wang J, Sastre M, Ono K, Sauve AA, Pasinetti GM. Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor-γ coactivator 1α regulated β-secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models. Neurobiol Aging. 2013 Jun;34(6):1581-8; https://pubmed.ncbi.nlm.nih.gov/23312803/

Niacin

Koh JH, Kim JY. Role of PGC-1α in the Mitochondrial NAD^+ Pool in Metabolic Diseases. Int J Mol Sci. 2021 Apr 27;22(9):4558; https://pubmed.ncbi.nlm.nih.gov/33925372/

Quercetin

Li X, Wang H, Gao Y, Li L, Tang C, Wen G, Yang Y, Zhuang Z, Zhou M, Mao L, Fan Y. Quercetin induces mitochondrial biogenesis in experimental traumatic brain injury via the PGC-1α signaling pathway. Am J Transl Res. 2016 Aug 15;8(8):3558-66; https://pubmed.ncbi.nlm.nih.gov/27648146/

Rayamajhi N, Kim SK, Go H, Joe Y, Callaway Z, Kang JG, Ryter SW, Chung HT. Quercetin induces mitochondrial biogenesis through activation of HO-1 in HepG2 cells. Oxid Med Cell Longev. 2013;2013:154279; https://pubmed.ncbi.nlm.nih.gov/24288584/

Li X, Wang H, Wen G, Li L, Gao Y, Zhuang Z, Zhou M, Mao L, Fan Y. Neuroprotection by quercetin via mitochondrial function adaptation in traumatic brain injury: PGC-1α pathway as a potential mechanism. J Cell Mol Med. 2018 Feb;22(2):883-891; https://pubmed.ncbi.nlm.nih.gov/29205806/

Xue H, Li P, Luo Y, Wu C, Liu Y, Qin X, Huang X, Sun C. Salidroside stimulates the Sirt1/PGC-1α axis and ameliorates diabetic nephropathy in mice. Phytomedicine. 2019 Feb 15;54:240-247; https://pubmed.ncbi.nlm.nih.gov/30668374/

... as can alpha-lipoic acid

Original Articles

The synergic effects of alpha-lipoic acid supplementation and electrical isotonic contraction on anthropometric measurements and the serum levels of VEGF, NO, sirtuin-1, and PGC1-α in obese people undergoing a weight loss diet

Majid Mohammadshahi, **Elahe Zakizadeh** ✉ Kambiz Ahmadi-Angali, Majid Ravanbakhsh & Bijan Helli

Pages 1195-1201 | Received 03 Mar 2020, Accepted 24 Apr 2020, Published online: 14 May 2020

Abstract

Background: The anti-obesity effects of Alpha-lipoic acid (α-LA) and isotonic contraction has been reported. However, the underlying mechanism is not fully understood. This study aimed to investigate the effect of 1200 mg/day α-LA supplementation and 3 sessions per week of Faradic (an electrical stimulating system) on anthropometric parameters, body composition, VEGF, Sirtuin-1, nitric oxide (NO), and PGC1-α in obese people undergoing a weight loss regime.

Methods: This randomised clinical trial was carried out on 100 obese adults. The subjects were randomly assigned to four groups of 25 subjects including Faradic, α-LA, α-LA + Faradic, and control. A Bio Impedance Analyser (BIA) was used to estimate anthropometric measurements including weight, body mass index (BMI), fat mass, and fat free mass. The serum levels of Sirtuin-1, PGC1-α, VEGF, and NO levels were measured. All measurements were done at baseline and after 8 weeks of the intervention.

Results: A significant weight reduction was observed in all four groups compared to baseline (p<.01). The placebo group had significantly higher weight, BMI, weight circumstance (WC), and body fat (BF) compared with the other groups. The α-LA + Faradic group had significantly lower weight, BMI, BF, WC than control, faradic, and α-LA groups and higher, Sirtuin and PGC than the control group (all p < .05).

Randomised controlled trial

Well-powered study with 4 groups of 25 subjects

A significant weight reduction was observed in all four groups compared to baseline (p<.01). The placebo group had significantly higher weight, BMI, weight circumstance (WC), and body fat (BF) compared with the other groups. The α-LA + Faradic group had significantly lower weight, BMI, BF, WC than control, faradic, and α-LA groups and higher, Sirtuin and PGC than the control group (all p < .05).

Source: Mohammadshahi M, Zakizadeh E, Ahmadi-Angali K, Ravanbakhsh M, Helli B. The synergic effects of alpha-lipoic acid supplementation and electrical isotonic contraction on anthropometric measurements and the serum levels of VEGF, NO, sirtuin-1, and PGC1-α in obese people undergoing a weight loss diet. Arch Physiol Biochem. 2022 Oct;128(5):1195-1201.

Peer-reviewed scientific journal evidence of EGCG and L-Carnitine as PGC-1a activators

Epigallotocatechin-3-Gallate (Green tea extract)

Lee MS, Lee S, Doo M, Kim Y. Green Tea (-)-Epigallotocatechin-3-Gallate Induces PGC-1α Gene Expression in HepG2 Cells and 3T3-L1 Adipocytes. Prev Nutr Food Sci. 2016 Mar;21(1):62-7; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4827637/

L-Carnitine

Pesce V, Fracasso F, Cassano P, Lezza AM, Cantatore P, Gadaleta MN. Acetyl-L-carnitine supplementation to old rats partially reverts the age-related mitochondrial decay of soleus muscle by activating peroxisome proliferator-activated receptor gamma coactivator-1alpha-dependent mitochondrial biogenesis. Rejuvenation Res. 2010 Apr-Jun;13(2-3):148-51; https://pubmed.ncbi.nlm.nih.gov/20370498/

Calvani, Menotti & Peluso, Gianfranco & Benatti, Paola & Nicolai, Raffaella & Reda, Elham. (2003). The role of carnitine system in maintaining muscle homeostasis. Basic Appl Myol. 13. 105-120; http://www.bio.unipd.it/bam/PDF/13-3/03541Calvani.pdf

MCCARTY, Mark; DINICOLANTONIO, James J.; O'KEEFE, James H.. The Ability of Carnitine to Act as a Type 1Histone Deacetylase Inhibitor May Explain the Favorable Impact of Carnitine Supplementation on Mitochondrial Biogenesis in the Elderly. **Medical Research Archives,** [S.l.], v. 8, n. 2, feb. 2020. ISSN 2375-1924; https://esmed.org/MRA/mra/article/view/2055

Copper also has an important role in mitochondrial biogenesis

Home > Journal of Bioenergetics and Biomembranes > Article

Role of Copper in Mitochondrial Biogenesis Via Interaction with ATP Synthase and Cytochrome *c* Oxidase

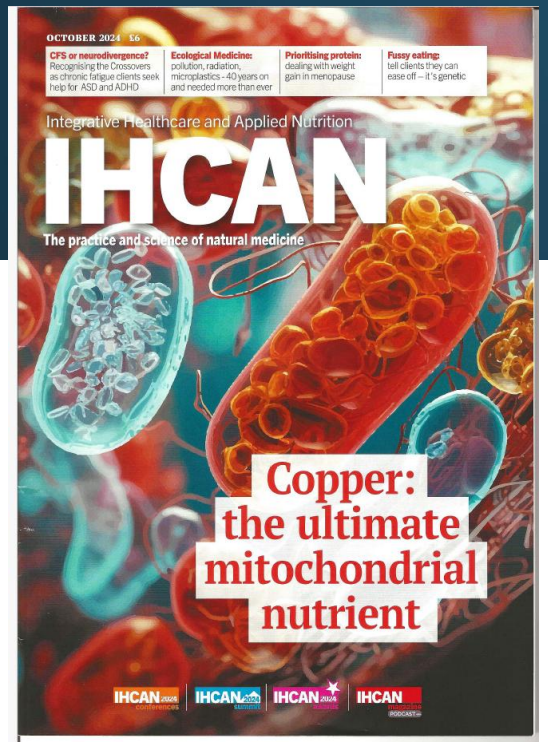
Published: October 2002

Volume 34, pages 389–395, (2002) Cite this article

Journal of Bioenergetics and Biomembranes

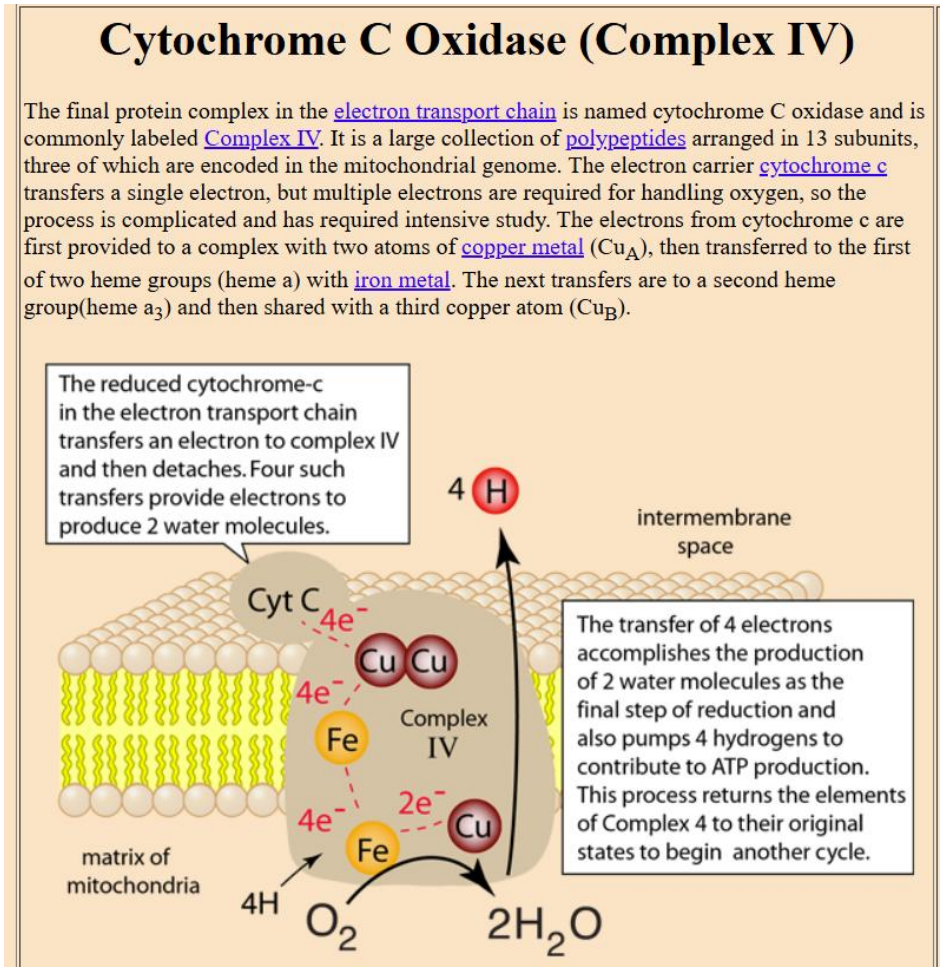
Aims and scope →

Submit manuscript →



Source: Ruiz LM, Libedinsky A, Elorza AA. Role of Copper on Mitochondrial Function and Metabolism. Front Mol Biosci. 2021 Aug 24;8:711227.

Mitochondria can't work without bioavailable copper – so their numbers are likely to be low if it is lacking



1

"The di-copper center Cu_A is an essential metal cofactor in cytochrome oxidase (Cox) of mitochondria and many prokaryotes, mediating one-electron transfer from cytochrome c to the site for oxygen reduction."[2]

"Mitochondria are critical copper-utilizing organelles that harbor an essential cuproenzyme cytochrome *c* oxidase, which powers energy production."[3]

Source: 1. http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/Complex4.html; 2. Canonica F, Hennecke H, Glockshuber R. Biochemical pathway for the biosynthesis of the Cu_A center in bacterial cytochrome c oxidase. FEBS Lett. 2019 Nov;593(21):2977-2989.; 3. Garza NM, Swaminathan AB, Maremanda KP, Zulkifli M, Gohil VM. Mitochondrial copper in human genetic disorders. Trends Endocrinol Metab. 2023 Jan;34(1):21-33.

Photobiomodulation may also have its role ... especially near infrared (NIR) light

MITOCHONDRION
Volume 14, 2014, pp. 42-48.

doi: 10.1016/j.mito.2013.11.001
LiteCure® Laser Used in Study

Mitochondrion
OFFICIAL JOURNAL OF THE Mitochondria® RESEARCH SOCIETY

"Our data indicates that NIR light alters mitochondrial biogenesis signalling and may represent a mechanistic link to the clinical benefits"

Effect of Near-Infrared Light Exposure on Mitochondrial Signaling in C_2C_{12} Muscle Cells

Linda M.-D. Nguyen, Angelina G. Malamo, Kelly A. Larkin-Kaiser, Paul A. Borsa, Peter J. Adhihetty

Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, United States

Highlights:

- NIR light exposure activates mitochondrial signaling in C_2C_{12} muscle cells.
- Chronic NIR light exposure (4d) activates mitochondrial regulatory proteins.
- Mitochondrial responses to NIR light may involve ROS signaling.
- Mitochondrial adaptations may contribute to NIR light therapeutic benefits.

Abstract: Near-infrared (NIR) light is a complementary therapy used to treat musculoskeletal injuries but the underlying mechanisms are unclear. Acute NIR light treatment (~800–950 nm; 22.8 J/cm^2) induced a dose-dependent increase in mitochondrial signaling (AMPK, p38 MAPK) in differentiated muscle cells. Repeated NIR light exposure (4 days) appeared to elevate oxidative stress and increase the upstream mitochondrial regulatory proteins AMPK (3.1-fold), p38 (2.8-fold), PGC-1α (19.7%), Sirt1 (26.8%), and reduced RIP140 (23.2%), but downstream mitochondrial regulation/content (Tfam, NRF-1, Sirt3, cytochrome c, ETC subunits) was unaltered. Our data indicates that NIR light alters mitochondrial biogenesis signaling and may represent a mechanistic link to the clinical benefits.

Source: Nguyen LM, Malamo AG, Larkin-Kaiser KA, Borsa PA, Adhihetty PJ. Effect of near-infrared light exposure on mitochondrial signaling in C2C12 muscle cells. Mitochondrion. 2014 Jan;14(1):42-8.

If too many, selective autophagy: Mitophagy

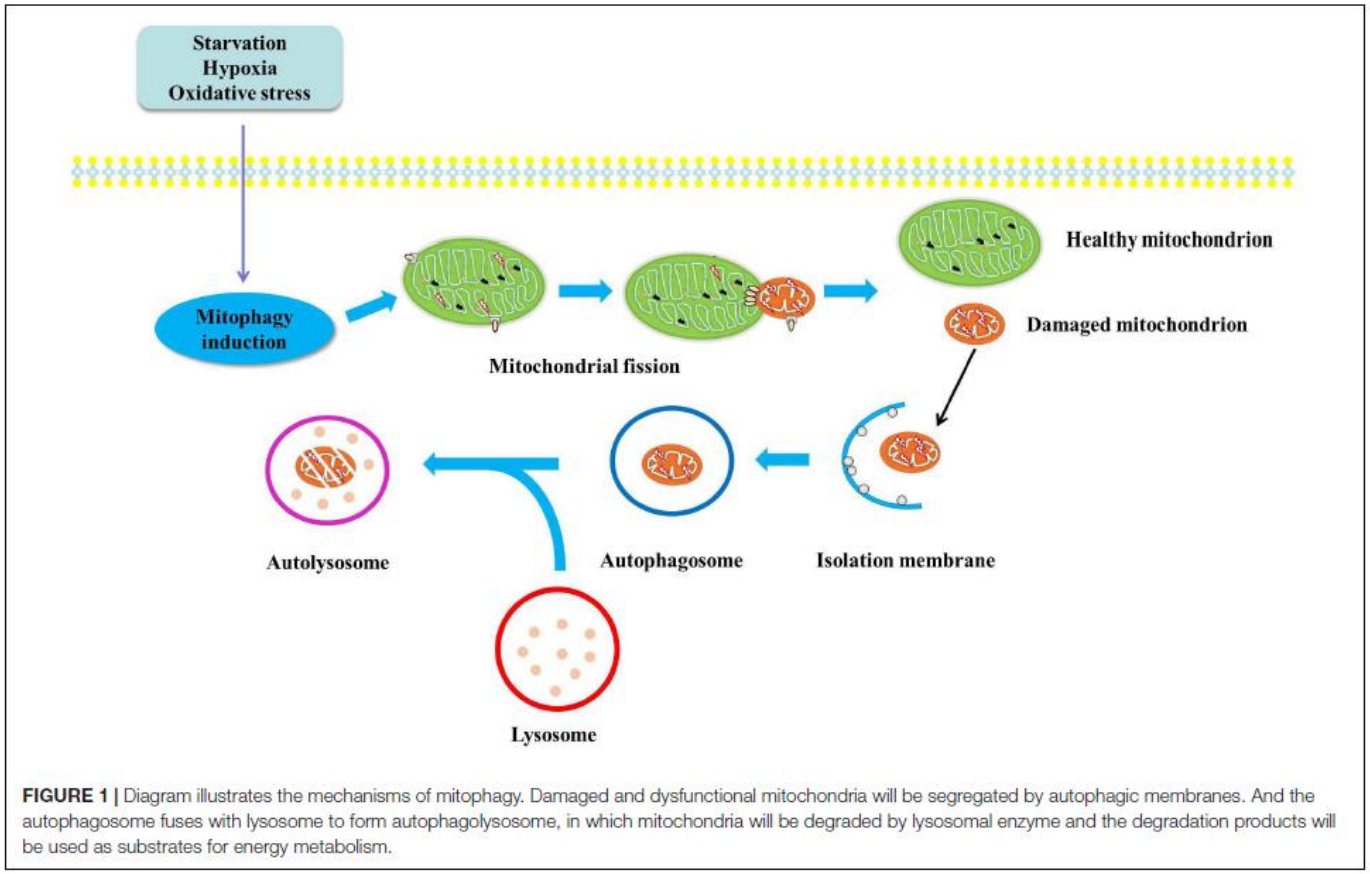


FIGURE 1 | Diagram illustrates the mechanisms of mitophagy. Damaged and dysfunctional mitochondria will be segregated by autophagic membranes. And the autophagosome fuses with lysosome to form autophagolysosome, in which mitochondria will be degraded by lysosomal enzyme and the degradation products will be used as substrates for energy metabolism.

