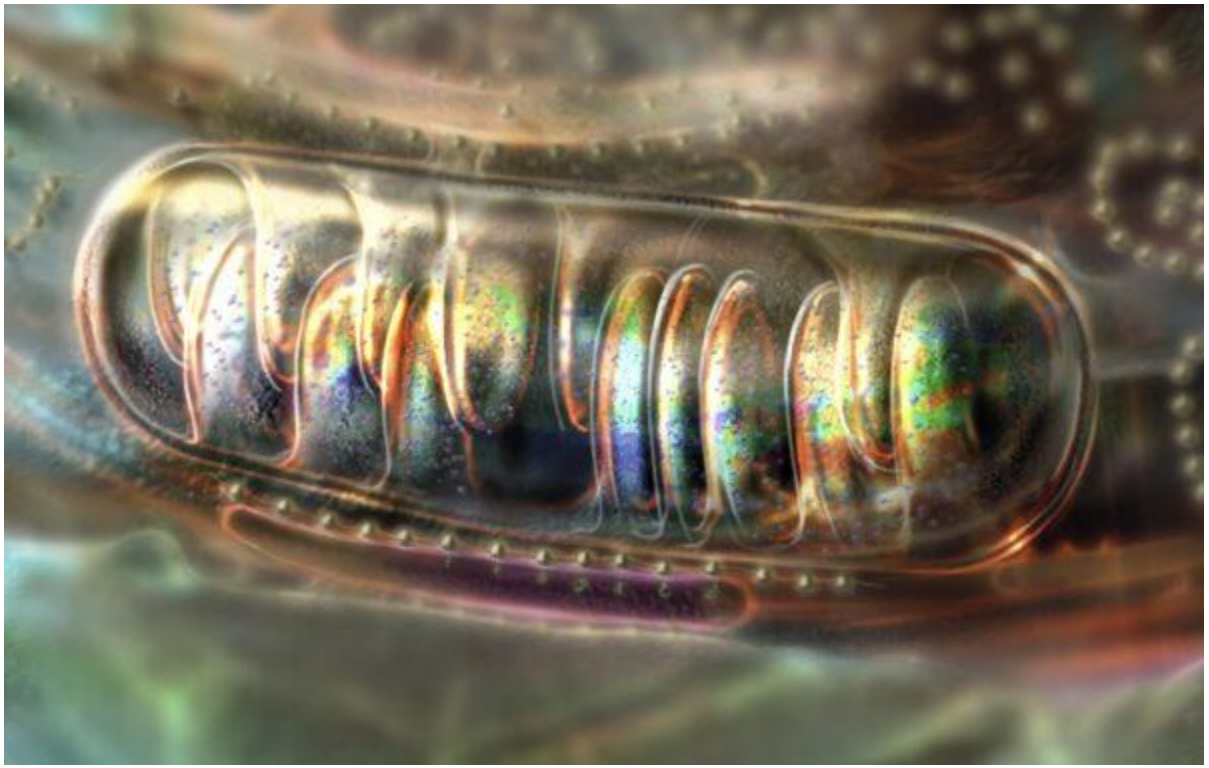


Mitochondrial Testing

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

Webinar AONM Mini-Series 5th April 2022

