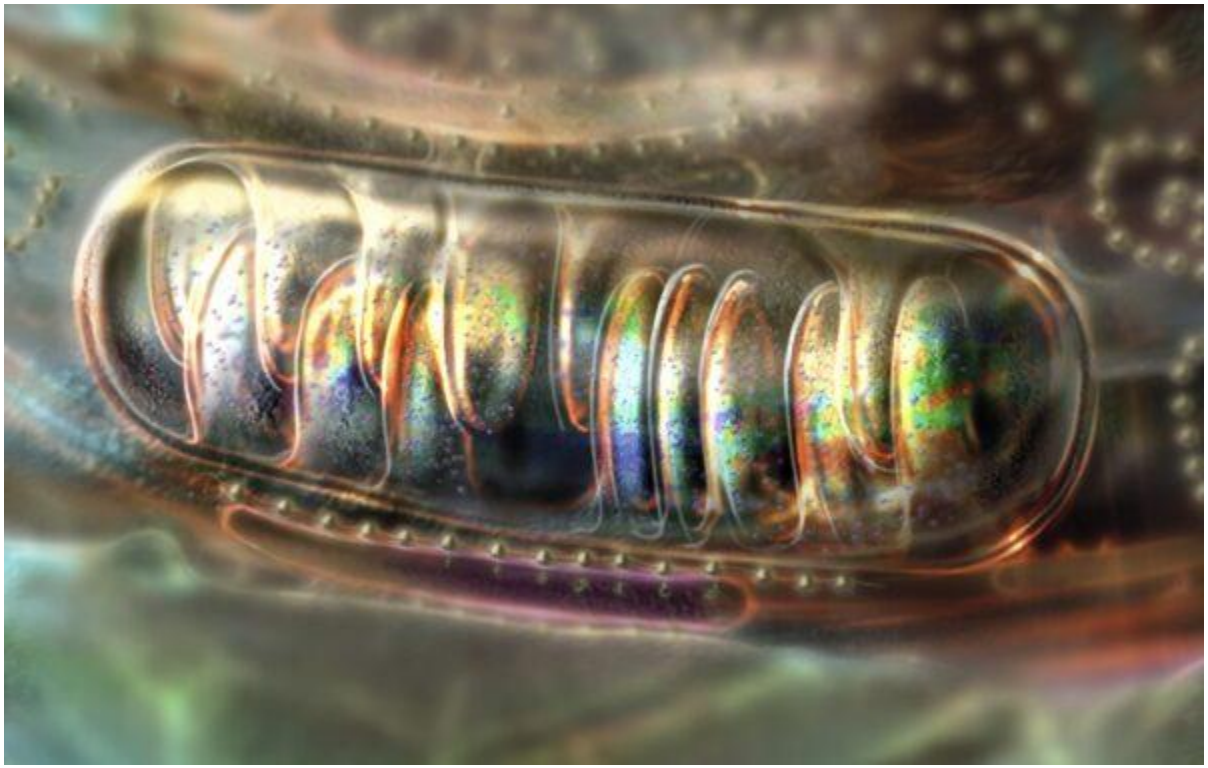


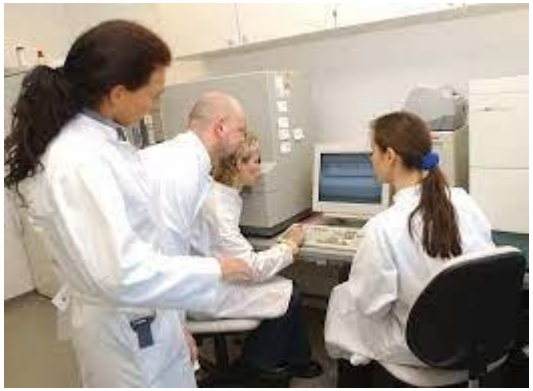
Mitochondrial Testing

Academy of Nutritional Medicine

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

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 7,200 peer-reviewed publications.



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-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate ratio
   Mitochondrial Fuel Pathways
   OxPhos

ATP Profile

XXX
Max-Mustermann Straße 5
xxx Berlin

MMD

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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

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

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.

RESULTS

Sample type: heparin blood

Quantitative Detection of Spike Protein in Plasma/Serum, Quantitative Detection of Spike Protein in Exosomes, Quantitative Detection of Spike Protein in Immune Cells (PBMC)

Spike protein in Plasma/Serum	**NEGATIVE**
Spike protein in Exosomes	**POSITIVE 18,42 pg/ml**
Spike protein in Immune cells (PBMC)	**POSITIVE 6,99 pg/ml**

Interpretation:

No evidence of the SARS-CoV-2 spike protein in plasma/serum
Indication of the SARS-CoV-2 spike protein in exosomes
Indication of the SARS-CoV-2 spike protein in immune cells (PBMC)

General note:

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), also known as 2019-nCoV (2019 Novel Coronavirus), is a virus that causes illnesses ranging from common cold symptoms to severe consequences such as shortness of breath. The SARS-CoV2 spike (S) protein plays the most important role in the attachment, fusion and entry of viruses and serves as a target for the development of antibodies, entry inhibitors and vaccines. The spike protein receptor binding domain (RBD, S-RBD) in SARS-CoV-2 spike protein binds strongly to human angiotensin-converting enzyme-2 receptors (ACE2).

The analytical sensitivity of the spike protein detection is 4.5 pg/mL

In plasma/serum, the free spike protein (unbound) is determined.

Recommendation: Determination of anti-SARS-CoV-2 antibodies against the spike protein. Determination of the neutralizing capacity of anti-SARS-CoV-2 antibodies (spike protein) against the different SARS-CoV-2 variants.

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 ADP > ATP + 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.

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 (next time!):**
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   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

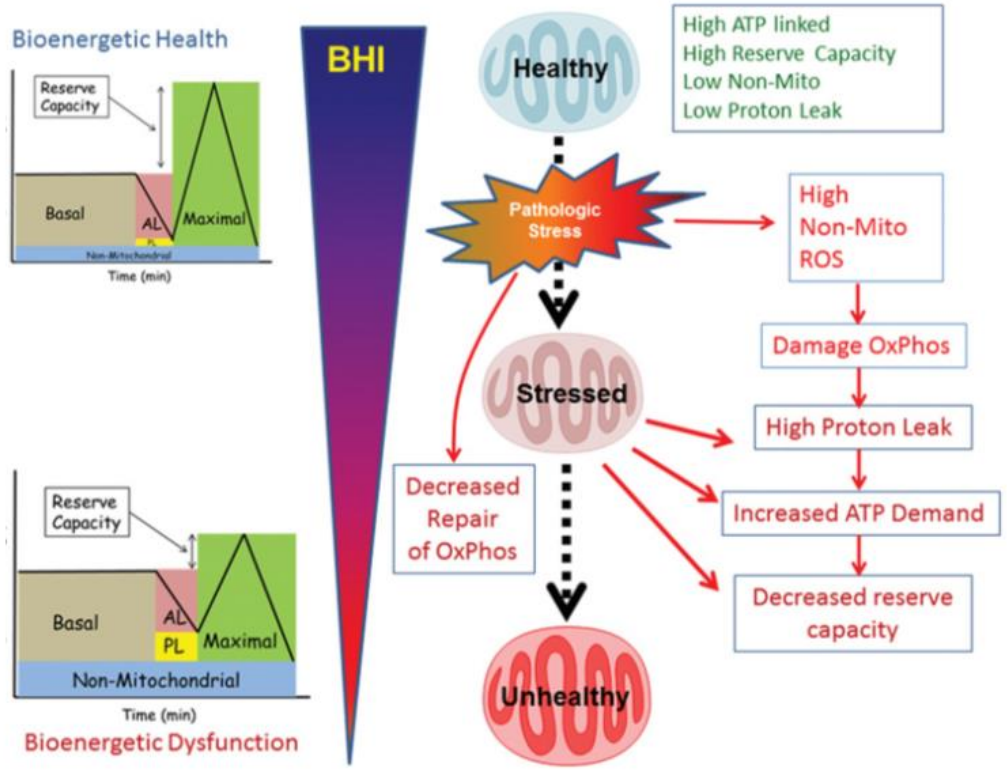


Figure 2: BMI as a dynamic measure of the response of the body to stress.