Mitophagy is a subcategory of autophagy – the clearing of mitochondrial debris; if the mitochondria are compromised it is best to activate mitophagy first, as radical autophagy risks clearing indwelling mitochondria that may still be functional.

Source: Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal. 2014 Jan 20;20(3):460-73.

Healthy mitochondrial fragments are reused

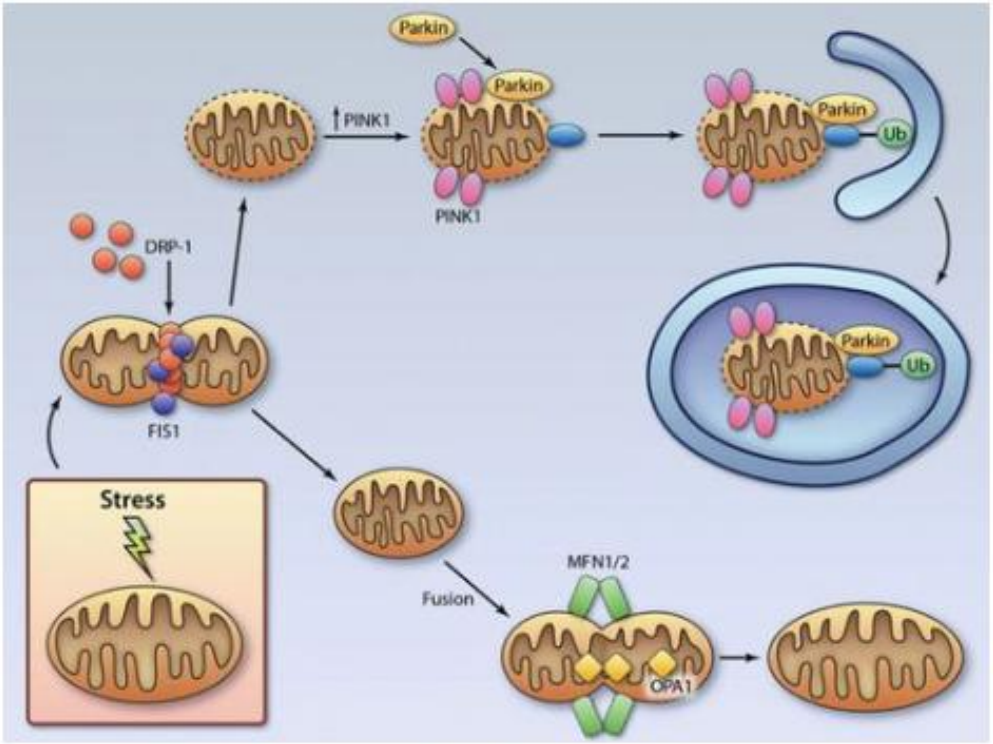


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Figure 3. Regulation of mitophagy. Damaged mitochondria undergo dynamin-related protein 1 (DRP1)-mediated fission before mitophagy. Reduced mitochondrial membrane potential leads to accumulation of phosphatase and tensin homolog–induced putative kinase 1 (PINK1) and subsequent recruitment of the E3 ubiquitin ligase Parkin to mitochondria. Parkin promotes ubiquitination of proteins in the mitochondrial membrane, which targets the damaged mitochondrion for removal by an autophagosome. The healthy mitochondrial fragment will undergo fusion mediated by mitofusin-1/2 (MFN1/2) and optic atrophy protein 1 (OPA1). (illustration credit: Ben Smith.)

Source: Kubli DA, Gustafsson ÅB. Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res. 2012 Oct 12;111(9):1208-21.

Calorie restriction drives autophagy, can prolong life and health span

JCI The Journal of Clinical Investigation

Essential role for autophagy in life span extension

Frank Madeo, … , Maria Chiara Maiuri, Guido Kroemer

J Clin Invest. 2015;125(1):85-93. https://doi.org/10.1172/JCI73946.

Review

Life and health span can be prolonged by calorie limitation c
restriction. Both starvation and the genetic inactivation of nu
cytoplasmic recycling process that counteracts the age-assc
improves the metabolic fitness of cells. Here we review expe
nutrient and growth-related signaling pathways as well as th
extension via the induction of autophagy. Furthermore, we d
only necessary but, at least in some cases, also sufficient fo

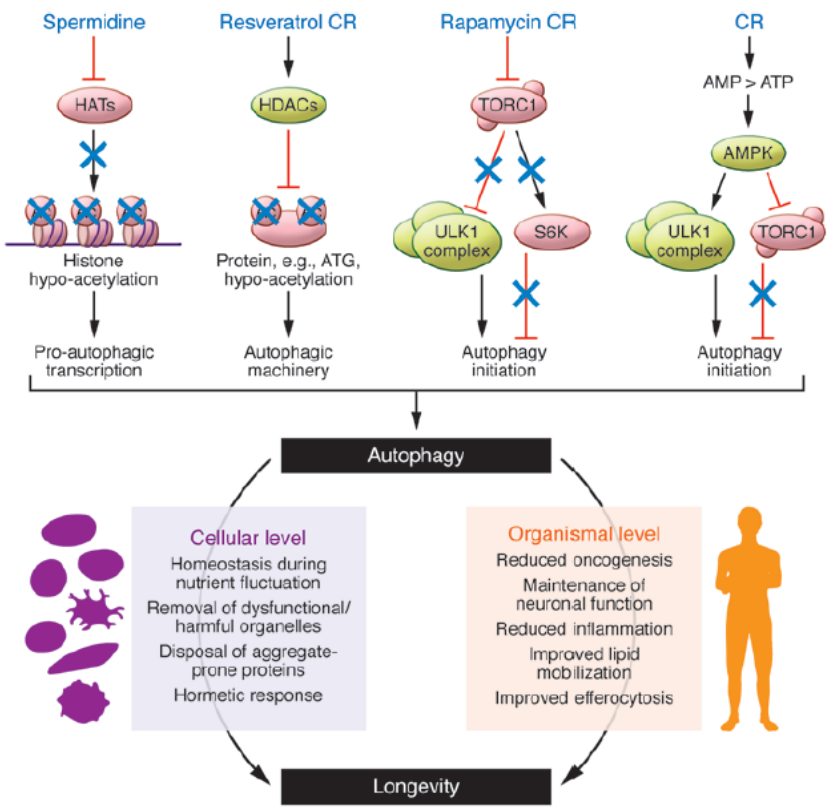


Figure 3. Autophagy inducers and functions of autophagy that prolong life span. Several pharmacologic and dietary interventions activate autophagy signaling and thereby promote beneficial effects at the cellular and organismal levels, contributing to prolonged life span and health span.

Exercise: mitochondrial fission and mitophagy are higher in skeletal muscle of physically active humans

› Front Physiol. 2019 Aug 22;10:1088. doi: 10.3389/fphys.2019.01088. eCollection 2019.

Regular Endurance Exercise Promotes Fission, Mitophagy, and Oxidative Phosphorylation in Human Skeletal Muscle Independently of Age

Estelle Balan [1], Céline Schwalm [1], Damien Naslain [1], Henri Nielens [2], Marc Francaux [1], Louise Deldicque [1]

Affiliations + expand

PMID: 31507451 PMCID: PMC6713923 DOI: 10.3389/fphys.2019.01088

Free PMC article

Abstract

This study investigated whether regular endurance exercise maintains basal mitophagy and mitochondrial function during aging. Mitochondrial proteins and total mRNA were isolated from vastus lateralis biopsies (n = 33) of young sedentary (YS), old sedentary (OS), young active (YA), and old active (OA) men. Markers for mitophagy, fission, fusion, mitogenesis, and mitochondrial metabolism were assessed using qRT-PCR, Western blot, and immunofluorescence staining. Independently of age, fission protein Fis1 was higher in active vs. sedentary subjects (+80%; $P < 0.05$). Mitophagy protein PARKIN was more elevated in OA than in OS (+145%; $P = 0.0026$). mRNA expression of Beclin1 and Gabarap, involved in autophagosomes synthesis, were lower in OS compared to YS and OA ($P < 0.05$). Fusion and oxidative phosphorylation proteins were globally more elevated in the active groups ($P < 0.05$), while COx activity was only higher in OA than in OS ($P = 0.032$). Transcriptional regulation of mitogenesis did not vary with age or exercise. In conclusion, physically active lifestyle seems to participate in the maintenance of lifelong mitochondrial quality control by increasing fission and mitophagy.

Keywords: OXPHOS; endurance exercise; fusion; mitochondria; mitogenesis; physical activity.

Study, biopsies and other markers from 33 participants

"In conclusion, the present study, based on mitochondrial extracts, demonstrates that expression of mitochondrial quality control and function markers does not depend on chronological age, but is improved by regular physical activity. These findings bring new pieces of evidence that **mitochondrial fission and mitophagy are higher in skeletal muscle of physically active humans**."

Source: Balan E, Schwalm C, Naslain D, Nielens H, Francaux M, Deldicque L. Regular Endurance Exercise Promotes Fission, Mitophagy, and Oxidative Phosphorylation in Human Skeletal Muscle Independently of Age. Front Physiol. 2019 Aug 22;10:1088; Guan Y, Drake JC, Yan Z. Exercise-Induced Mitophagy in Skeletal Muscle and Heart. Exerc Sport Sci Rev. 2019 Jul;47(3):151-156;

Mechanisms and molecular targets of mitophagy-triggering phytochemicals explained

Oxidative Medicine and Cellular Longevity

Forward Series

Review Article | Open Access

Effects of Plant and Animal Natural Products on Mitophagy

Farzaneh Shakeri, Vanessa Bianconi, Matteo Pirro, Amirhossein Sahebkar

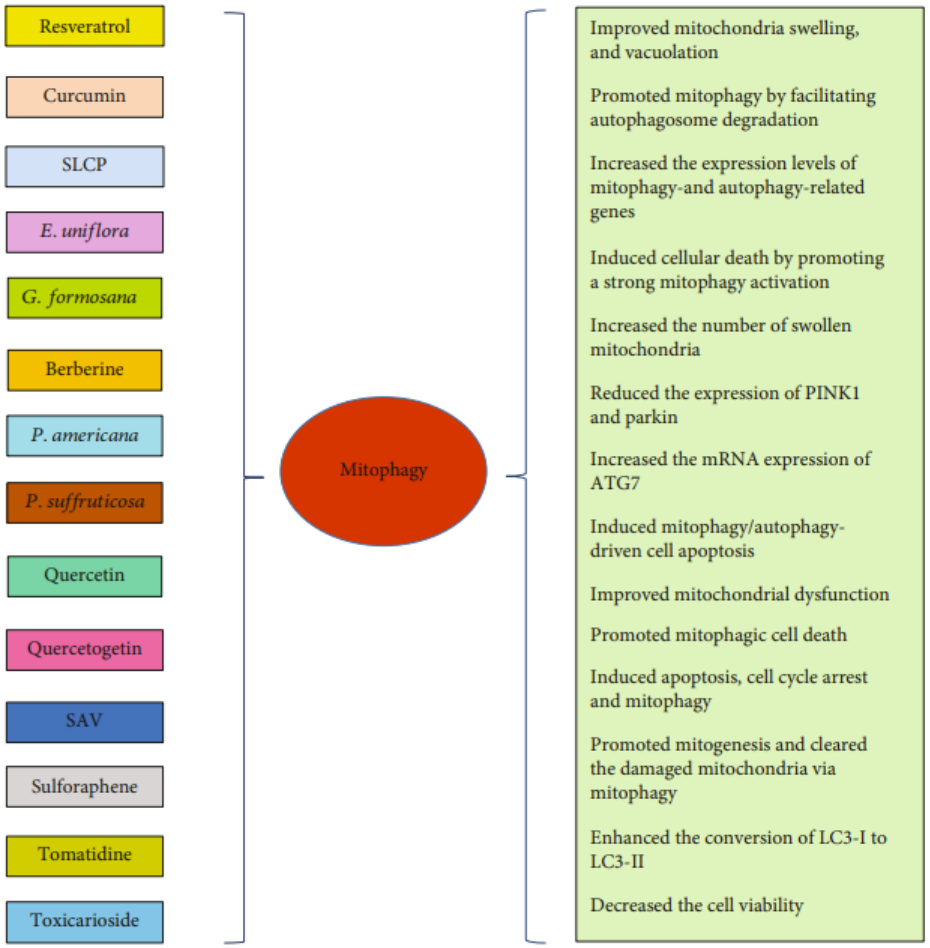


FIGURE 2: Effects of natural compounds on mitophagy.

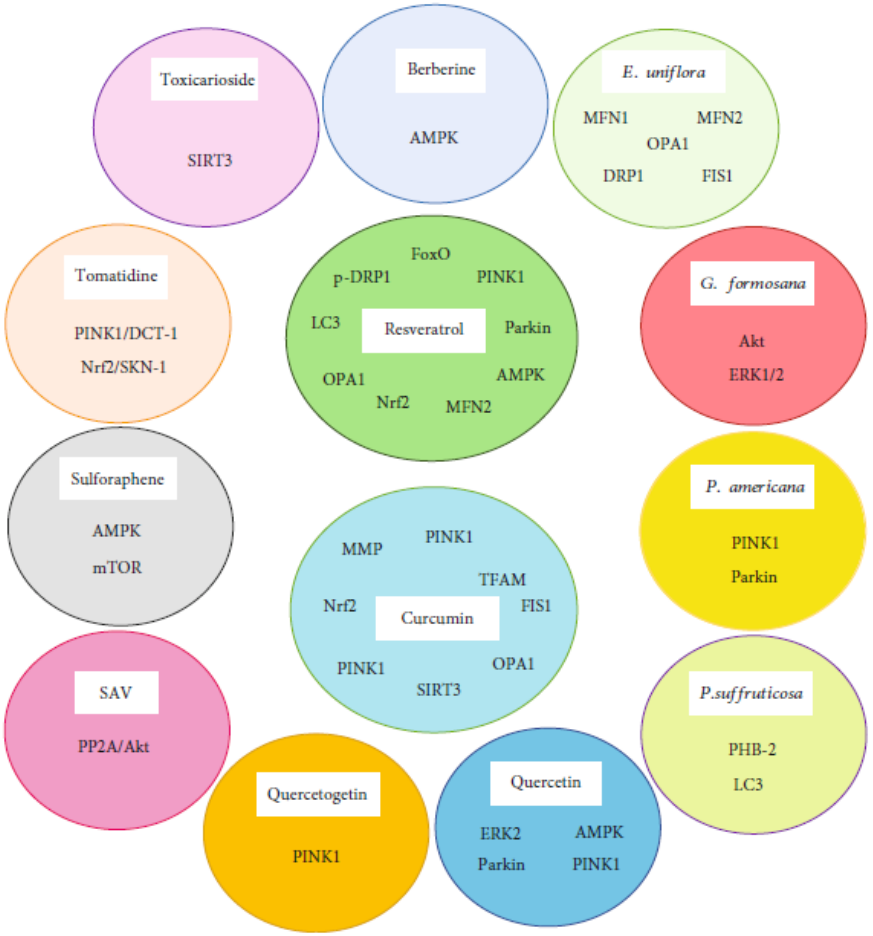


FIGURE 3: Specific molecular targets of natural compounds in the mitophagy pathway.

