

Mitochondrial Testing

Academy of Nutritional Medicine
with Gilian Crowther and Professor Brigitte Koenig

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

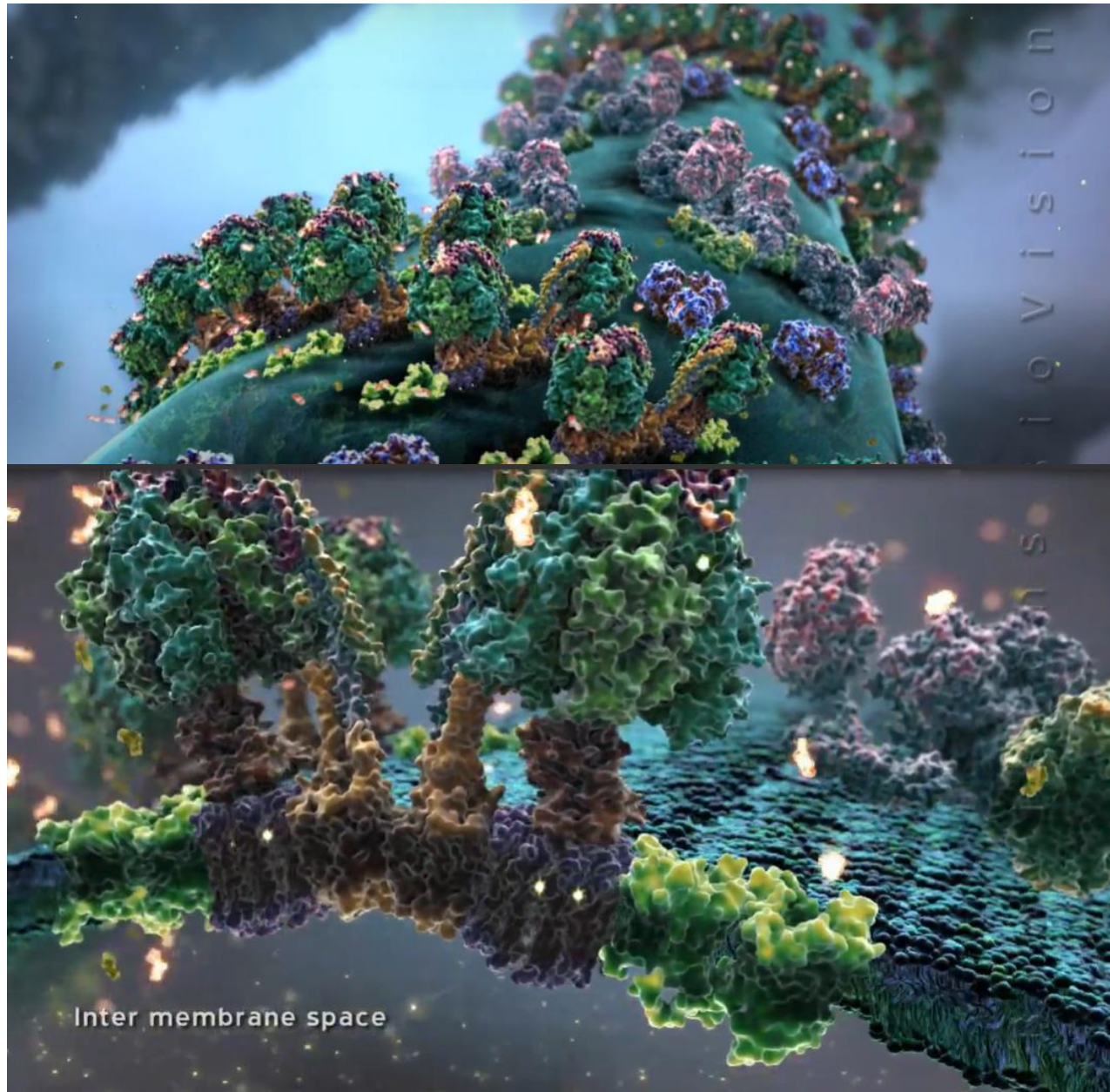
Mitochondria: Powering our cells



Biovisions Powering the Cell, Mitochondria, biovisions.mcb.harvard.edu
<https://www.youtube.com/watch?v=RrS2uROUjk4>

BioVisions
at Harvard University

The electron transport chain (ETC) – 5 complexes



What pathways may have become compromised?

Macronutrients

Are macronutrient fuels accessing the cell sufficiently?

Are the fuels the most efficient for generating mitochondrial energy?

Membranes

Are the cellular membranes of the right composition/sufficiently intact?

Any blockages in the membranes?

Micronutrients/cofactors

Correct substrates for the Krebs cycle?

Are the mitochondria able to metabolise the substrates? If not, why not?

Do the complexes of the electron transport chain have the right substrates?

Oxidative stress

High ROS within the cell?

High ROS within the mitochondria?

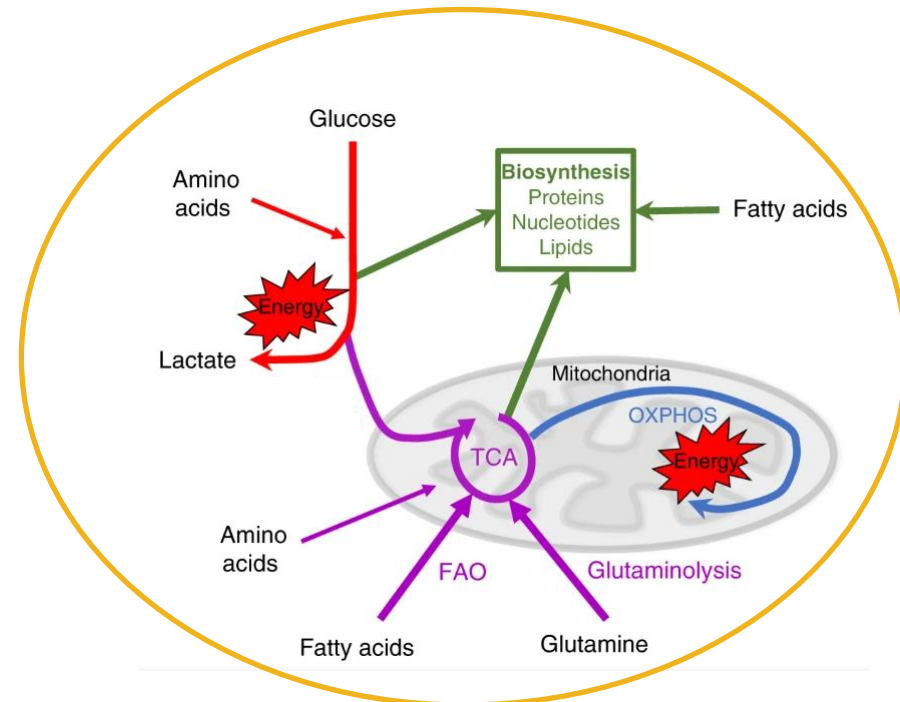
Mitochondrial mass/composition

Insufficient numbers of mitochondria, or non-intact?

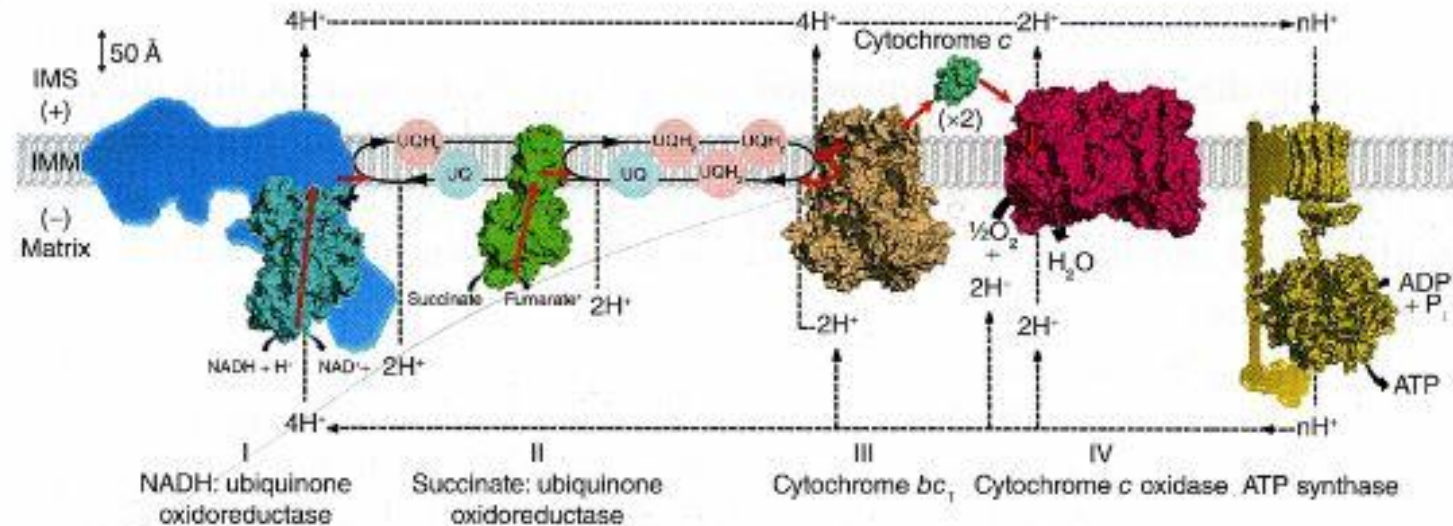
Obstacles along the electron transport chain

Blocks?

Proton leak?