Source: Source: Chacko BK, Kramer PA, Ravi S, Benavides GA, Mitchell T, Dranka BP, Ferrick D, Singal AK, Ballinger SW, Bailey SM, Hardy RW, Zhang J, Zhi D, Darley-Usmar VM. The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci (Lond). 2014 Sep;127(6):367–73;* https://pubmed.ncbi.nlm.nih.gov/24895057/

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

Overall MHI, derived from the multiple parameters

MITOCHONDRIAL HEALTH INDEX (MHI)

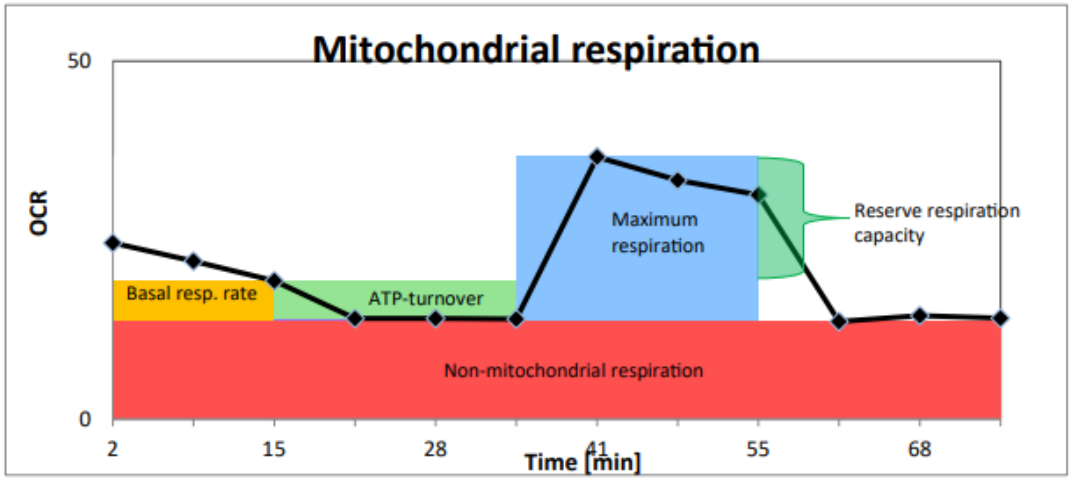
The MHI is a sensitive indicator of the reaction of immune cells (PBMCs) to oxidative stress, and for the changing metabolic programmes that they serve depending on the role they need to play in the case of inflammation, immune defence and immune health. The MHI is is also an indicator for the current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

Coupling efficiency, %	slightly low
Reserve respiration capacity, %	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 %
Coupling efficiency, %	Considerably high	98-100	
	Slightly enhanced	95-98	
	Optimal	90-95	
	Slightly low	85-90	86
	Moderately low	80-85	
	Considerably low	70-80	
	Extremely low	<70	

Interpretation of your results:

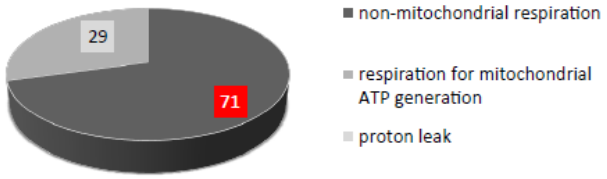
The coupling efficiency is slightly low.

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

Optimal
Slightly high
Moderately high
Very high
Extremely high

How the oxygen is being consumed, %



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

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

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.

Seite | 7

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

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
Maximum possible oxygen consumption rate, pmol oxygen/min	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 enery 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 %
Reserve respiration capacity, %	Optimal	>400	
	Slightly low	300-400	
	Moderately low	250-300	
	Considerably low	200-250	
	Extremely low	<200	0