Professor Brigitte Koenig, Magdeburg Molecular Detections

Mitochondrial testing with AONM/MMD

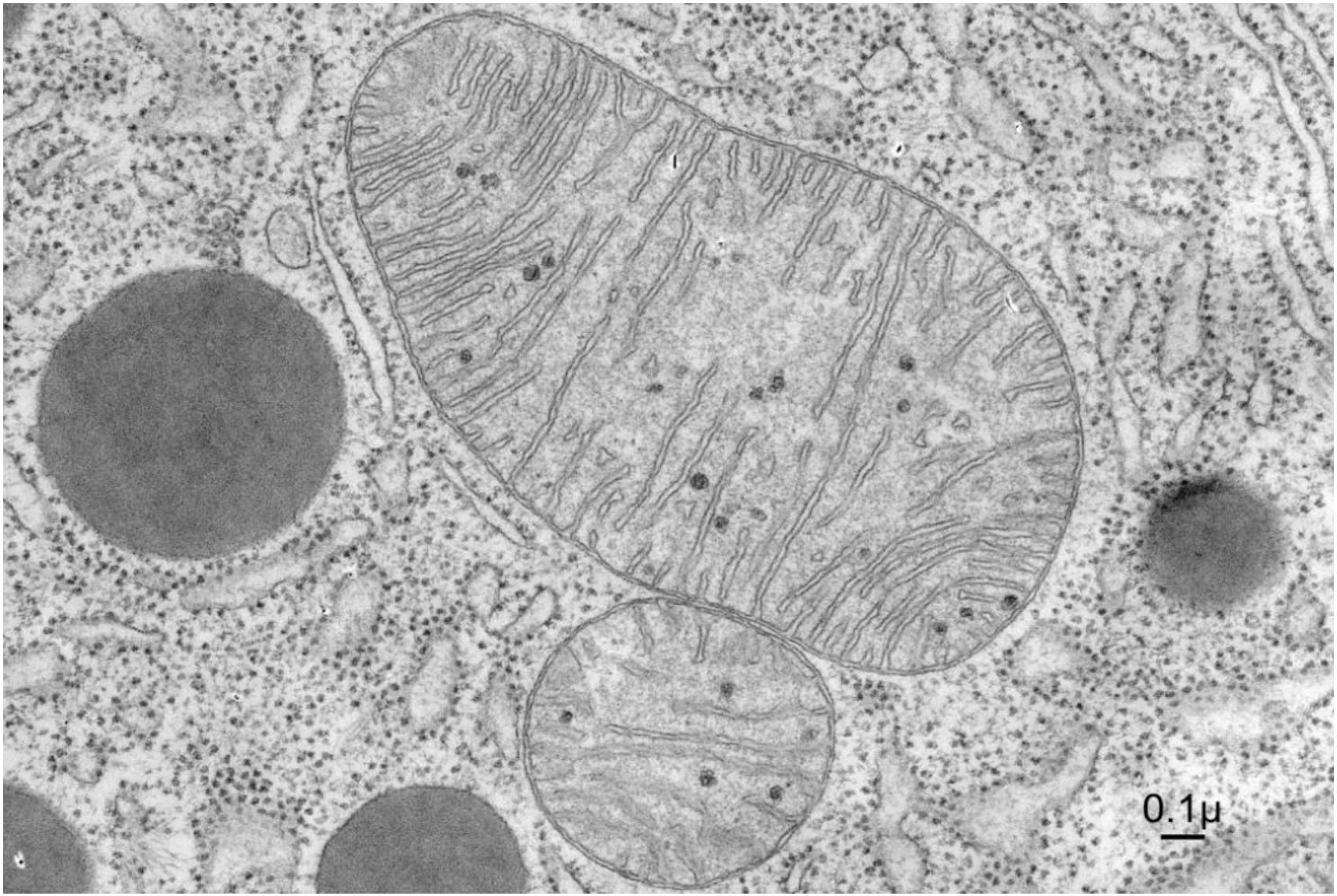
1. **Brief introduction to the mitochondria**