Five complexes along the electron transport chain



Complex 1

NADH

- FMN from B2
- Iron
- Sulphur
- NADH from B3
- CoQ

Complex 2

Succinate Dehydrogenase

- FAD from B2
- Iron
- Sulphur
- CoQ
- Hydroxocobalamin to activate succinate/heme synthesis
- DHA to form cardiolipin

Complex 3

Cytochrome reductase

- Sulphur
- Iron
- CoQ
- B1 to form Coenzyme A

Complex 4

Cytochrome oxidase

- Copper
- Iron in cytochrome A

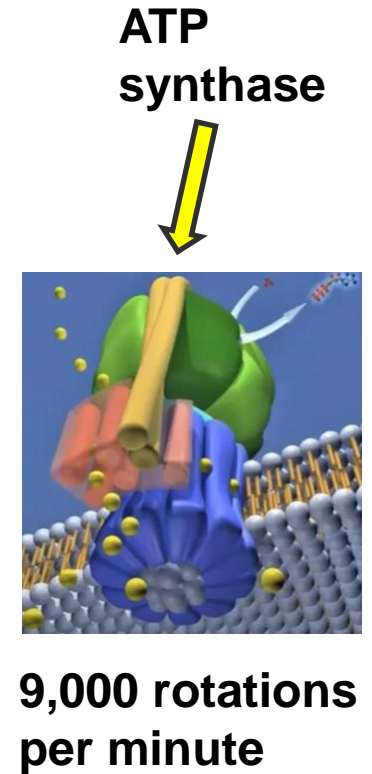
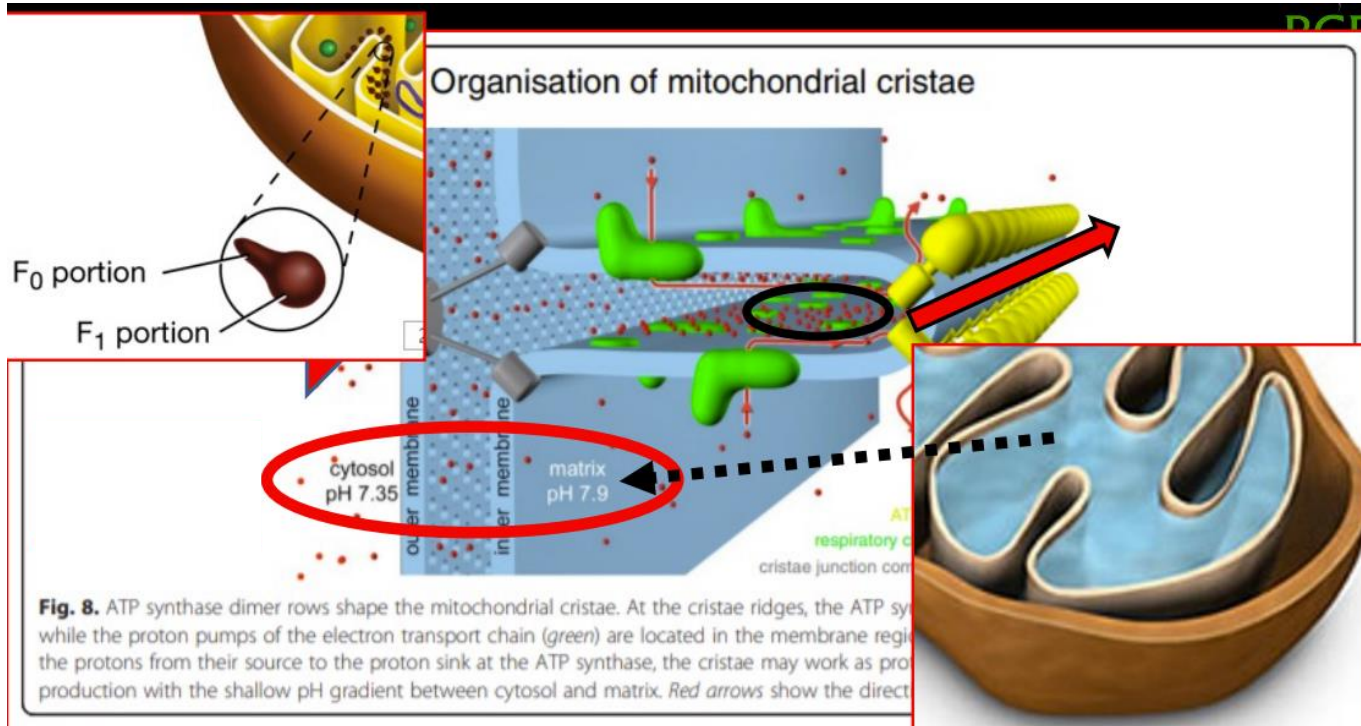
Complex 5

ATP synthase

- Mg

Source: Rich, R.P., Marechal, A., "The mitochondrial chain", in "Essays in Biochemistry: Mitochondrial Function", by Brown, G.C. et al., Portland Press Ltd., 2010, London, with permission; Vasquez, A., Mitochondrial nutrition and mitochondrial medicine for primary care conditions

ATP synthase is hugely delicate ...



✓ there are ~8000 ATP synthase nanomotors in each side of a single crypt of the mitochondrial matrix

Nanomotors/mitochondria

✓ on average, both bends of 20 membranes give 320000 nanomotors/mitochondria

Production of metabolic water in the mitochondria



László G. Boros: Metabolic protonation of matrix water entails deuterium depletion...



Watch later



Share

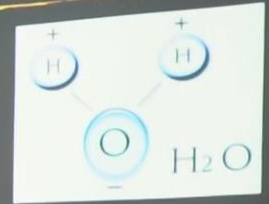
Water coequal of protons

✓ 4.83×10^{29} protons would yield 2.42×10^{29} water molecules

✓ $2.42 \times 10^{29} / 6.022 \times 10^{23} = 401727$ mol water

✓ $401727 \text{ mol} \times 18 \text{ gr} = 7231086 \text{ gr water}$

✓ Roughly 7231 liter (1910 gallons) water worth of protons are pumped per day



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NOVOTEL BUDAPEST DANUBE

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Mute (m)



14:20 / 55:08



YouTube

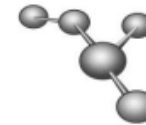


Mitochondrial testing with AONM/MMD

- 1. Brief introduction to the mitochondria**
- 2. ATP Profile: Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve Capacity**
- 3. 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
- 4. Supplementary biomarkers:**
Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1 α
Nrf-2
Mitochondrial 4977 deletion mutant (mt4977del)
Lactate/pyruvate ratio
Mitochondrial Fuel Pathways
OxPhos

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
Sample type: CPDA vacutainer
Results status: **Final report**
Validated by: Prof. Dr. Brigitte König
Cell type: PBMC
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
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Reference range mitochondrial ATP capacity

fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4
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Reference range glycolytic ATP capacity

fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4
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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
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Total ATP

ATP profile

Test	Result	Unit	Reference range	Result [%]
Total ATP	0.8	fmol/cell		

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

Total ATP

This is the quantity of ATP that the cells produce at rest via both mitochondrial and non-mitochondrial pathways. Total ATP is all the adenosine triphosphate (our cells' energy currency) available to the cell. This makes it possible to assess the relative performance of mitochondrial respiration (mitochondrial ATP capacity) versus anaerobic glycolysis (glycolytic ATP capacity).

Mitochondrial and glycolytic ATP capacity

Test	Result	Unit	Reference range	Result [%]
Mitochondrial ATP capacity	0.4	fmol/cell		50

Reference range mitochondrial ATP capacity

fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4

Mitochondrial ATP capacity measures the capacity to synthesise adenosine triphosphate (ATP) in the patient's mitochondria in a defined basal state. This is calculated by determining the absolute ATP production that is inhibited by addition of the ATP synthase inhibitor oligomycin (see figure above).

Glycolytic ATP capacity	0.5	fmol/cell		63
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Reference range glycolytic ATP capacity

fmol/cell	<0.8	0.8 - 1.0	1.0 - 1.2	1.2 - 1.4	>1.4

ATP can also be produced in the cytosol, outside the mitochondria (though still inside the cell). This parameter measures the glycolytic capacity for ATP production: the maximum quantity of ATP that the cells are able to produce at rest via non-mitochondrial pathways, i.e. anaerobic glycolysis. This makes it possible to assess the relative performance of anaerobic glycolysis versus mitochondrial respiration. It is important to have a high glycolytic capacity in the cells so that sufficient precursors for the Krebs Cycle can be made to then be cycled into the ETC, and also so that the cytosolic production of ATP (glycolysis) can be upregulated if needed, when immune cells need to address pathogens, etc.

Reserve ATP capacity

ATP profile									
Test	Result	Unit	Reference range	Result [%]					
Reserve ATP capacity	0.10	fmol/cell		13					
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

ATP synthesis is generally presumed to be coupled almost entirely to two metabolic processes: oxidative phosphorylation and glycolysis. There is however another essential metabolic process that interconverts the three adenine nucleotides (ATP, ADP and AMP) using adenylate kinase according to metabolic needs. Adenylate kinase catalyses a reversible reaction: $2 \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$. This is a vital factor in regulating the energy charge in cells, providing an open system able to accept, store and supply energy to cells as needed. The marker “Reserve ATP capacity” indicates how dynamically the cell is able to perform this catalytic interconversion.

Here, the reserve ATP capacity is 13 %/ 0.10 fmol/ cell. The patient's result is in the very low range. The optimal would be between 0.6 to 0.9 fmol/cell. 13 % means that the cell is unable to perform dynamic catalytic interconversion between the three adenine nucleotides (ATP, ADP and AMP) according to metabolic needs.

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3. **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
4. **Supplementary biomarkers (next time!):**
 Ratio of mtDNA to nDNA (mtDNA:nDNA)
 PGC-1 α
 Nrf-2
 Mitochondrial 4977 deletion mutant (mt4977del)
 Lactate/pyruvate ratio
 Phase 2:
 Number of mitochondria
 Intact mitochondria versus Non-intact mitochondria

Mitochondrial Health Index

The Mitochondrial Health Index (MHI) is an index composed of all the parameters below, based on the science developed at the University of Alabama that went into the evolution of this metric and the Seahorse XF measurements. It can be used to measure improvement in mitochondrial function, and to help identify where the block to optimal functioning might lie.

- Basal respiration rate
- Mitochondrial ATP turnover
- Proton leak
- Max. respiration rate
- Reserve capacity
- Non-mitochondrial respiration rate
- Calculation of the overall MHI

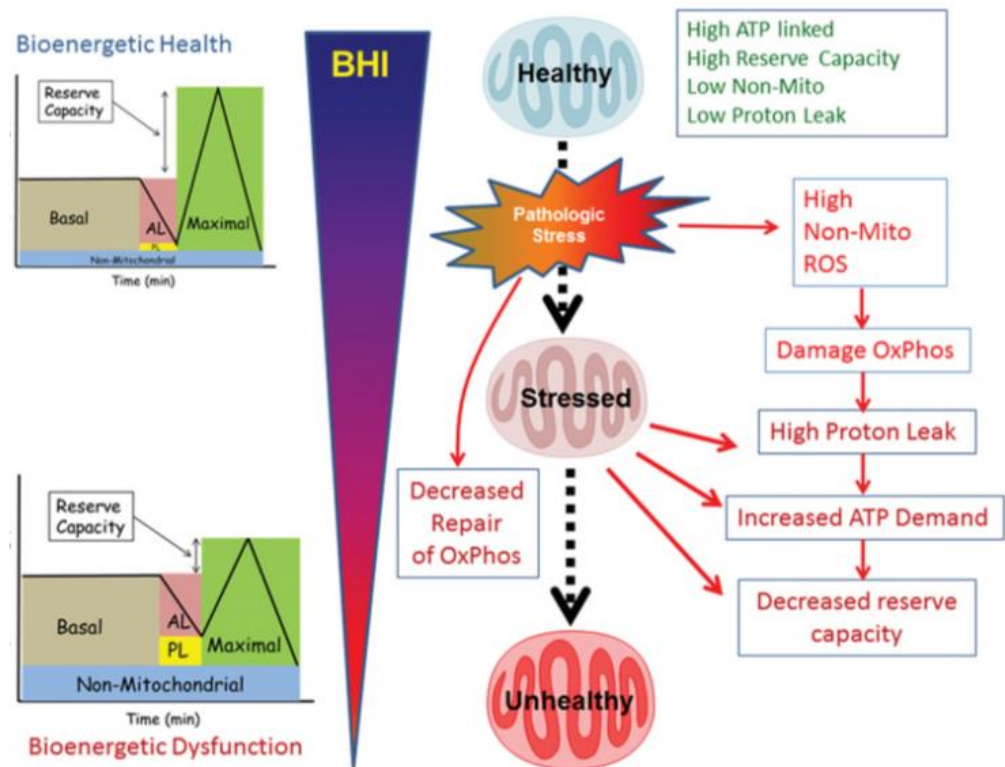


Figure 2: BMI as a dynamic measure of the response of the body to stress.

