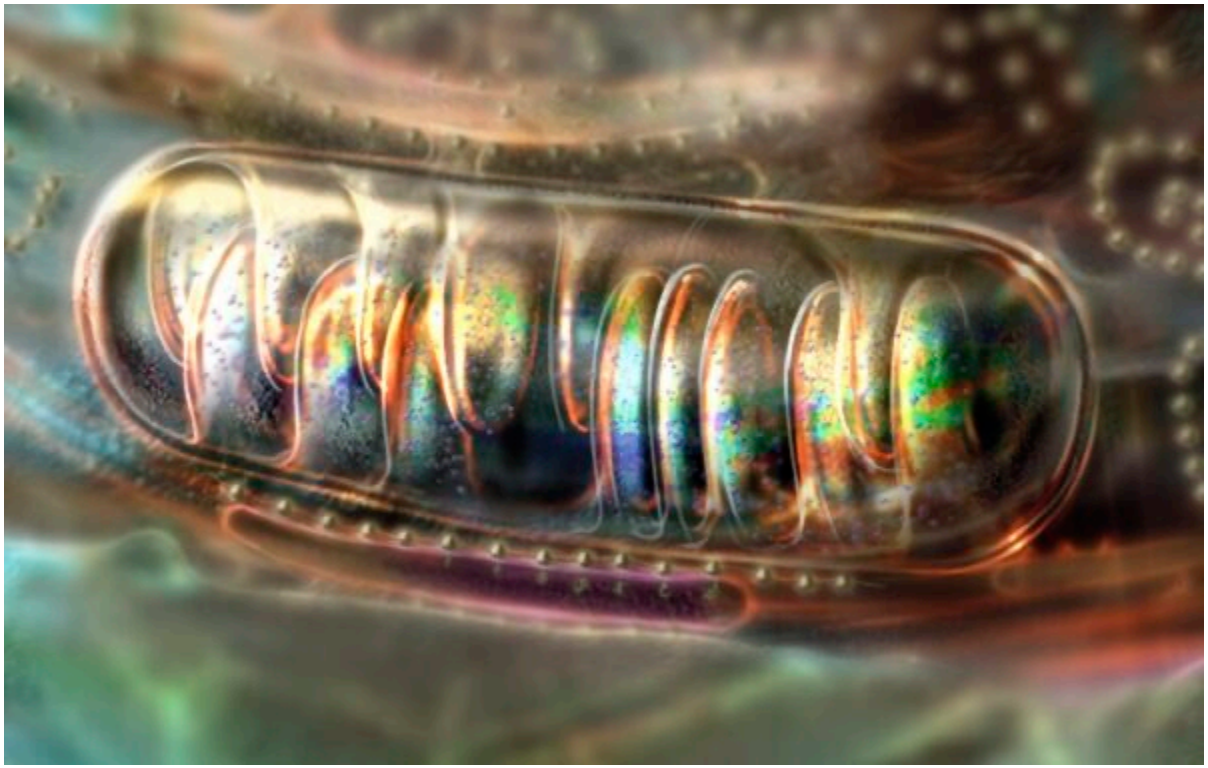


Advanced Mitochondrial Testing

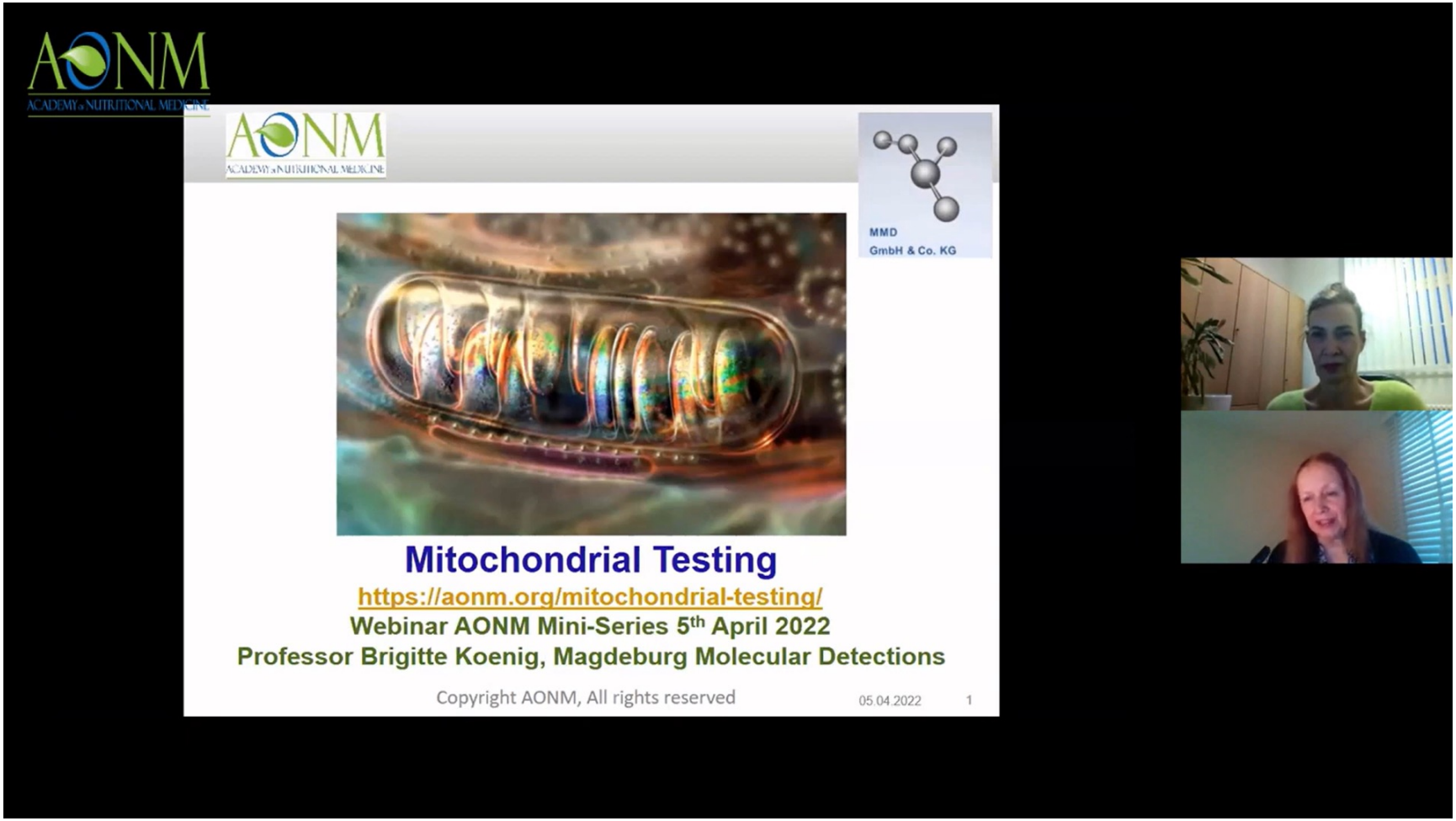
https://aonm.org/mitochondrial-testing/

Professor Brigitte Koenig, Magdeburg Molecular Detections

March 8th 2023

See AONM's past webinars for Part 1

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



Mitochondrial testing with AONM/MMD

1. **ATP Profile:** Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve Capacity

2. **Mitochondrial Health Index:**
   Basal respiration rate, mitochondrial ATP turnover, proton leak, maximum respiration rate, reserve capacity, non-mitochondrial rate, calculation of the overall Mitochondrial Health Index

3. **Supplementary biomarkers:**
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate index

4. **Overview of therapeutic initiatives**

ATP Profile – the mitochondria at rest

XXX
Max-Mustermann Straße 5
xxx Berlin

MMD

MMD GmbH & Co. KG
Breiter Weg 10a
39104 Magdeburg
Prof. Dr. Brigitte König
CEO/ Scientific Director
Prof. Dr. Gerhard Jorch
Medical Director

Tel. office: +49 391 535 37 97
Tel. laboratory: +49 391 611 72 09
Fax: +49 391 535 38 45
E-Mail: info@mmd-web.de
Web: www.mmd-web.de

Patient	AW	Date of birth	01.01.1990
		Entry on	23.07.2021
Order No.:			
Date of sample	22.07.2021	Validated by	Prof. Dr. Brigitte König
Sample type	CPDA vacutainer	Cell type	PBMC
Results status	**Final report**	Results status on	23.07.2021

ATP profile

Test	Result	Unit	Reference range	Result [%]
Total ATP	0.8	fmol/cell		
Mitochondrial ATP capacity	0.4	fmol/cell		50
Glycolytic ATP capacity	0.5	fmol/cell		63
Reserve ATP capacity	0.10	fmol/cell		13

Reference range total ATP									
fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	1.4 - 1.6	1.6 - 2.0	2.0 - 2.5	2.5 - 3.0	3.0 - 5.0
Reference range mitochondrial ATP capacity									
fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4				
Reference range glycolytic ATP capacity									
fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4				
Reference range reserve ATP capacity									
fmol/cell	<0.2	0.2 - 0.3	0.3 - 0.4	0.4 - 0.6	0.6 - 0.9	0.9 - 1.0	1.0 - 1.2	1.2 - 1.5	>1.5

Mitochondrial testing with AONM/MMD

1. **ATP Profile:** Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve Capacity

2. **Mitochondrial Health Index:**
   Basal respiration rate, mitochondrial ATP turnover, proton leak, maximum respiration rate, reserve capacity, non-mitochondrial rate, calculation of the overall Mitochondrial Health Index

3. **Supplementary biomarkers:**
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate index

4. **Overview of therapeutic initiatives**

Mitochondrial Health Index: an 11-page report, partially showing the mitochondria under pressure

RESULTS

Sample type: Blood in CPDA vials

Requisition:
Mitochondrial Health Index / PBMCs

Summary

	Patient´s value	Target value (optimal)
Mitochondrial Health Index (MHI)	0.93	>2.5
Mitochondrial Bioenergetics		
Coupling efficiency, %	81	90 - 95%
Reserve respiration capacity, %	65	>400
Cellular oxygen consumption profile		
Non-mitochondrial respiration as a share of total respiration, %	24	<10
Proton leak as a share of total respiration, %	15	5 -10%
Share of respiration used for mitochondrial ATP generation, %	61	>90
ATP turnover rate (mitochondrial oxygen utilisation)		
ATP base turnover, %	49	<20
ATP reserve, %	51	>80
Potential maximum oxygen consumption rate in pmol oxygen/min	62	>300
Cellular energy phenotype		
At rest	Resting	Resting
On energy demand	Energetic/glycolytic	Energetic/aerobic
Metabolic potential, mitochondrial percentage	149	>350
Metabolic potential, glycolysis percentage	222	>350
Oxygen consumption/glycolysis on energy demand	Strong preference for anaerobic glycolysis	