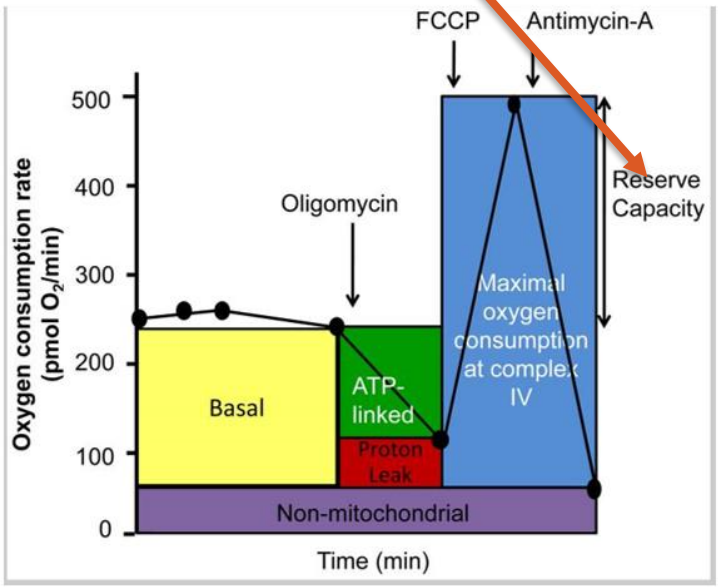


Figure 3: The cellular bioenergetic profile

Comparison of various tests

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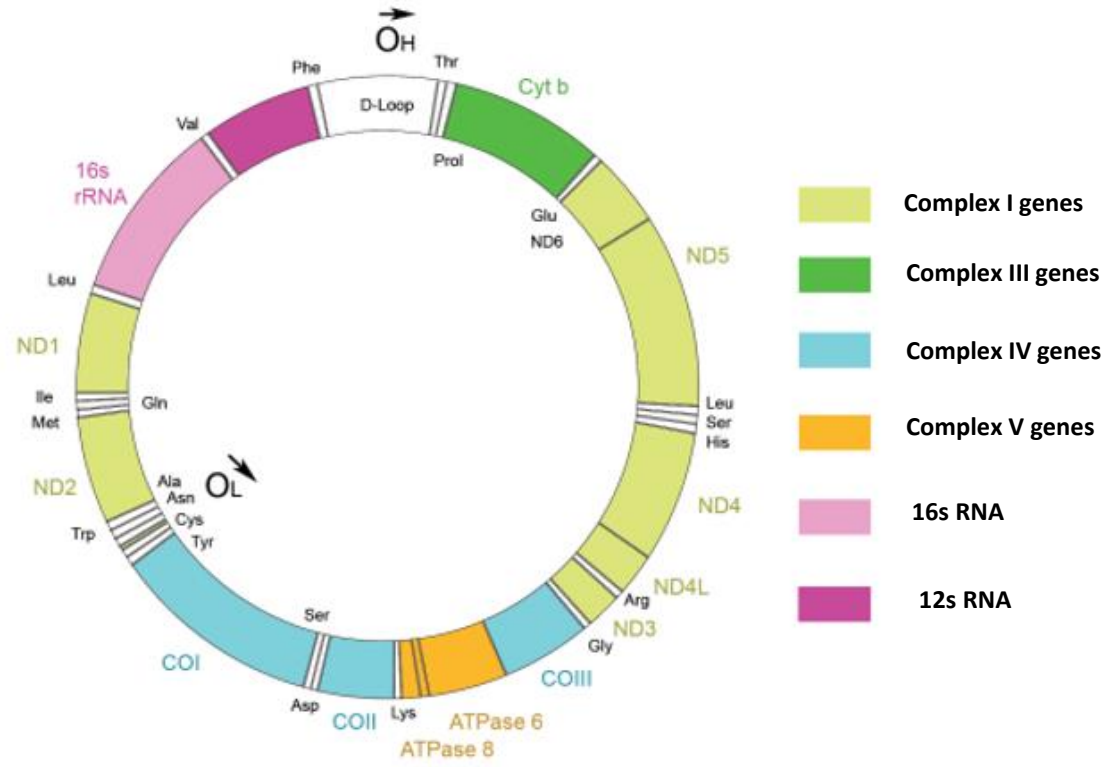
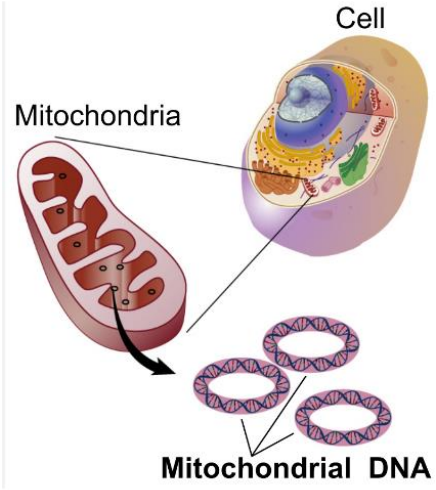
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate ratio

Mitochondria have their own DNA

mtDNA:nDNA

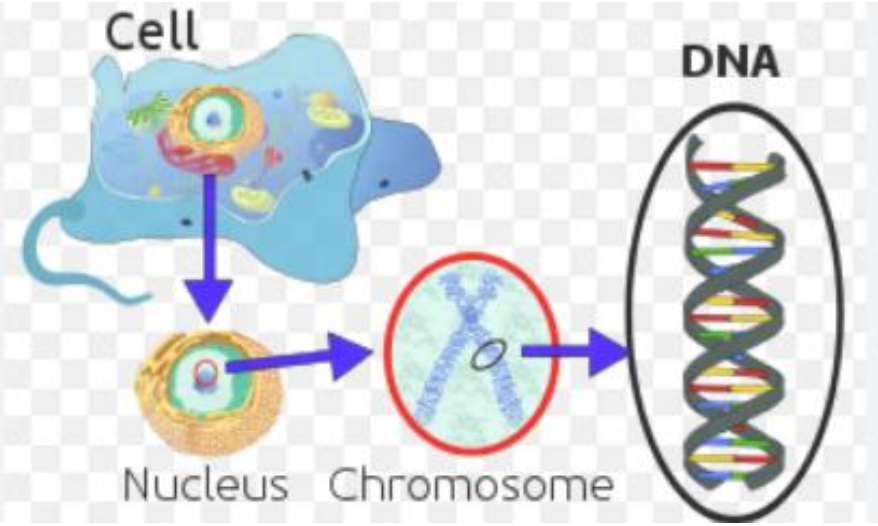


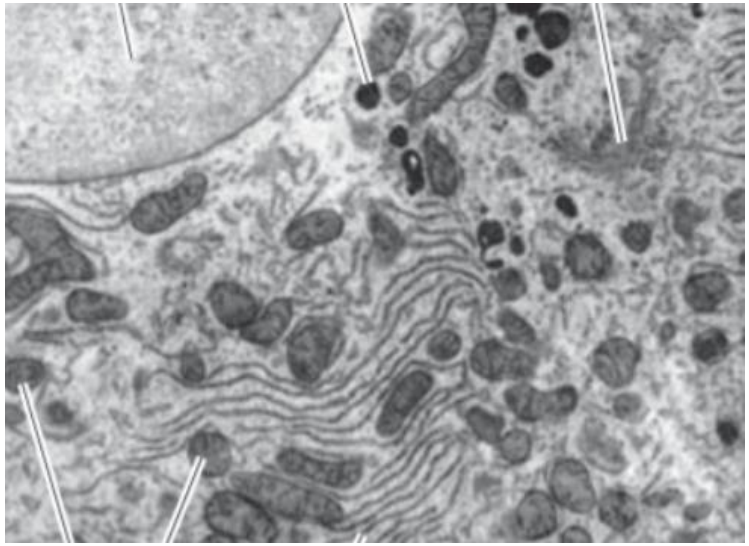


Source: MMD GmbH & Co KG Author Prof. Dr. Brigitte König; Hoffmann A, Spengler D. The Mitochondrion as Potential Interface in Early-Life Stress Brain Programming. Front Behav Neurosci. 2018 Dec 6;12:306; https://en.wikipedia.org/wiki/Mitochondrial_DNA: Images free to use under Commons License

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

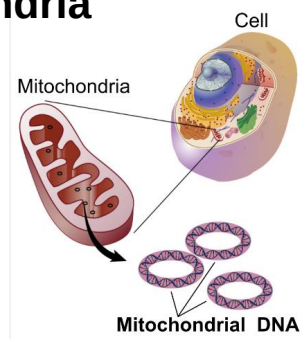
mtDNA:nDNA





The cell nucleus has only one copy of DNA

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



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

Example 2:

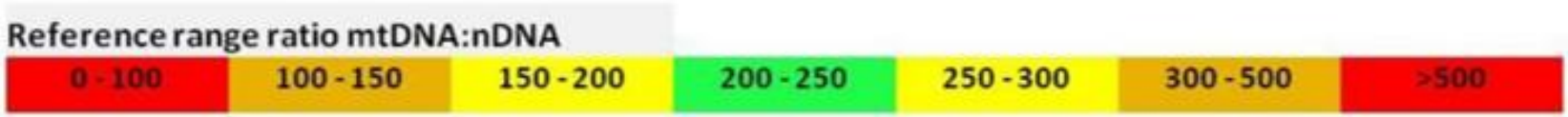
Ratio mtDNA:nDNA 1039

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



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

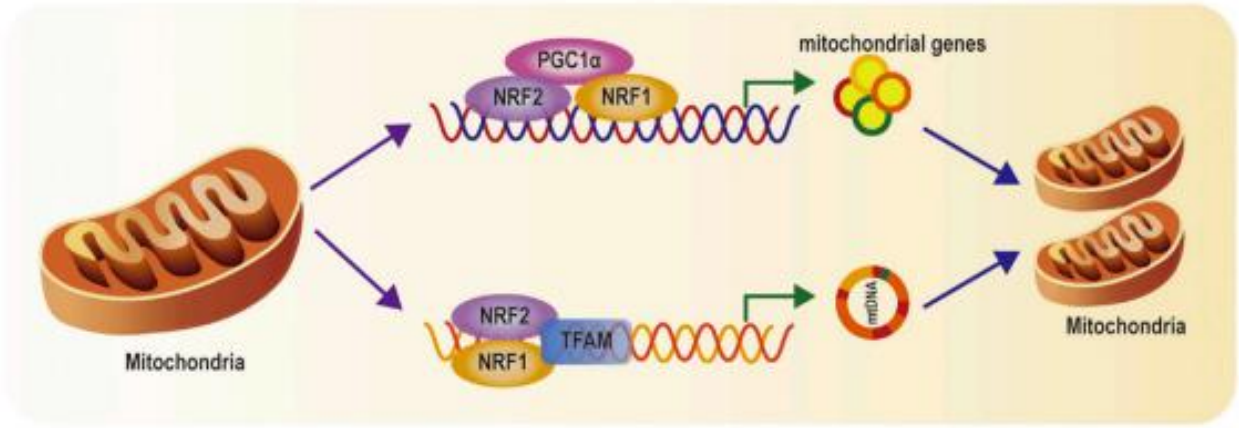


FIGURE 2 | Mitochondrial biogenesis pathways: When PGC-1α is activated, PGC-1a activates NRF1 and NRF2, and subsequently TFAM, which regulate genes involved in subunits of mitochondrial respiratory chain complexes, import of nuclear-encoded mitochondrial proteins, and mtDNA replication and transcription.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

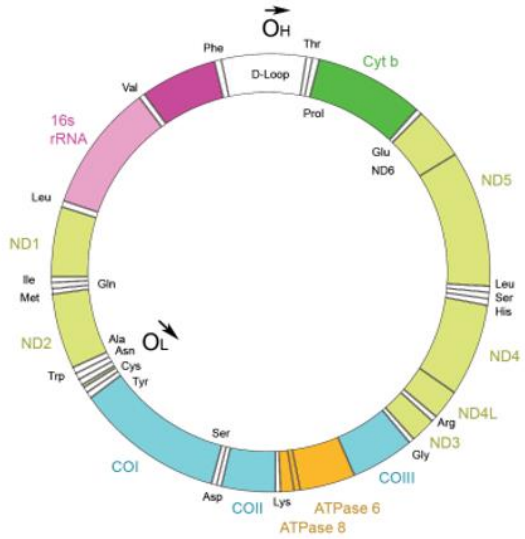
Interpretation: "Basic values of the peripheral blood leucocytes"

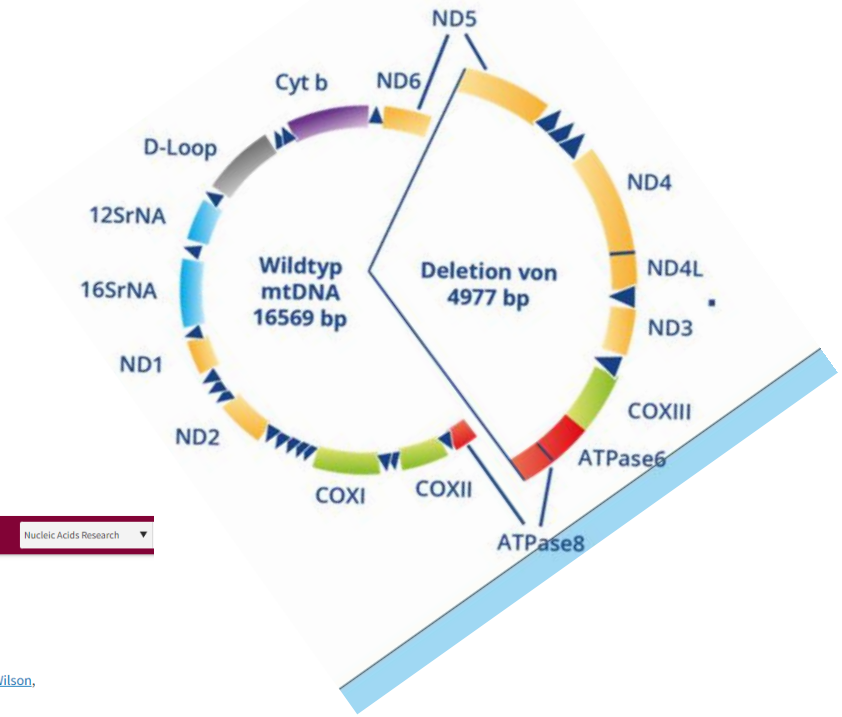
PGC-1-alpha expression is barely detectable. This indicates extremely low/absent new mitochondrial formation.

If this is the case, and mtDNA:nDNA is low too, then initiatives should be taken to increase PGC-1-alpha *(list of inducers available)*

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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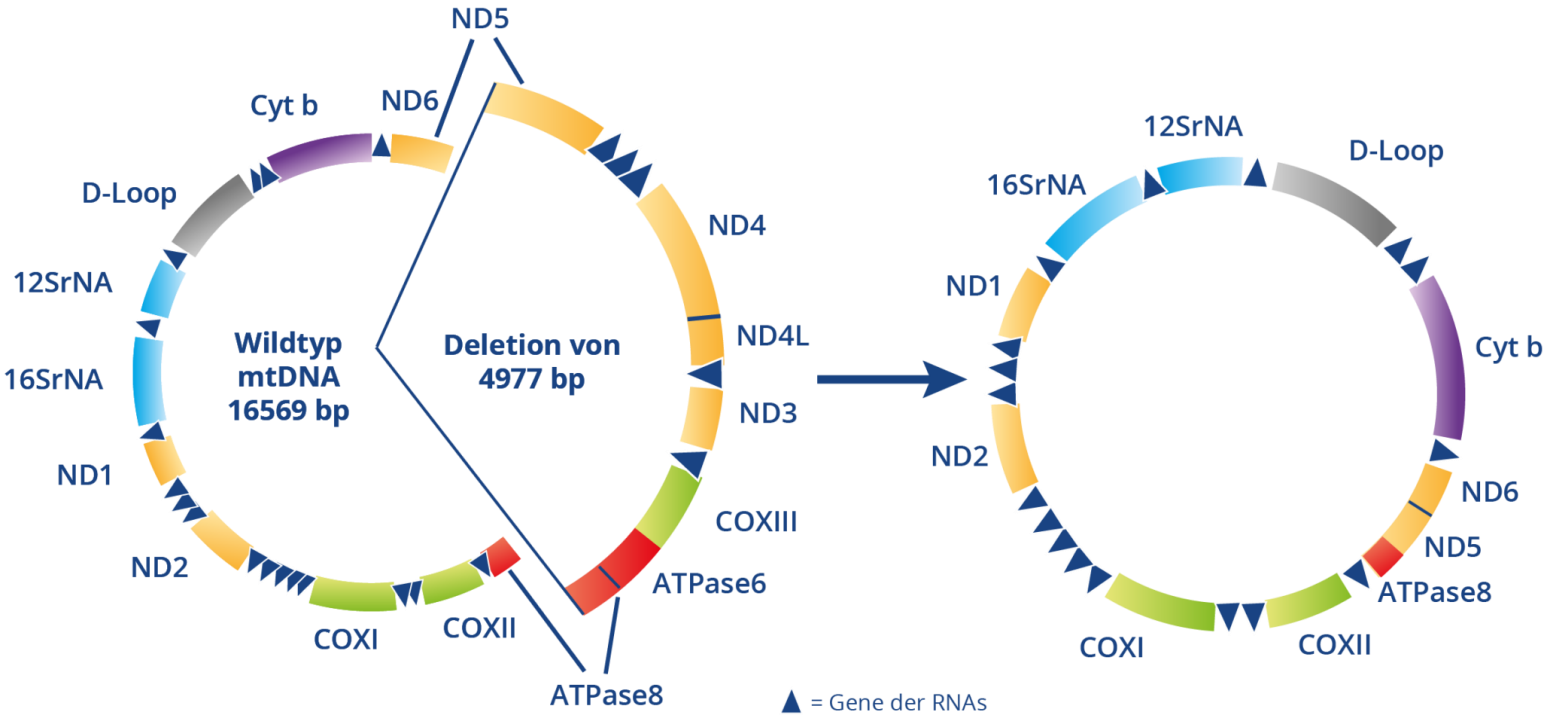
Abstract