Mitochondrial Health Index: top page

Requisition: Mitochondrial Health Index / PBMCS

Sample type: Blood in CPDA vials

Summary

	Patient's value	Target value (optimal)
Mitochondrial Health Index (MHI)	0.00	>2.5
Mitochondrial Bioenergetics		
Coupling efficiency, %	86	90-95
Reserve respiration capacity, %	0	>400
Cellular oxygen consumption profile		
Non-mitochondrial respiration as a share of total respiration, %	32	<10
Proton leak as a share of total respiration, %	10	5-10
Share of respiration used for mitochondrial ATP generation, %	58	>90
ATP turnover rate (mitochondrial oxygen utilisation)		
ATP base turnover, %	100	<20
ATP reserve, %	0	>80
Basal oxygen consumption rate in pmol oxygen/min	28.75	
Potential maximum oxygen consumption rate in pmol oxygen/min	22	>500
Cellular energy phenotype		
At rest	Resting	Resting
On energy demand	Resting	Energetic/Aerobic
Metabolic potential, mitochondrial percentage	84	>350
Metabolic potential, glycolysis percentage	151	>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
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Overall MHI, derived from the multiple parameters

MITOCHONDRIAL HEALTH INDEX (MHI)

The MHI is a sensitive indicator of the reaction of immune cells (PBMCs) to oxidative stress, and for the changing metabolic programmes that they serve depending on the role they need to play in the case of inflammation, immune defence and immune health. The MHI is also an indicator for the current "health" of the cell. It is interactively composed of the following parameters.

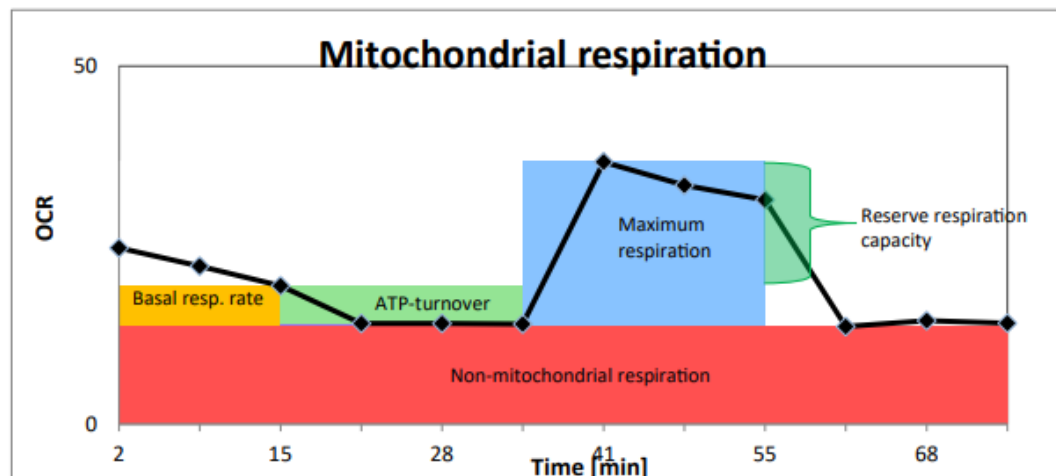
YOUR RESULTS

Mitochondrial Health Index (MHI)

Extremely low

Parameter	Evaluation	Reference values	Results
Mitochondrial Health Index (MHI)	Optimal	>2.5	
	Slightly low	2.0-2.5	
	Moderately low	1.5-2.0	
	Considerably low	1.0-1.5	
	Extremely low	<1.0	0.77

YOUR RESULTS PROFILE



Summary relating to mitochondrial dysfunction: selected markers

	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 functionally intact mitochondria		✓	No Yes	Acute inflammation, active chronic inflammation/ autoimmune disease	✓ No Yes

Further diagnostic opportunities for personalised therapy

Investigate minerals and further mitochondrial cofactors

Investigate mitochondrial mass (mtDNA:nDNA/number of mitochondria) and analyse mitochondrial mutations that influence ATP generation (e.g., the common deletion mt4977bp).

- **Upregulated ROS in the cells**
- **Compromised function of the electron transport chain**
- **Limited no. of functionally intact mitochondria**
- **Insufficient ATP on demand**

Proton leak/coupling efficiency

MITOCHONDRIAL BIOENERGETICS

<i>Coupling efficiency, %</i>	slightly low
<i>Reserve respiration capacity, %</i>	Extremely low

COUPLING EFFICIENCY

Coupling efficiency is a metric for the transformation of oxygen into the energy currency ATP. The cause of reduced coupling efficiency is a proton leak. A proton leak accounts for any oxygen in the mitochondria that is not being used for ATP synthesis. (see also p. 6, oxygen consumption profile).

YOUR RESULTS

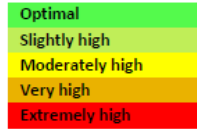
Parameter	Evaluation	Reference values in %	Result in %
<i>Coupling efficiency, %</i>	Considerably high	98-100	86
	Slightly enhanced	95-98	
	Optimal	90-95	
	Slightly low	85-90	
	Moderately low	80-85	
	Considerably low	70-80	
	Extremely low	<70	

Interpretation of your results:

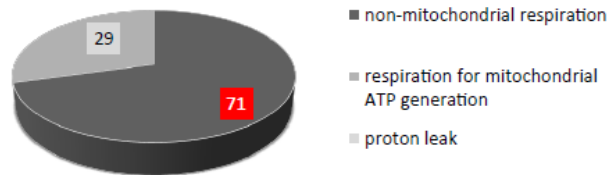
The coupling efficiency is slightly low.

A proton leak accounts for any oxygen in the mitochondria that is not being used for ATP synthesis. The causes of a proton leak are for example a) high concentration of damaging free radicals; b) a lack of redox equivalents; c) inhibitors of ATPases, including ATP synthase; d) a fatty acid composition of the mitochondria that is suboptimal.

Non-mitochondrial respiration



How the oxygen is being consumed, %



Extremely high non-mitochondrial respiration as a share of total respiration

Parameter	Evaluation	Reference values, %	Result, %
<i>Non-mitochondrial respiration as a share of total respiration, %</i>	Optimal	0-10	70.59
	Slightly high	10-20	
	Moderately high	20-30	
	Very high	30-50	
	Extremely high	>50	

Increased non-mitochondrial respiration can be due to intracellular pathogens such as EBV, Borrelia, etc. Significantly increased values can be caused by heavy metals, xenobiotics and other contaminants

Interpretation of your results

Your immune cells are using only 29% of the oxygen directly for generating mitochondrial energy. 71% of the oxygen is being used for non-mitochondrial processes. The non-mitochondrial oxygen consumption, independent of whether it is used for respiration at the surface of the cell and/or prooxidative processes, is having a negative effect on the MHI (see the MHI). No proton leak is detectable.

Recommendation

Investigate oxidised lipids, proteins, nuclear and mitochondrial DNA in the immune cells to assess the damage that has already occurred, and for targeted use of antioxidants.

Maximum possible oxygen consumption rate

Patient: Xxxx
 Date of birth: Xxxx
 Sample taken: 21.03.2018
 Receipt of sample: 26.03.2018
 Test completed: 26.03.2018
Final result
 Validated by: Prof. Dr. Brigitte König

Parameter	Evaluation	Reference values, pmol/min	Result, pmol/min
<i>Maximum possible oxygen consumption rate, pmol oxygen/min</i>	Optimal	>500	
	Slightly low	300-500	
	Moderately low	200-300	
	Very low	100-200	
	Extremely low	<100	22.99

Interpretation of your result:

Your immune cells are using 25.6 % of their possible oxygen consumption capacity for their base energy balance. This value is slightly high. This indicates a load on the immune cells that is disrupting cell regulation.

The maximum useable oxygen volume (in pmol oxygen/min) that can be converted into energy (ATP) by the mitochondria is 22.99 pmol/min. This potential oxygen consumption rate is, from an absolute perspective, considered to be extremely low. On energy demand, after subtraction of the basal cellular oxygen consumption (5.88 pmol/min) noch 17.32 pmol oxygen/min remaining for mitochondrial ATP generation. This means the absolute potential ATP turnover rate is extremely low.

Against the backdrop of the other results, several factors may be responsible for the non-optimal absolute potential ATP turnover rate, either alone or in combination: a) insufficient mitochondrial mass; b) the limited utilisation of fatty acids and particularly of glucose; c) insufficient provision of the immune cells with the requisite minerals, vitamins, etc.; d) a defective electron transport chain.

Further diagnostic options

Investigate the mitochondrial mass (mtDNA:nDNA, i.e. number of mitochondria), and analyse the mitochondrial mutations that are influencing ATP generation (e.g., common deletion mt4977bp; full sequencing).

Investigate the mitochondrial use of fatty acids and glucose as fuels.

Maximum possible oxygen consumption rate extremely low

Reserve respiration capacity very low in this patient

Summary

	Patient's value	Target value (optimal)
Mitochondrial Health Index (MHI)	0.00	>2.5
Mitochondrial Bioenergetics		
Coupling efficiency, %	86	90-95
Reserve respiration capacity, %	0	>400

RESERVE RESPIRATION CAPACITY

Reserve respiration capacity shows the extent to which the existing mitochondria can use further oxygen for generating energy. Low reserve respiration capacity can be due to a) insufficient utilisation of fuels (glucose, fatty acids); b) high resting metabolism due to ROS and RNS; c) non-intact complexes of the electron transport chain; d) altering metabolic status due for example to the immune cells adapting their role as a result of infection (viral, bacterial), anti-tumour immune responses, autoimmune disease, etc.