Optimal	Slightly high / low	Moderately high/low	Very high/low	Extremely high/low

Seite | 1

Summary relating to mitochondrial dysfunction: selected markers

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 √			

- **Upregulated ROS in both the cell and mitochondria**
- **Compromised electron transport chain**
- **Limited number of intact mitochondria**
- **Insufficient ATP on demand**

Comparison of various tests

Patient	xxxxxxx
Date of birth	19.07.1941
Sample taken	15.06.2021
Receipt of sample	16.06.2021
Test completed	16.06.2021
Final result	**16.06.2021**
Validated by	Prof. Dr. Brigitte König
Medical Director	Prof. Dr. Gerhard Jorch

Comparison with previous values

	28.10.2020	19.05.2021	Current value 16.06.2021	Target value (optimal)
Mitochondrial Health Index (MHI)	1.87	1.54	1.90	>2.5
Mitochondrial bioenergetics				
Coupling efficiency, %	94.76	84.62	93.80	100
Reserve respiration capacity, %	242.93	291.35	468.73	>400
Cellular oxygen consumption profile				
Non-mitochondrial respiration as a share of total respiration, %	33.66	32.09	35.32	<10
Proton leak as a share of total respiration, %	3.76	10.45	4.96	
Share of respiration for mitochondrial ATP generation, %	62.58	57.46	59.72	>90
ATP turnover rate (mitochondrial oxygen utilisation)				
ATP base turnover, %	27.35	21.62	16.30	<20
ATP reserve, %	72.65	78.38	83.70	>80
Maximum possible oxygen consumption rate, pmol oxygen/min	90.78	123.10	180.06	>300
Cellular energy phenotype				
At rest	Resting	Resting	Resting	Resting
On energy demand	Energetic	aerobic	aerobic	Energetic/aerobic
Metabolic potential, % - Mitochondria	262.44	297.81	401.74	>350
Metabolic potential, % - glycolysis	312.43	252.29	334.84	>350
Oxygen consumption/glycolysis ratio on energy demand	Slight preference for anaerobic glycolysis	Slight preference for the mitochondria	Slight preference for the mitochondria	

Maximum possible oxygen consumption rate has doubled; many markers are showing improvement

Mitochondrial testing with AONM/MMD

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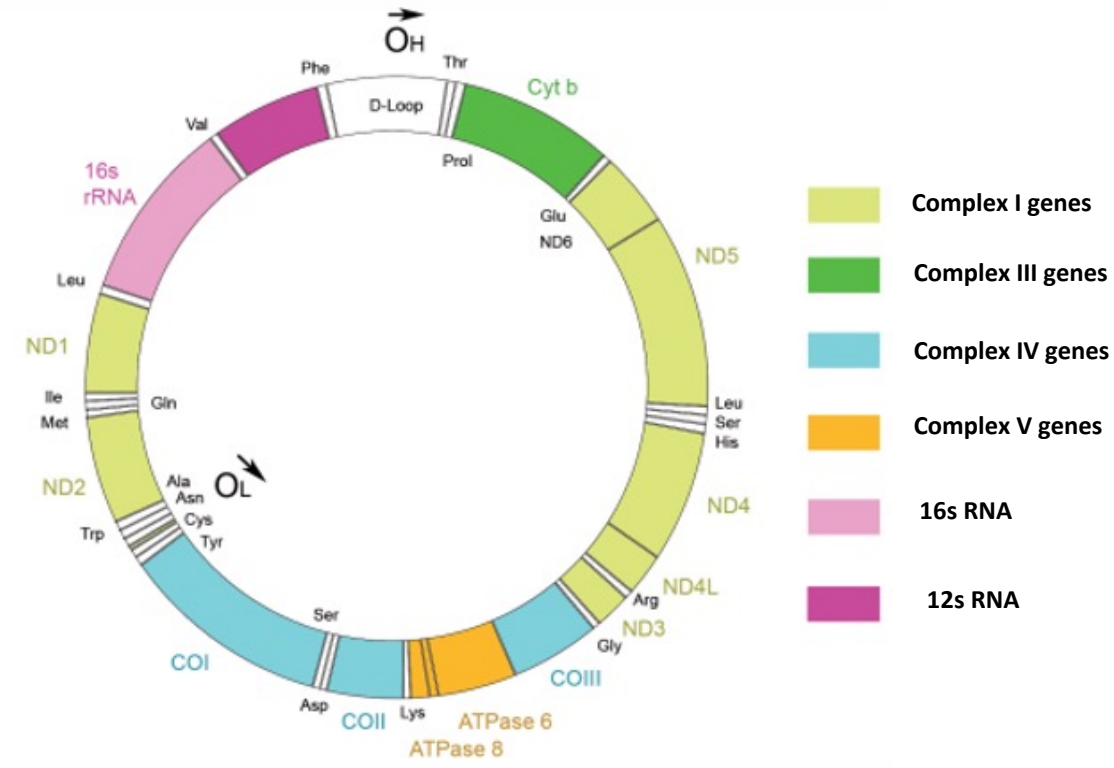
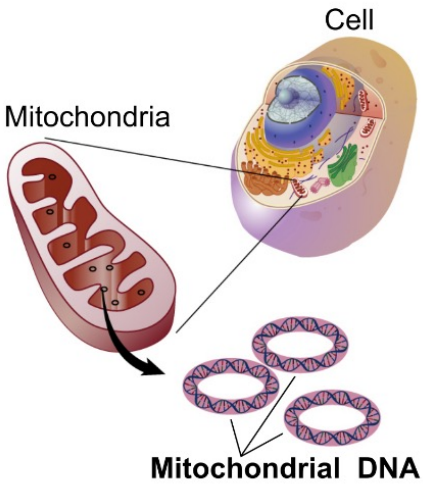
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate index

4. **Overview of therapeutic initiatives**

Mitochondria have their own DNA

mtDNA:nDNA

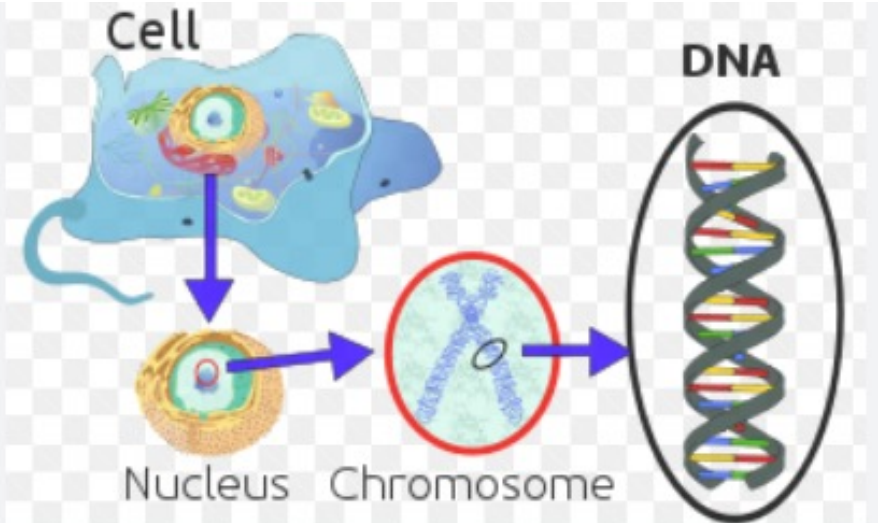


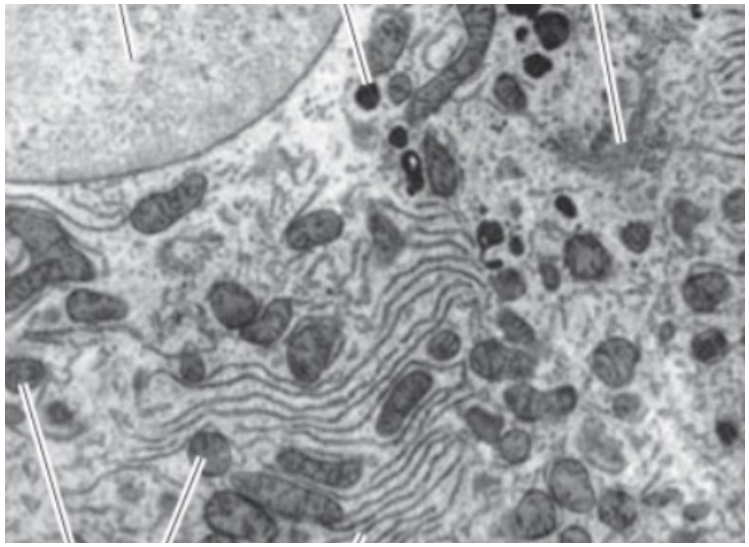


Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König; Hoffmann A, Spengler D. The Mitochondrion as Potential Interface in Early-Life Stress Brain Programming. Front Behav Neurosci. 2018 Dec 6;12:306; https://en.wikipedia.org/wiki/Mitochondrial_DNA: Images free to use under Commons License

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

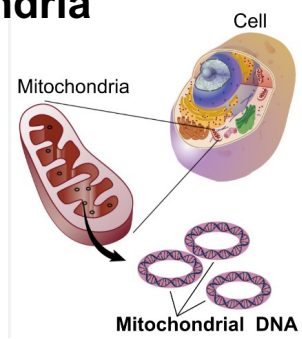
mtDNA:nDNA





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 all the mitochondria detected are healthy/intact.**

mtDNA:nDNA – numbers pathologically high/low

mtDNA:nDNA

Example 2:

ratio mtDNA:nDNA **580**

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Example 3:

ratio mtDNA:nDNA **115**

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Too high (see example 2):

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

Too low (see example 3):

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

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

(Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1Alpha)

