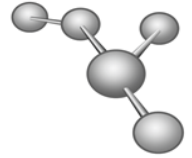


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Patient JD Date of birth 01.01.1990
 Entry on 23.07.2021

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	1.5	fmol/cell		
Mitochondrial ATP capacity	1.3	fmol/cell		87
Glycolytic ATP capacity	1.2	fmol/cell		80
Reserve ATP capacity	0.7	fmol/cell		47

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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Interpretation/ Comments

ATP profile:

The ATP Profile determines total ATP production and allows a quantitative comparison of energy engines (mitochondria and glycolysis) in a basal state.

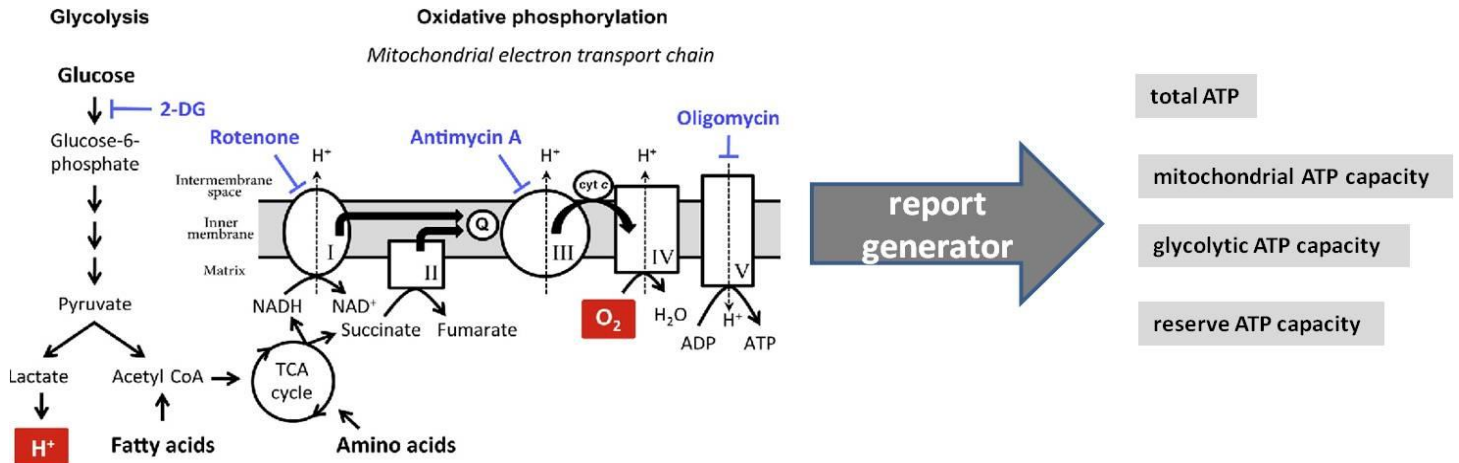


Figure 1: Schematic representation of cellular bioenergetic pathways. The capacity of the individual bioenergetic pathways can be analyzed by using inhibitors of glycolysis as well as inhibitors of the mitochondrial respiratory chain, either individually or in combination. The glucose analogue 2-deoxyglucose (2-DG) cannot undergo conversion to glucose-6-phosphate, thereby competitively inhibiting glycolytic ATP production. Rotenone and antimycin A block Complex I and Complex III of the mitochondrial electron transport chain (ETC), respectively, while oligomycin inhibits Complex V by blocking its proton channel. The metrics of the assay are absolute ATP quantities in fmol/cell. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Q, coenzyme Q; cyt c, cytochrome c; H⁺, proton; TCA, tricarboxylic acid; ETC, electron transport chain; acetyl CoA, acetyl coenzyme A; 2-DG, 2-deoxyglucose; complex I, NADH dehydrogenase; complex II, succinate dehydrogenase; complex III, coenzyme Q: cytochrome-c oxidoreductase; complex IV, cytochrome-c oxidase; complex V, ATP synthase (modified from Pelletier, M., Billingham, L., Ramaswamy, M., & Siegel, R. (2014). *Extracellular flux analysis to monitor glycolytic rates and mitochondrial oxygen consumption. Methods in enzymology*, 542, 125-49.)

ATP is measured by a chemiluminescent (light) reaction using the Luciferin/Luciferase reagent.