Source: : https://www.hindawi.com/journals/omcl/2020/6969402/;

Mitophagy agents: known effects on the nervous system, including human studies

Table 1

Mechanism of action and known effects on the nervous system.

Urolithin A (UrA) • activates Nrf2 regularizing mitophagy and mitochondrial biogenesis; • activates AMPK pathway.	• In AD models of SH-SY5Y cells and in vivo, UrA prevented destruction of the AIP-AhR complex and suppressed expression of APP and BACE1; • In microglia, UrA alleviates neuroinflammation and enhances phagocytosis by enhancing mitophagy, leading to a decrease in the accumulation of tau-protein and beta-amyloid; • In BV2 microglial cell PD model, UrA, due to mitophagy, reduced neuroinflammation and provided a neuroprotective effect; • UrA attenuates memory impairment and neuroinflammation in APP/PS1 mice; • UrA appeared to be safe and induced the molecular signature of improved mitochondrial and cellular health in the elderly.	[44] [43,83] [84] [85] [86] MoA ref: [66,67,68,69,85,87]
Resveratrol (RES) • reduces oxidative stress by suppressing the formation of ROS, derivatives of NADPH oxidase; • activates Nrf2 regularizing mitophagy and mitochondrial biogenesis; • in endothelial dysfunction, resveratrol activated mitophagy through Bnip3.	• In a model of AD cells, RES promoted mitophagy, attenuating oxidative damage to neurons caused by Aβ-peptide; • In a cadmium-induced mitophagy model, RES attenuated the overexpression of p62 and PINK1/Parkin, which suppressed mitophagy and ultimately restored mitochondrial homeostasis	[88] [89] MoA ref: [89,90,91]
Quercetin (QU) • activates AMPK and Nrf2, contributing to the modulation of the **Spermidine (SP)** • activates ATM, induces mitochondrial membrane depolarization promoting the PINK1/Parkin-dependent mitophagy pathway; • inhibits mTOR; • activates AMPK.	• QU decreased levels of hyperphosphorylated Tau and amyloid plaques in AD models, leveled out neuroinflammation, inhibited microglia and astrocyte activation, and provided a mitoprotective effect, an increase in ATP production, and a decrease in ROS levels; • In PD rat models, QU reduced oxidative stress, increased PINK1 and Parkin, and reduced α-synuclein expression in vitro, and in vivo, QU • SP improved olfactory memory in models with age-related memory impairment drosophila flies; • In C. elegans models of AD and PD, SP inhibited memory loss in AD models and improved behavioral performance in PD models; • In accelerated aging SAMP8 model of mice, SP increased the expression of neurotrophic factors in neurons and reduced memory loss in the ORT and the open field test OFT; • In older adults, SP improved episodic memory in MST.	[92,93] [94] [106] [107] [108] [109,110] MoA ref: [54,107,111,112,113]

Source: Makarov M, Korkotian E. Differential Role of Active Compounds in Mitophagy and Related Neurodegenerative Diseases. Toxins (Basel). 2023 Mar 6;15(3):202.

Urolithin A proven to activate mitophagy in several well-powered studies (1/2)

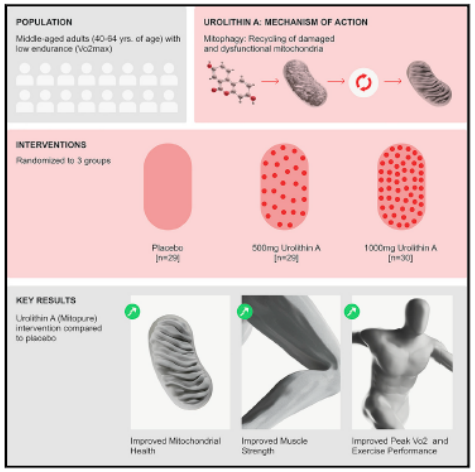
Randomised controlled trial

Cell Reports Medicine

Article

Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults

Graphical abstract



Authors

Anurag Singh, Davide D'Amico, Pénélope A. Andreux, ..., Patrick Aebischer, Johan Auwerx, Chris Rinsch

Correspondence

asingh@amazentis.com

In brief

Singh et al. investigate the impact of oral supplementation with Urolithin A, a gut microbiome postbiotic known to activate mitophagy, in a randomized clinical trial in middle-aged adults. Results show that supplementation results in improvements in muscle strength and exercise-performance measures along with an impact on mitochondrial biomarkers.

Highlights

- Oral supplementation with Urolithin A increases muscle strength
- High dose of Urolithin A positively impacts exercise-performance measures
- An increase in mitophagy proteins in human skeletal muscle observed in parallel
- Supplementation is safe and increases circulating levels of Urolithin A

- *Urolithin A (UA) is a microbiome metabolite of elligitannins and polyphenolic compounds especially from pomegranates, berries and walnuts*
- *It was tested in a blinded random-controlled trial in a cohort of 88 middle-aged subjects (40-64 yrs) who were carefully screened to meet specific inclusion and exclusion criteria*
- *The trial lasted 4 months, and was divided into 3 groups: placebo, 500 mg daily UA intake, and 1,000 mg daily intake*
- *Subjects underwent extensive testing at baseline, after 2 months, and at the end of the study*
- *Subjects taking UA showed statistically significant improvement in physical performance parameters, e.g. peak VO2 and estimated VO2max. Total cycling distance increased, time to fatigue during exercise, with statistically significant increase in walking distance and gait speed in the 6-min walking test, especially in the higher intake group.*
- *Also UA induced improvements in various mitochondrial biomarkers, e.g. increased protein levels related to improved mitophagy, and expression of genes belonging to mitochondria.*
- *Intake also improved exercise performance: subjects showed statistically significant increase in quadriceps muscle strength and knee flexion*

Source: Singh A, D'Amico D, Andreux PA, Fouassier AM, Blanco-Bose W, Evans M, Aebischer P, Auwerx J, Rinsch C. Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults. Cell Rep Med. 2022 May 17;3(5):100633; https://www.nutrition-evidence.com/search?term=Urolithin+A+improves+muscle+strength&search=search

Can be detrimental to stimulate mitophagy if too few mitochondria/they are too weak; in anorexia, amenorrhoea …

Orthomolecular and mitochondrial medicine

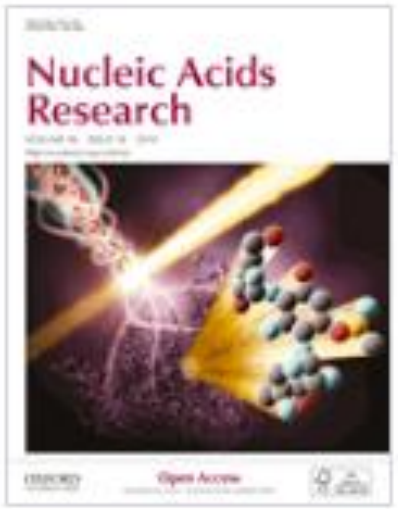
DNA

Test	Result	Reference range
ratio mtDNA:nDNA	2	

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Reference range ratio mtDNA:nDNA

0 - 100	100 - 150	150 - 200	200 - 250	250 - 300	300 - 500	>500



Volume 46, Issue 18

12 October 2018

"A study investigated the effect of ciprofloxacin on mitochondria, the important cell organelles in our body that produce the energy for cellular function. **Ciprofloxacin stopped normal maintenance and transcription of mitochondrial DNA by changing mtDNA topology**, causing impaired mitochondrial energy production and blocking cellular growth and differentiation." …. "While **fluoroquinolones are designed to inhibit the bacterial topoisomerase gyrase**, which leads to the death of the bacterium, **they also inhibit the topoisomerase 2 of our own cells**."[2]

JOURNAL ARTICLE

Ciprofloxacin impairs mitochondrial DNA replication initiation through inhibition of Topoisomerase 2

Anu Hangas, Koit Aasumets, Nina J Kekäläinen, Mika Paloheinä, Jaakko L Pohjo Joachim M Gerhold, Steffi Goffart

Nucleic Acids Research, Volume 46, Issue 18, 12 October 2018, Pages 9625–9636, https://doi.org/10.1093/nar/gky793

Published: 31 August 2018 **Article history**

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Abstract

Maintenance of topological homeostasis is vital for gene expression and genome replication in all organisms. Similar to other circular genomes, also mitochondrial DNA (mtDNA) is known to exist in various different topological forms, although their functional significance remains unknown. We report here that both known type II topoisomerases Top2α and Top2β are present in mammalian mitochondria, with especially Top2β regulating the supercoiling state of mtDNA. Loss of Top2β or its inhibition by ciprofloxacin results in accumulation of positively supercoiled mtDNA, followed by cessation of mitochondrial transcription and replication initiation, causing depletion of mtDNA copy number. These mitochondrial effects block both cell proliferation

Source: MMD testing laboratory, private correspondence with Professor Brigitte Koenig; 2. Hangas A, Aasumets K, Kekäläinen NJ, Paloheinä M, Pohjoismäki JL, Gerhold JM, Goffart S. Ciprofloxacin impairs mitochondrial DNA replication initiation through inhibition of Topoisomerase 2. Nucleic Acids Res. 2018 Oct 12;46(18):9625-9636.

Balance between the two very important

Liu *et al.*
Journal of Biomedical Science (2023) 30:86
https://doi.org/10.1186/s12929-023-00975-7

Journal of Biomedical Science

REVIEW Open Access

Crosstalk between mitochondrial biogenesis and mitophagy to maintain mitochondrial homeostasis

Lei Liu[1,2,3*], Yanjun Li[4], Guo Chen[4] and Quan Chen[4*]

Abstract

Mitochondrial mass and quality are tightly regulated by two essential and opposing mechanisms, mitochondrial biogenesis (mitobiogenesis) and mitophagy, in response to cellular energy needs and other cellular and environmental cues. Great strides have been made to uncover key regulators of these complex processes. Emerging evidence has shown that there exists a tight coordination between mitophagy and mitobiogenesis, and their defects may cause many human diseases. In this review, we will first summarize the recent advances made in the discovery of molecular regulations of mitobiogenesis and mitophagy and then focus on the mechanism and signaling pathways involved in the simultaneous regulation of mitobiogenesis and mitophagy in the response of tissue or cultured cells to energy needs, stress, or pathophysiological conditions. Further studies of the crosstalk of these two opposing processes at the molecular level will provide a better understanding of how the cell maintains optimal cellular fitness and function under physiological and pathophysiological conditions, which holds promise for fighting aging and aging-related diseases.

Keywords Mitochondrial biogenesis, Mitophagy, Mitophagy receptors, Mitochondrial quality, Aging, Aging-related diseases

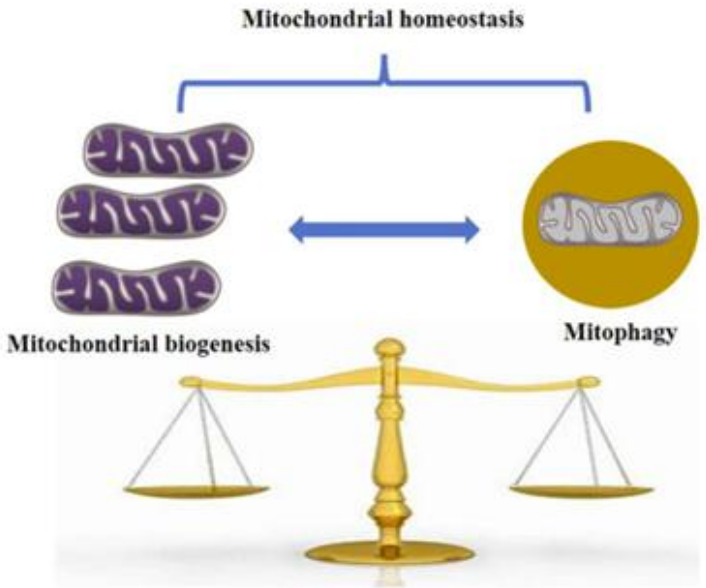


Fig. 1 Coordination between mitophagy and mitobiogenesis. Mitophagy and mitobiogenesis are two opposing processes that work together to maintain mitochondrial quality and quantity in response to various physiological and environmental signals

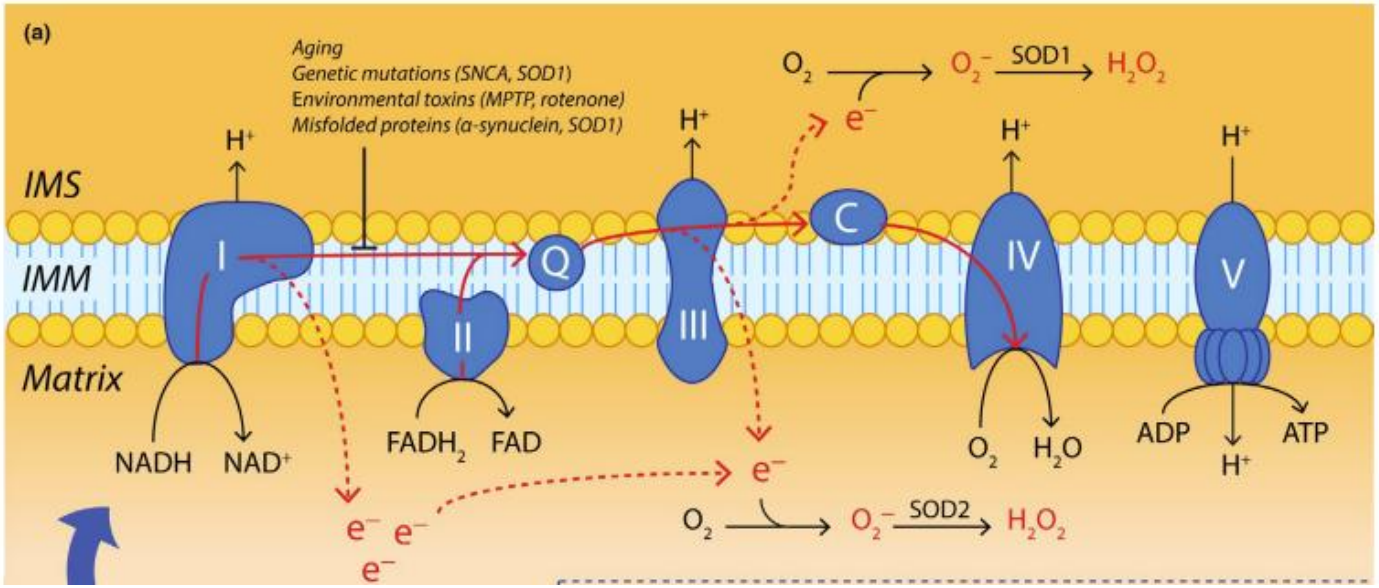
Source: Liu L, Li Y, Chen G, Chen Q. Crosstalk between mitochondrial biogenesis and mitophagy to maintain mitochondrial homeostasis. J Biomed Sci. 2023 Oct 12;30(1):86.

Agenda

Supplementary biomarkers:

Mitochondrial mass: Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1α, mitobiogenesis regulator
What to do to increase/decrease mitochondrial mass?
Mitochondrial stress test
Nrf-2, antioxidant regulator
Mitochondrial fuel markers

Mitochondria are the key source of oxidative stress in most cells



1

"Oxidative stress2023 occurs when reactive oxygen species (ROS) accumulates in mitochondria and cytosol. Superoxide anion (O2• –), hydrogen peroxide (H2O2), and hydroxyl radical (HO•) normally produced by ETC complex I and III are scavenged by endogenous antioxidants [70], such as superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and glutathione peroxidase (GPx)."[2]

"Mitochondria are the **most important source of ROS** in most mammalian cells"[3]

Source: 1. : Trist BG et al. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. Aging Cell. 2019 Dec;18(6):e13031.; 2. Wong KY et al. Relationships between Mitochondrial Dysfunction and Neurotransmission Failure in Alzheimer's Disease. Aging Dis. 2020 Oct 1;11(5):1291-1316; 3. Bhatti JS et al. Mitochondrial dysfunction and oxidative stress in metabolic disorders – A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis. 2017 May;1863(5):1066-1077.