YOUR RESULTS

Parameter	Evaluation	Reference values in %	Result in %
<i>Reserve respiration capacity, %</i>	Optimal	>400	
	Slightly low	300-400	
	Moderately low	250-300	
	Considerably low	200-250	
	Extremely low	<200	0

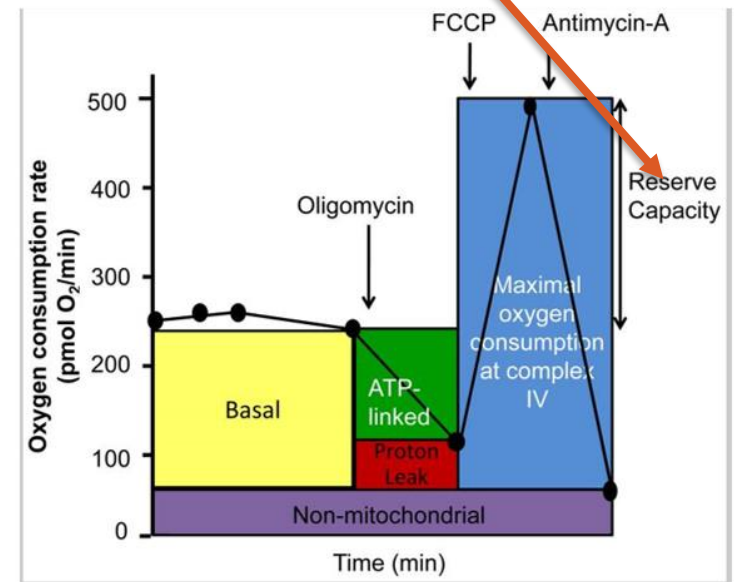


Figure 3: The cellular bioenergetic profile

Comparison of various tests

Patient: xxxxxxxx
 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	

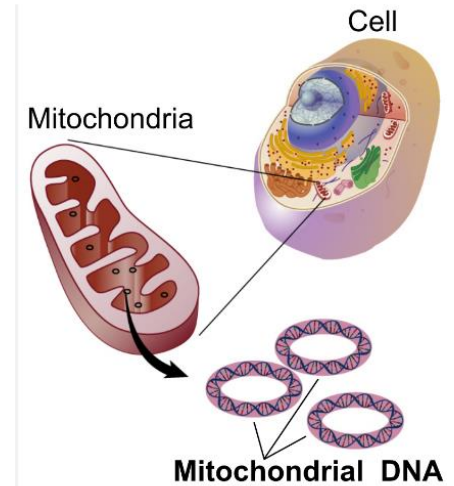
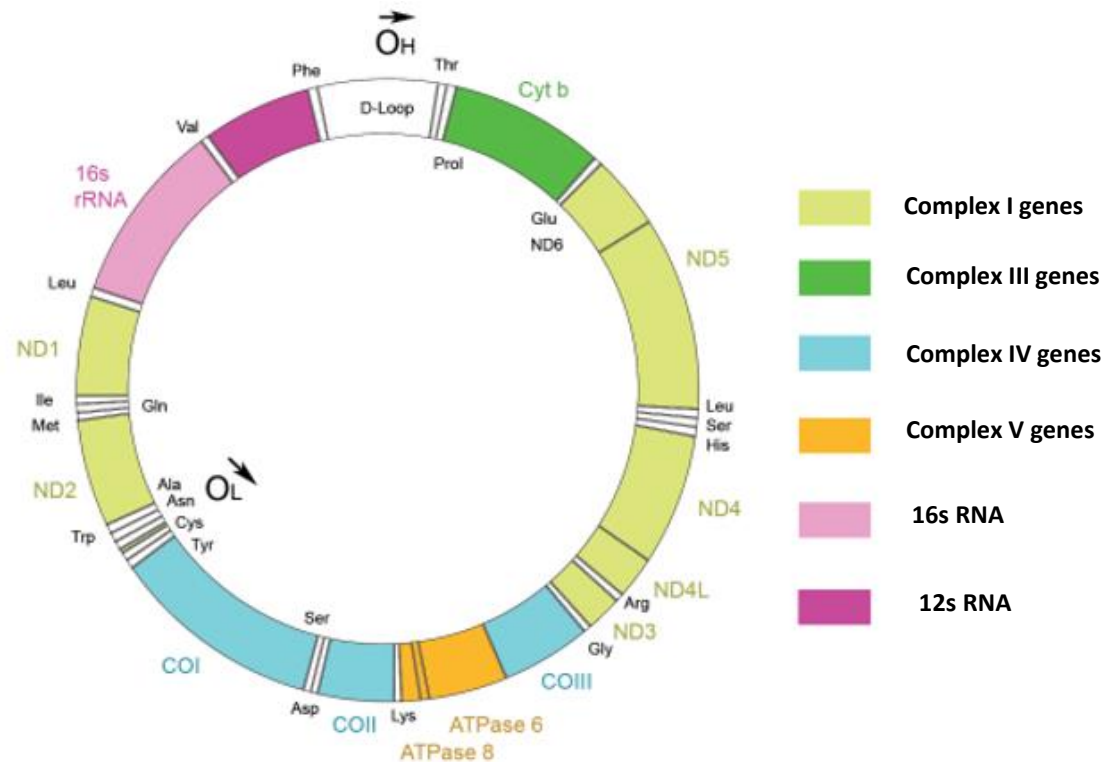
Maximum possible oxygen consumption rate has doubled; many markers are showing improvement

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Ratio of mtDNA to nDNA (mtDNA:nDNA)
PGC-1 α
Nrf-2
Mitochondrial 4977 deletion mutant (mt4977del)
Lactate/pyruvate ratio

Mitochondria have their own DNA

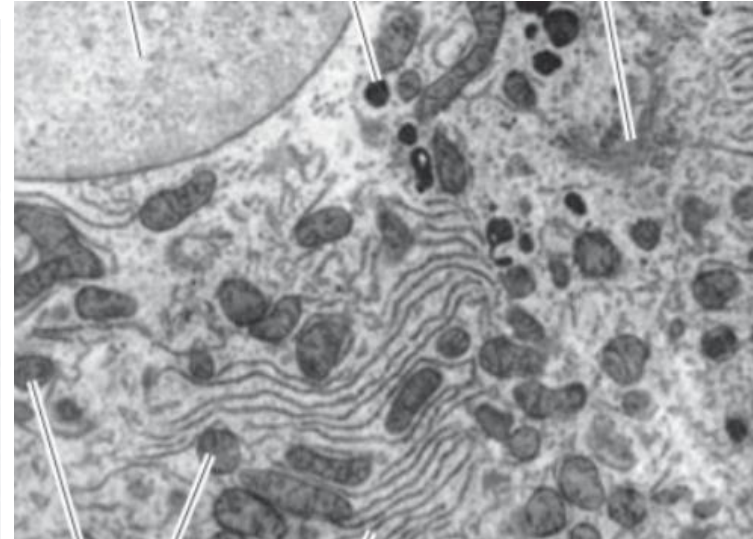
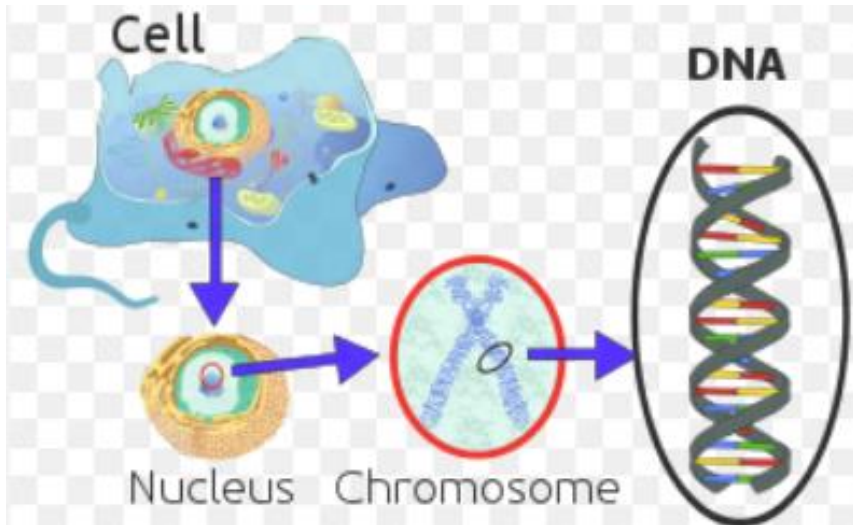
mtDNA:nDNA



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

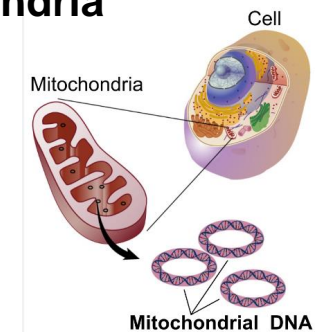
It is possible to compare nuclear DNA to sets of mitochondrial DNA per cell: one to many

mtDNA:nDNA



The cell nucleus has only one copy of DNA

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



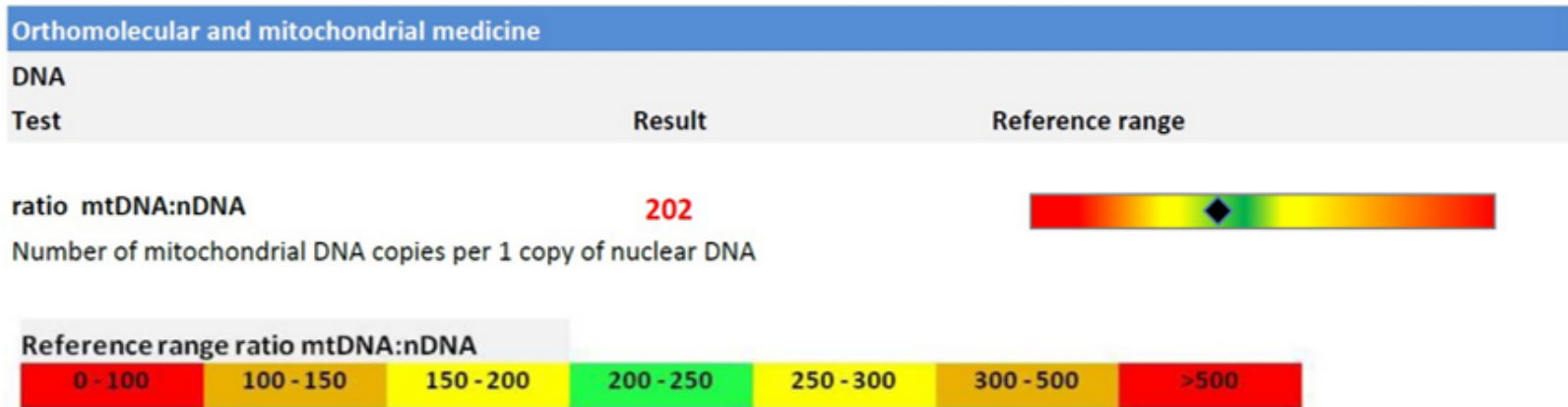
Ratio of mitochondrial DNA to nuclear DNA shows the mitochondrial mass in the cell

DNA tests:

Ratio of mitochondrial DNA to nuclear DNA

mtDNA:nDNA

Example 1:



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

Example 2:

Ratio mtDNA:nDNA **1039**
Number of mitochondrial DNA copies per 1 copy of nuclear DNA