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

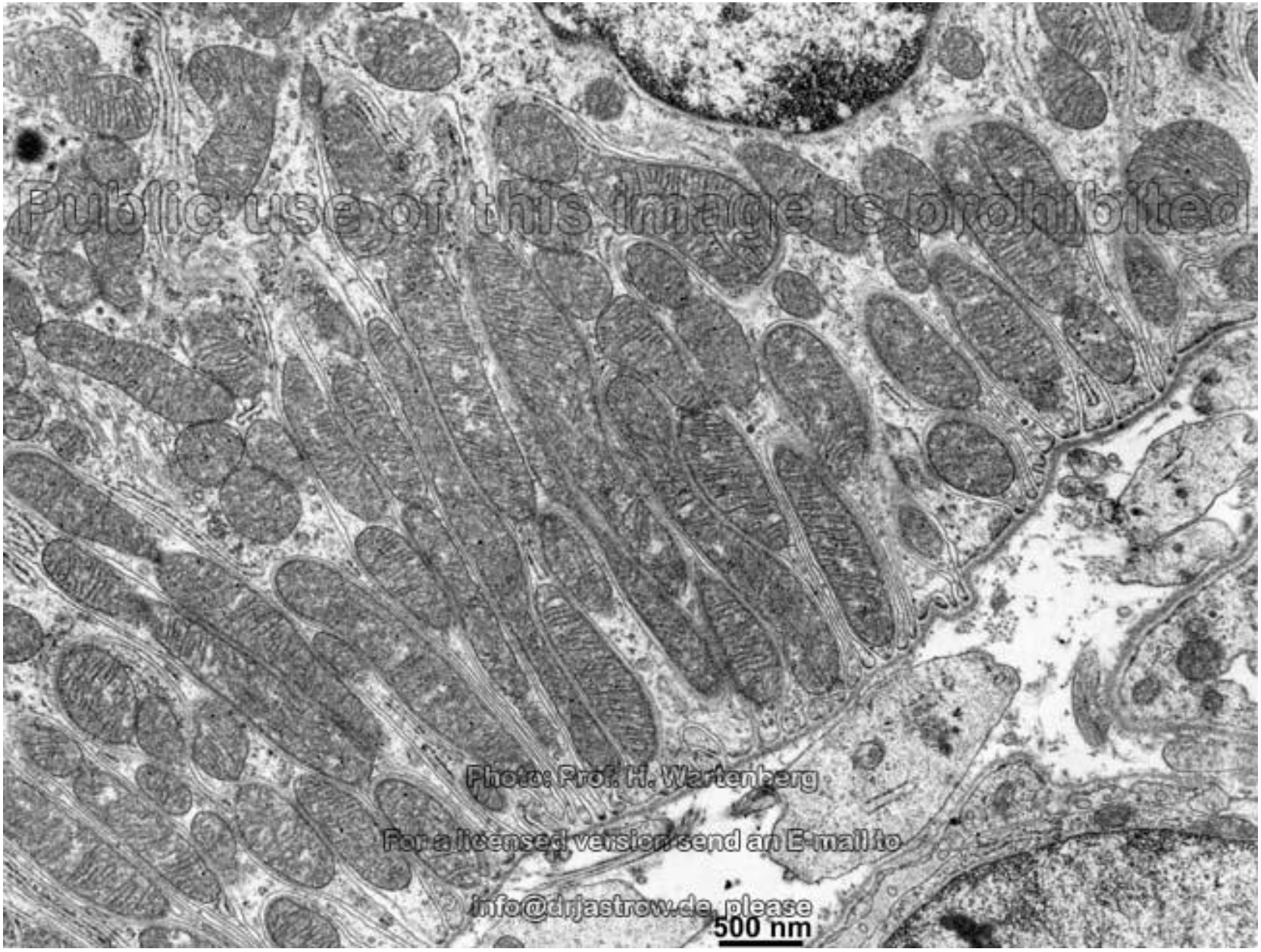
4. **Supplementary biomarkers (next time!):**
   Ratio of mtDNA to nDNA (mtDNA:nDNA)
   PGC-1α
   Nrf-2
   Mitochondrial 4977 deletion mutant (mt4977del)
   Lactate/pyruvate ratio
   Phase 2:
   Number of mitochondria
   Intact mitochondria versus Non-intact mitochondria

Our mitochondria are the powerhouses of our cells ...