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

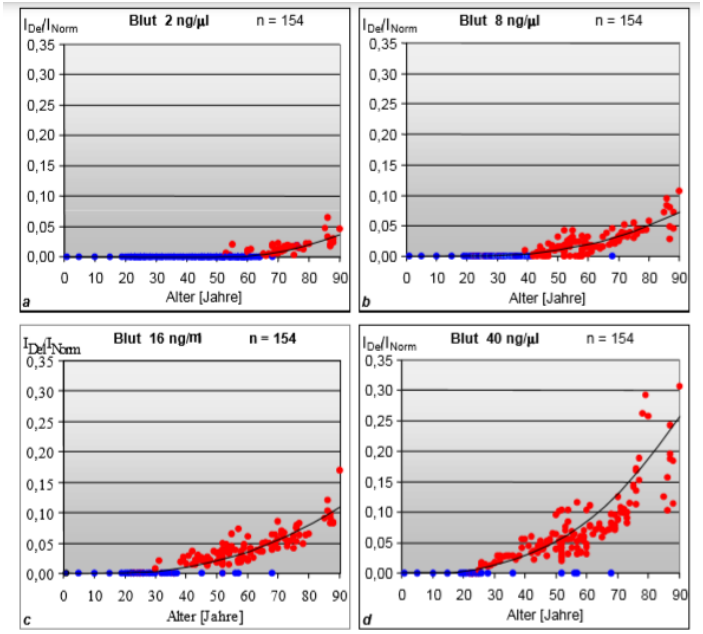
The mitochondrial deletion mutant mt4977bp is noticeably enhanced. This indicates oxidative stress and damage to mitochondrial DNA.

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.**

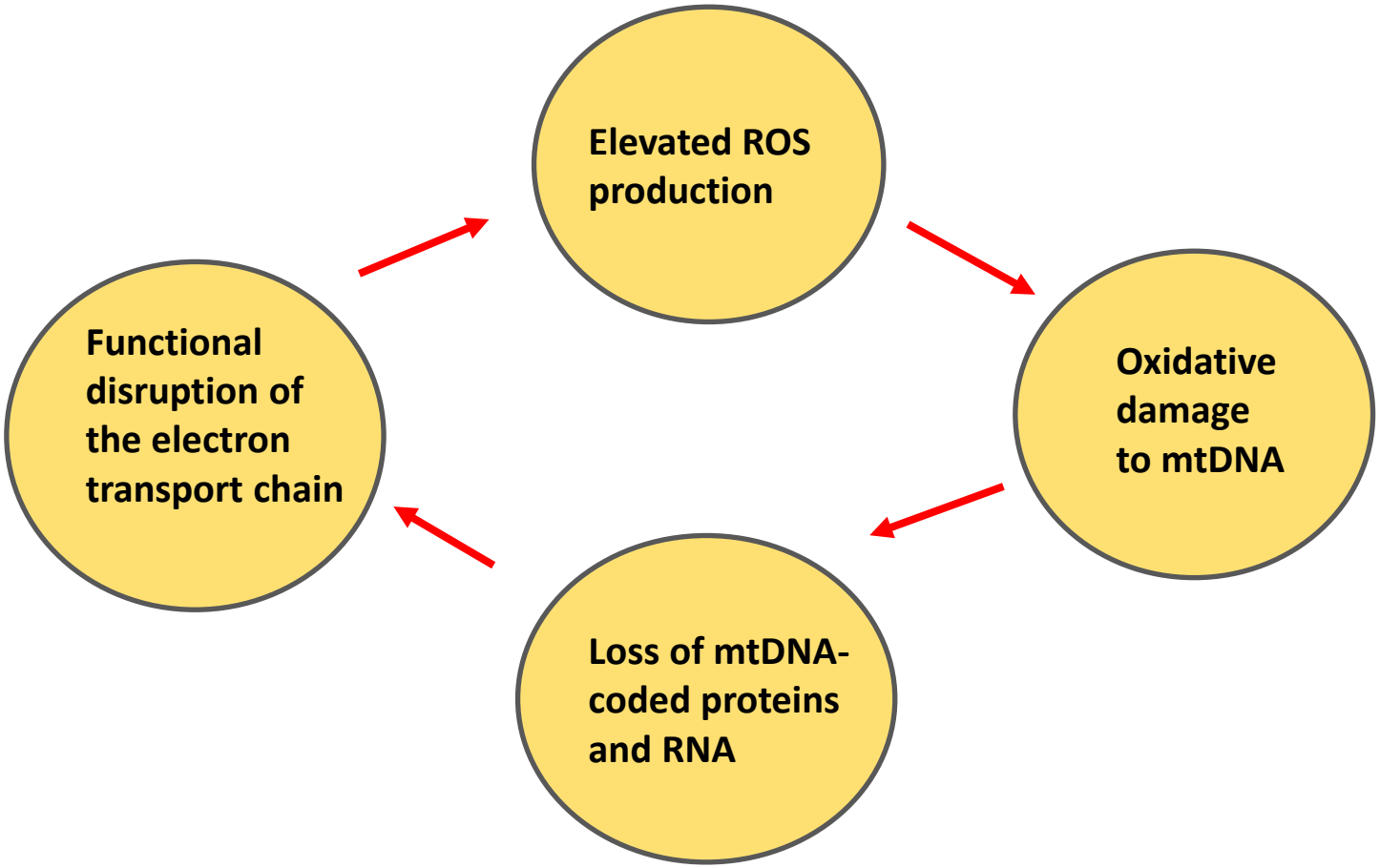
Significantly increased **Undetectable**