Numerous diseases are associated with enhanced ROS generation in mitochondria and reduced antioxidant defence

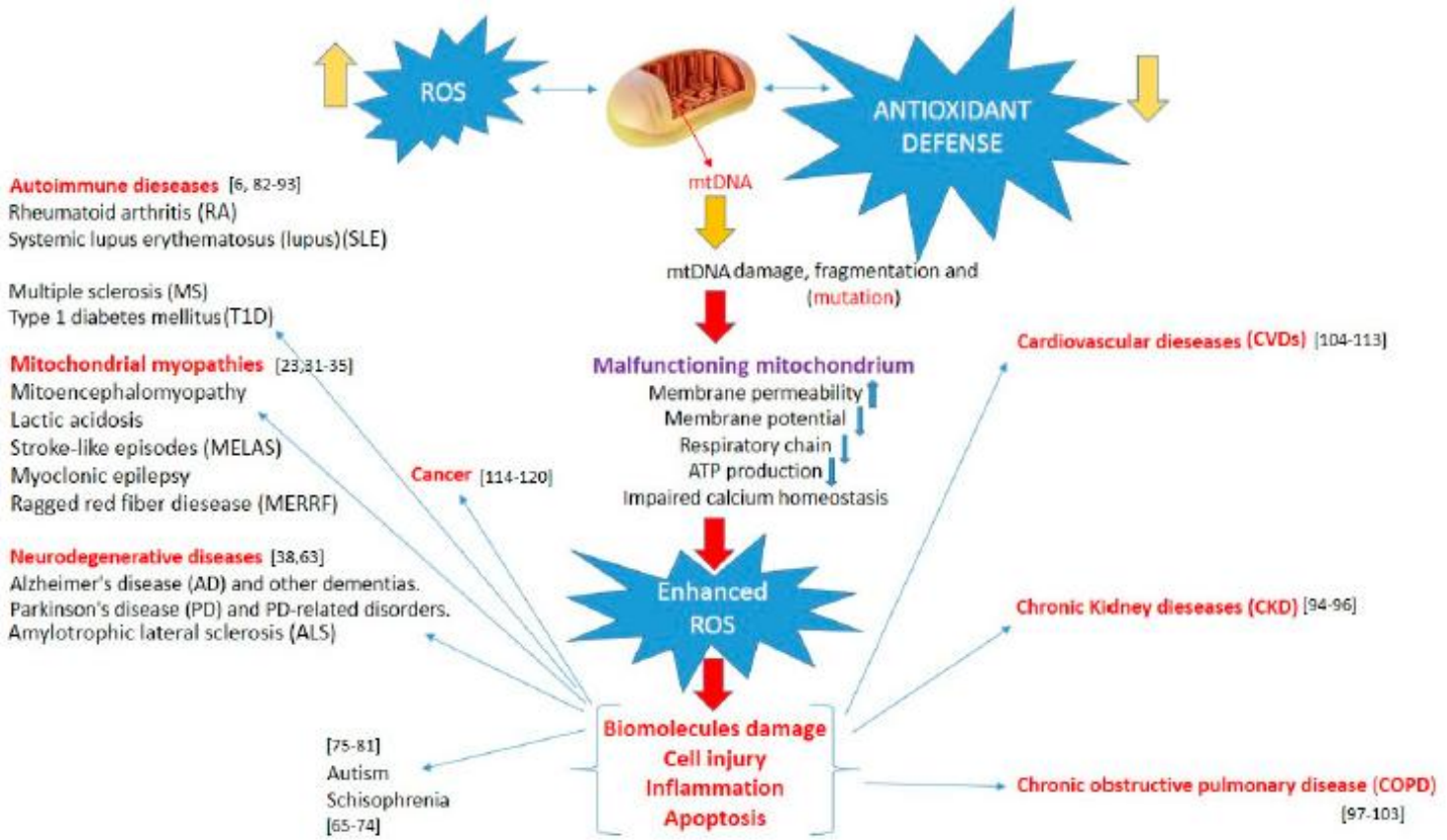
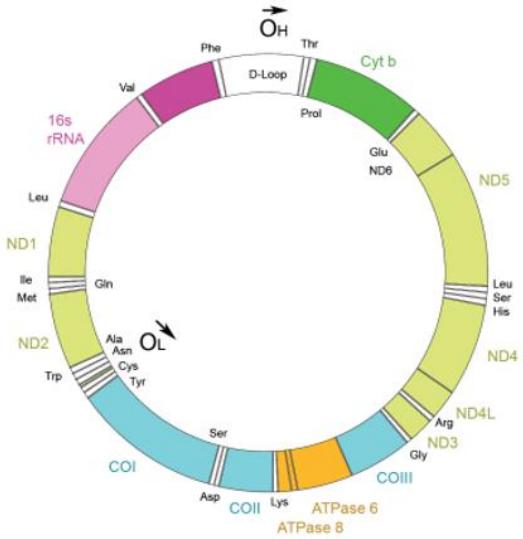


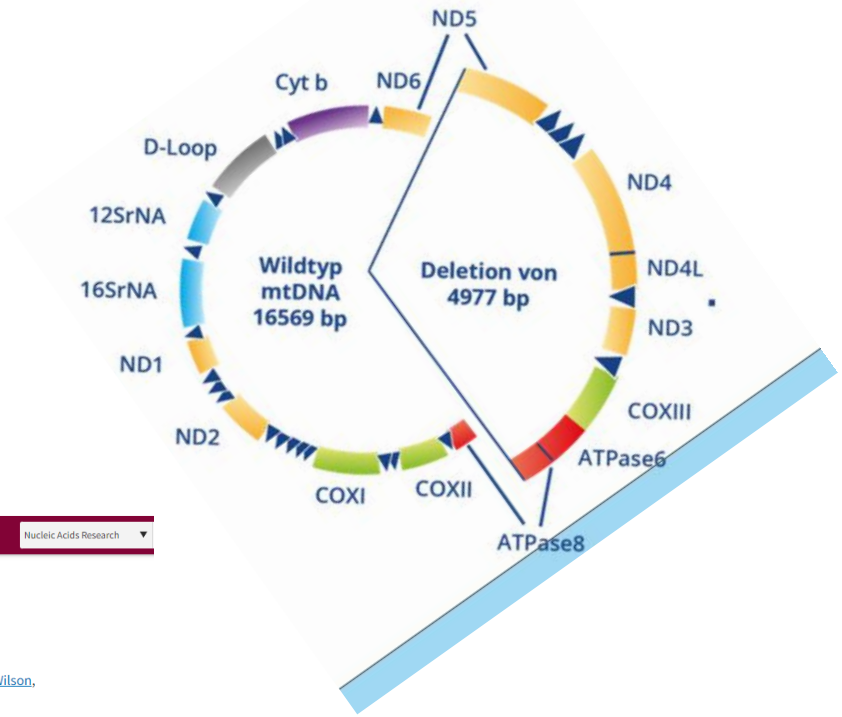
Figure 1. Increased reactive oxygen species, overwhelming antioxidant defenses, induce mtDNA damage, and mitochondrial dysfunction lead to enhanced oxidative stress. This, in turn, can induce biomolecule and cell damage, apoptosis, and inflammation, triggering various pathologies.

Source: Kowalczyk P, Sulejczak D, Kleczkowska P, Bukowska-Ośko I, Kucia M, Popiel M, Wietrak E, Kramkowski K, Wrzosek K, Kaczyńska K. Mitochondrial Oxidative Stress-A Causative Factor and Therapeutic Target in Many Diseases. Int J Mol Sci. 2021 Dec 13;22(24):13384.

The “common deletion” mDNA^{4977} is caused by oxidative stress

Oxidative stress





Nucleic Acids Research

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Volume 37, Issue 8
1 May 2009

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JOURNAL ARTICLE

Oxidative stress induces degradation of mitochondrial DNA

Inna Shokolenko, Natalia Venediktova, Alexandra Bochkareva, Glenn L. Wilson, Mikhail F. Alexeyev

Nucleic Acids Research, Volume 37, Issue 8, 1 May 2009, Pages 2539–2548,
https://doi.org/10.1093/nar/gkp100
Published: 05 March 2009 **Article history**

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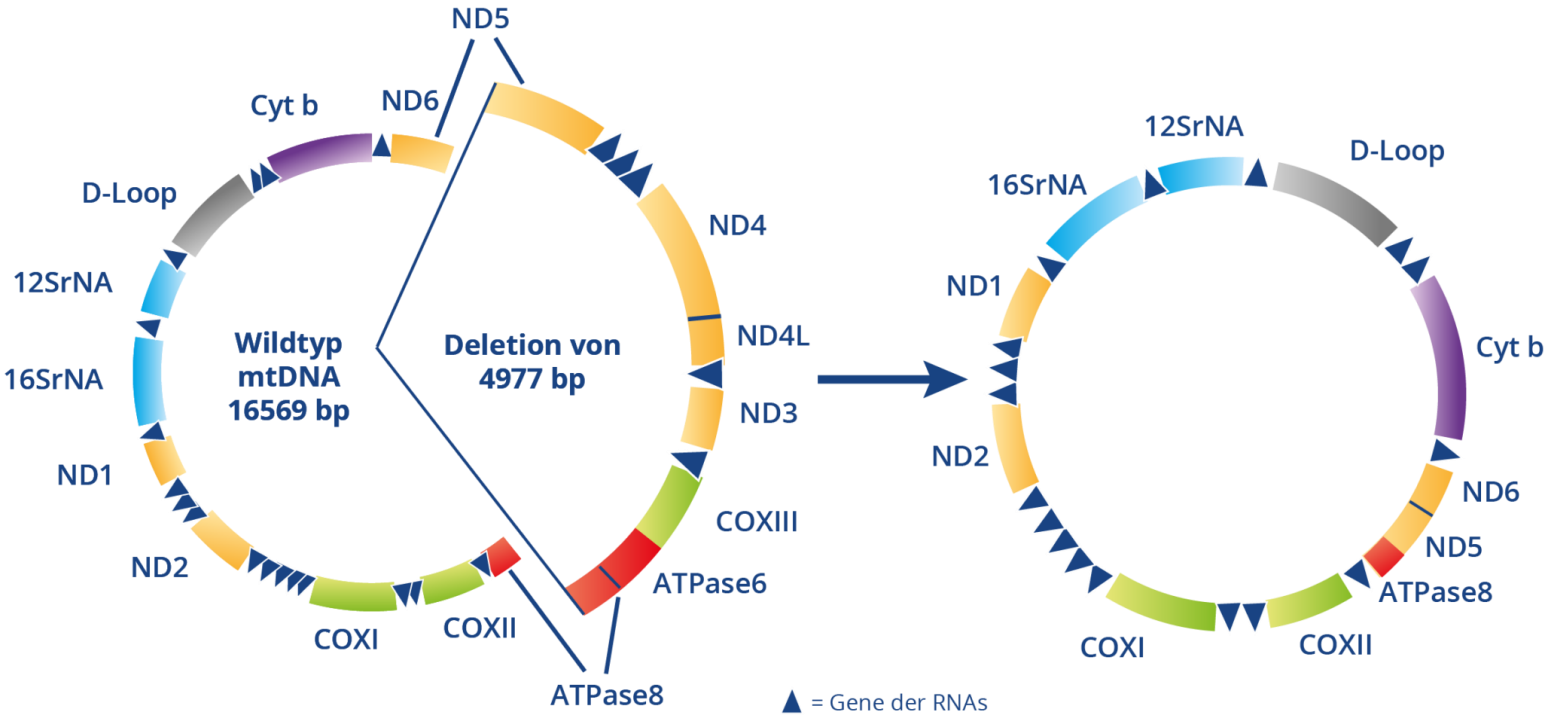
Abstract

Mitochondrial DNA (mtDNA) is located in close proximity of the respiratory chains, which are the main cellular source of reactive oxygen species (ROS). ROS can induce oxidative base lesions in mtDNA and are believed to be an important cause of the mtDNA mutations, which accumulate with aging and in diseased states. However, recent studies indicate that cumulative levels of base substitutions in mtDNA can be very low even in old individuals. Considering the reduced complement of DNA repair pathways available in mitochondria and higher susceptibility of mtDNA to oxidative damage than nDNA, it is presently

Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König

This can be measured, and shows the degree of oxidative stress the mitochondria are suffering …

Oxidative stress



Before deletion
Wildtype mtDNA = 16569 base pairs

After deletion
mtDNA = 11562 base pairs

Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König

… as well as any damage to mitochondrial DNA

Oxidative stress

Deletion mutant 4977

Example 1:

Mitochondrial 4977 Deletion mutant (mt4977del) **1.03E+06**

Number of copies of non-mutated mtDNA to 1 copy mt4977del

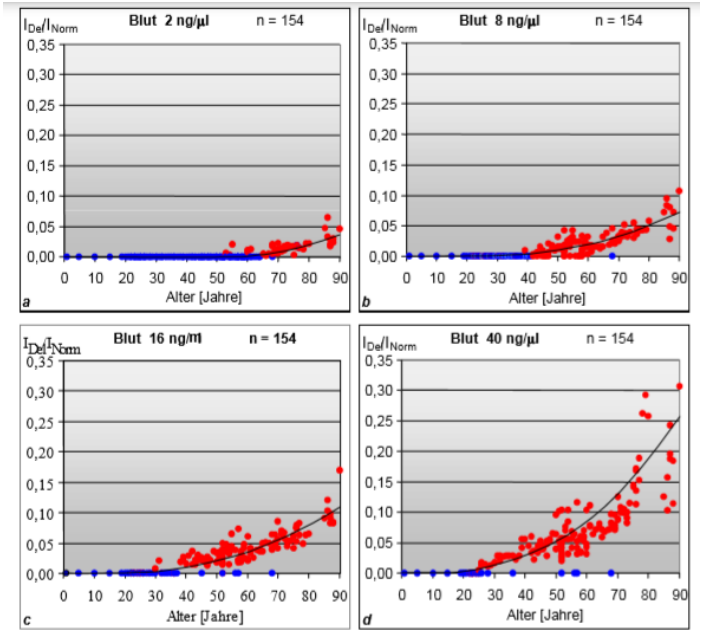
The mitochondrial deletion mutant mt4977bp is noticeably enhanced. This indicates oxidative stress and damage to mitochondrial DNA.

Among mtDNA deletions, one of the most vital that causes huge destruction of almost one third in length of the mitochondrial genome is the 4977-bp mtDNA deletion ($mDNA^{4977}$). This is one of the best-described large-scale mtDNA deletions, and has been found to accumulate in numerous disorders (literature available upon request). It is often known as a “common deletion” due to the frequency with which it has been reported. The deleted region encodes seven polypeptides essential for the OXPHOS pathway: four for Complex I, one for Complex IV, and two for Complex V. **This can cause complete failure of ATP production in the mitochondria affected.**

Action can be taken: it can be reversed …

Oxidative stress

Mitochondrial DNA – common deletion



DNA test	result	reference range
Mitochondrial 4977 Deletion mutant (mt4977del)	6.44E+05	

Number of copies of non-mutated mtDNA to 1 copy mt4977del

Reference range mt4977del			
<1E+06	1E+06 - 1E+07	>1E+07	>1E+08

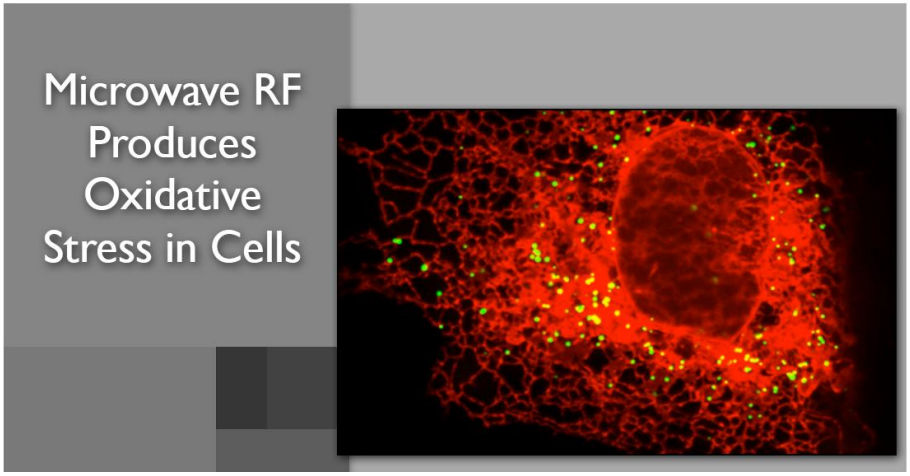
The mitochondrial deletion mutant mt4977bp **is detectable at a greatly increased level. This indicates mitochondria with a lack of ability to generate ATP and greatly increased oxidative stress.**

Significantly increased **Undetectable**

This is not an inherited polymorphism: it arises due to endogenous and exogenous factors, especially oxidative stress. This is why checking for it can be very useful, as measures can be taken to reduce the levels, and repeat tests document a decline in levels if the initiatives are successful.