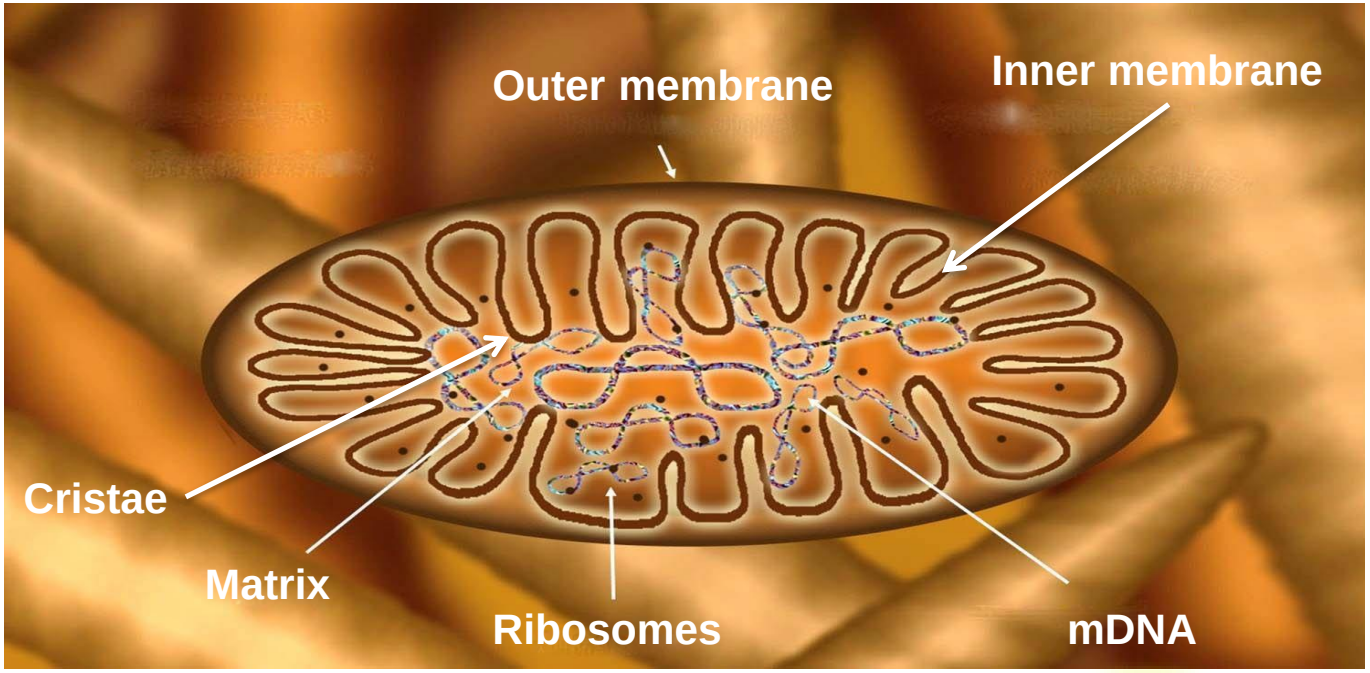


Source: The Keith R. Porter Collection, Center for Biological Science Archives, University of Maryland, Baltimore Country, with permission

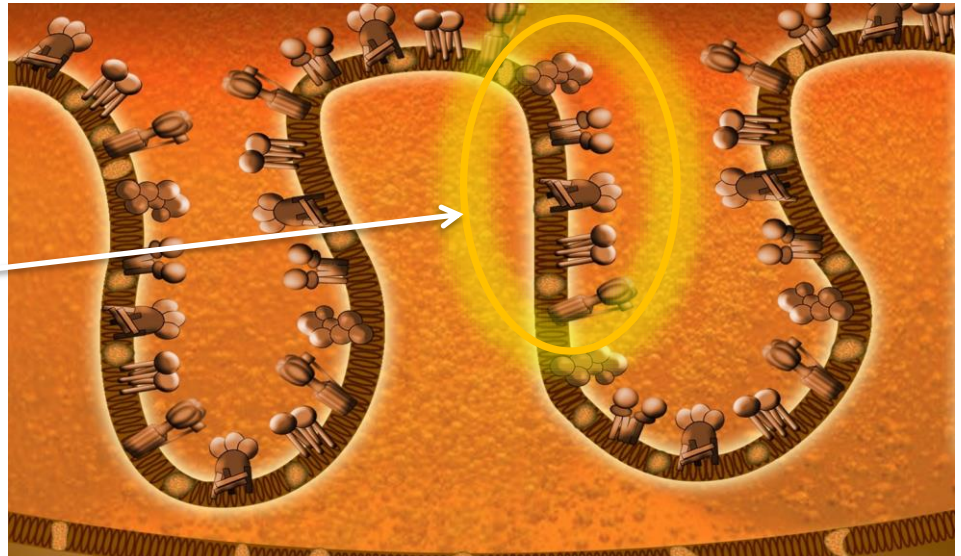
... sometimes hundreds in one cell, sometimes thousands or even millions ...



Structure of a mitochondrion
Mitos = thread | Chondros = grain/seed



The **electron transport chains** are along the cristae – many thousands in each mitochondrion



The cristae allow efficient use of space

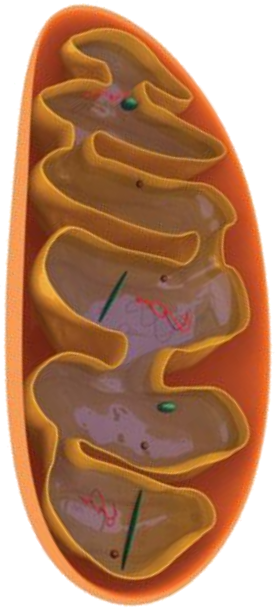
Source: http://www.exatest.com/MITOCHONDRIA%20SEMINAR/Mitochondria%20Seminar%20%20P1-1%20INTRO.htm

Perfect symbiosis: anaerobic and aerobic respiration combined

Source: The MitoBiome Concept, https://www.tisso.de/Veranstaltung/live-stream-seminar-mitobiom-concept-module-i/?instance_id=361; http://rkutchjm.files.wordpress.com/2012/12/mitochondria.jpg

What are our mitochondria responsible for?