PGC-1-alpha

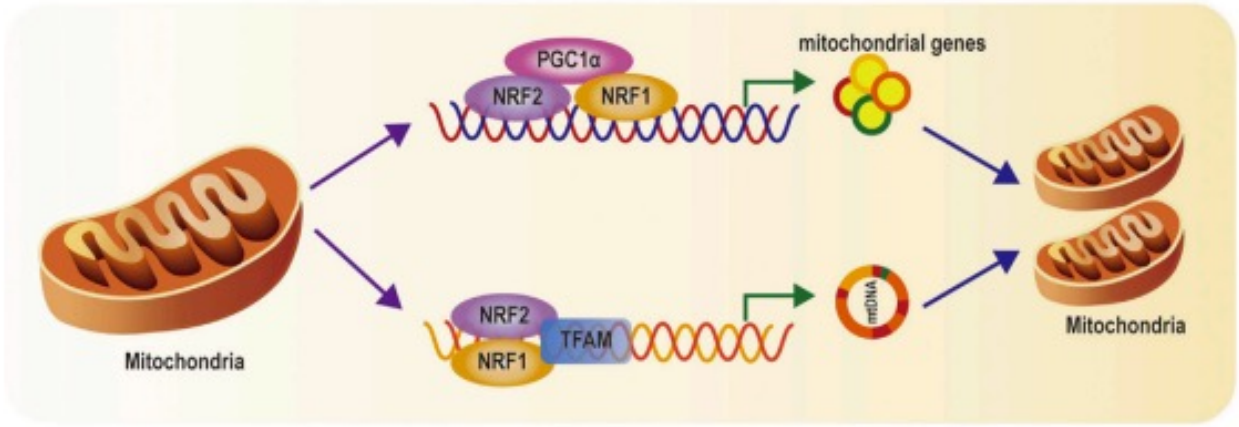


FIGURE 2 | Mitochondrial biogenesis pathways: When PGC-1α is activated, PGC-1a activates NRF1 and NRF2, and subsequently TFAM, which regulate genes involved in subunits of mitochondrial respiratory chain complexes, import of nuclear-encoded mitochondrial proteins, and mtDNA replication and transcription.

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)	0.000953

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

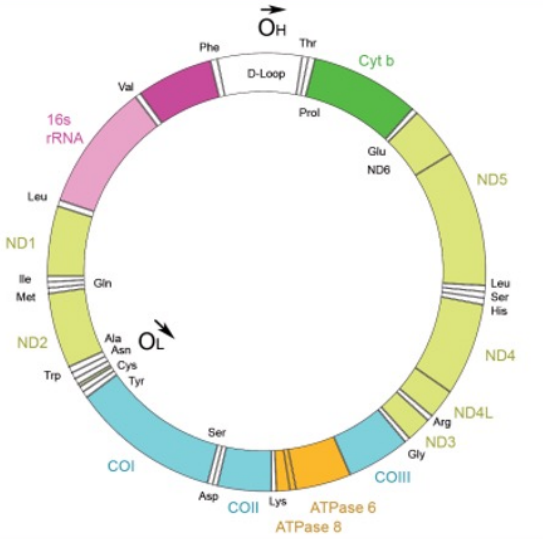
Interpretation: "Basic values of the peripheral blood leucocytes"

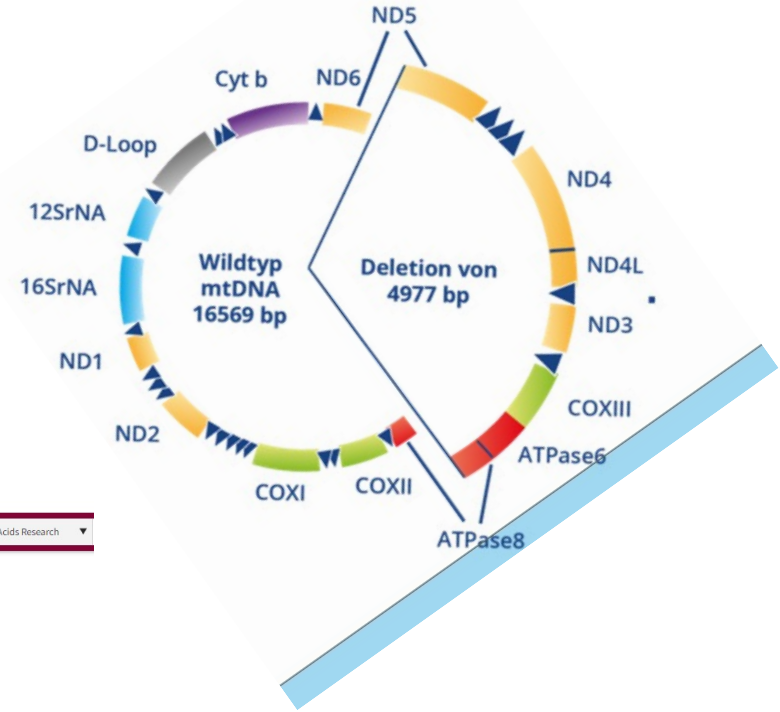
PGC-1-alpha expression is barely detectable. This indicates extremely low/absent new mitochondrial formation.

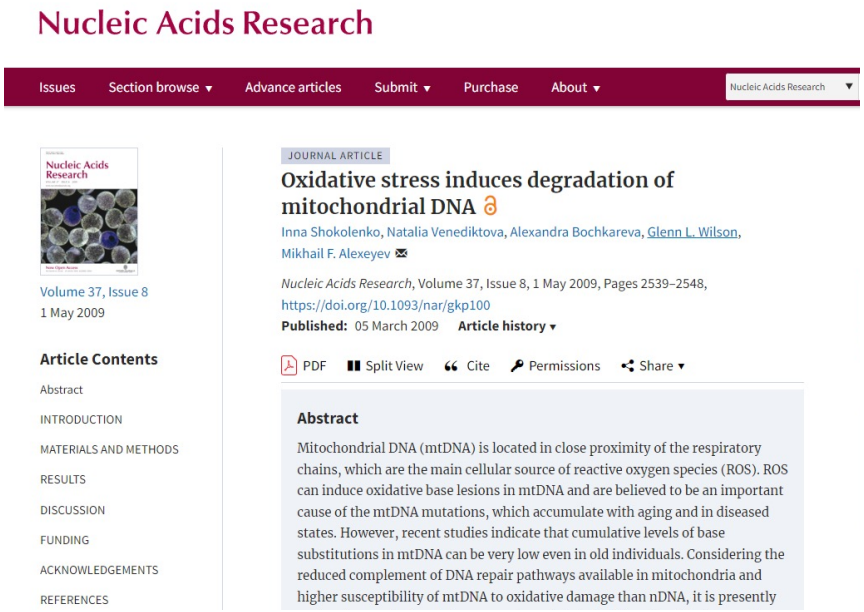
If this is the case, and mtDNA:nDNA is low too, then initiatives should be taken to increase PGC-1-alpha *(Gilian will discuss briefly later)*

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

Oxidative stress







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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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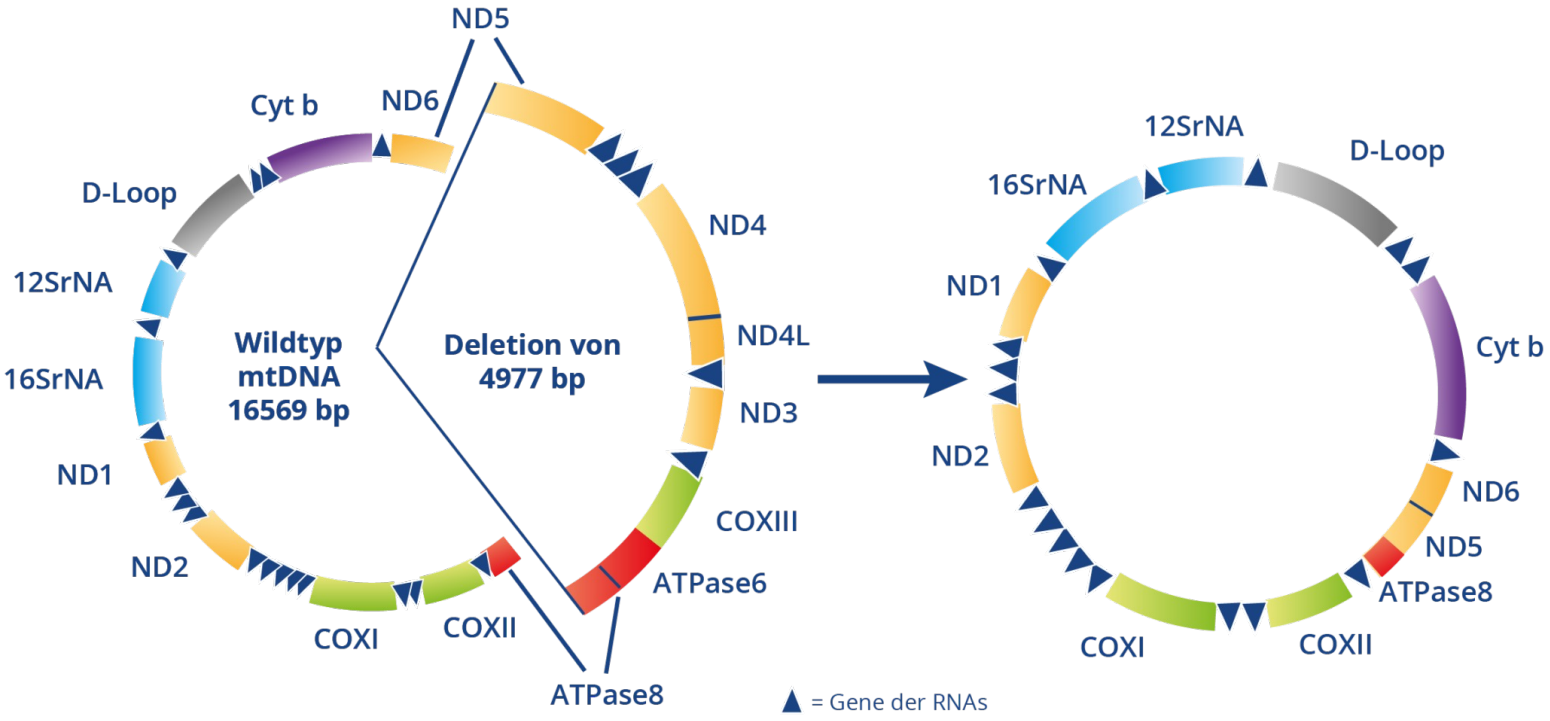
Abstract

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

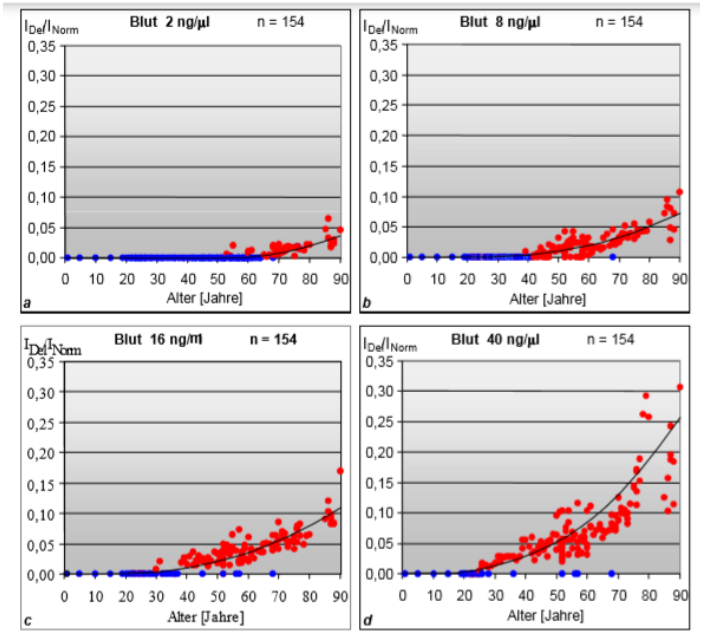
The mitochondrial deletion mutant mt4977bp is noticeably enhanced. This indicates oxidative stress and damage to mitochondrial DNA.