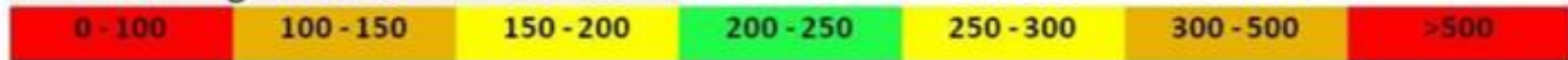


Example 3:

ratio mtDNA:nDNA **115**
Number of mitochondrial DNA copies per 1 copy of nuclear DNA



Reference range ratio mtDNA:nDNA



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

PGC-1-alpha

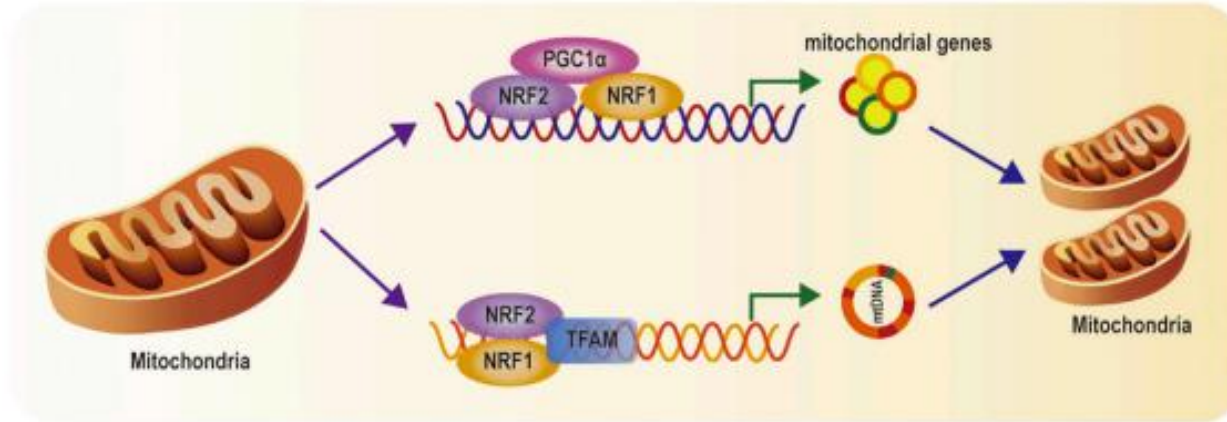


FIGURE 2 | Mitochondrial biogenesis pathways: When PGC-1 α is activated, PGC-1 α activates NRF1 and NRF2, and subsequently TFAM, which regulate genes involved in subunits of mitochondrial respiratory chain complexes, import of nuclear-encoded mitochondrial proteins, and mtDNA replication and transcription.

1

- PGC-1 α 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-1 α .
- PGC-1 α -mediated regulation of mitochondrial quality may affect age-related mitochondrial dysfunction and insulin sensitivity.

2

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

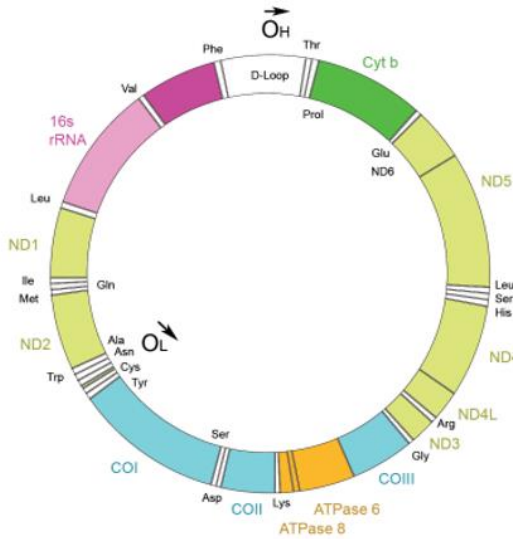
Interpretation: "Basic values of the peripheral blood leucocytes"

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 (*list of inducers available*)

The “common deletion” mDNA⁴⁹⁷⁷ is caused by oxidative stress

Oxidative stress



Nucleic Acids Research

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Volume 37, Issue 8
1 May 2009

Article Contents

- Abstract
- INTRODUCTION
- MATERIALS AND METHODS
- RESULTS
- DISCUSSION
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- ACKNOWLEDGEMENTS
- REFERENCES

JOURNAL ARTICLE
Oxidative stress induces degradation of mitochondrial DNA
Inna Shokolenko, Natalia Venediktova, Alexandra Bochkareva, Glenn L. Wilson, Mikhail F. Alexeyev

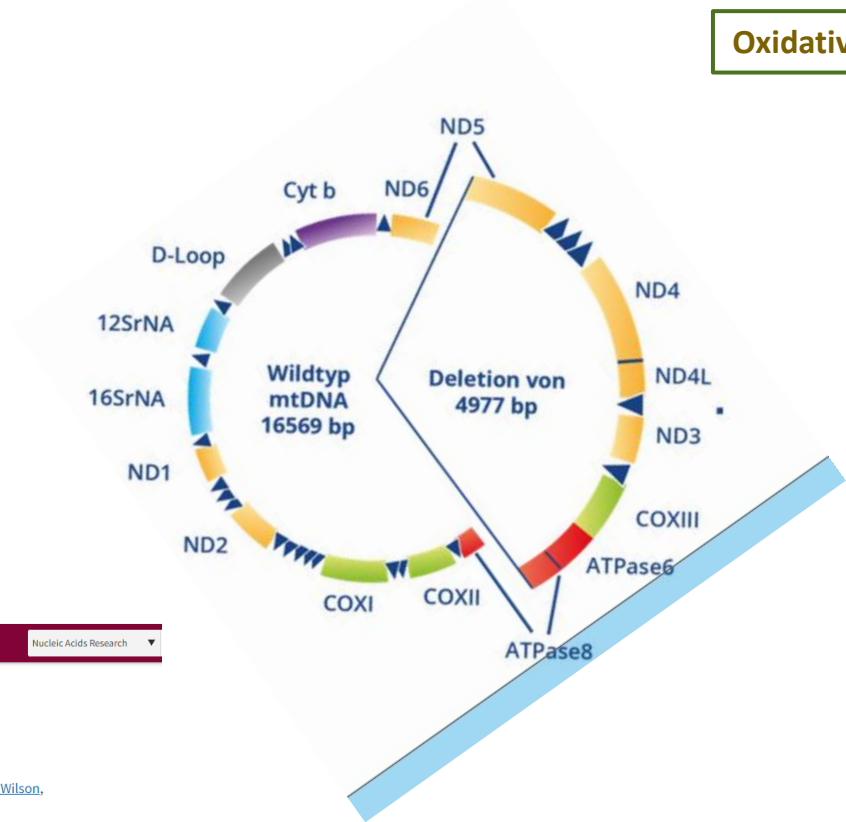
Nucleic Acids Research, Volume 37, Issue 8, 1 May 2009, Pages 2539–2548,
<https://doi.org/10.1093/nar/gkp100>

Published: 05 March 2009 Article history

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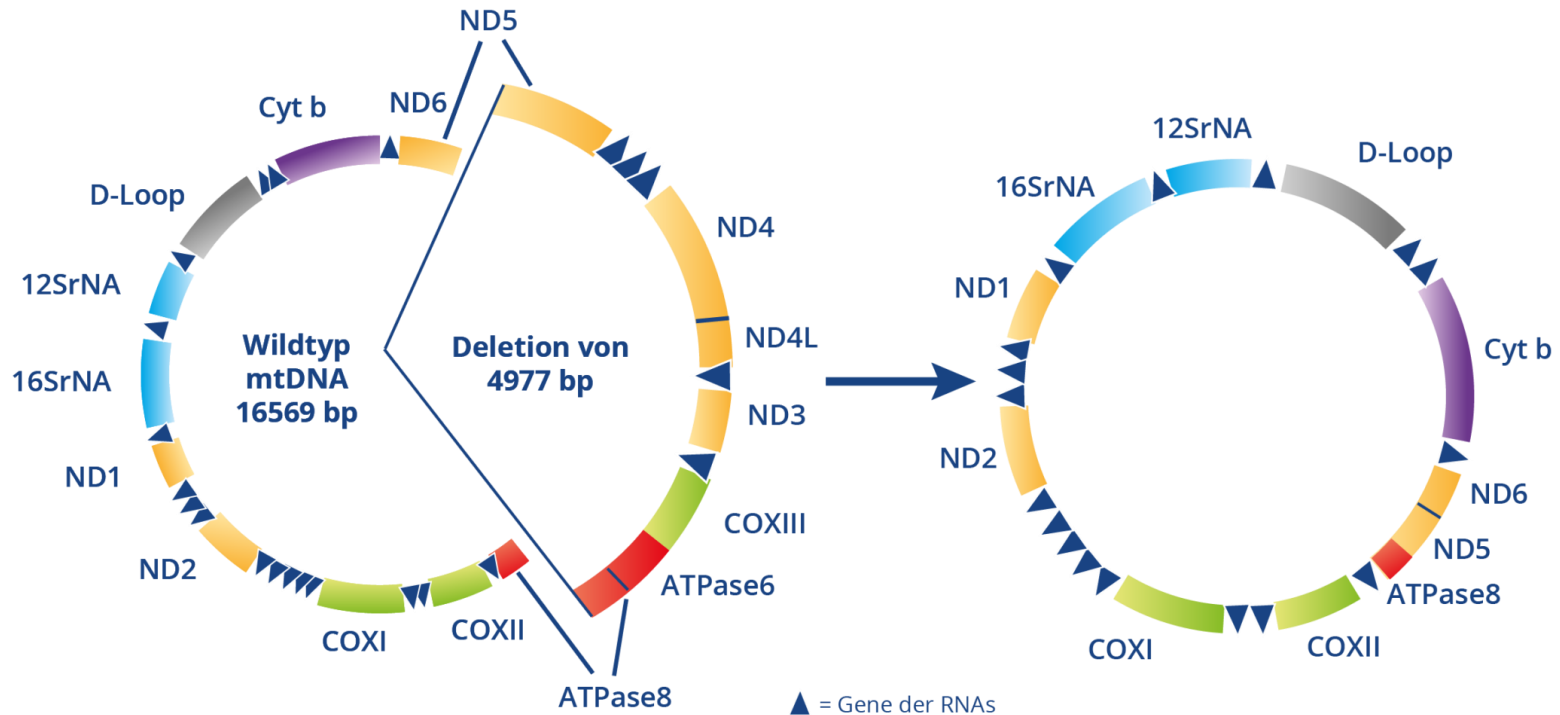
Abstract

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



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

Oxidative stress



Before deletion
Wildtyp mtDNA = 16569 base pairs

After deletion
mtDNA = 11562 base pairs

... as well as any damage to mitochondrial DNA

Deletion mutant 4977

Oxidative stress

Example 1:

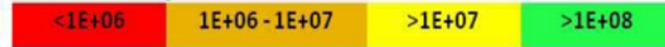
Mitochondrial 4977 Deletion mutant
(mt4977del)

1.03E+06



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

Reference range mt4977del



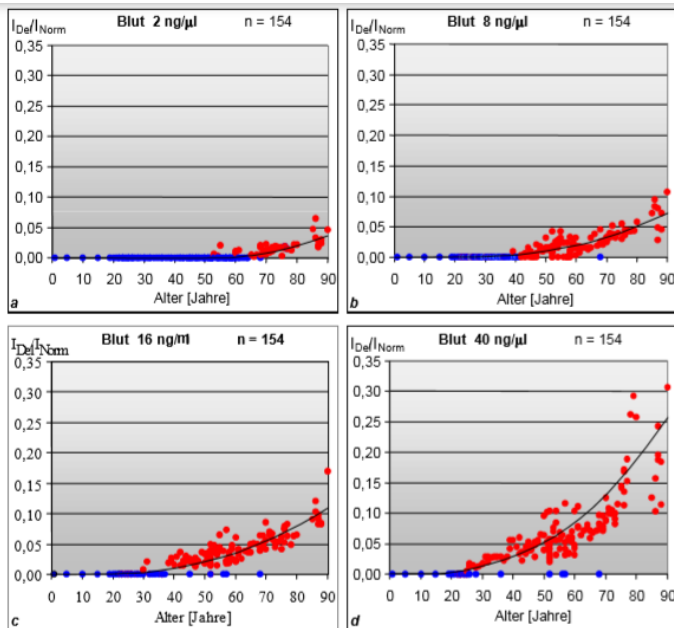
The mitochondrial deletion mutant mt4977bp is noticeably enhanced. This indicates oxidative stress and damage to mitochondrial DNA.

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 (mDNA⁴⁹⁷⁷). 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
--------	---------------	--------	--------

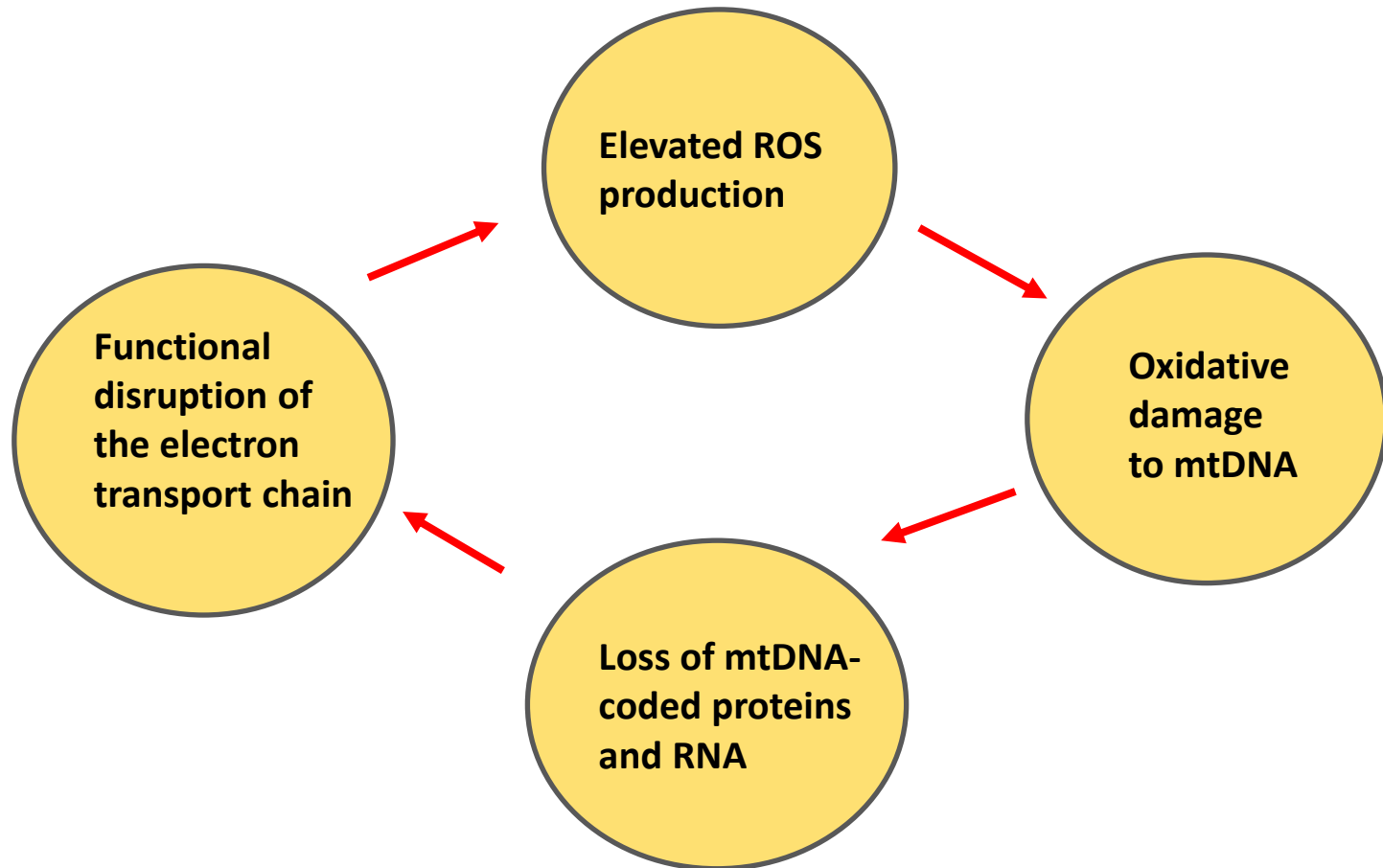
The mitochondrial deletion mutant mt4977bp is detectable at a greatly increased level. This indicates mitochondria with a lack of ability to generate ATP and greatly increased oxidative stress.



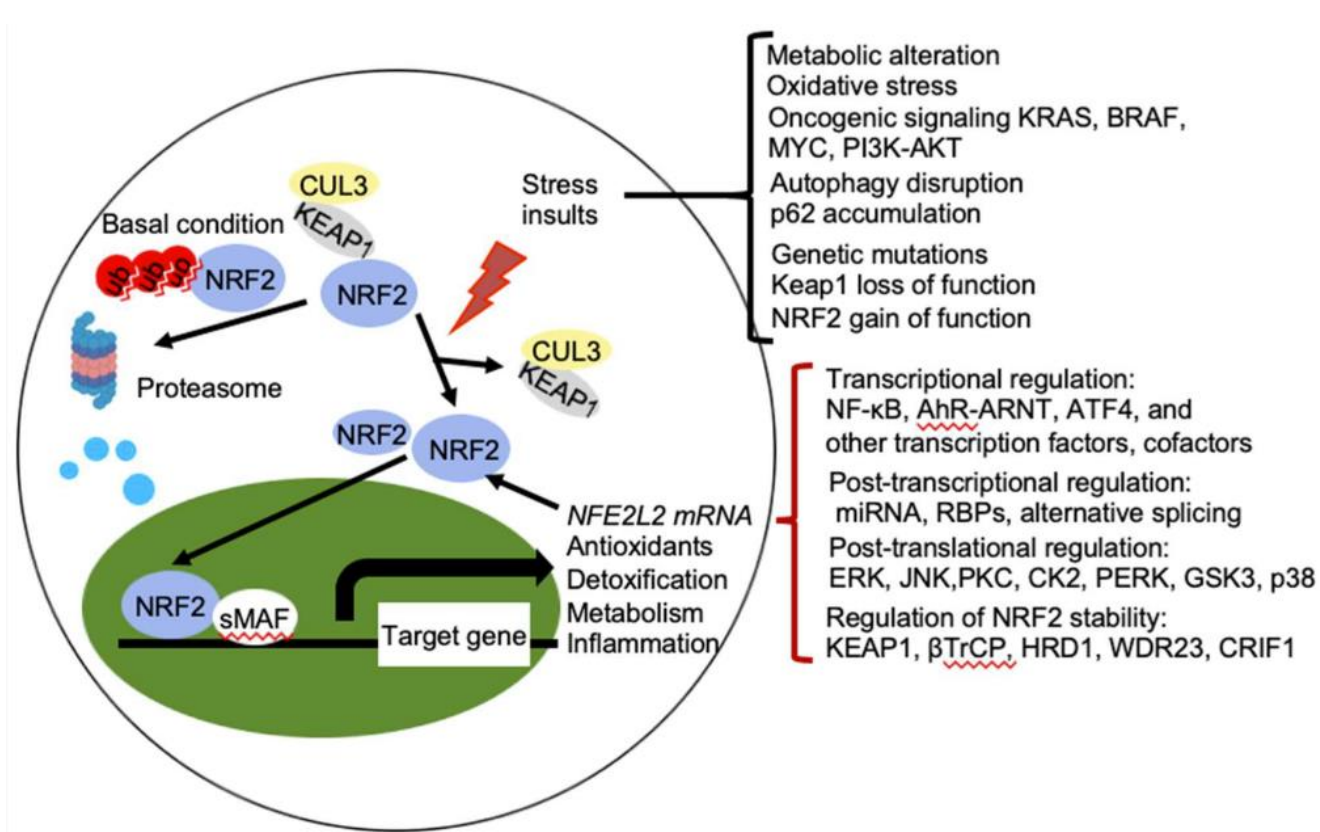
This is not an inherited polymorphism: it arises due to endogenous and exogenous factors, especially oxidative stress. This is why checking for it can be very useful, as measures can be taken to reduce the levels, and repeat tests document a decline in levels if the initiatives are successful.