Examples



Bioenergetics → **High-intensity energy metabolism and nutrient sensing**

Biosynthesis →
- **Heme synthesis**
- **Progesterone and thus all our downstream steroids**
- **Primary source of single-carbon groups (methylation) for the folate pathway**
- **Specific phospholipids (PE, PG, PA) and cardiolipin**

Signaling →
- **Apoptosis (release of Cytochrome C)**
- **T cell function, neutrophil activation**
- **O_2 sensor for vasoconstriction**
- **Sensing of blood sugar levels (O-GlcNAc)**
- **Release of ROS for multiple cell-cell communication activities**
- **Photon modulation**

Sources: Ajioka, RS et al, The Biosynthesis of Heme in Mammals. Biochimica et Biophysica Acta 1763 (2006) 723–736; Kuklinski, B. Cryptopyrroluria, nitrosative stress and mitochondrial disease; Black, SM, et al, The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scc, Proc Natl Acad Sci USA, 1994; Synthesis of Steroid Hormones web.calstatela.edu/faculty/mchen/439Lectures/439Ensteroids4.ppt; Deth RC. 2003. Molecular Origins of Human Attention: the Dopamine-Folate Connection. Springer Science + Business Media, New York

Mitochondrial testing with AONM/MMD

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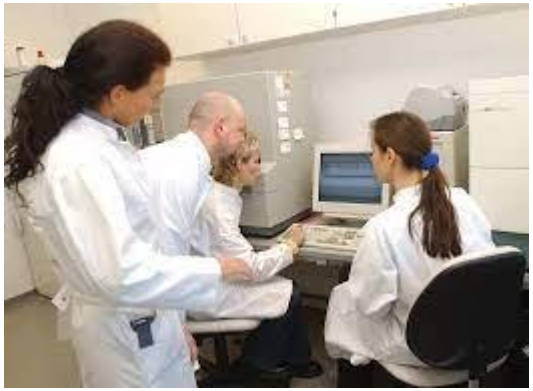
   Phase 2:
   Number of mitochondria
   Intact mitochondria versus Non-intact mitochondria

MMD - Magdeburg Molecular Detections

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



ATP Profile

XXX
Max-Mustermann Straße 5
xxx Berlin

MMD

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Prof. Dr. Brigitte König
CEO/ Scientific Director
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Fax: +49 391 535 38 45
E-Mail: info@mmd-web.de
Web: www.mmd-web.de

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

ATP profile

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

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

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.

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.

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   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