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

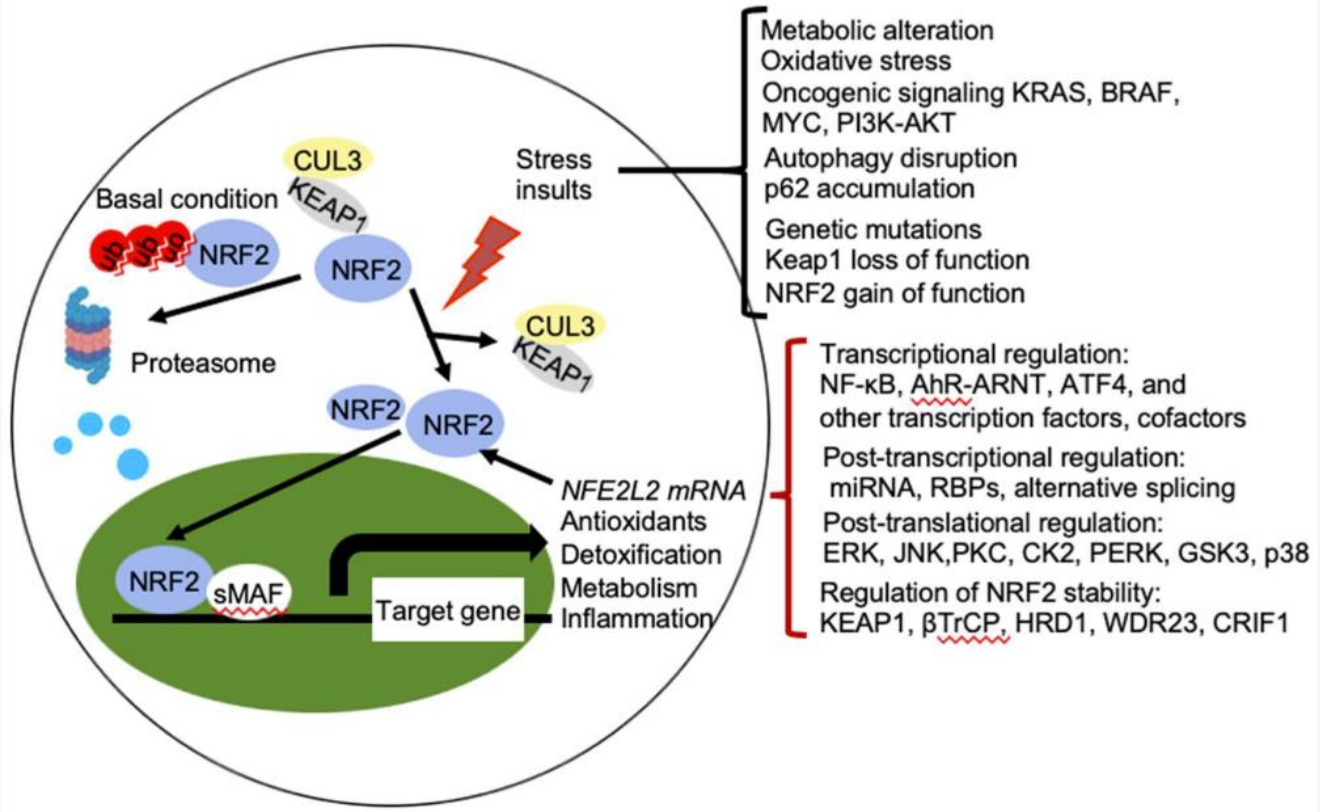
Oxidative stress



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

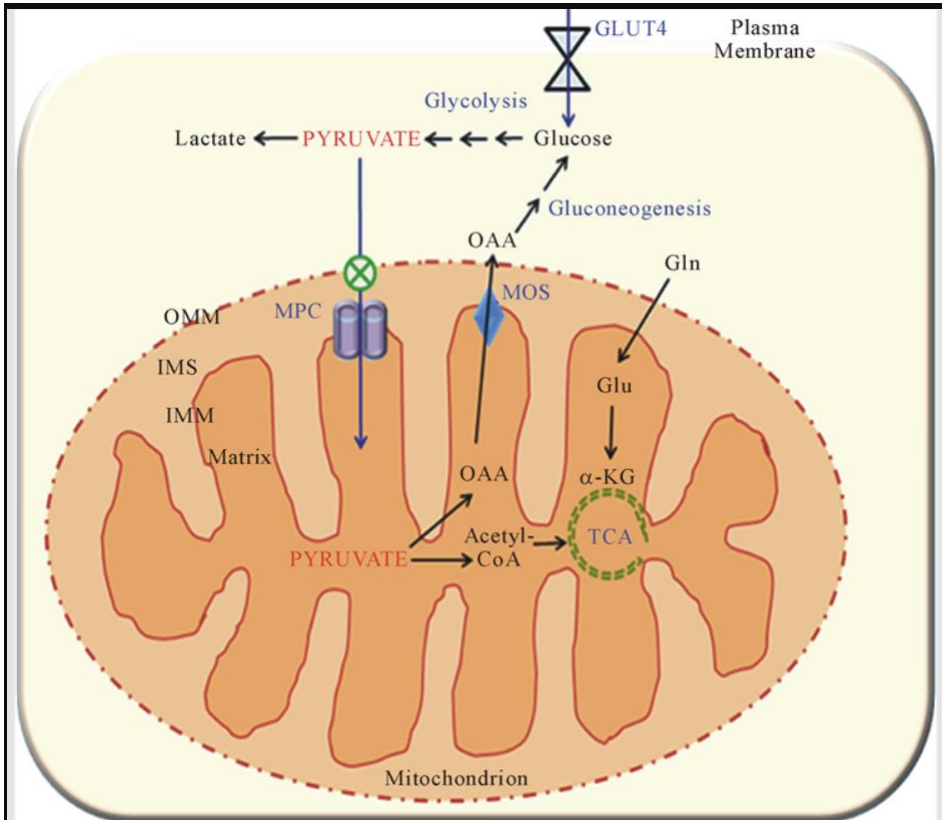
Exogenous antioxidants

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.

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.

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 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

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<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.

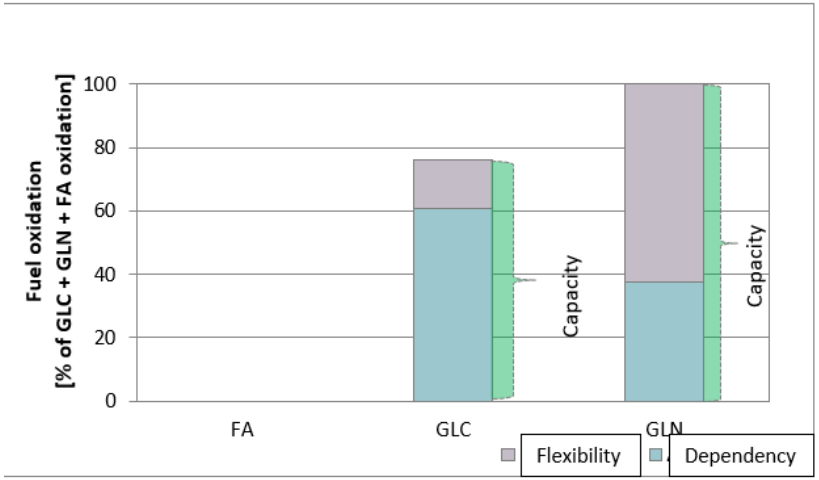
- **So, ideally, the PBMCs should not show much dependence on any one fuel.**