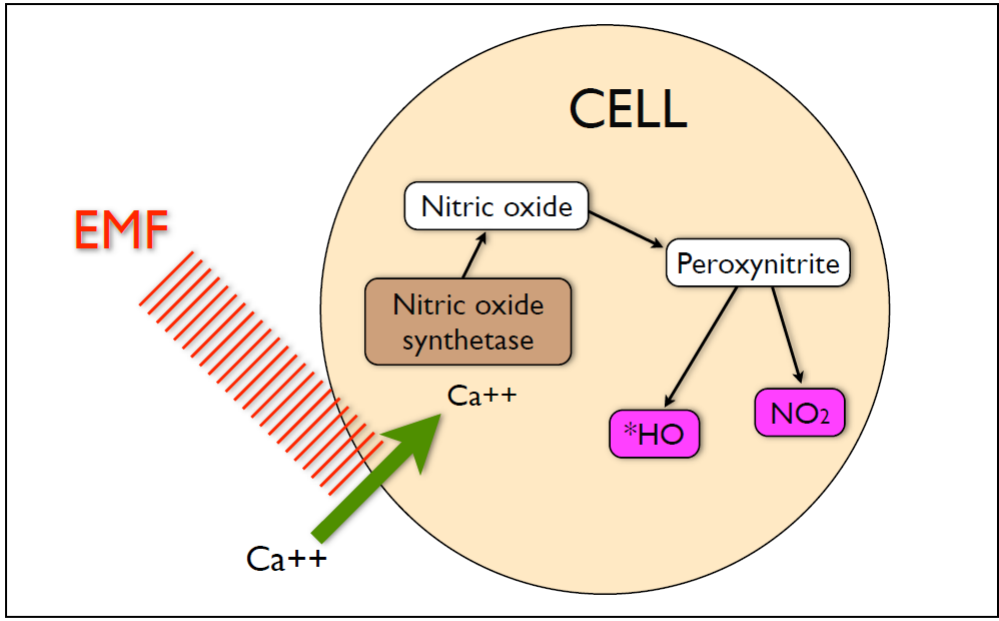
Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König

EMFs are a major cause of oxidative stress – they increase mitochondrial membrane depolarisation



"Kahya et al. (2014) exposed a breast cancer cell line derived from metastatic site (MDAMB-231) to 900 MHz modulated at 217 Hz for 1 h at an average calculated SAR of 0.36±0.02 W/kg. They found induction of apoptosis, including increased caspases 3 and 9 activities, and **increased mitochondrial membrane depolarization** in compared to control cells. **Moreover, oxidative stress was also induced (increased ROS formation)**. These levels decreased towards controls when treatment of 1h with sodium selenite (200 nM), a well-known antioxidant agent, was carried out before RF exposure."[2]

EMF Activation of VGCCs Increases Free Radical Production



Peroxynitrite produces free radicals, including hydroxyl radical and NO_2.

This increase in free radicals then leads to inflammation, oxidant stress, and damage to cell structures, including DNA.

Source: Pall ML. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. J Cell Mol Med. 2013 Aug;17(8):958-65; https://skyvisionsolutions.files.wordpress.com/2013/11/dart-presentation.pdf;2. https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_041.pdf; https://www.bsem.org.uk/articles/56-read-the-2020-nir-consensus-statement

Heavy metals can induce ROS production within the mitochondria

Open Access Review

Mechanisms of Metal-Induced Mitochondrial Dysfunction in Neurological Disorders

by Hong Cheng [1], Bobo Yang [2], Tao Ke [2], Shaojun Li [3], Xiaobo Yang [1,4], Michael Aschner [2,*] and Pan Chen [2,*]

"Metals can cause neurodegeneration by disrupting mitochondrial function, and thereby deplete ATP, **induce ROS production**, and ultimately lead to cell death through apoptotic and/or necrotic mechanisms"

biomolecules

MDPI

Review

The Role of Reactive Oxygen Species in Arsenic Toxicity

Yuxin Hu [1], Jin Li [2], Bin Lou [2], Ruirui Wu [2], Gang Wang [1], Chunwei Lu [1], Huihui Wang [2], Jingbo Pi [2] and Yuanyuan Xu [2,*]

1 Experimental Teaching Center, School of Public health, China Medical University, No.77 Puhe Road, Shenyang North New Area, Shenyang 110122, China; yxhu@cmu.edu.cn (Y.H.); gwang@cmu.edu.cn (G.W.); cwlu@cmu.edu.cn (C.L.)

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* Correspondence: yyxu@cmu.edu.cn or laurel1214@hotmail.com

"**Arsenic inhibits complex I in the mitochondrial electron transport chain, which leads to excessive generation of ROS**, giving rise to lipid peroxidation and protein damage and the subsequent formation of mitochondrial permeability transition (MPT)"

Iranian Journal of Pharmaceutical Research : IJPR

Brieflands

Repeated Administration of Mercury Intensifies Brain Damage in Multiple Sclerosis through Mitochondrial Dysfunction

Farzad Kahrizi, Ahmad Salimi, [...], and Jalal Pourahmad

"Our findings proved that repeated exposure with mercury accelerates progression of MS through **mitochondrial damage related to oxidative stress**"

SARS-CoV-2 creates havoc in the mitochondria via ROS production, too

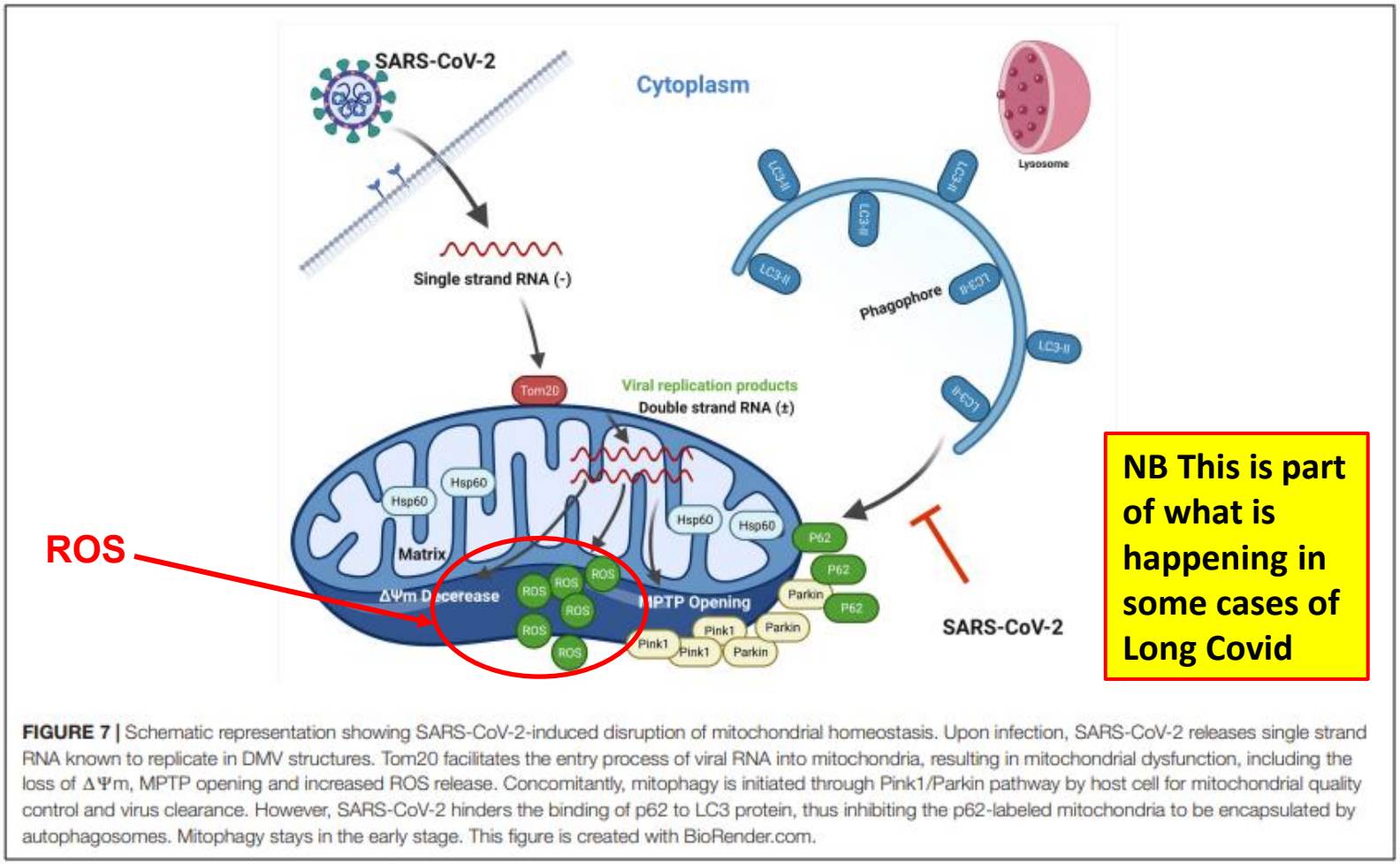


FIGURE 7 | Schematic representation showing SARS-CoV-2-induced disruption of mitochondrial homeostasis. Upon infection, SARS-CoV-2 releases single strand RNA known to replicate in DMV structures. Tom20 facilitates the entry process of viral RNA into mitochondria, resulting in mitochondrial dysfunction, including the loss of $\Delta\Psi m$, MPTP opening and increased ROS release. Concomitantly, mitophagy is initiated through Pink1/Parkin pathway by host cell for mitochondrial quality control and virus clearance. However, SARS-CoV-2 hinders the binding of p62 to LC3 protein, thus inhibiting the p62-labeled mitochondria to be encapsulated by autophagosomes. Mitophagy stays in the early stage. This figure is created with BioRender.com.

* Double-membrane vesicles

"We demonstrate that SARS-CoV-2 induces mitochondrial damage of mitochondrial membrane depolarization, mitochondrial permeability transition pore opening and increased ROS release."

Source: Shang C, Liu Z, Zhu Y, Lu J, Ge C, Zhang C, Li N, Jin N, Li Y, Tian M, Li X. SARS-CoV-2 Causes Mitochondrial Dysfunction and Mitophagy Impairment. Front Microbiol. 2022 Jan 6;12:780768.

Hyperglycaemia also a big driver of mitochondrial as well as overall cellular oxidative stress

REVIEW article
Front. Physiol., 17 January 2019
Sec. Redox Physiology
Volume 9 - 2018 |
https://doi.org/10.3389/fphys.2018.01857

This article is part of the Research Topic
Oxidative Stress Revisited - Major Role in Vascular Diseases
View all 13 Articles >

Therapeutic Options Targeting Oxidative Stress, Mitochondrial Dysfunction and Inflammation to Hinder the Progression of Vascular Complications of Diabetes

João S. Teodoro[1†], Sara Nunes[2†], Anabela P. Rolo[1], Flávio Reis[2*] and Carlos M. Palmeira[1*]

"Hyperglycemia induces oxidative stress, namely via mitochondrial dysfunction and enhanced reactive oxygen species (ROS) generation"

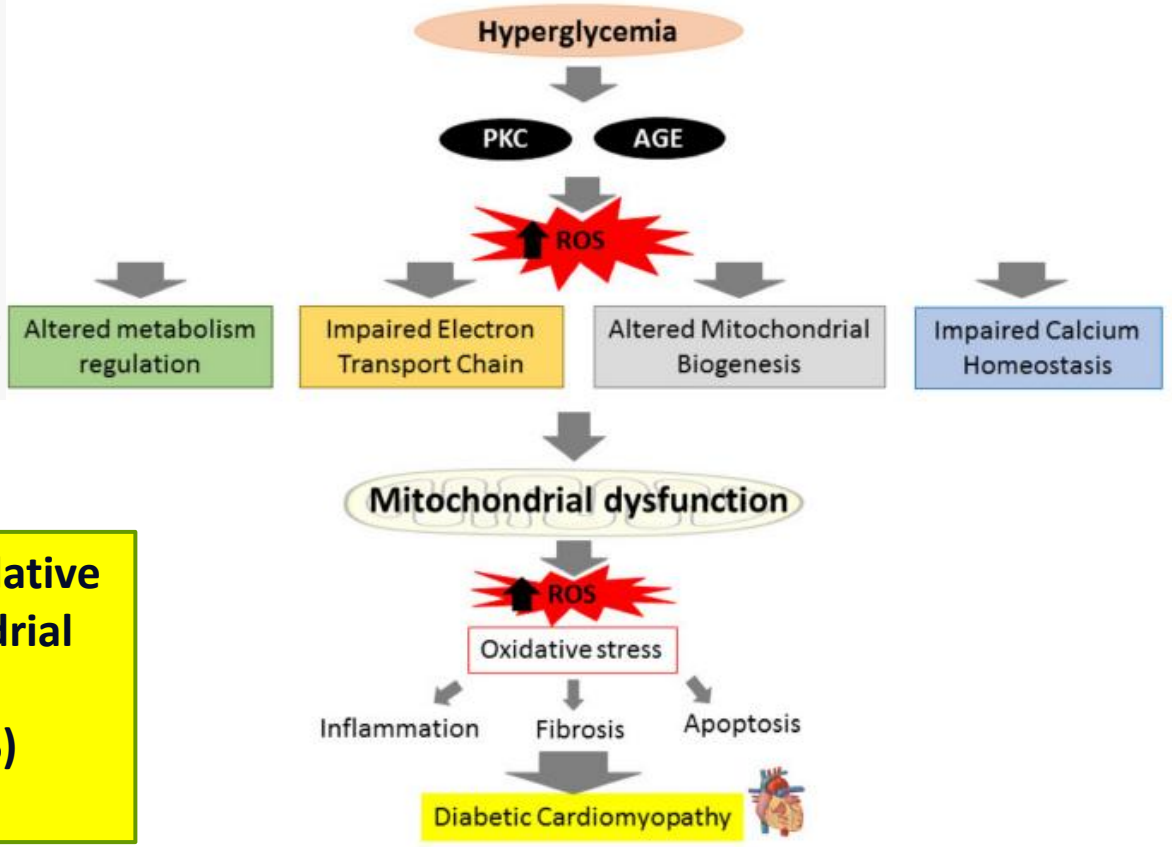


Figure 1. Prolonged hyperglycemia can produce reactive oxygen species (ROS) via activation of protein kinase C (PKC) pathways and advanced glycation end products (AGEs) production, leading to altered metabolism regulation, altered mitochondrial biogenesis, impaired mitochondrial calcium handling, and impaired electron transport chain. These actions will cause mitochondrial to deteriorate and generate more ROS. The ROS produced results in oxidative stress, which can initiate inflammation, fibrosis, and apoptosis, causing diabetic cardiomyopathy (DCM).

Source: Teodoro JS, Nunes S, Rolo AP, Reis F, Palmeira CM. Therapeutic Options Targeting Oxidative Stress, Mitochondrial Dysfunction and Inflammation to Hinder the Progression of Vascular Complications of Diabetes. Front Physiol. 2019 Jan 17;9:1857; Sapian S, Taib IS, Latip J, Katas H, Chin KY, Mohd Nor NA, Jubaidi FF, Budin SB. Therapeutic Approach of Flavonoid in Ameliorating Diabetic Cardiomyopathy by Targeting Mitochondrial-Induced Oxidative Stress. Int J Mol Sci. 2021 Oct 27;22(21):11616.

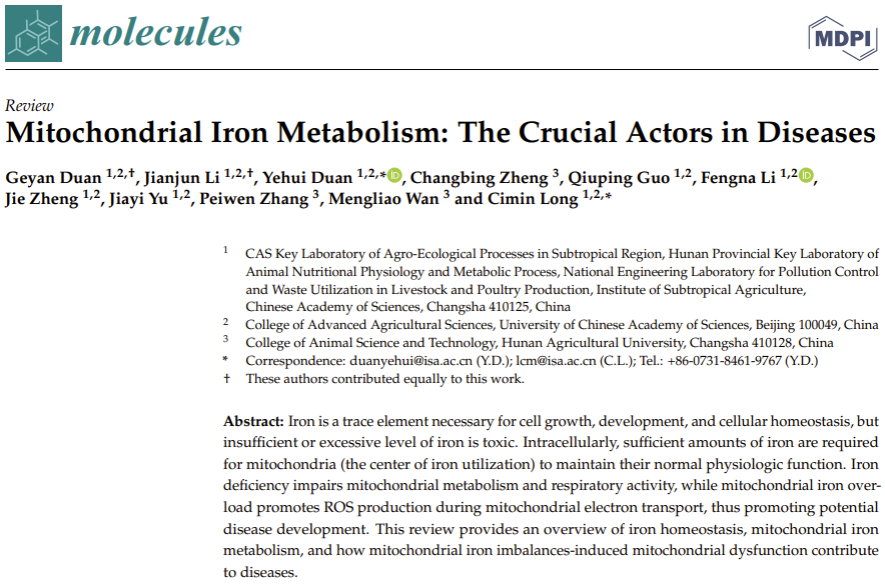
Mitochondrial iron overload/dysregulation promotes ROS production

Role of Iron-Related Oxidative Stress and Mitochondrial Dysfunction in Cardiovascular Diseases

Fang Yan, Kaifeng Li, [...], and Haifeng Zhang

"Mitochondrial Dysfunction Is Related to Iron-Induced Oxidative Stress.
As mitochondria have fundamental roles in creating ROS via oxygen metabolism to produce energy and iron utilization for iron-sulfur cluster assembly and heme synthesis, they tend to be vulnerable to damage from oxidative stress induced by iron."