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.**

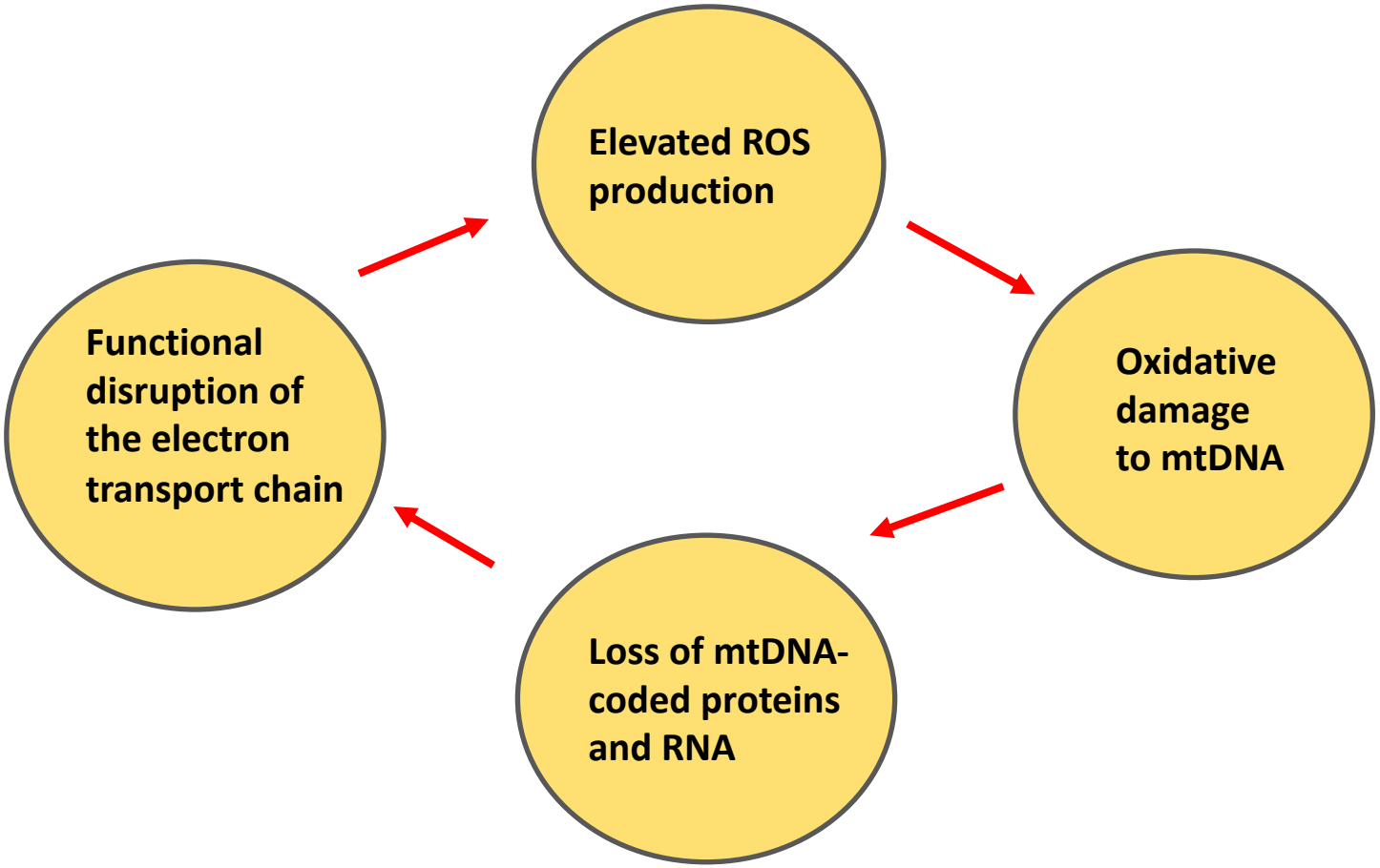
Significantly increased **Undetectable**

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

Vicious cycle of reactive oxygen species (ROS) production and oxidative damage

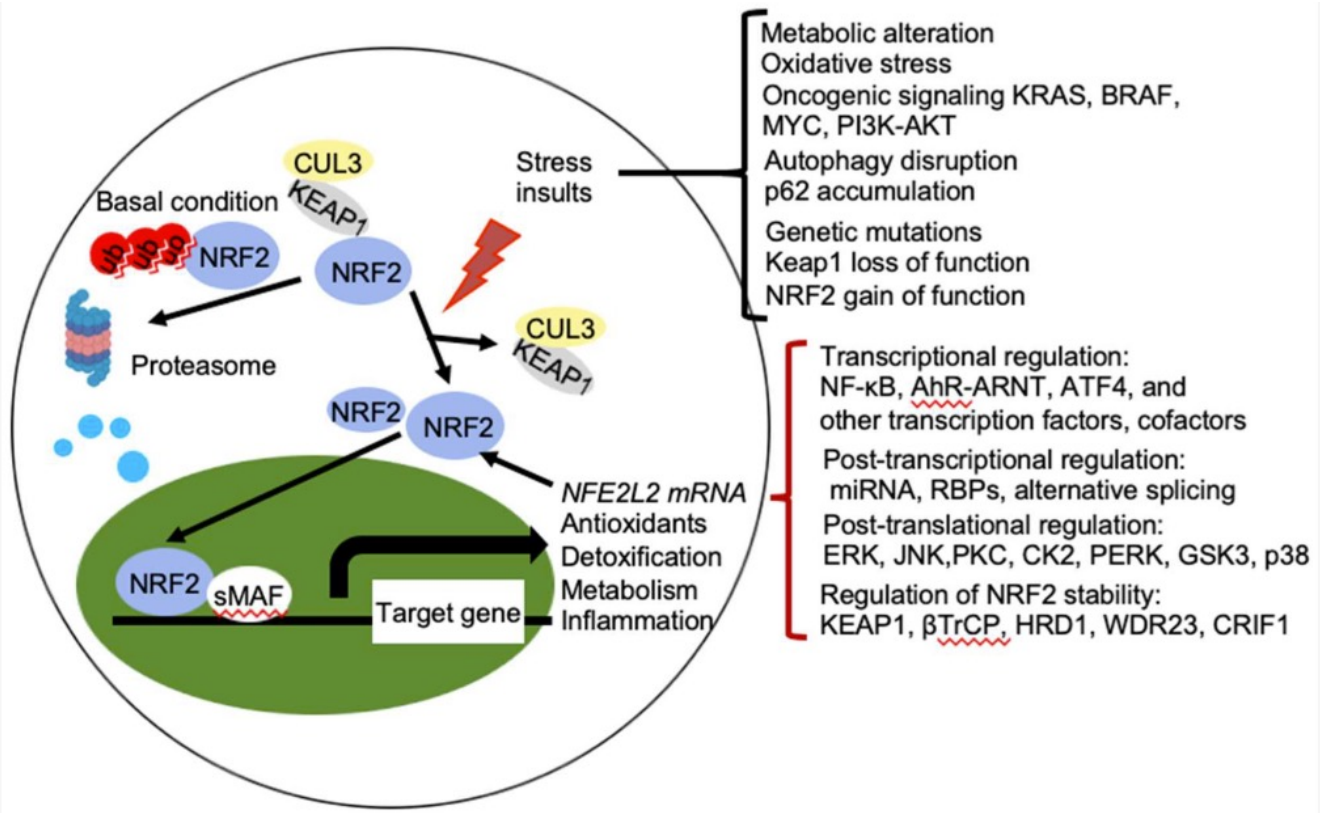
Oxidative stress



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

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:

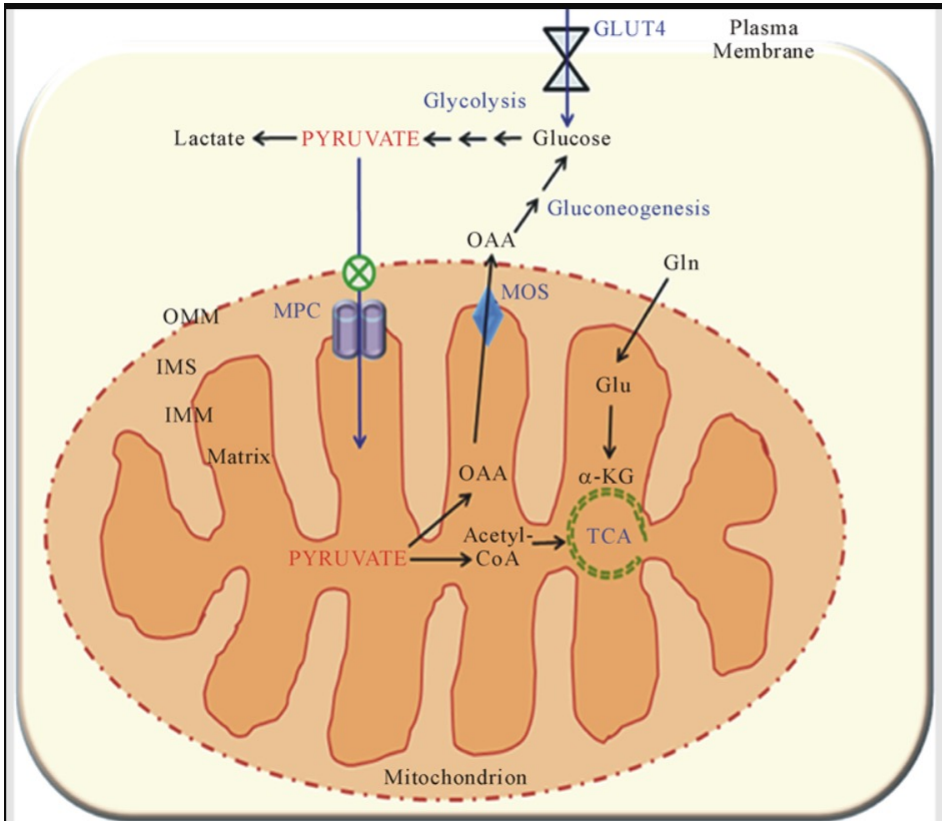
Endogenous antioxidants (Nrf-2 activation) and

Exogenous antioxidants

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

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.