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%.**



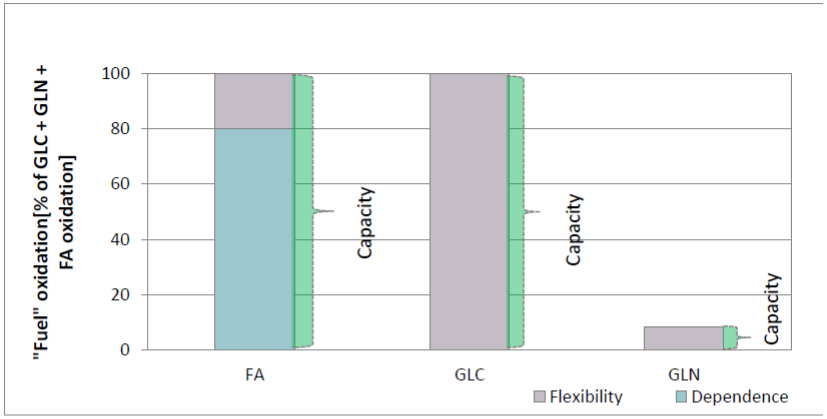
FA: fatty acids; GLC: glucose; GLN: glutamine (amino acids)

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



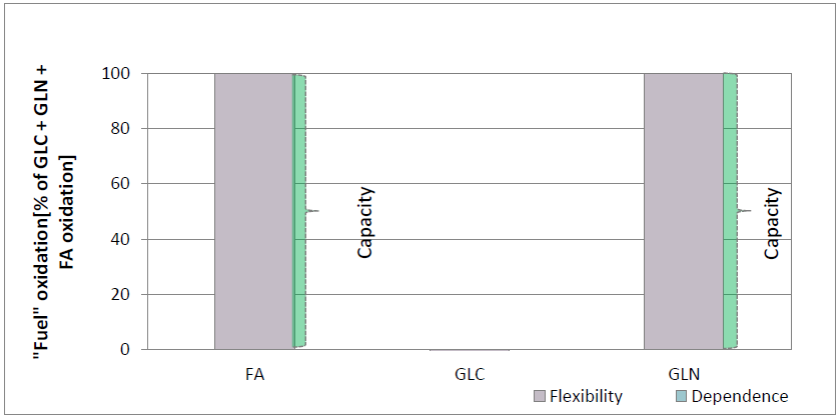
FA: Fatty acids; GLC: Glucose; GLN: Glutamine (Amino acids)

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.

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)	**0.2** (>2.5), extremely low <1.0
Mitochondrial oxygen utilisation:	
1a) Baseline oxygen consumption rate	**38** pmol/min (?)
1b) Maximum oxygen consumption rate	**47** pmol/min (>300), extremely low <100
1c) Reserve respiration capacity	**+25%** (> 400%), extremely low <200
Oxygen consumption profile:	
2a) Oxygen used for mitochondria ATP gen.	**59%** (>90%)
2b) Oxygen used for non-mitochondria resp.	**29%** (<10%), moderately high 20-30
2c) Proton leak	**12%** (5-10%)
3) Coupling efficiency	**83%** (90-95%), moderately low 80-85
Cellular energy:	
4a) Base setting of immune cells	**Resting** (resting)
4b) Metabolic potential – mitochondria	**117%** (>350%), extremely low <200
4c) Metabolic potential – glycolysis	**265%** (>350%), moderately low 250-300
Mitochondria DNA	
5) Total number of mitochondria	212 (200-250), normal 200-250
6) Non-intact mitochondria	[Very low?]
7) Mitochondria DNA damage (mt4977del)	[Moderately - Very high?]
Lactate-pyruvate index	
8) Lactate/pyruvate in dormant cells	1.14 (0.7 – 1.0)
9) Lactate/pyruvate in activated cells	2.41 (0.7 – 1.0), 90% converted to lactate
Mitochondria RNA	
10) PGC-1α	0.00021 (~0.01000), very low
11) Nrf-2	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:
• *The mitochondria are being inactivated physically/directly* (e.g. by parasite)	Focus on removing the parasite / endotoxin. Further testing.
• *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.	Focus on identifying the threat (virus, bacteria, immune response, toxins, etc). Low Nrf-2: could be a successful strategy (oxidating the cell to fight off virus), or it could be a failure in the antioxidant system. Low PGC-1α: could be a successful strategy (new mitochondria could react to the environment in the same way, so no benefit) or it could be a failure in the biogenesis/mitophagy system.
• *The mitochondria are incorrectly 'stuck'* in their current state – even after the external threat has passed.	It may be helpful to try to 'kick-start' the mitochondria? Try Nrf-2 up-regulators (e.g. Tisso herbal supplements).

(Etcetera)

MITOCHONDRIAL TESTS

ACADEMY of NUTRITIONAL MEDICINE

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

☑	#TEST NUMBER	NAME OF TEST		MATERIAL	PRICE
☐	M1	ATP Profile:	Total ATP, Mitochondrial ATP, Glycolytic ATP, Reserve ATP	CPDA x1	£125
☐	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
☐	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)

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

MMD

AONM ACADEMY of NUTRITIONAL MEDICINE

MITOCHONDRIAL TESTS USA 3

CAPILLARY BLOOD DRAW (FINGERPRICK)

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

☑	#Test number	Name of test	Price (individual tests)	Price (ALL)
☐	MFP1	Oxidative stress measured using the mt4977 deletion mutant	£80	
☐	M6	Nrf-2 (Master antioxidant regulator)	£50	
☐	M15	8-OH-dG-DNA (a predominant ROS lesion)	£58	£352
☐	MFP8	Telomere length	£149	
☐	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

MMD

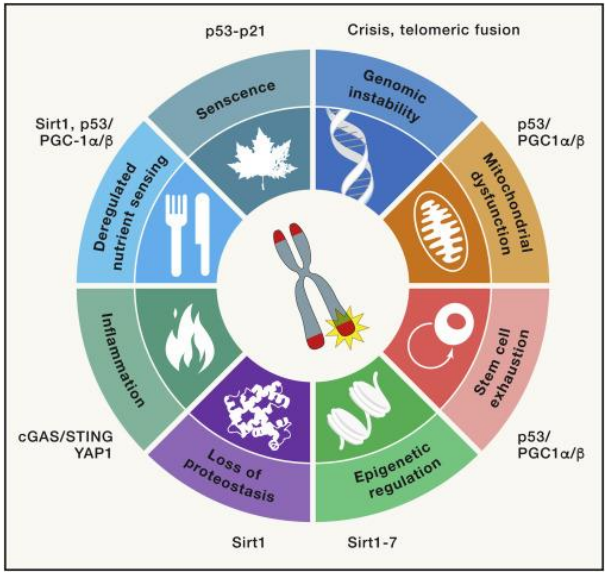
[Clinic]