""mitochondrial iron overload promotes ROS production during mitochondrial electron transport, thus promoting potential disease development"



molecules MDPI

Review

Mitochondrial Iron Metabolism: The Crucial Actors in Diseases

Geyan Duan [1,2,†], Jianjun Li [1,2,†], Yehui Duan [1,2,*], Changbing Zheng [3], Qiuping Guo [1,2], Fengna Li [1,2], Jie Zheng [1,2], Jiayi Yu [1,2], Peiwen Zhang [3], Mengliao Wan [3] and Cimin Long [1,2,*]

1 CAS Key Laboratory of Agro-Ecological Processes in Subtropical Region, Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
2 College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
3 College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China
* Correspondence: duanyehui@isa.ac.cn (Y.D.); lcm@isa.ac.cn (C.L.); Tel.: +86-0731-8461-9767 (Y.D.)
† These authors contributed equally to this work.

Abstract: Iron is a trace element necessary for cell growth, development, and cellular homeostasis, but insufficient or excessive level of iron is toxic. Intracellularly, sufficient amounts of iron are required for mitochondria (the center of iron utilization) to maintain their normal physiologic function. Iron deficiency impairs mitochondrial metabolism and respiratory activity, while mitochondrial iron overload promotes ROS production during mitochondrial electron transport, thus promoting potential disease development. This review provides an overview of iron homeostasis, mitochondrial iron metabolism, and how mitochondrial iron imbalances-induced mitochondrial dysfunction contribute to diseases.

Source: Yan F, Li K, Xing W, Dong M, Yi M, Zhang H. Role of Iron-Related Oxidative Stress and Mitochondrial Dysfunction in Cardiovascular Diseases. Oxid Med Cell Longev. 2022 Sep 7;2022:5124553; www.rootcauseprotocol.com

KPU also worth considering: this glitch in the production of heme is associated with oxidative stress, too

International Journal of *Molecular Sciences*

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Perspective

Pyrroles as a Potential Biomarker for Oxidative Stress Disorders

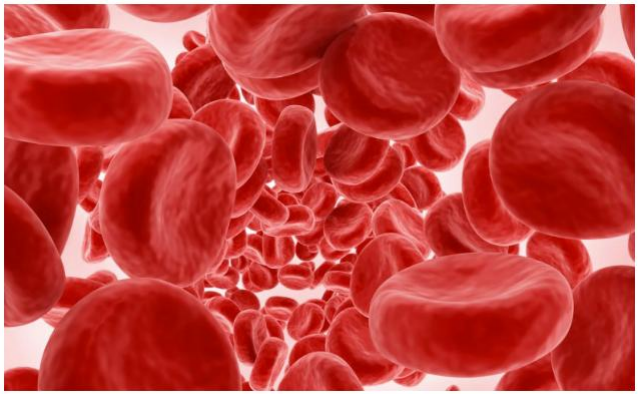
Brett Lambert [1], Annalese Semmler [2], Cristina Beer [3] and Joanne Voisey [3,*]

[1] Applied Analytical Laboratories, Logandowns Dr, Meadowbrook, QLD 4131, Australia
[2] School of Clinical Sciences, Faculty of Health, Queensland University of Technology, Kelvin Grove, QLD 4059, Australia
[3] Centre for Genomics and Personalised Health, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Kelvin Grove, QLD 4059, Australia
* Correspondence: j.voisey@qut.edu.au

Abstract: Redox imbalance or oxidative stress that results from both environmental and genetic factors is observed in patients with schizophrenia. Therefore, identifying markers of oxidative stress in the early stages of psychosis and using antioxidant treatments as an adjuvant to antipsychotics has important implications. The reaction of *p*-*N*,*N*-dimethylaminobenzaldehyde (DMAB) with pyrrole moieties has been well studied for well over a century for use as a marker of oxidative stress dysregulation. Throughout this time, pyrroles have been investigated with varying veracity in urine extracts to identify elevated levels in patients diagnosed with schizophrenia. Since the 1960's, various claims have been made with respect to what causes the colour change when DMAB is added to urine extracts. Whilst the substances from this reaction have not been fully elucidated, an objective look at most studies indicates that urobilinogen is likely to be one them. Urobilinogen has also been identified as a major interferent in our results. Both pyrroles and urobilinogen condense the DMAB reaction system (form condensation products) and are quite different. The urobilinogen detected in urine forms when gut microflora chemically reduces the bilirubin content of bile acids. In comparison, evidence suggests that the pyrrole fraction originates from the fragmentation of regulatory haem by reactive oxygen species (ROS) such as hydrogen peroxide and super and nitrous oxides. Clinical studies in our laboratories have established that pyrroles as a urine biomarker have specificity in detecting schizophrenia; however, caution must be applied as the readings are subject to interference by other DMAB active compounds that are present, such as urobilinogen. This review highlights the initial chemistry in isolating pyrroles and provides recommendations for standardised laboratory testing to ensure pyrroles are correctly measured and distinguished from other by-products.

check for updates

Citation: Lambert, B.; Semmler, A.; Beer, C.; Voisey, J. Pyrroles as a Potential Biomarker for Oxidative



Kryptopyrroluria (KPU) is a metabolic disorder characterised by a disorder in the breakdown of heme that leads to the excessive excretion of pyrroles in the urine. Pyrroles are a group of chemical compounds derived from the breakdown of heme, a component of haemoglobin. As a result, important nutrients such as vitamin B6 (pyridoxine), zinc and manganese become bound to the pyrroles and are lost through the urine. This can lead to deficiencies of these essential nutrients and disrupt various metabolic processes in the body.

https://aonm.org/kryptopyrroluria-the-elephant-in-the-room/

Source: Lambert B, Semmler A, Beer C, Voisey J. Pyrroles as a Potential Biomarker for Oxidative Stress Disorders. Int J Mol Sci. 2023 Feb 1;24(3):2712.

Fluoroquinolones can cause huge mitochondrial damage/oxidative stress: Ciprofloxacin and others

sports

MDPI

Review

Antibiotic Therapy and Athletes: Is the Mitochondrial Dysfunction the Real Achilles' Heel?

Valentina Puccini

Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy; valentinapuccini20@gmail.com

Original

The mitochondria targeted antioxidant MitoQ protects against fluoroquinolone-induced oxidative stress and mitochondrial membrane damage in human Achilles tendon cells

Damon A. Lowes, Carol Wallace, Michael P. Murphy, Nigel R. Webster & **Helen F. Galley**

Pages 323-328 | Received 30 Sep 2008, Published online: 09 Sep 2009

Download citation https://doi.org/10.1080/10715760902736275

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Abstract

Tendinitis and tendon rupture during treatment with fluoroquinolone antibiotics is thought to be mediated via oxidative stress. This study investigated whether ciprofloxacin and moxifloxacin cause oxidative stress and mitochondrial damage in cultured normal human Achilles' tendon cells and whether an antioxidant targeted to mitochondria (MitoQ) would protect against such damage better than a non-mitochondria targeted antioxidant. Human tendon cells from normal Achilles' tendons were exposed to 0–0.3 mM antibiotic for 24 h and 7 days in the presence of 1 μM MitoQ or an untargeted form, idebenone. Both moxifloxacin and ciprofloxacin resulted in up to a 3-fold increase in the rate of oxidation of dichlorodihydrofluorescein, a marker of general oxidative stress in tenocytes ($p<0.0001$) and loss of mitochondrial membrane permeability ($p<0.001$). In cells treated with MitoQ the oxidative stress was less and mitochondrial membrane potential was maintained. Mitochondrial damage to tenocytes during fluoroquinolone treatment may be involved in tendinitis and tendon rupture.

"This study confirmed that both ciprofloxacin and moxifloxacin caused oxidative stress and damage to the mitochondrial membrane contributing to the development of tendonitis and tendon rupture in some patients."

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

11 March 2019
EMA/175398/2019

Disabling and potentially permanent side effects lead to suspension or restrictions of quinolone and fluoroquinolone antibiotics

On 15 November 2018, EMA finalised a review of serious, disabling and potentially permanent side effects with quinolone and fluoroquinolone antibiotics given by mouth, injection or inhalation. The

Source: https://www.ema.europa.eu/en/documents/referral/quinolone-fluoroquinolone-article-31-referral-disabling-potentially-permanent-side-effects-lead_en.pdf

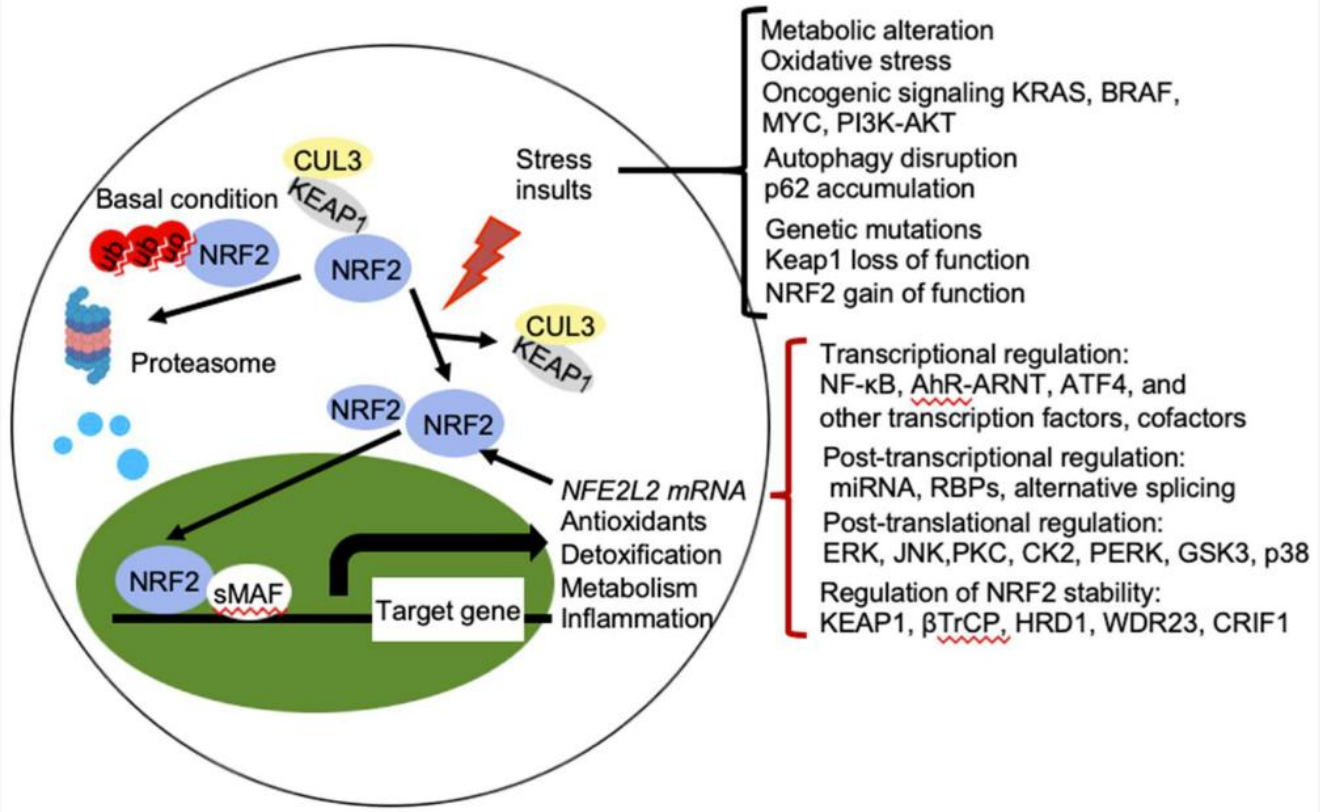
Agenda

Supplementary biomarkers:

Mitochondrial mass: Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1α, mitobiogenesis regulator
What to do to increase/decrease mitochondrial mass?
Mitochondrial stress test
Nrf-2, antioxidant regulator
How to quell excess oxidative stress?
Mitochondrial fuel utilisation

One initiative is to check Nrf-2: our cells' master antioxidant regulator

Nrf-2



"Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) is a critical transcription factor that regulates the expression of over 1000 genes in the cell under normal and stressed conditions. **Nrf2 has been historically considered as a crucial regulator of antioxidant defense to protect against various insult-induced organ damage"**

Source: Zang H, Mathew RO, Cui T. The Dark Side of Nrf2 in the Heart. Front Physiol. 2020 Jul 9;11:722.

Problem if it is undetectable and you have evident oxidative stress

Nrf-2

RESULTS

Sample type: Blood in CPDA vials

Requisition:
RNA

Summary

RNA profile

Test	Unit	Result
Nrf-2	Relative expression (to GAPDH)	Not detectable

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

Interpretation: "Basic values of the peripheral blood leucocytes"

Nrf-2 expression is not detectable, indicating extremely low/absent defence against reactive oxygen metabolites in the cell.

Nrf-2

NRF-2, nuclear factor erythroid 2-related factor 2, is the master regulator of our antioxidant system to protect cells from reactive oxygen species. Nrf-2 activates Phase II detoxification – particularly glutathione-S-transferase and other antioxidant enzymes, including SOD-2, catalase and glutathione peroxidase. It is crucial to have adequate levels of this in the mitochondria.

Important to compare with the MHI – is there oxidative stress both in the cell and in the mitochondria?

Nrf-2 vs. oxidative stress

Sample taken	16.08.2022
Receipt of sample	18.08.2022
Test completed	18.08.2022
Final result	**18.08.2022**
Validated by	Prof. Dr. Brigitte König
Medical Director	Prof. Dr. Gerhard Jorch

Interpretation

	None	Slight	Moderate	Considerable	Extreme
Mitochondrial dysfunction				√	
Cellular imbalance			√		

Indications of

Increased formation of oxygen radicals in the cell		No √ Yes	Insufficient ATP formation on energy demand		No √ Yes
Increased formation of oxygen radicals in the mitochondria		No √ Yes	Limited glucose utilisation		No Yes
Restricted function of the electron transport chain in the mitochondria		No √ Yes	Limited fatty acid oxidation		No Yes
Limited number of intact mitochondria		No √ Yes			

If the Nrf-2 level is low or undetectable and the 4977 deletion mutant is elevated, it is vital to initiate action to support:

Endogenous antioxidants (Nrf-2 activation) and

Exogenous antioxidants

[Gilian will give an overview of those a bit later]

Agenda

Supplementary biomarkers:

Mitochondrial mass: Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1α, mitobiogenesis regulator
What to do to increase/decrease mitochondrial mass?
Mitochondrial stress test
Nrf-2, antioxidant regulator
How to quell excess oxidative stress?
Mitochondrial fuel utilisation

Many inducers of Nrf2

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Review

Nrf2, the Master Regulator of Anti-Oxidative Responses

Sandra Vomund [1,†], Anne Schäfer [2,†], Michael J. Parnham [1], Bernhard Brüne [1,2] and Andreas von Knethen [1,2,*]

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† These authors contributed equally to this work.

Received: 17 November 2017; Accepted: 16 December 2017; Published: 20 December 2017

Abstract: Tight regulation of inflammation is very important to guarantee a balanced immune response without developing chronic inflammation. One of the major mediators of the resolution of inflammation is the transcription factor: the nuclear factor erythroid 2-like 2 (Nrf2). Stabilized following oxidative stress, Nrf2 induces the expression of antioxidants as well as cytoprotective genes, which provoke an anti-inflammatory expression profile, and is crucial for the initiation of healing. In view of this fundamental modulatory role, it is clear that both hyper- or hypoactivation of Nrf2 contribute to the onset of chronic diseases. Understanding the tight regulation of Nrf2 expression/activation and its interaction with signaling pathways, known to affect inflammatory processes, will facilitate development of therapeutic approaches to prevent Nrf2 dysregulation and ameliorate chronic inflammatory diseases. We discuss in this review the principle mechanisms of Nrf2 regulation with a focus on inflammation and autophagy, extending the role of dysregulated Nrf2 to chronic diseases and tumor development.