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*

Lactate/pyruvate Plus also 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

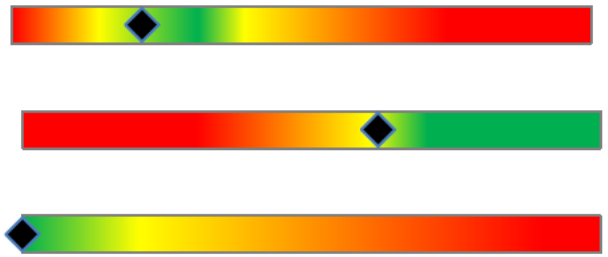
Index	Basal metabolic rate
>2.0	The cell is primarily using carbohydrates and preferentially converting them to lactate.
>1.2 – 2.0	The cell is primarily using carbohydrates and partially converting them to lactate.
1 – 1.2	The cell is primarily using carbohydrates and transporting them into the mitochondria.
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).

AONM has a new MMD test that shows whether the mitochondria in the cells are intact or not, and the proportions

NEW

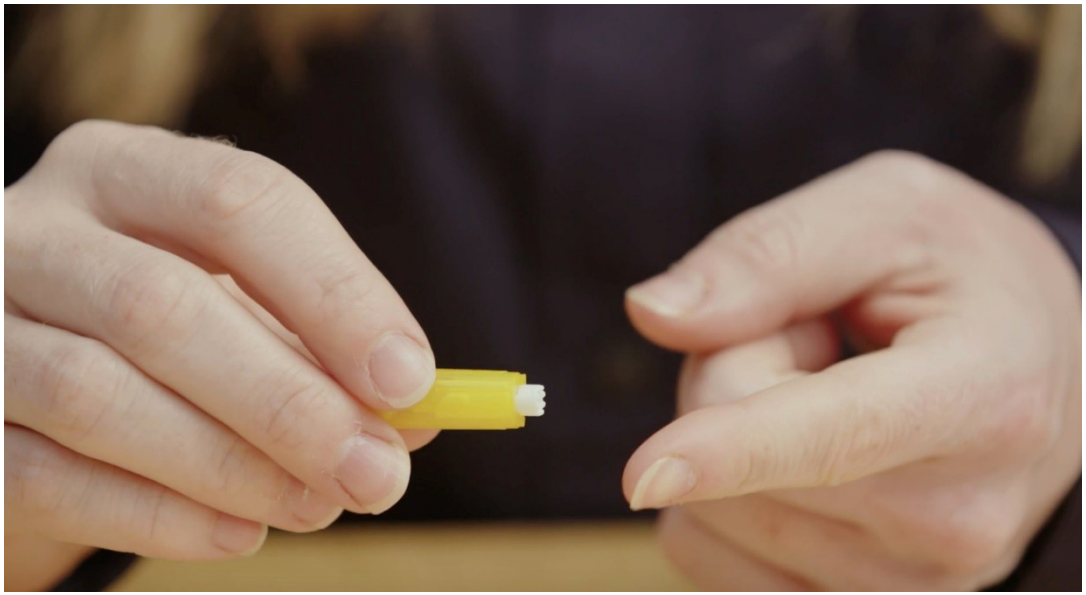
Number of mitochondria	67
intact mitochondria	6.74E+07
non-intact mitochondria	0.00



… a useful add-on to the mitochondrial mass mtDNA:nDNA

We will soon have the test for mitochondrial oxidation available as a fingerprick test

MitoOx



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

Mitochondrial testing with AONM/MMD

1. **ATP Profile:** Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve Capacity

2. **Mitochondrial Health Index:**
   Basal respiration rate, mitochondrial ATP turnover, proton leak, maximum respiration rate, reserve capacity, non-mitochondrial rate, calculation of the overall Mitochondrial Health Index

3. **Supplementary biomarkers:**
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate index

4. **Overview of therapeutic initiatives**

If too few mitochondria, check your PGC-1-alpha – there are many nutrients and nutraceuticals/herbs that can activate that

mtDNA:nDNA vs. PGC1 alpha

ratio mtDNA:nDNA **115**

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Test	Unit	Result
PGC-1-alpha	Relative expression (to GAPDH)	0.000953

Resveratrol (Japanese Knotweed)

L-Carnitine

Epigallotocatechin-3-Gallate (EGCG)

Gotu kola (Centella Asiatica)

Grape seed proanthocyanidin extract

Nicotinamide riboside

Niacin

Pyrroloquinoline quinone, PQQ

Quercetin

Salidroside (Rhodiola rosea extract)

R-Lipoic Acid

Ellagic acid

Cyanidin-rich fruits: Red berries including grapes, bilberry, blackberry, blueberry, cherry, chokeberry, cranberry, elderberry, hawthorn, loganberry, açai berry, raspberry

Ecklonia cava polyphenol extract

Curcumin

Sources available on request

If too many mitochondria and they are are damaged, consider mitophagy …

mtDNA:nDNA

ratio mtDNA:nDNA **580**

Number of mitochondrial DNA copies per 1 copy of nuclear DNA

Limited number of intact mitochondria — No / √ Yes

Mechanisms and molecular targets of phytochemicals that induce mitophagy explained

Oxidative Medicine and Cellular Longevity

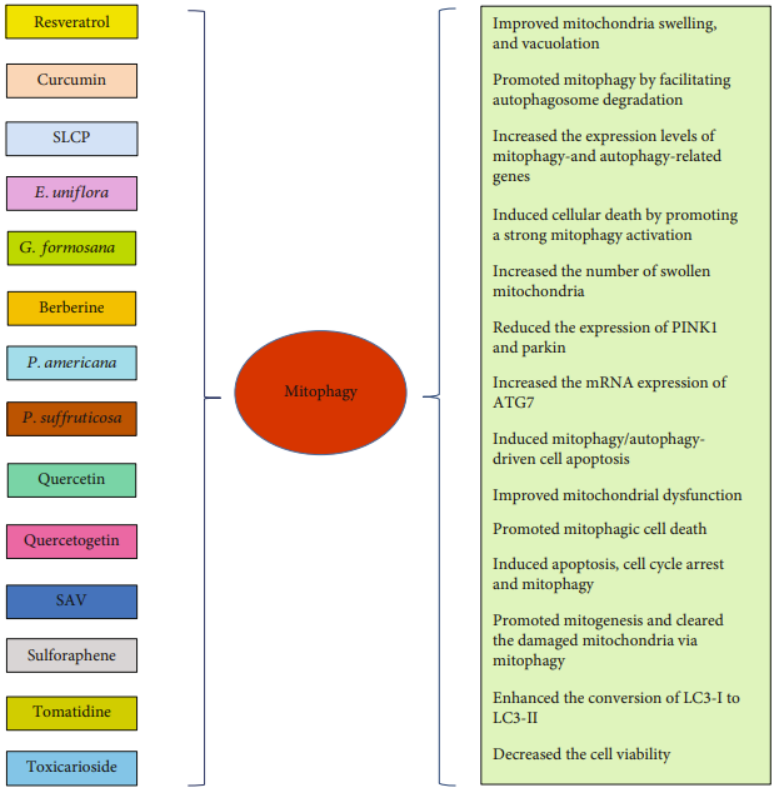


FIGURE 2: Effects of natural compounds on mitophagy.