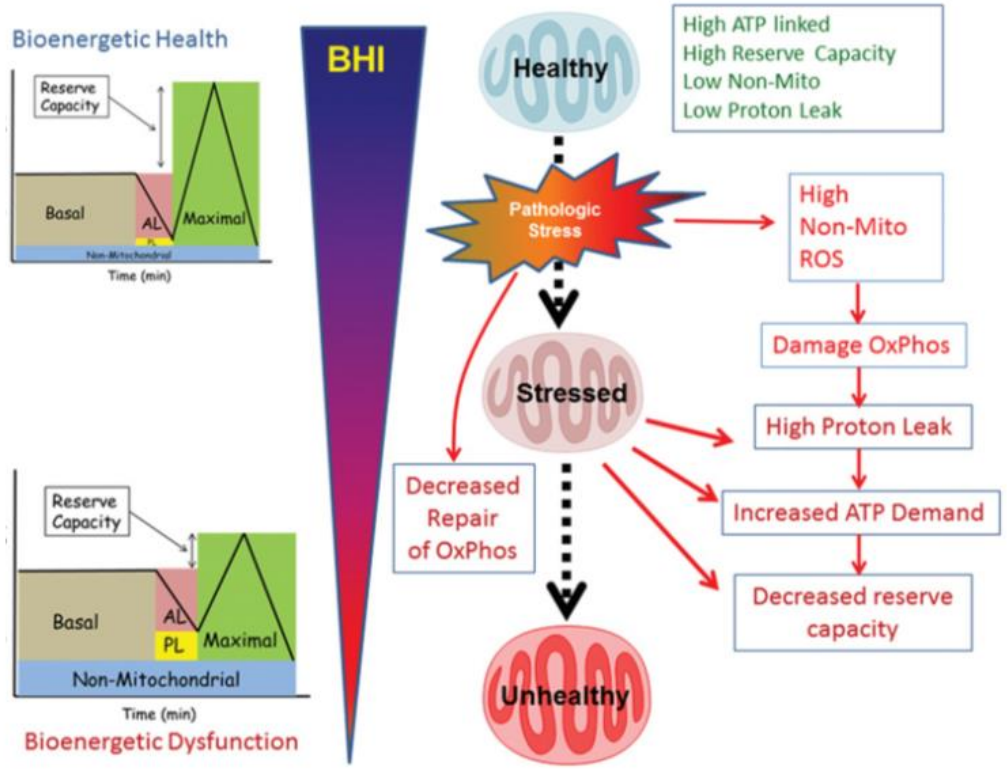


Figure 2: BMI as a dynamic measure of the response of the body to stress.

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/

MHI: Key markers

1) Basal respiration rate (orange)

This parameter shows how much oxygen is being used in the mitochondria at rest (in pmol O2/min)

2) ATP turnover (light green)

This is the rate of mitochondrial ATP synthesis in a defined basal state after the proton leak has been deducted.

3) Coupling efficiency/proton leak (purple)

A proton leak is a physiological process in which oxidative phosphorylation is uncoupled from ATP synthesis, and energy is dissipated as heat.

4) Non-mitochondrial respiration (red)

This parameter is an index of oxygen-consuming processes that are not mitochondrial.

5) Maximum respiration (blue)

This is measured by putting the cell under stress, into a (standardised) state of increased energy demand. This reveals the maximum oxygen consumption that can take place at Cytochrome c oxidase (Complex IV of the mitochondria).

6) Reserve respiration capacity

The difference between the basal respiration rate and maximum respiration rate is the reserve capacity of the mitochondrion.

7) Energetic phenotype

Differentiating ATP generation from glycolysis and oxidative phosphorylation is vital for cellular bioenergetics but has traditionally been hard to quantify. The Seahorse XF is able to calculate the ratio of ATP generation by each pathway from simultaneous measurements of extracellular acidification and oxygen consumption in the basal state as well as on energy demand.

8) Calculation of the MHI

The Mitochondrial Health Index itself is calculated from the various parameters measured in the test.

Mitochondrial Health Index: top page

RESULTS

Sample type: Blood in CPDA vials

Requisition:
Mitochondrial Health Index / PBMCs

Summary

	Patient's value	Target value (optimal)
Mitochondrial Health Index (MHI)	0.77	>2.5
Mitochondrial Bioenergetics		
Coupling efficiency, %	100.00	100
Reserve respiration capacity, %	324.52	>400
Cellular oxygen consumption profile		
Non-mitochondrial respiration as a share of total respiration, %	71.00	<10
Proton leak as a share of total respiration, %	0.00	
Share of respiration used for mitochondrial ATP generation, %	29.00	>90
ATP turnover rate (mitochondrial oxygen utilisation)		
ATP base turnover, %	26.00	<20
ATP reserve, %	74.00	>80
Potential maximum oxygen consumption rate in pmol oxygen/min	22.99	>300
Cellular energy phenotype		
At rest	Resting/glycolytic	Resting
On energy demand	energetic	Energetic
Metabolic potential, mitochondrial percentage	189.87	>350
Metabolic potential, glycolysis percentage	135.97	>350
Oxygen consumption/glycolysis on energy demand	Moderate mitochondrial preference	

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

Overall MHI, derived from the multiple parameters

MITOCHONDRIAL HEALTH INDEX (MHI)