Nrf2 inducers:

Oleanic acid (from olive oil)
Sulphurophane, brassica-derived glucosinolates
Alpha lipoic acid
Carnosol (found in rosemary and sage – Salvia carnosa)
Quercetin
Capsaicin
Curcumin
Reishi mushroom
Sodium butyrate
Auranofin (organogold compound/a gold salt)
EPA & especially DHA
Bifidobacterium, *Lactobacillus*, and *Bacteroides*

Nrf2 can in turn induce glutathione [2]

Source: Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A. Nrf2, the Master Regulator of Anti-Oxidative Responses. Int J Mol Sci. 2017 Dec 20;18(12):2772; 2. Staurengo-Ferrari L, Badaro-Garcia S, Hohmann MSN, Manchope MF, Zaninelli TH, Casagrande R, Verri WA Jr. Contribution of Nrf2 Modulation to the Mechanism of Action of Analgesic and Anti-inflammatory Drugs in Pre-clinical and Clinical Stages. Front Pharmacol. 2019 Jan 11;9:1536.; Houghton CA, Fassett RG, Coombes JS. Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality? Oxid Med Cell Longev. 2016;2016:7857186.

Sulphurophane and oleanolic acid particularly valuable as Nrf2 inducers

Review Article

Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality?

Christine A. Houghton, Robert G. Fassett, and Jeff S. Coombes

School of Human Movement and Nutrition Science, The University of Queensland, Brisbane, Australia

Correspondence should be addressed to Jeff S. Coombes; jcoombes@uq.edu.au

Received 13 October 2015; Accepted 6 December 2015

Academic Editor: Ahmed Abdel Moneim

The recognition that food-derived nonnutrient molecules can modulate gene expression to influence intracellular molecular mechanisms has seen the emergence of the fields of nutrigenomics and nutrigenetics. The aim of this review is to describe the properties of nutrigenomic activators of transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), comparing the potential for sulforaphane and other phytochemicals to demonstrate clinical efficacy as complementary medicines. Broccoli-derived sulforaphane emerges as a phytochemical with this capability, with oral doses capable of favourably modifying genes associated with chemoprevention. Compared with widely used phytochemical-based supplements like curcumin, silymarin, and resveratrol, sulforaphane more potently activates Nrf2 to induce the expression of a battery of cytoprotective genes. By virtue of its lipophilic nature and low molecular weight, sulforaphane displays significantly higher bioavailability than the polyphenol-based dietary

"Both its high bioavailability and significant Nrf2 inducer capacity contribute to the therapeutic potential of sulforaphane-yielding supplements."

Biochemical pharmacology

Author Manuscript HHS Public Access

Oleanolic Acid Activates Nrf2 and Protects from Acetaminophen Hepatotoxicity via Nrf2-Dependent and Nrf2-independent Processes

Scott A. Reisman, Lauren M. Aleksunes, and Curtis D. Klaassen

"Collectively, increased nuclear accumulation of Nrf2, increased mRNA expression of Nrf2- target genes, and upregulation of Nqo1 protein and activity provide strong evidence that oleanolic acid activates the Nrf2-Keap1 pathway. … Oleanolic acid also activates Nrf2-independent cytoprotective mechanisms. Thus, this study establishes oleanolic acid as an Nrf2 activator"

Source: Houghton CA, Fassett RG, Coombes JS. Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality? Oxid Med Cell Longev. 2016;2016:7857186; Reisman SA, Aleksunes LM, Klaassen CD. Oleanolic acid activates Nrf2 and protects from acetaminophen hepatotoxicity via Nrf2-dependent and Nrf2-independent processes. Biochem Pharmacol. 2009 Apr 1;77(7):1273-82.

Many natural Nrf2 activators in juices and other beverages too: broccoli, pomegranate, beetroot, blueberry, noni ...

Table 2. List of phytochemicals present in fruit juices as Nrf2 activators.

Sources	Bioactive Natural Compound/s	Study Observations	Therapeutic Indications	Reference
Apple juice	Chlorogenic acid, 4-coumaroylquinic acid, epicatechin, procyanidin B2	In distal colon of mice GPX2, GSR, CAT and in liver GPX1 and NOQ-1 mRNA were significantly up-regulated.	Protection against ROS associated toxicity	[62]
Beetroot juices	Betanin	Activation and translocation of Nrf2 and significantly increased the expression of GSTP, GSTM, GSTT and NQO-1.	Cytoprotective, Anticarcinogenic Hepatoprotective	[69]
Blueberry juice	Chlorogenic acid, vanillic acid, syringic acid, trans-ferulic acid, = protocatechuic acid, *p*-coumaric acid	Activated Nrf2 and up-regulation of HO-1 and glutamate-cysteine ligase modifier subunit (GCLM)	Beneficial to endothelial cell activity and vascular function	[61]
Broccoli sprout crude juice	N/A	Protected against β-amyloid peptide –induced cytotoxicity and apoptosis; up-regulated the intracellular glutathione content and mRNA levels or activity of HO-1, thioredoxin, thioredoxin reductase (TrxR), and NAD(P)H:quinone (NQO-1)	Neuroprotective	[66]
Cabbage and sauerkraut juices	N/A	Sauerkraut juice significantly increased the activity of GST and NQO-1; translocation of Nrf2 and up-regulation of GST and NQO-1 by both juices.	Chemo-preventive	[68]
Fruit juice of *Actinidia chinensis*	N/A	Increased Keap1 and Nrf2 activity; up-regulation of SOD and GSH and down-regulation of ALT and AST diabetic patients	Antidiabetic Anti-inflammatory	[70]
Garlic juice	N/A	Reduces the ROS in presence of toxic heavy metal Cd; significantly induce the SOD and CAT activity; Nrf2 and NQO-1 expression was significantly increased; HO-1 expression not significant.	Prevents heavy metal (Cd) induced liver damages	[67]
Noni fruit juice	N/A	Aqueous and chloroform fractions protect cells from *tert*-Butyl hydroperoxide (TBHP)-induced cell damage; significantly decreases the TBHP cytotoxicity, ROS generation, mitochondrial membrane depolarization and apoptotic; nuclear accumulation of Nrf2 and HO-1, CAT, SOD-1.	Neuroprotective	[64]
	4-Methyl catechol, 4-Ethyl catechol, 4-Vinyl catechol, Scopoletin	Increased Nrf2 nuclear translocation, expression of HO-1, NQO-1 and glucose 6-phosphate dehydrogenase activity	Diabetic wound healing Cytotoxicity	[47,65]
Orange juice	Lycopene, phytoene, all-E-β-carotene, and other carotenoids	Up-regulated the expression of GCS-1. GST-4, SOD-4, HSP-16.2 genes; significantly increased the ROS reductions, gene expression activation, oxidative stress resistance; induces GST-4::GFP expressions and increased SKN-1/Nrf2 transcription factor.	Neuroprotective and Suppress oxidative stress	[56]
Pomegranate juice	Anthocyanin and hydrolysable tannins	Decreased in SOD, GST, CAT and membrane-ATPases; significant increase in Nrf2 and NF-κB expression in nitrosodiethylamine (NDEA)-induced fibrotic rats.	Liver fibrosis Hepatoprotective	[58]
Pomegranate juice	Punicalagin	Increased Nrf2 translocation and up-regulation of HO-1; decreased the generation of RONS, NO; increased the production of SOD activity	Intestinal injuries	[59]

N/A: Not available; Cd: Cadmium.

Source: Xu, J.; Yang, Y.; Liu, Y.; Cao, S. Natural Nrf2 Activators from Juices, Wines, Coffee, and Cocoa. Beverages 2020, 6, 68.

Exogenous mitochondrial antioxidants for which there is good evidence

Alpha lipoic acid
Acetyl-L-carnitine
Ginseng (especially Korean)
CoQ10
Resveratrol
Vitamin C
Melatonin
Apolactoferrin

Foods containing glutathione
Hydrogen-rich water

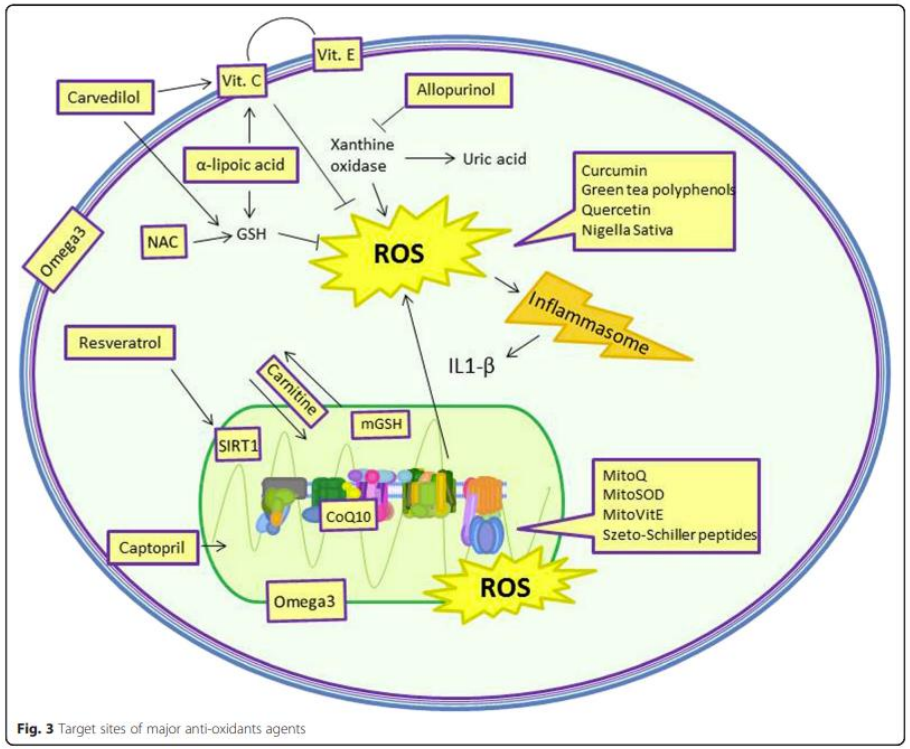


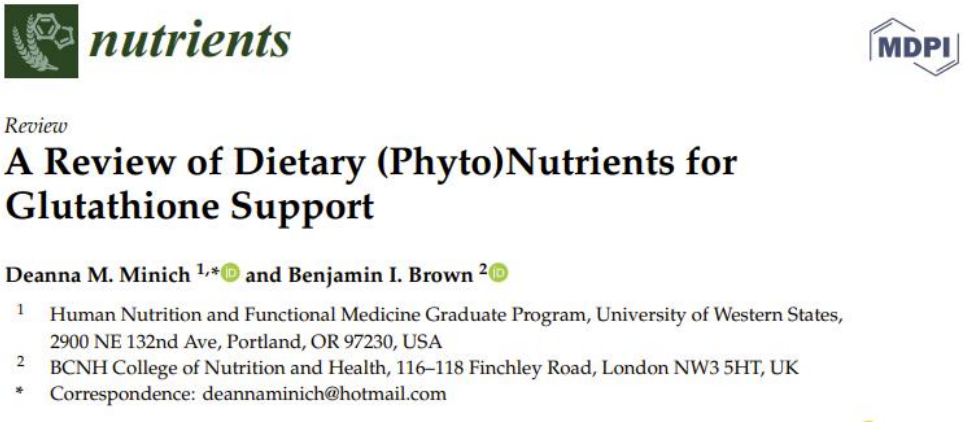
Fig. 3 Target sites of major anti-oxidants agents

Sources available upon request; image; Granata S, Dalla Gassa A, Tomei P, Lupo A, Zaza G. Mitochondria: a new therapeutic target in chronic kidney disease. Nutr ;etab (Lond). 2015 Nov 25;12:49.

Glutathione our key intracellular antioxidant – crucial for the mitochondria

By evaluating levels of GSH and GSSG, as well as the GSH/GSSG ratio in blood, one can get a glimpse into the degree of mitochondrial dysfunction at a tissue level. An increased GSSG:GSH ratio is an indication of oxidative stress.

Glutathione, free (GSH)		185 mg/l	150 - 460	[*.....]
Glutathione, total		380 mg/l		
Glutathione, oxidized	**+**	**195 mg/l**	**15 - 90**	**[..... *>**



nutrients

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Review

A Review of Dietary (Phyto)Nutrients for Glutathione Support

Deanna M. Minich [1,*] and Benjamin I. Brown [2]

1 Human Nutrition and Functional Medicine Graduate Program, University of Western States, 2900 NE 132nd Ave, Portland, OR 97230, USA
2 BCNH College of Nutrition and Health, 116–118 Finchley Road, London NW3 5HT, UK
* Correspondence: deannaminich@hotmail.com

"… recent research suggests that when glutathione is administered in liposomal or sublingual forms it may be made more bioavailable and favorably impact systemic glutathione levels.[1]"

Source: 1. Minich DM, Brown BI. A Review of Dietary (Phyto)Nutrients for Glutathione Support. Nutrients. 2019 Sep 3;11(9):2073; Enns GM, Cowan TM. Glutathione as a Redox Biomarker in Mitochondrial Disease-Implications for Therapy. J Clin Med. 2017 May 3;6(5):50.

Dragons Blood (Sangre de Grado) a little known remedy with a very high ORAC* value

molecules MDPI

Molecules. 2018 Oct 15;23(10):2641. doi: 10.3390/molecules23102641

Dragon's Blood Sap: Storage Stability and Antioxidant Activity

Juan D Escobar [1], Cristina Prieto [2,*], Maria Pardo-Figuerez [1], José M Lagaron [2,*]

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https://doi.org/10.1007/s12210-022-01122-4

REVIEW

Dragon's Blood: antioxidant properties for nutraceuticals and pharmaceuticals

Isabella S. A. Peres[1] · Kiara A. O. Conceição[1] · Larissa A. F. Silva[1] · Nadia G. Khouri[2] · Cristiana M. P. Yoshida[1] · Viktor O. C. Concha[1] · Massimo Lucarini[3] · Alessandra Durazzo[3] · Antonello Santini[4] · Eliana B. Souto[5,6] · Patricia Severino[7]

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ELSEVIER

Journal of Ethnopharmacology

Volume 115, Issue 3, 12 February 2008, Pages 361-380

Review

Dragon's blood: Botany, chemistry and therapeutic uses

Deepika Gupta [a], Bruce Bleakley [b], Rajinder K. Gupta [a]

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https://doi.org/10.1016/j.jep.2007.10.018 Get rights and content

Abstract

Dragon's blood is one of the renowned traditional medicines used in different cultures of world. It has got several therapeutic uses: haemostatic, antidiarrhetic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory, antioxidant, etc. Besides these medicinal applications, it is used as a coloring material, varnish and also has got applications in folk magic. These red saps and resins are derived from a number of disparate taxa. Despite its wide uses, little research has been done to know about its true source, quality control and clinical applications. In this review, we have tried to overview different sources of Dragon's blood, its source wise chemical constituents and therapeutic uses. As well as, a little attempt has been done to review the techniques used for its quality control and safety.

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Review article https://doi.org/10.12980/jclm.5.2017J7-75

Meta-analysis of dragon's blood resin extract as radio-protective agent

Subaika Mahmood[1], Toseef Fatima[1], Hina Zulfaqar[1], Romana Saher[1], Muhammad Rafiq[1], Abdul Rehman[1], Rongji Dai[2*], Muhammad Ashfaq[1], Murtaza Hasan[1*]

[1]*Department of Biochemistry & Biotechnology (Baghdad-ul-Jadeed Campus), the Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*

[2]*School of life Sciences, Beijing Institute of Technology, Beijing, 100081, People's Republic of China*

Source: https://mypuras.com/blogs/articles/dragons-blood-super-antioxidant-properties?srsltid=AfmBOoogOajNIFUz4z3tvN87ALbqNT2ObPaRLtpOXvWZU-agxGhiDpMB; * oxygen radical absorption capacity, dev. by USDA & Tufts University

Acai berries and Astaxanthin also very potent antioxidants

nutrients

MDPI

Review

Açaí (*Euterpe oleracea* Mart.) in Health and Disease: A Critical Review

Lucas Fornari Laurindo [1,2], Sandra Maria Barbalho [1,3,4], Adriano Cressoni Araújo [1,3], Elen Landgraf Guiguer [1,3,4], Arijit Mondal [5], Gabrielle Bachtel [6] and Anupam Bishayee [6,*]

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Abstract: The açaí palm (*Euterpe oleracea* Mart.), a species belonging to the *Arecaceae* family, has been cultivated for thousands of years in tropical Central and South America as a multipurpose dietary plant. The recent introduction of açaí fruit and its nutritional and healing qualities to regions outside its origin has rapidly expanded global demand for açaí berry. The health-promoting and disease-preventing properties of this plant are attributed to numerous bioactive phenolic compounds present in the leaf, pulp, fruit, skin, and seeds. The purpose of this review is to present an up-to-

marine drugs

MDPI

Mar Drugs. 2014 Jan 7;12(1):128–152. doi: 10.3390/md12010128

Astaxanthin: Sources, Extraction, Stability, Biological Activities and Its Commercial Applications—A Review

Ranga Rao Ambati [1,*], Phang Siew Moi [1], Sarada Ravi [2], Ravishankar Gokare Aswathanarayana [3]

"Astaxanthin is a potent antioxidant to terminate the induction of inflammation in biological systems."