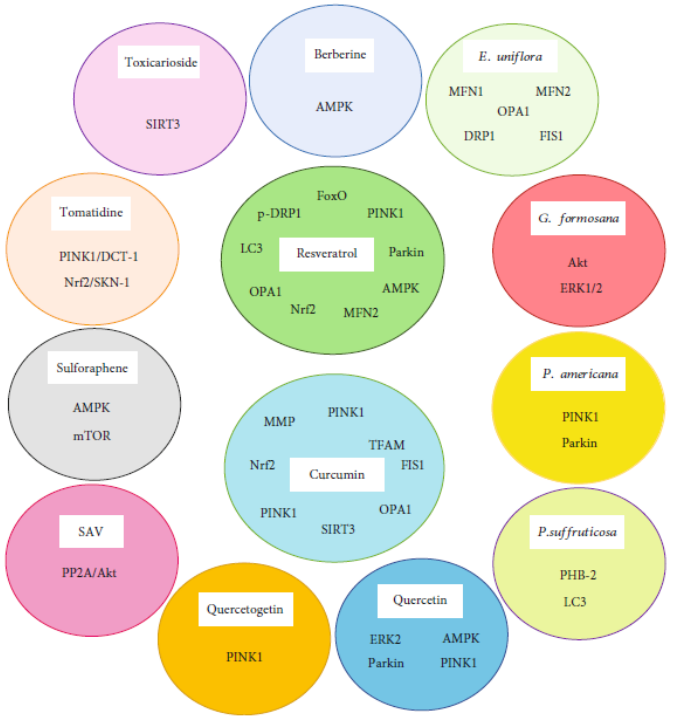


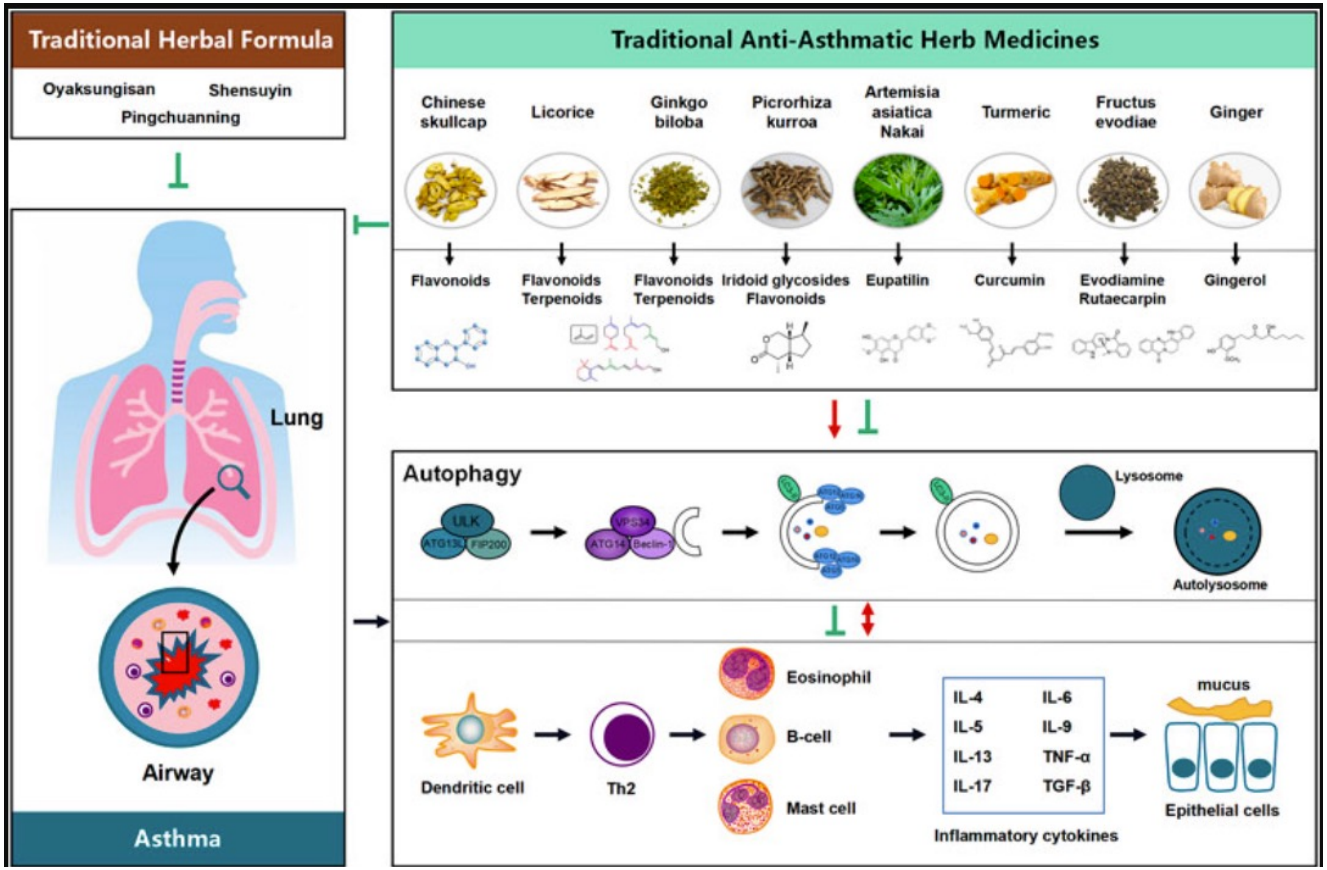
FIGURE 3: Specific molecular targets of natural compounds in the mitophagy pathway.

Source: : https://www.hindawi.com/journals/omcl/2020/6969402/; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4242674/pdf/pone.0113682.pdf

… and autophagy

mtDNA:nDNA

Autophagy Modulators From Chinese Herbal Medicines



Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4248894/pdf/zjv12715.pdf; https://www.frontiersin.org/articles/10.3389/fphar.2021.710679/full; https://www.frontiersin.org/files/Articles/710679/fphar-12-710679-HTML/image_m/fphar-12-710679-g001.jpg

If you have high oxidative stress in the mitochondria, check your Nrf-2 activity …

MitoOx vs. Nrf2

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

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

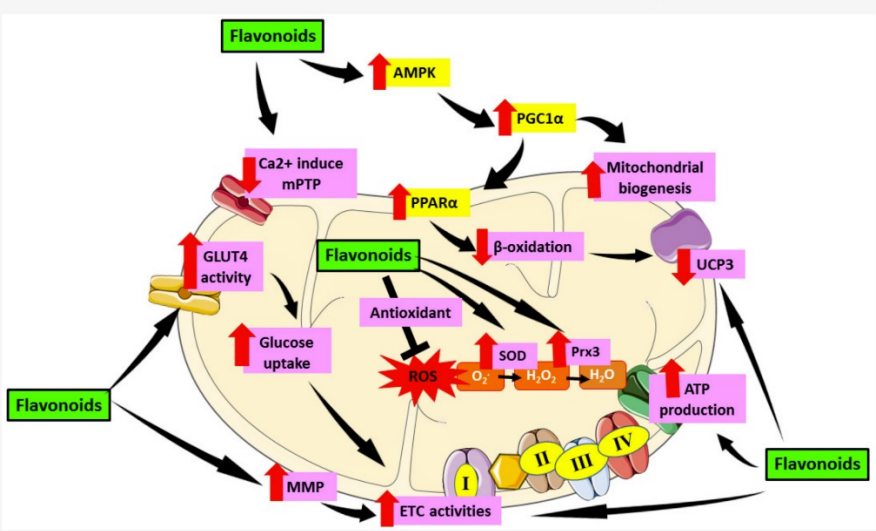
Reference range mt4977del			
<1E+06	1E+06 - 1E+07	>1E+07	>1E+08

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.**

Nrf-2	Relative expression (to GAPDH)	Not detectable

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

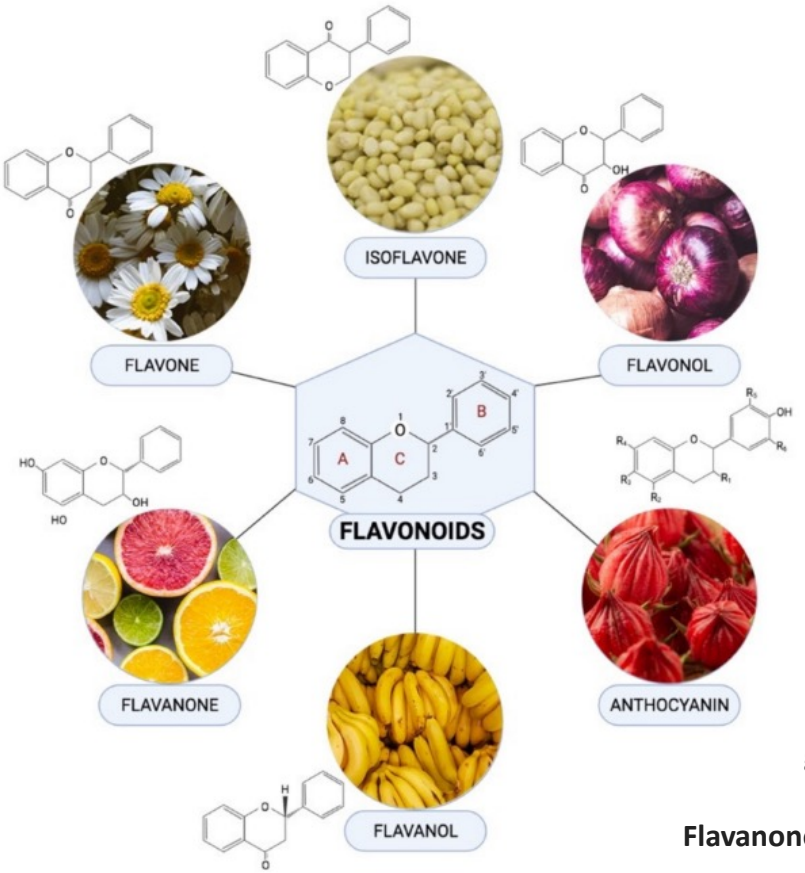
… time to consider taking action against those free radicals



Source: Sapian S et al. Therapeutic Approach of Flavonoid in Ameliorating Diabetic Cardiomyopathy by Targeting Mitochondrial-Induced Oxidative Stress. Int J Mol Sci. 2021 Oct 27;22(21):11616

Flavonoids have numerous antioxidant properties

MitoOx vs. Nrf2



Flavones: glycosides in celery, parsley, red peppers, mint, and ginkgo biloba. This group of flavonoids includes luteolin, apigenin, and tangeritin

Isoflavones: e.g. genistein, daidzein, glycitein, formononetin, biochanin A, and equol

Flavonols: Flavonoids with a ketone group, e.g. green leafy vegetables, including onions, kale, lettuce, apples, and berries, rutin, myricetin, and quercetin, and herbs such as dill, chives, and tarragon. Flavonols can upregulate Nrf2

Anthocyanins: red, purple, and blue colours in flowers, seeds and fruits. Induce enzymes (superoxide dismutase, catalase) that remove ROS or modulate ROS-forming enzymes (NADPH oxidase) in mitochondria

Flavanols: bananas, pears, apples, blueberries, peaches, green tea, epicatechin (in e.g. dark chocolate

Flavanones: citrus fruits, including oranges, lemons, and grapes

Figure 4. Chemical structures and example of sources where they are found abundant in for each flavonoid subclasses.