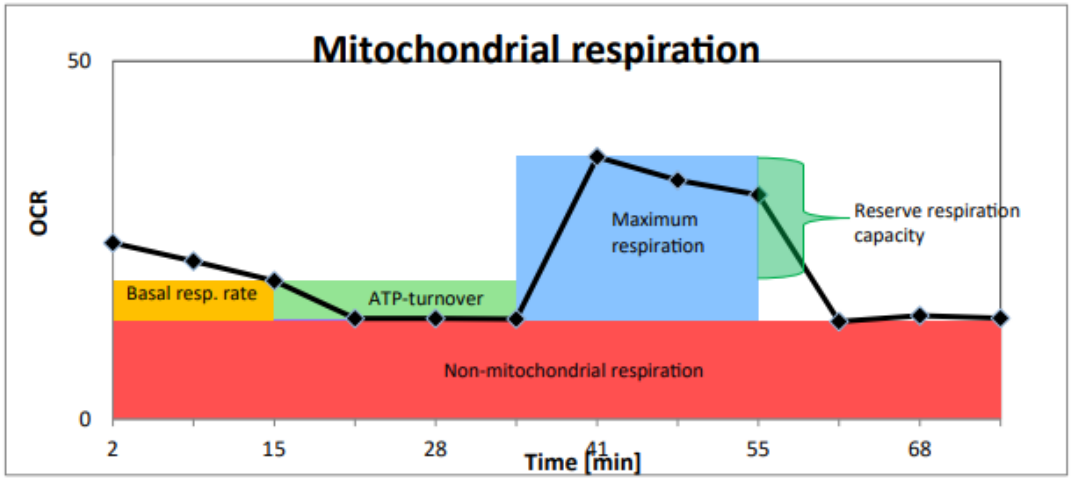
The MHI is a sensitive indicator of the reaction of immune cells (PBMCs) to oxidative stress, and for the changing metabolic programmes that they serve depending on the role they need to play in the case of inflammation, immune defence and immune health. The MHI is is also an indicator for the current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

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

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	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 cell**
- **Compromised electron transport chain**
- **Limited no. of intact mitochondria**
- **Insufficient ATP on demand**
- **Signs of active inflammation/ possible autoimmune disease**

Photon leak/coupling efficiency

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

YOUR RESULTS PROFILE – MITOCHONDRIAL HEALTH INDEX

Coupling efficiency, %	Moderately low
Non-mitochondrial respiration as a share of total respiration, %	Very high
Reserve respiration capacity, %	Optimal

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 (see p. 6, oxygen consumption profile).

Parameter	Evaluation	Reference values in %	Ergebnis in %
Coupling efficiency, %	optimal	99-100	
	Slightly low	95-99	
	Moderately low	90-95	93.80
	Considerably low	70-90	
	Extremely low	<70	

Interpretation of your results:

The coupling efficiency is moderately low.

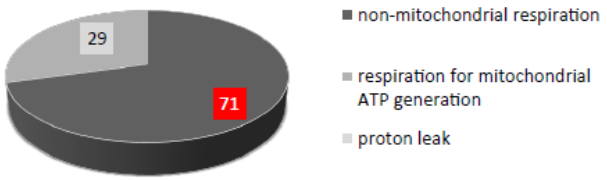
During oxidative phosphorylation, the electrons removed from biological fuels such as glucose and fatty acids go through a series of electron carriers located in the intermitochondrial membrane (IMM), widely known as mitochondrial electron transport chain (ETC). Ultimately these electrons reduce molecular oxygen and energy (ATP – adenosine triphosphate) is generated.

Electron leak refers to the exit of electrons from the ETC before they get reduced to water at Complex IV, and is the major causative factor for the production of mitochondrial superoxide