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

Oxidative stress



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”

Problem if it is undetectable and you have evident oxidative stress

RESULTS

Nrf-2

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

	<u>Interpretation</u>				
	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

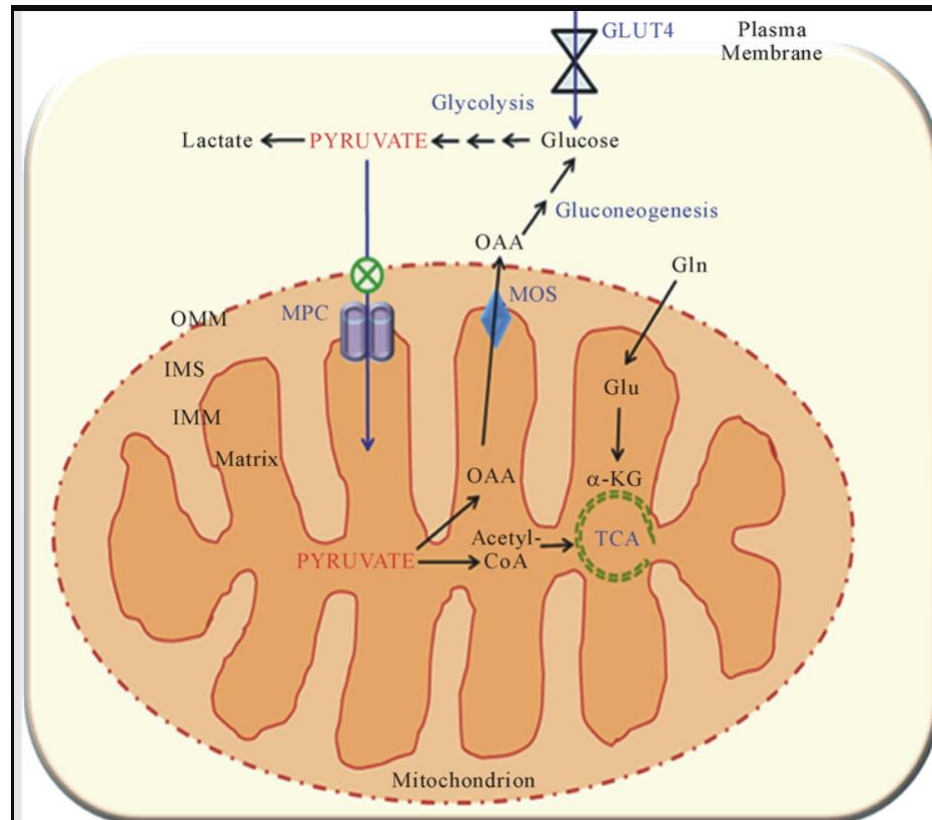
This test of mitochondrial oxidation levels can also be done as a fingerprick test



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.

Lactate/pyruvate ratio Plus: shows what nutrients are being used as fuel for the mitochondria

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

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.

Lactate/pyruvate ratio Plus: shows what macronutrients are being used as fuel for the mitochondria

Cell type:

Peripheral blood mononuclear cells (PBMC)

Lactate/Pyruvate 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

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).

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.
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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.

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.

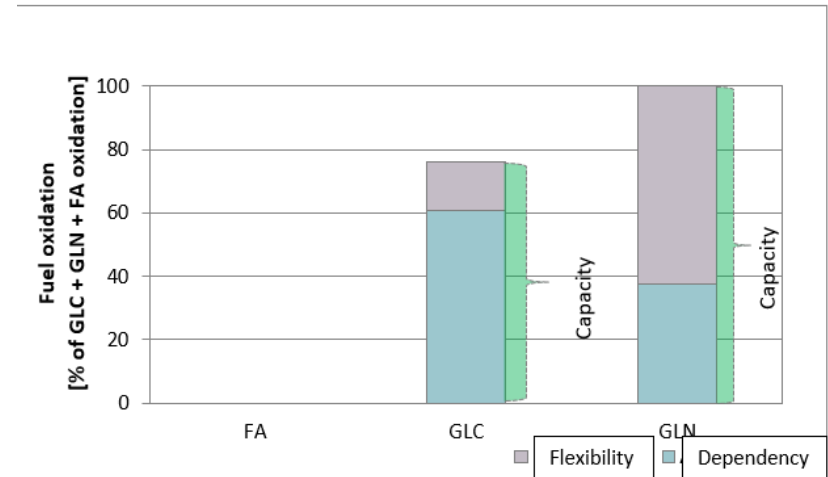
- So, ideally, the PBMCs should not show much dependence on any one fuel.

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%.



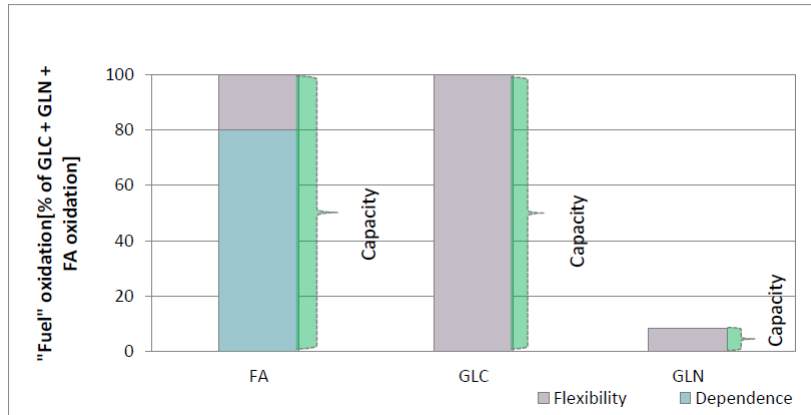
A: fatty acids; GLC: glucose; GLN: glutamine (amino acids)

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



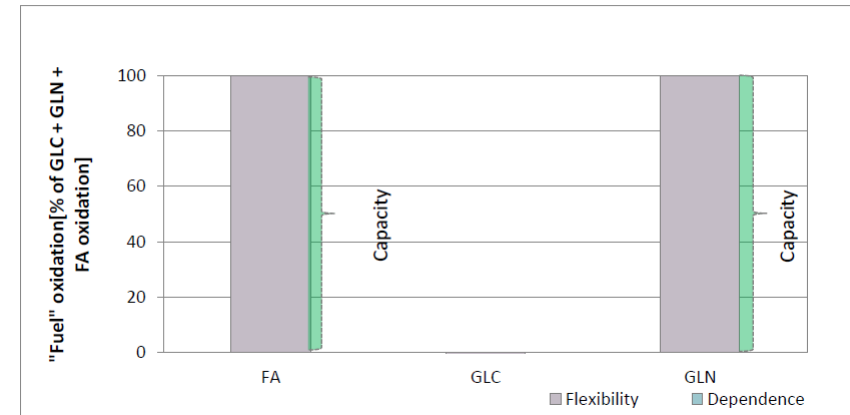
FA: Fatty acids; GLC: Glucose; GLN: Glutamine (Amino acids)

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.

Summary also possible (1/2)

May 2024 – Mitochondrial Tests – MMD lab., Germany.

PBMC (Peripheral Blood Mononuclear Cells) tested ^[a]

Test	Result (optimal range)
MHI (Mitochondria Health Index) <i>Mitochondrial oxygen utilisation:</i> 1a) Baseline oxygen consumption rate 1b) Maximum oxygen consumption rate 1c) Reserve respiration capacity <i>Oxygen consumption profile:</i> 2a) Oxygen used for mitochondria ATP gen. 2b) Oxygen used for non-mitochondria resp. 2c) Proton leak 3) Coupling efficiency <i>Cellular energy:</i> 4a) Base setting of immune cells 4b) Metabolic potential – mitochondria 4c) Metabolic potential – glycolysis	0.2 (>2.5), extremely low <1.0 38 pmol/min (?) 47 pmol/min (>300), extremely low <100 +25% (> 400%), extremely low <200 59% (>90%) 29% (<10%), moderately high 20-30 12% (5-10%) 83% (90-95%), moderately low 80-85 Resting (resting) 117% (>350%), extremely low <200 265% (>350%), moderately low 250-300
Mitochondria DNA 5) Total number of mitochondria 6) Non-intact mitochondria 7) Mitochondria DNA damage (mt4977del)	212 (200-250), normal 200-250 [Very low?] [Moderately - Very high?]
Lactate-pyruvate index 8) Lactate/pyruvate in dormant cells 9) Lactate/pyruvate in activated cells	1.14 (0.7 – 1.0) 2.41 (0.7 – 1.0), 90% converted to lactate
Mitochondria RNA 10) PGC-1α 11) Nrf-2	0.00021 (~0.01000), very low 0.00099 (~0.01000), very low

Summary also possible (1/2)

Diagnoses options

Potential diagnosis options	Treatment / considerations
A) There is a threat/toxicity which is causing the mitochondria to limit their energy production:	If so, then:
<ul style="list-style-type: none">• <i>The mitochondria are being inactivated physically/directly (e.g. by parasite)</i>	Focus on removing the parasite / endotoxin. Further testing.
<ul style="list-style-type: none">• <i>The mitochondria are reacting 'correctly' to an actual or perceived threat in their immediate environment (e.g. viral infection, or immune response) and are down-regulating into a hibernation state.</i>	<p>Focus on identifying the threat (virus, bacteria, immune response, toxins, etc).</p> <p>Low Nrf-2: could be a successful strategy (oxidating the cell to fight off virus), <u>or</u> it could be a failure in the antioxidant system.</p> <p>Low PGC-1α: could be a successful strategy (new mitochondria could react to the environment in the same way, so no benefit) <u>or</u> it could be a failure in the biogenesis/mitophagy system.</p>
<ul style="list-style-type: none">• <i>The mitochondria are incorrectly 'stuck' in their current state – even after the external threat has passed.</i>	It may be helpful to try to 'kick-start' the mitochondria? Try Nrf-2 up-regulators (e.g. <u>Tisso</u> herbal supplements).

(Etcetera)

MITOCHONDRIAL TESTS

PATIENT INFORMATION		BARCODE (Lab use only)	Please send results to: <input type="checkbox"/> myself <input type="checkbox"/> my practitioner	
Patient FIRST NAME*:			ORDERING DR/PRACTITIONER INFORMATION	
Patient SURNAME*:		Dr. / Practitioner name:		
DATE OF BIRTH (DD/MM/YYYY)*:		Time of Blood Draw*:		Clinic:
Sex assigned at birth* (please circle): male female		Date of blood draw (DD/MM)*:		Street Address:
Street Address:		Material/Quantity <input type="checkbox"/> CPDA		Postcode: City:
Postcode:	City:	County: Country:		
County:	Country:	Tel no:		
Tel no:		Email:		
Email*:		AONM HELPLINE: +44 (0) 3331 210 305		

<input checked="" type="checkbox"/>	#TEST NUMBER	NAME OF TEST		MATERIAL	PRICE
<input type="checkbox"/>	M1	ATP Profile:	Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve ATP	CPDA x1	£125
<input type="checkbox"/>	M2	Mitochondrial Health Index (MHI):	Basal respiration rate, mitochondrial ATP turnover, proton leak, maximum respiration rate, reserve capacity, non-mitochondrial rate, calculation of the overall Mitochondrial Health Index	CPDA x1	£195
<input type="checkbox"/>	M3 (M1+M2)	Combination of ATP Profile and MHI (M1 & M2)		CPDA x2	£285

SUPPLEMENTARY BIOMARKERS ON REQUEST (can normally only be done along with the ATP Profile and/or MHI)

<input type="checkbox"/>	M4	Ratio of mtDNA to nDNA		1 additional CPDA (max. 2)	£70
<input type="checkbox"/>	M5	PGC-1 α		1 additional CPDA (max. 2)	£50
<input type="checkbox"/>	M6	Nrf-2		1 additional CPDA (max. 2)	£50
<input type="checkbox"/>	M7 (M4+M5+M6)	Combination of Ratio of mtDNA to nDNA, PGC-1 α , and Nrf-2 (M4, M5, M6)		1 additional CPDA (max. 2)	£135
<input type="checkbox"/>	M8	Lactate/pyruvate ratio (must be ordered at same time as MHI)		1 additional CPDA (max. 2)	£70
<input type="checkbox"/>	M9	Mitochondrial 4977 deletion mutant (mt4977del)		1 additional CPDA (max. 2)	£70
<input type="checkbox"/>	M10 (M3+M7+M8+M9)	Combination of all above (M3, M7, M8, M9)		CPDA x2	£485
<input type="checkbox"/>	M11	Intact vs. non-intact mitochondria (must be ordered at same time as MHI + M4 + M9)		CPDA x2	£25
<input type="checkbox"/>	M12	Mitochondrial Fuel Pathways (must be ordered at same time as MHI + M4 + M9)		CPDA x2	£195

Add £50 for courier delivery (to send from UK). Please Request shipping prices from elsewhere.