Source: 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; https://www.mdpi.com/1422-0067/22/21/11616;

Other antioxidants for which there is good evidence

MitoOx vs. Nrf2

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

Melons contain superoxide dismutase
Foods containing glutathione (see below)
Hydrogen-rich water

Sources available upon request

If there is too much lactate …

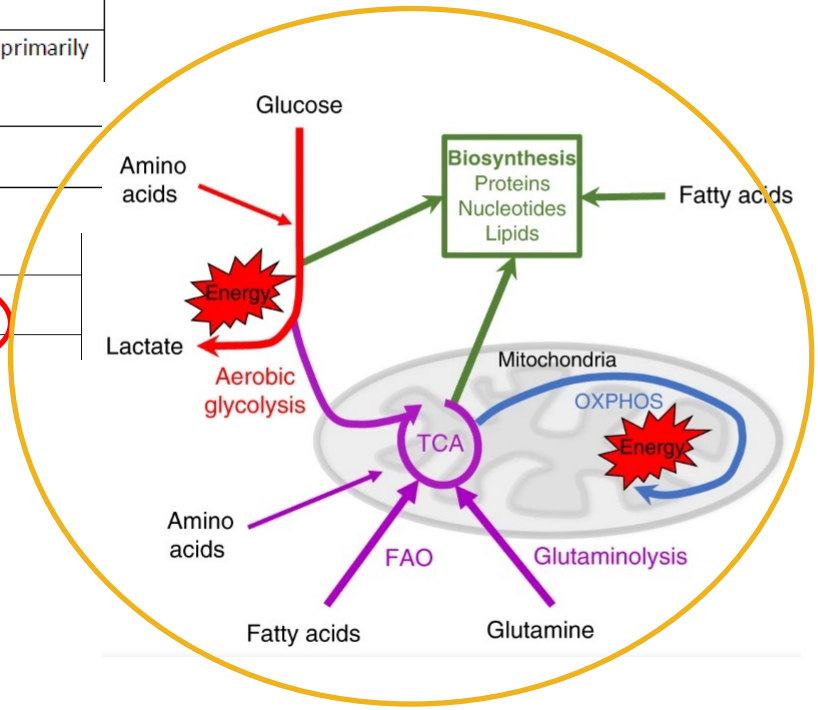
Lactate/Pyruvate in activated cells	3.10	The cells are primarily using carbohydrates and converting around 80% of them to lactate

Lactate/Pyruvate Plus

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

Ratio	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.

Cellular oxygen consumption profile		
Non-mitochondrial respiration as a share of total respiration, %	56	<10



… what is stopping the fuel from getting into the mitochondria?

Source: *MMD, O&M und Ernaehrung,* Mitochondrien – texts from 2016/No. 156 through to 2020/No. 171 Prof. Dr. rer. nat. Brigitte König; *mitochondrial research by Martin D. Brand and others (e.g.* https://pubmed.ncbi.nlm.nih.gov/28270511/*);* https://www.nature.com/articles/s41467-019-10015-4

B vitamins vital throughout the entire cycle ...

Lactate/Pyruvate Plus

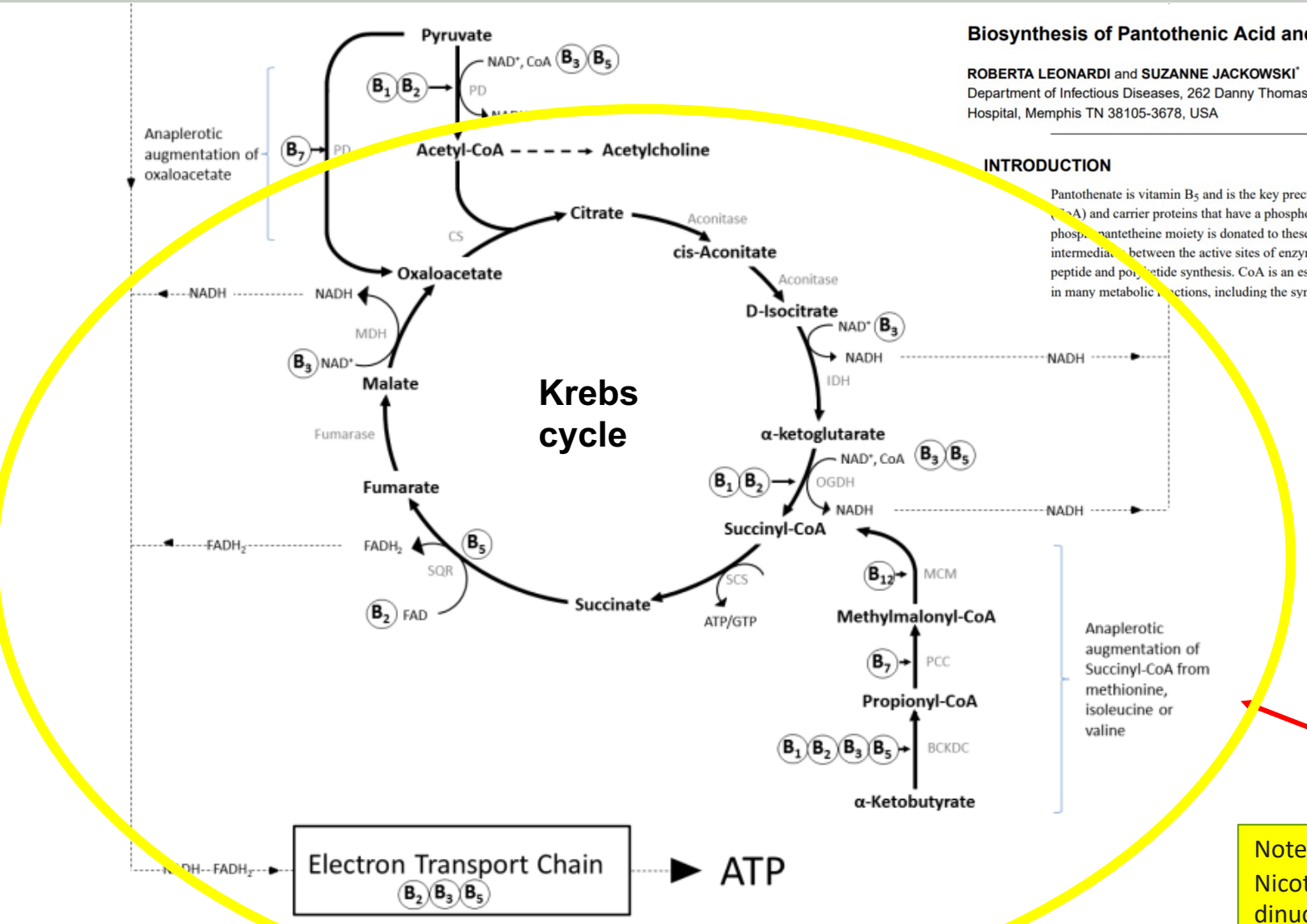


Figure 1. The role of B-vitamins in mitochondrial energy production. The citric acid cycle

Biosynthesis of Pantothenic Acid and Coenzyme A

ROBERTA LEONARDI and **SUZANNE JACKOWSKI***
Department of Infectious Diseases, 262 Danny Thomas Place, St. Jude Children's Research Hospital, Memphis TN 38105-3678, USA

INTRODUCTION

Pantothenate is vitamin B_5 and is the key precursor for the biosynthesis of coenzyme A (CoA) and carrier proteins that have a phosphopantetheine prosthetic group. The phosphopantetheine moiety is donated to these proteins by CoA and is used to shuttle intermediates between the active sites of enzymes involved in fatty acid, non-ribosomal peptide and polyketide synthesis. CoA is an essential cofactor for cell growth and is involved in many metabolic reactions, including the synthesis of phospholipids, synthesis and

B1
B2
B3
B5
B7 (Biotin)
B12

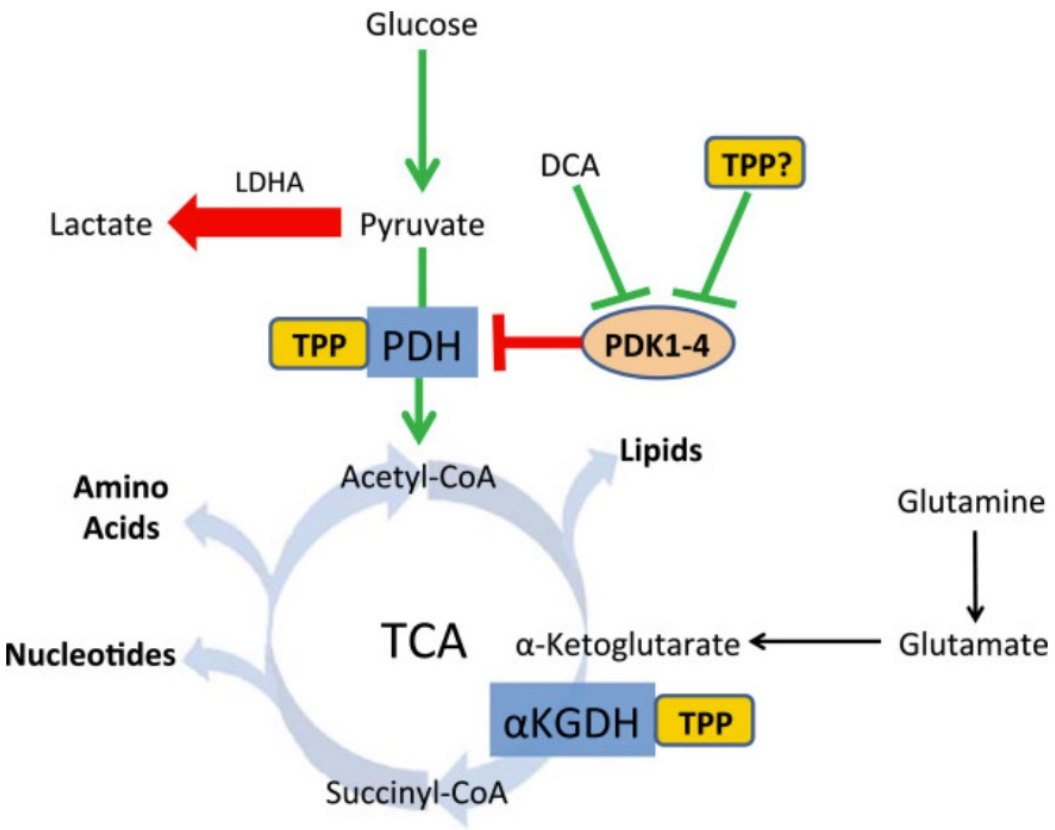
Mitochondrion

Note the huge role of Nicotinamide adenine dinucleotide (NAD)

Source: https://www.mdpi.com/2072-6643/8/2/68; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4950986/

… especially B1; you can't make mitochondrial energy without it

Lactate/Pyruvate Plus



The pyruvate dehydrogenase complex (PDH/PDC) is dependent on thiamine = B1 (TPP - thiamine pyrophosphate)
Blocked = ⇧ lactate

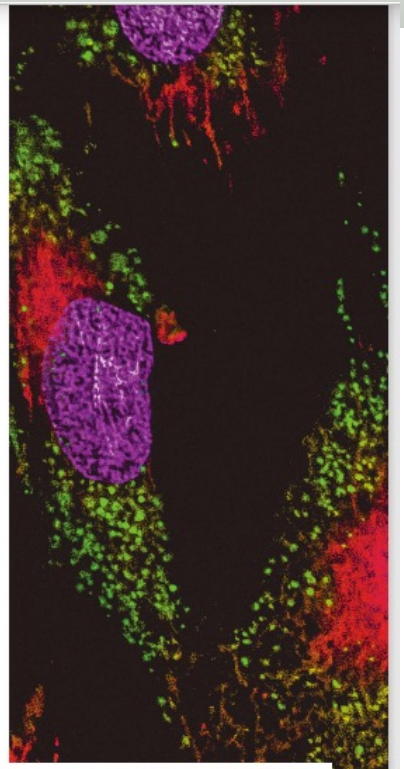
What can block PDH?:
B1 deficiency
Lack of Mg
Hyperglycaemia
High carbohydrate/sugar diets, coffee, tea, alcohol, tobacco
Most pharmaceutical, environmental, and industrial chemicals
Arsenic, mercury and aluminium block ALA (important gene) and impair the PDC

Source: Zastre JA, Sweet RL, Hanberry BS, Ye S. Linking vitamin B1 with cancer cell metabolism. Cancer Metab. 2013 Jul 24;1(1):16; ALA-D = delta-aminolevulinate dehydratase

Protective downregulation of the mitochondria always worth considering if excess lactate discovered

IHCAN mitochondrial medicine

The Cell Danger Response: a new paradigm for understanding chronic disease?