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

Source: https://doi.org/10.1016/j.cell.2020.12.028

Spike protein also possible

RESULTS

Sample type: heparin blood

Quantitative Detection of Spike Protein in Plasma/Serum, Quantitative Detection of Spike Protein in Exosomes, Quantitative Detection of Spike Protein in Immune Cells (PBMC)

Spike protein in Plasma/Serum	**NEGATIVE**
Spike protein in Exosomes	**POSITIVE 18,42 pg/ml**
Spike protein in Immune cells (PBMC)	**POSITIVE 6,99 pg/ml**

Interpretation:

No evidence of the SARS-CoV-2 spike protein in plasma/serum
Indication of the SARS-CoV-2 spike protein in exosomes
Indication of the SARS-CoV-2 spike protein in immune cells (PBMC)

General note:

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), also known as 2019-nCoV (2019 Novel Coronavirus), is a virus that causes illnesses ranging from common cold symptoms to severe consequences such as shortness of breath. The SARS-CoV2 spike (S) protein plays the most important role in the attachment, fusion and entry of viruses and serves as a target for the development of antibodies, entry inhibitors and vaccines. The spike protein receptor binding domain (RBD, S-RBD) in SARS-CoV-2 spike protein binds strongly to human angiotensin-converting enzyme-2 receptors (ACE2).

The analytical sensitivity of the spike protein detection is 4.5 pg/mL

In plasma/serum, the free spike protein (unbound) is determined.

Recommendation: Determination of anti-SARS-CoV-2 antibodies against the spike protein. Determination of the neutralizing capacity of anti-SARS-CoV-2 antibodies (spike protein) against the different SARS-CoV-2 variants.

RESULTS

Sample type: heparin blood

Quantitative Detection of Spike Protein in Plasma/Serum, Quantitative Detection of Spike Protein in Exosomes, Quantitative Detection of Spike Protein in Immune Cells (PBMC)

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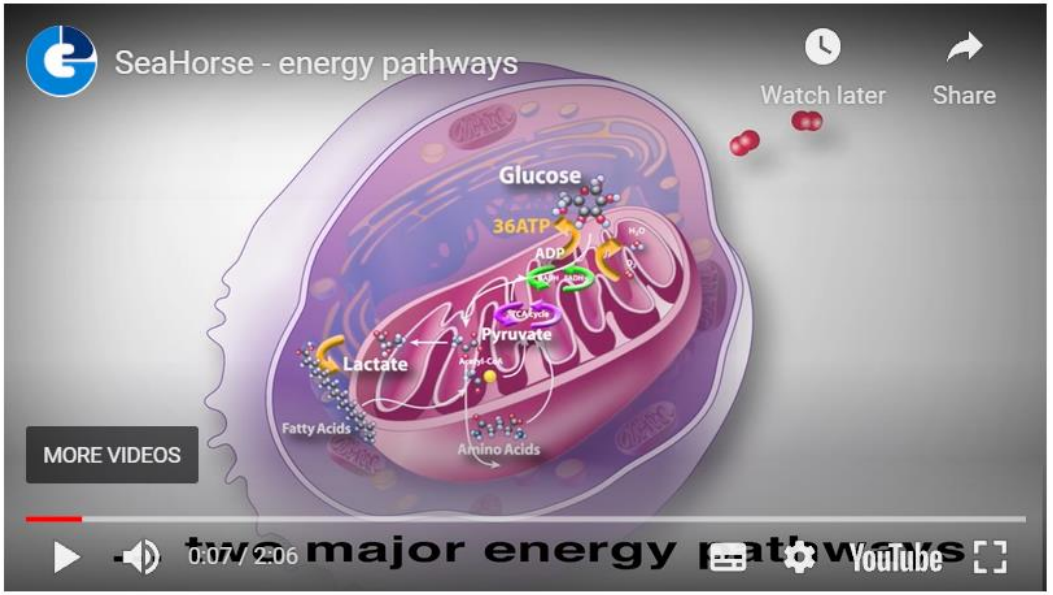
Many videos about the Seahorse technology available, and over 7,200 studies* for which the Seahorse has been used

HOW THE SEAHORSE XF WORKS

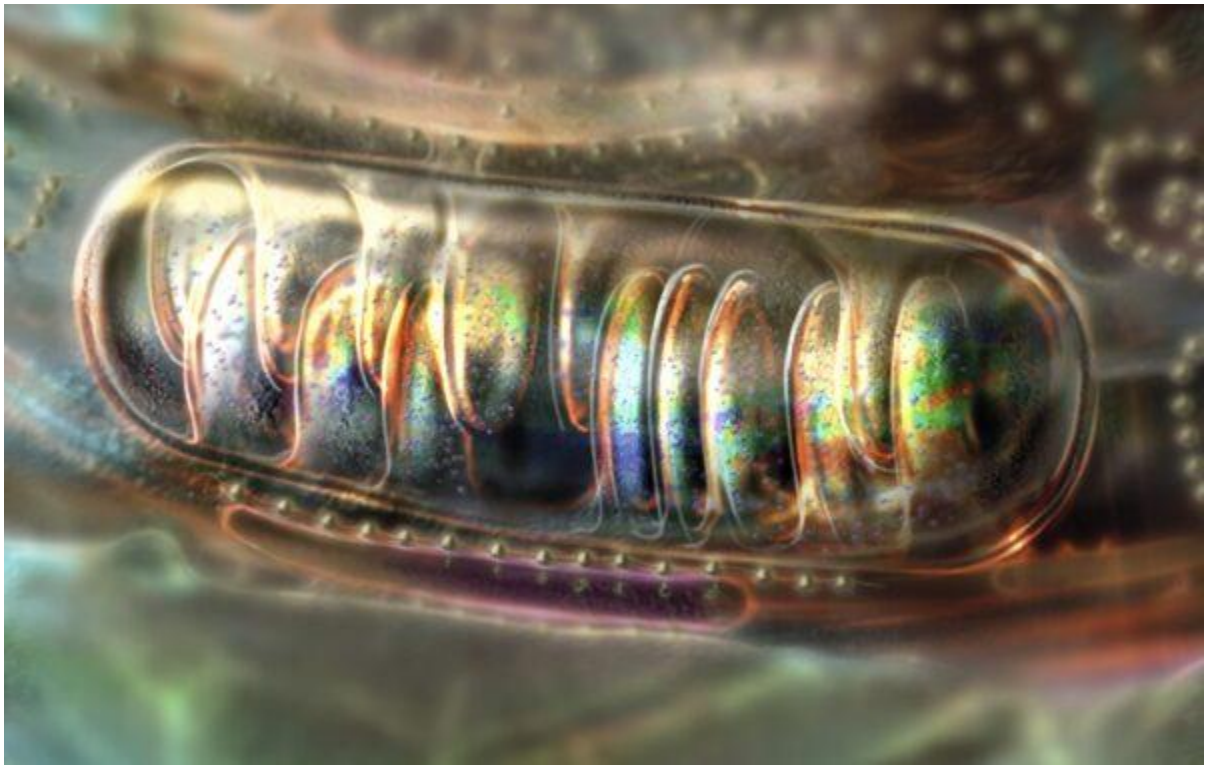
Videos are at the bottom of this page:
https://aonm.org/mitochondrial-testing/

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



Thanks very much for your attention!