Tests plus courier. Total:

Fingerprick tests also available



MITOCHONDRIAL TESTS USA 3 CAPILLARY BLOOD DRAW (FINGERPRICK)

PATIENT INFORMATION		BARCODE (Lab use only)	Please send results to: <input type="checkbox"/> myself <input type="checkbox"/> my practitioner	
Patient FIRST NAME*:			ORDERING DR/PRACTITIONER INFORMATION	
Patient SURNAME*:		Date of Fingerprick (DD/MM)*:	Dr. / Practitioner name:	
DATE OF BIRTH (DD/MM/YYYY)*:			Clinic:	
Sex* (please circle): male female		Please fill 2x rings for each test, up to a total of 9 rings for ALL tests.	Street Address:	
Street Address:			ZIP: _____ City: _____	
ZIP: _____	City: _____	AONM HELPLINE: +44 (0) 3331 210 305	County: _____ Country: _____	
County: _____	Country: _____		Tel no: _____	
Tel no: _____		Email: _____		
Email*:				

<input checked="" type="checkbox"/>	#Test number	Name of test	Price (individual tests)	Price (ALL)
<input type="checkbox"/>	MFP1	Oxidative stress measured using the mt4977 deletion mutant	£80	£352
<input type="checkbox"/>	M6	Nrf-2 (Master antioxidant regulator)	£50	
<input type="checkbox"/>	M15	8-OH-dG-DNA (a predominant ROS lesion)	£58	
<input type="checkbox"/>	MFP8	Telomere length	£149	
<input type="checkbox"/>	MFP7	Fingerprick Test Kit (incl. delivery to USA by Royal Mail)	£15	

Courier (shipping) costs to be determined. Tests plus courier. Total: _____

Tests of telomere length indicate a patient's biological (rather than chronological) age



Patient	
Geburtsdatum	28.11.1967
Probennahme	05.03.2024
Probeneingang	06.03.2024
Untersuchungsende	02.04.2024
Endbefund	02.04.2024
Validiert durch	Prof. Dr. Brigitte König
Ärztliche Leitung	Prof. Dr. Gerhard Jorch

MMD GmbH & Co. KG | Breiter Weg 10 a | 39104 Magdeburg

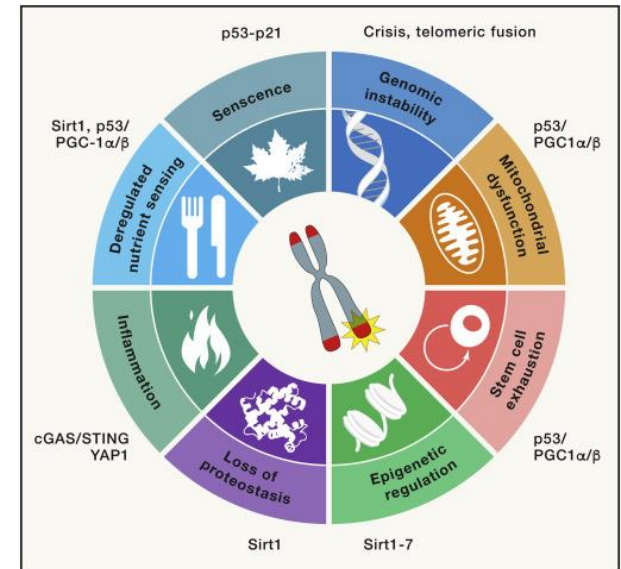
[Clinic]

RESULTS

Sample type: Whole blood Test requested: Telomere length

Relative telomere length (telomere to single copy gene ratio, T/S)	0.724
Absolute telomere length	7.23 kb
Chronological age	57
Age calculated by telomere length, i.e. biological age	33

Test results/interpretation: The calculated absolute telomere length (7.23 kilobases) corresponds to an age of between 30-35 years. The result means that the telomeres are longer than for 50% of people in the respective age group. This is a very encouraging result.



Relevance of telomere dysfunction for features of cellular aging

Many videos about the Seahorse technology available, and over 7,200 studies* for which the Seahorse has been used

HOW THE SEAHORSE XF WORKS

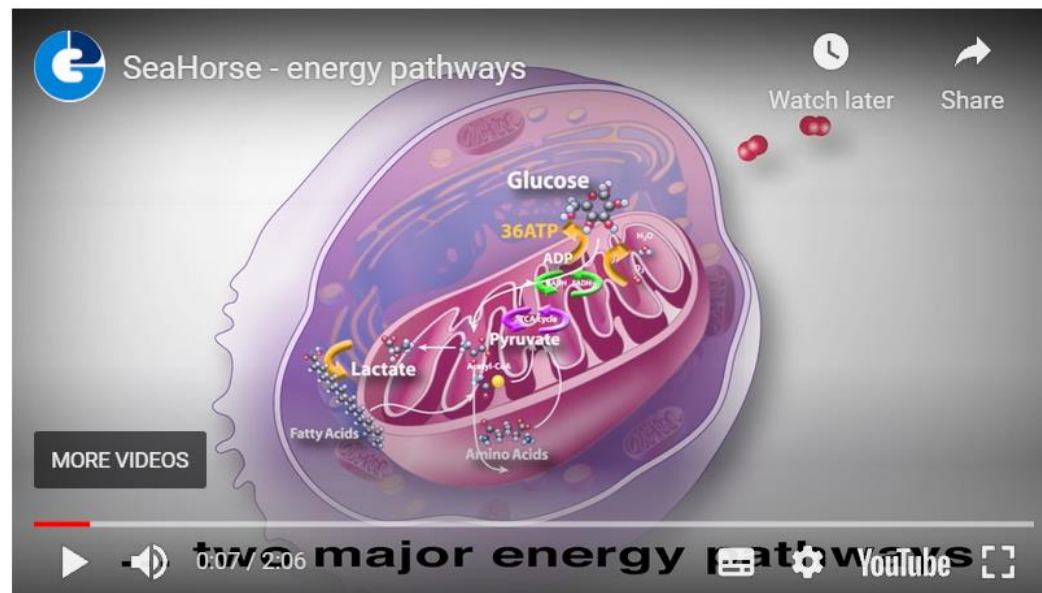


Videos are at the bottom of this page:

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

SEAHORSE: ENERGY PATHWAYS

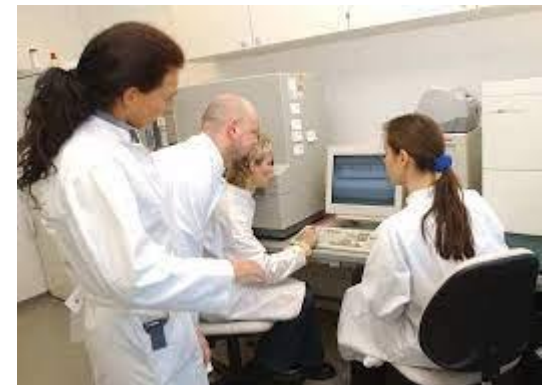
The tests require only one vial of blood in a CPDA[i] tube. The laboratory uses

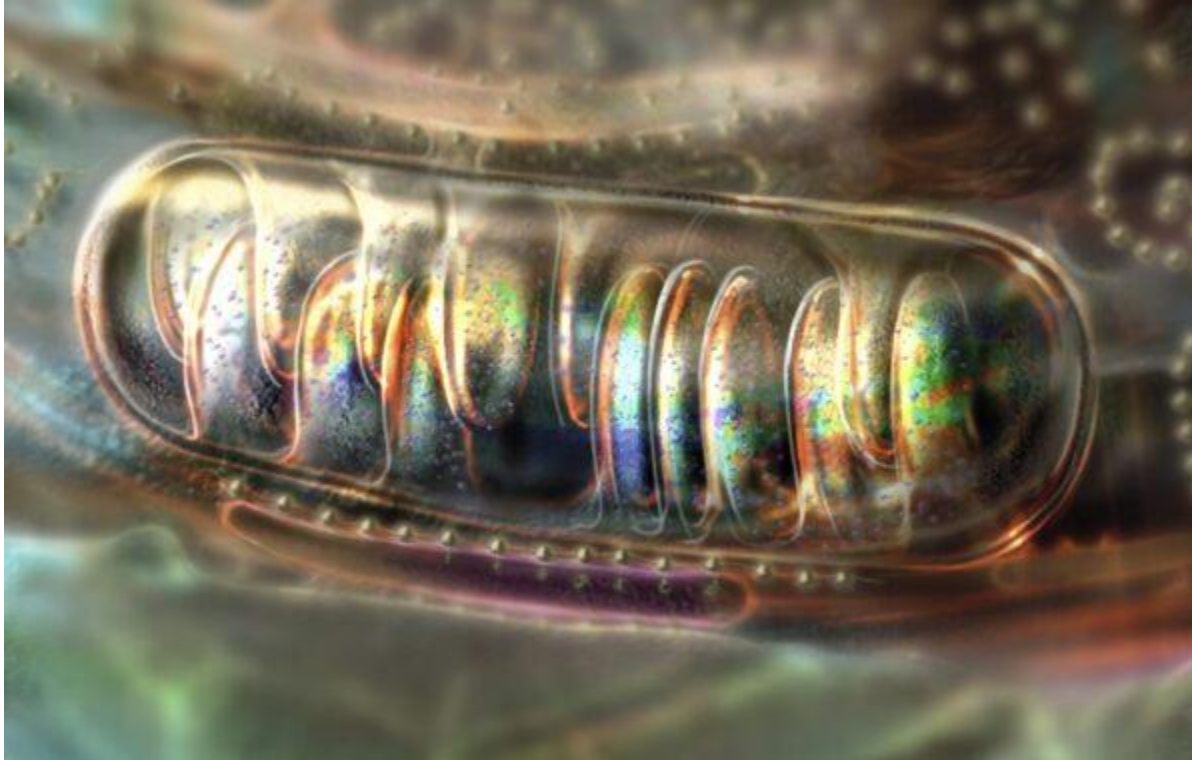


* <https://www.agilent.com/search/?N=4294836537>

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 7,200 peer-reviewed publications. Professor Koenig's publication list:

<https://aonm.org/references/>





Thanks very much for your attention!

info@aonm.org, 0333 121 0305

gilian@aonm.org, 0786 772 6387

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