Non-mitochondrial respiration

Optimal
Slightly high
Moderately high
Very high
Extremely high

How the oxygen is being consumed, %



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

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

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

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

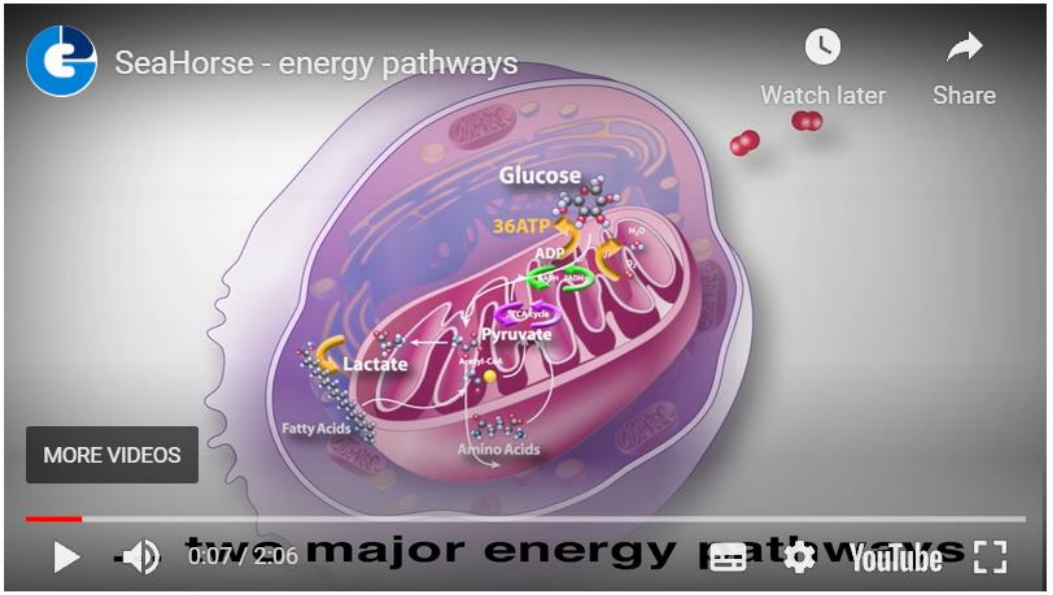
Many videos about the Seahorse technology available, and over 7,400 studies* for which the Seahorse has been used

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

Mitochondrial testing with AONM/MMD

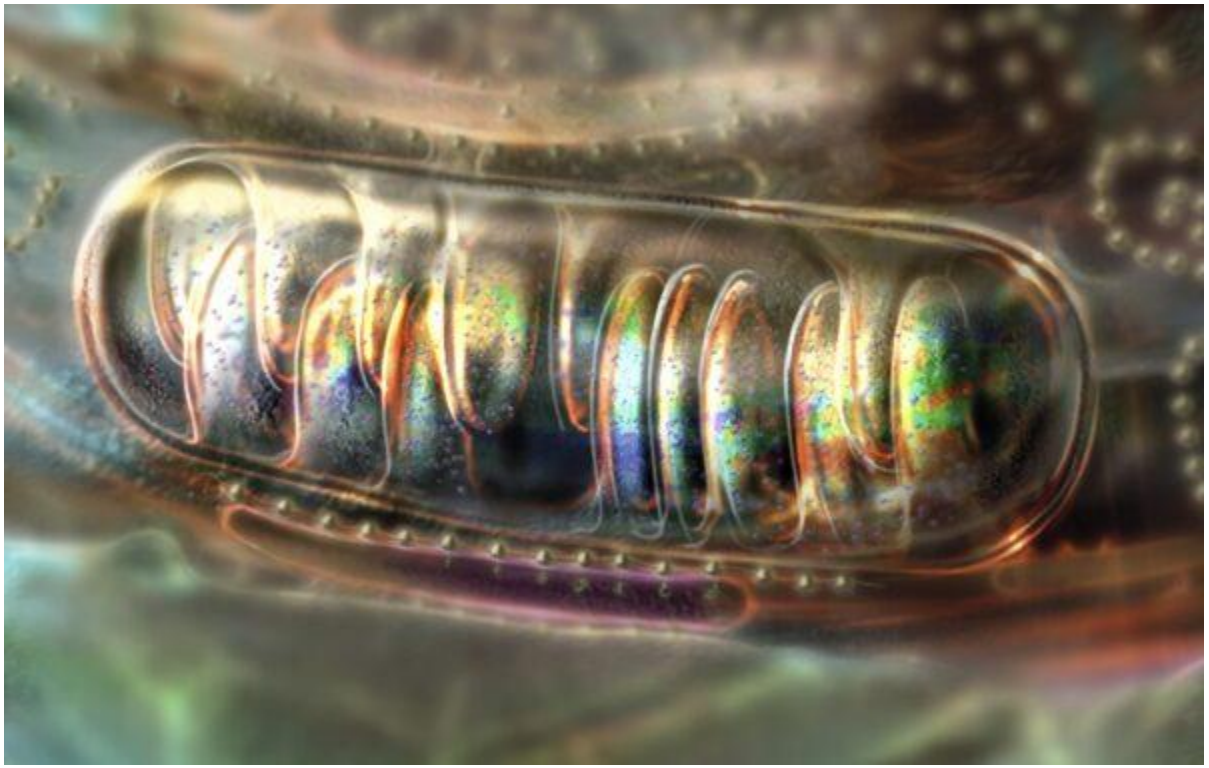
Supplementary biomarkers (next time!):

Already available:

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



Thanks very much for your attention!

mitochondria@aonm.org