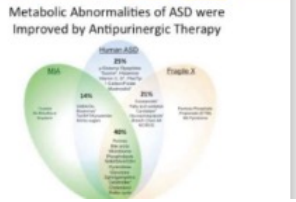
When danger threatens, mitochondria alter their cellular metabolism to shield cells from injury – triggering a cascade of responses affecting methylation, energy production and more. **GILIAN CROWTHER**, Director of Research for the Academy of Nutritional Medicine, explains how the ground-breaking research of Prof Robert Naviaux has unlocked a new understanding of mitochondria's pivotal role in chronic disease.

Dr Robert Naviaux, MD, PhD, who runs the Mitochondrial and Metabolic Disease Centre at the University of California, first introduced the concept of the Cell Danger Response in an article in *Mitochondrion* in 2013: "Metabolic features of the Cell Danger Response". (1)

Dr Naviaux is a Professor of Genetics, in the Departments of Medicine, Paediatrics, and Pathology. He directs a core laboratory for metabolomics at UC San Diego. He is an internationally known expert in human genetics, inborn errors of metabolism, metabolomics, and mitochondrial medicine. He is the discoverer of the cause of Alpers syndrome – the oldest homeostasis". This is a response activated when a cell encounters a threat that could injure or kill it.

Threats that he cites include:

- **Biological** – viruses, bacteria, fungi, parasites.
- **Chemical** – eg heavy and trace metals like lead, mercury, cadmium, arsenic, and nickel, certain electrophilic aromatic chemicals like the plasticiser bisphenol A, chemical flame retardants like brominated diphenyl ethers (BDEs), and certain halogenated pesticides like chlorpyrifos and DDT.
- **Physical** – eg heat, salt, pH shock, UV/ionising radiation.
- And also **psychological**, because psychological trauma also has physiological repercussions.



Source: *Naviaux RK. Metabolic features of the Cell Danger Response. Mitochondrion. 2014 May;16:7-17*
https://www.ihcan-mag.com/imag/aonm.pdf

UPCOMING WEBINARS

14 MARCH 2023, 7PM GMT

PROF. DR. JOHN LAMBERT

Long COVID from a Clinical Perspective - Follow up Q&As

16 MARCH 2023, 7PM GMT

DR. CRAIG D. SHIMASAKI

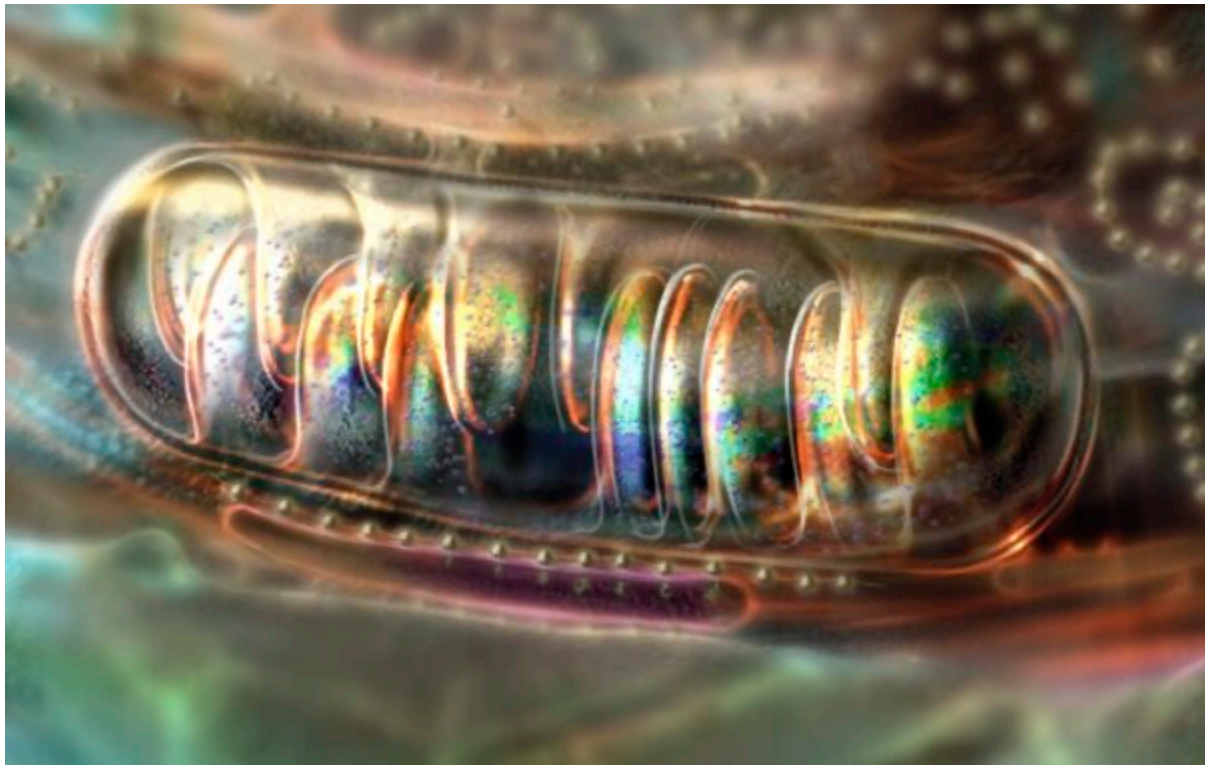
Infections and our Immune Response: *The Rise in Group A Strep, RSV, and Influenza Post-COVID - What Impact can these Infections Have on our Health?*

30 MARCH 2023, 7PM GMT

KUNAL GARG

Toxiplex Basic - A direct mycotoxin detection assay for human serum/plasma

aonm.org/upcoming_events



Thanks very much for your attention!

mitochondria@aonm.org

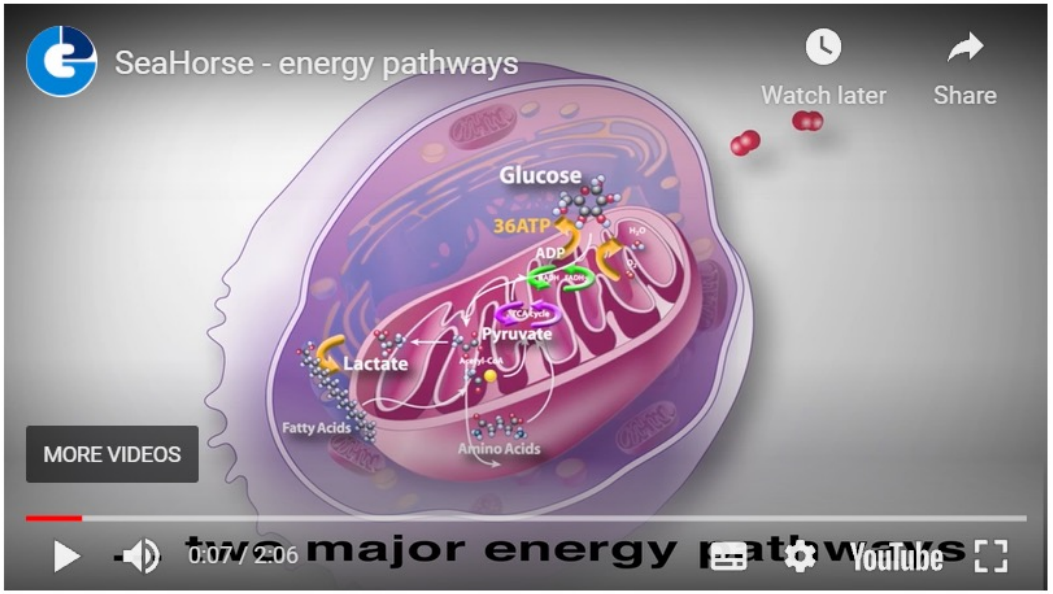
Many videos about the Seahorse technology available, and over 7,600 studies* for which the Seahorse has been used

HOW THE SEAHORSE XF WORKS

Videos are at the bottom of this page:
https://aonm.org/mitochondrial-testing/

The tests require only one vial of blood in a CPDA[i] tube. The laboratory uses

SEAHORSE: ENERGY PATHWAYS



* https://www.agilent.com/search/?N=4294836537

MMD - Magdeburg Molecular Detections

MMD, **M**agdeburg **M**olecular **D**etections, specialises in mitochondrial testing. The ATP Profile measures ATP capacity via a chemiluminescent (light) reaction using a Luciferin/Luciferase reagent. MMD is also a pioneer in the use of the Seahorse XF. Seahorse Biosciences has developed a unique extracellular flux analyser that is able to measure multiple parameters in the cell and mitochondria with huge precision. They use a microplate-based system with unprecedented throughput to make these measurements very sensitively, with extremely rapid kinetics. This technology has come to be considered the gold standard for measuring mitochondrial function in cellular systems. Since its introduction in 2006, Seahorse XF technology has been used in over 7,400 peer-reviewed publications.