Haematococcus lacustris cysts full of astaxanthin

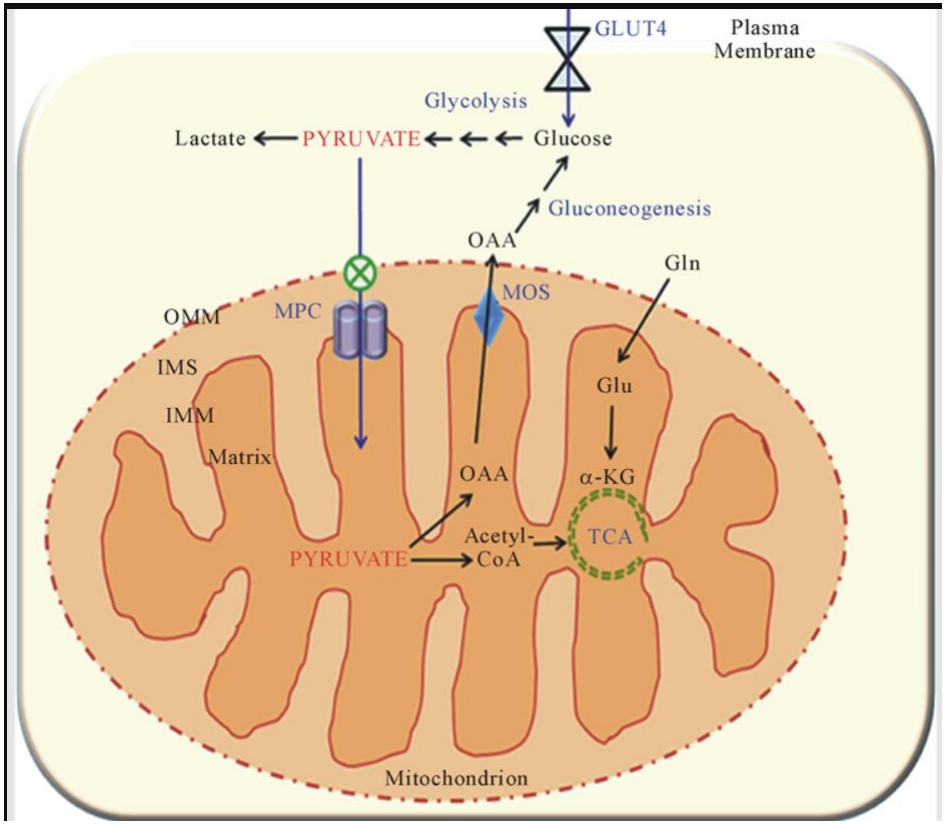
Source: https://nutritionfacts.org/video/clinical-studies-on-acai-berries/, https://en.wikipedia.org/wiki/Haematococcus_lacustris

This test of mitochondrial oxidation levels can also be done as a fingerprick test

Simple, can be done as a follow-up, or to check on your physical workup regime: are you over-training?

Pyruvate is the product of glycolysis, and can either be transformed into lactate or transported into the mitochondria

Lactate/Pyruvate Plus



Glucose in cells is converted to pyruvate. It can then be converted to lactate in the cytoplasm or transported into the mitochondria via the mitochondrial pyruvate carrier (MPC). Ideally most of it gets into the mitochondria. Here, you can see that the MPC is blocked, so lactate will build up in the cytosol.

Figure 1. Schematic diagram of a mitochondrion illustrating the cellular components associated with pyruvate transport and metabolism.

Source: Tang, B. (2014) The Mitochondrial Pyruvate Carrier and Metabolic Regulation. *CellBio*, **3**, 111-117.

Agenda

Supplementary biomarkers:

Mitochondrial mass: Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1α, mitobiogenesis regulator
What to do to increase/decrease mitochondrial mass?
Mitochondrial stress test
Nrf-2, antioxidant regulator
How to quell excess oxidative stress?
Mitochondrial fuel utilisation

Lactate/pyruvate Plus gives insight into what nutrients are being used as fuel for the mitochondria

Lactate/Pyruvate Plus

Cell type:
Peripheral blood mononuclear cells (PBMC)

Lactate/Pyruvate Index PLUS

Lactate/Pyruvate ratio PLUS

Test	Result	Interpretation
Lactate/Pyruvate in dormant cells	1.61	Your immune cells are primarily metabolising carbohydrates and partially (30%) converting them to lactate
Lactate/Pyruvate in activated cells	2.43	The cells are primarily using carbohydrates and converting around 80% of them to lactate

The normal range for immune cells usually ranges from 1.0 – 0.7. Examples are calculated below

Index	Basal metabolic rate
>2.0	The cell is primarily using carbohydrates and preferentially converting them to lactate.
>1.2 – 2.0	The cell is primarily using carbohydrates and partially converting them to lactate.
1 – 1.2	The cell is primarily using carbohydrates and transporting them into the mitochondria.
0.8 – 1.0	The cell is using carbohydrates, fatty acids and amino acids. The carbohydrates are primarily being transported into the mitochondria.
<0.8	The cell is primarily using fatty acids as fuel.

This result:
Under pressure, the fuel is largely not going into the mitochondria, it is being recycled into lactate. The buildup can be very painful (fibromyalgia-type symptoms).

Lactate/pyruvate Plus: shows what level of lactate is being produced by the mitochondria both at rest and under pressure

Lactate/Pyruvate Plus

The higher the value of lactate compared to pyruvate, the more glycolysis is occurring. A higher level of pyruvate compared to lactate is a prerequisite for successful transfer of substrates in the mitochondria for oxidative phosphorylation.

The normal range for immune cells usually ranges from 1.0 – 0.7. Examples are calculated below

Index	Basal metabolic rate
>2.0	The cell is primarily using carbohydrates and preferentially converting them to lactate.
>1.2 – 2.0	The cell is primarily using carbohydrates and partially converting them to lactate.
1 – 1.2	The cell is primarily using carbohydrates and transporting them into the mitochondria.
0.8 – 1.0	The cell is using carbohydrates, fatty acids and amino acids. The carbohydrates are primarily being transported into the mitochondria.
<0.8	The cell is primarily using fatty acids as fuel.

Source: https://www.agilent.com/cs/library/usermanuals/public/103344-400.pdf; Prof. Dr. rer. nat. Brigitte König, MMD Labor; *mitochondrial research by Martin D. Brand and others*

Mitochondrial Fuel Utilisation shows up unusual results sometimes – here, no use of fatty acids at all

MITOCHONDRIAL FUEL UTILIZATION

PBMC mitochondria normally take glucose and fatty acids as fuels for ATP generation in approximately equal proportions. Glutamine finds little utilization for ATP generation in PBMC This assay determines dependence, capacity, and flexibility of cells to burn (oxidize) one of the three fuels for energy production using the mitochondria: Glucose, glutamine, or fatty acids.

The following three parameters can be used to assess mitochondrial and cellular health as well as immune status (e.g., chronic inflammation, autoimmune disease):

Dependency: The "Dependency" measured value determines which fuels must necessarily be used for the metabolism of the PBMC. The PBMC are very flexible and should not be directly dependent on any fuel.

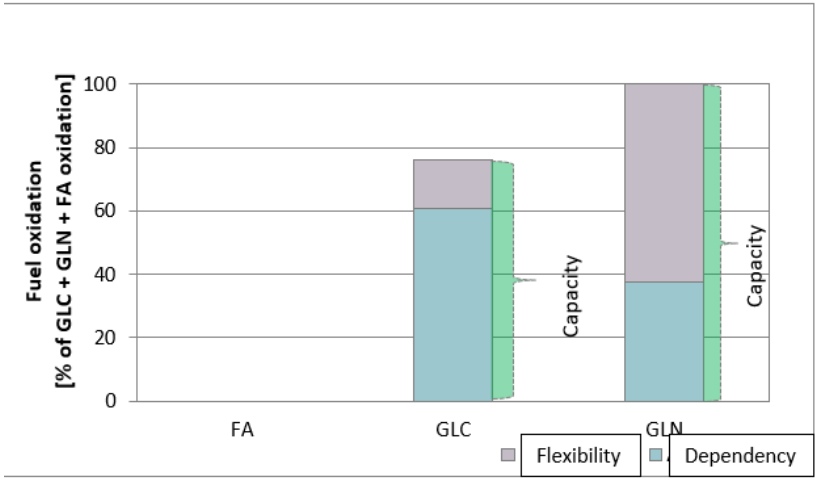
- **So, ideally, the PBMCs should not show much dependence on any one fuel.**

Flexibility: For each fuel, the "flexibility" reading shows the difference between the fraction used for metabolism and the fraction available for metabolism (capacity minus dependence). When one fuel is eliminated for energy production, PBMCs should be able to fall back on another fuel.

- **Ideally, for glucose and fatty acids, the flexibility is 100%.**

Capacity: The measured value "Capacity" is composed additively of dependence and flexibility. The measured value "Capacity" shows the ability to use a certain fuel to meet the energy demand for metabolism.

- **Ideally, for glucose and fatty acids, the capacity is 100%.**



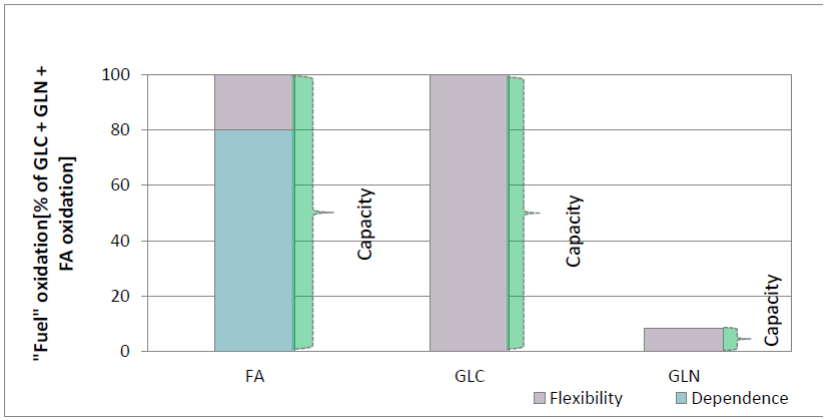
FA: fatty acids; GLC: glucose; GLN: glutamine (amino acids)

CATEGORY	CAPACITY (%)	DEPENDENCY (%)	FLEXIBILITY (%)
ABILITY TO UTILISE GLC / GLUCOSE	76.15	60.91	15.24
ABILITY TO UTILISE GLN / GLUTAMINE	100.00	37.63	62.37
ABILITY TO UTILISE FAS / FATTY ACIDS	0.00	0.00	0.00

Mitochondrial Fuel Utilisation here with very little glutamine (a), and no glucose at all (b)

a)

RESULT



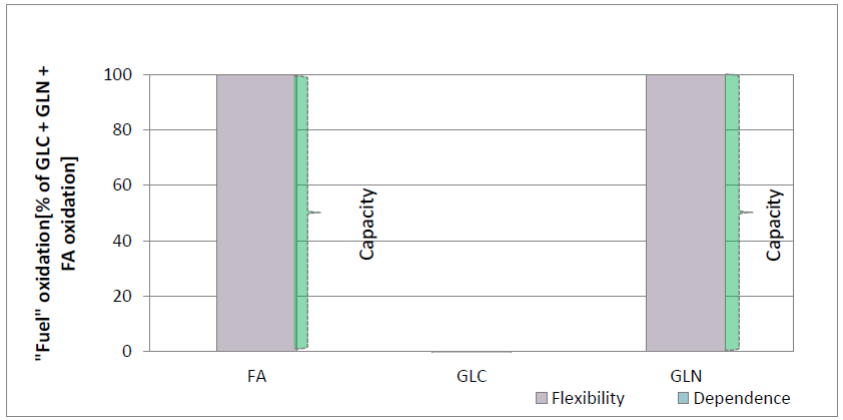
FA: Fatty acids; GLC: Glucose; GLN: Glutamine (Amino acids)

Dependency Group	Capacity (%)	Dependence (%)	Flexibility (%)
Glc-dependence / Glucose	100,00	0,00	100,00
Gln-dependence / Glutamine	8,40	0,00	8,40
FA-dependence fatty acids	100,00	80,23	19,77

Interpretation of your mitochondrial fuel profile result.

b)

RESULT

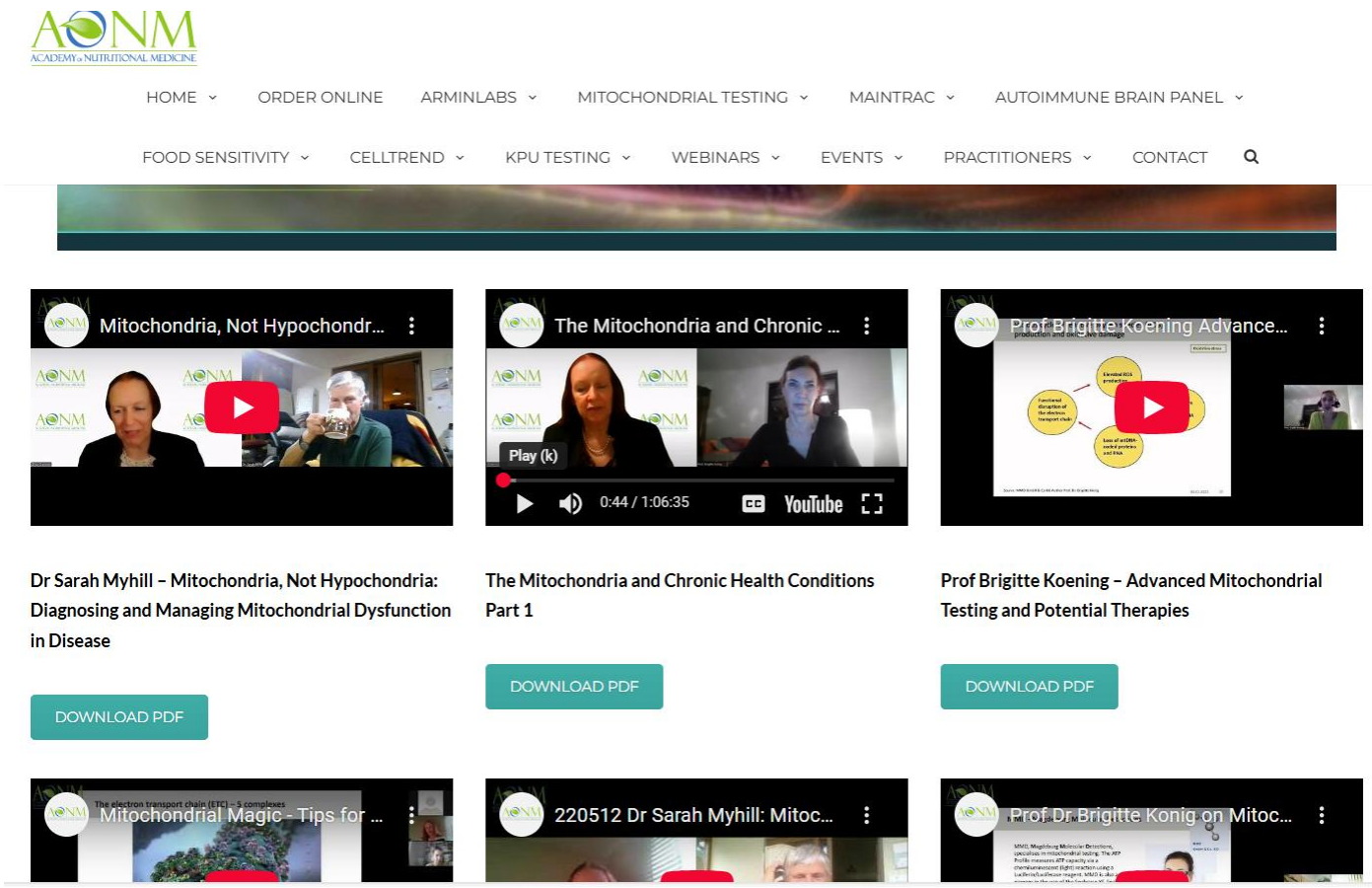


FA: Fatty acids; GLC: Glucose; GLN: Glutamine (Amino acids)

Dependency Group	Capacity (%)	Dependence (%)	Flexibility (%)
Glc-dependence / Glucose	0.00	0.00	0.00
Gln-dependence / Glutamine	100.00	0.00	100.00
FA-dependence fatty acids	100.00	0.00	100.00

Interpretation of your mitochondrial fuel profile result.

Please see Part 1 if you haven‘t yet



https://aonm.org/mitochondria-webinars/

Thank you – Q&A

www.aonm.org