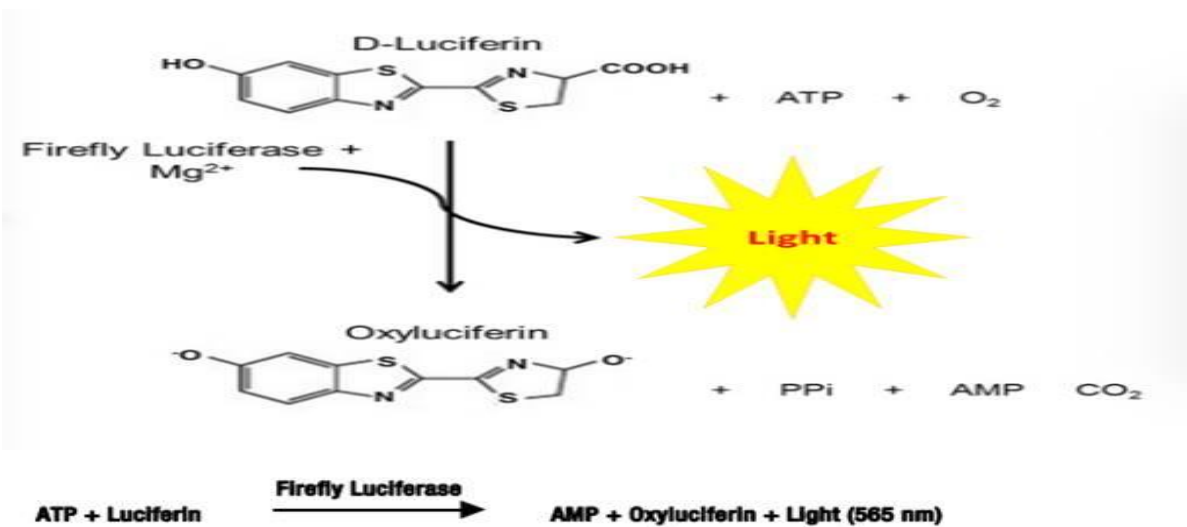


Figure 2: One-Step Luciferase Assay System. (modified from www.tebu-bio.com)

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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. The test measures the total ATP production rate in cells and distinguishes between the capacity for ATP production from mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis. This makes it possible to assess the relative performance of mitochondrial respiration (mitochondrial ATP capacity) versus anaerobic glycolysis (glycolytic ATP capacity). See Figure 1 above.

Here, the patient's Total ATP (entire cell) at rest is 1,5 fmol/ cell. The patient's result is in the optimal range.

Mitochondrial ATP capacity:

"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). Mitochondrial-generated ATP, if production is functioning smoothly, has a very high harvest: ~ 34 ATP from one molecule of glucose, and far more from fats (e.g., ~ 146 ATP from one molecule of oleic acid). The metric is given as both a percentage and in femtomoles/cell. See Figure 1 above.

Here, mitochondrial ATP is 87 %/ 1,3 fmol/ cell. The patient's result is in the upper end of the moderate range. The optimal would be > 1.4 fmol/cell.

This is a useful marker of mitochondrial function; it can be compared with glycolytic ATP capacity and reserve ATP capacity to see how well the mitochondria are functioning in a basal state. Lower than optimal mitochondrial ATP capacity suggests a lack of substrate availability, damage to the complexes of the electron transport chain or the mitochondrial membranes, contaminants such as pesticides or heavy metals blocking substrate exchange, etc.

Glycolytic ATP capacity:

ATP can also be produced in the cytosol, outside the mitochondria (though still inside the cell). This is produced largely from glucose, and the amount of ATP per molecule of glucose is very low (just 2 ATP per molecule of glucose). 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. This metric, again, is expressed as a percentage as well as in femtomoles/cell. See Figure 1 above.

Here, the glycolytic ATP capacity is 80 %/ 1,2 fmol/ cell. The patient's result is in the upper end of the moderate range. The optimal would be > 1.4 fmol/cell.

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 electron transport chain, and also so that the cytosolic production of ATP (glycolysis) can be upregulated if needed, when the immune cells need to address pathogens, etc.

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Reserve ATP capacity:

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. See Figure 1 above.

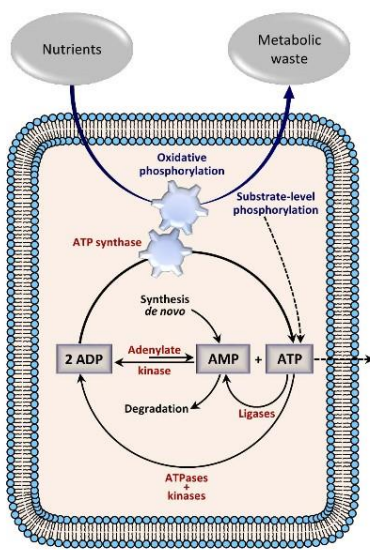


Figure 3: The Adenylate energy system. Oxidative phosphorylation and substrate-level phosphorylation generate ATP which is degraded by kinases (also ATPases) and ligases yielding ADP and AMP, respectively. The three adenine nucleotides are catalytically interconverted by adenylate kinase according to the needs of the metabolic system. AMP is also subjected to processes of synthesis/degradation, some AMP molecules are de novo biosynthesized, and a part of AMP is hydrolyzed. According to experimental observations, a very small number of ATP molecules may not remain in the adenylate reactive structure. The system (thermodynamically open) needs a permanent input of nutrients as primary energy source and a consequent output of metabolic waste. The biochemical energy system depicted in the figure represents some key essential parts of the adenylate energy system. *De la Fuente, I. M. et al. (2014). On the dynamics of the adenylate energy system: homeorhesis vs homeostasis. PloS one, 9(10), e108676*

Here, the reserve ATP capacity is 47 %/ 0,7 fmol/ cell. The patient's result is in the optimal range.

47 % means that the cell is able to perform dynamic catalytic interconversion between the three adenine nucleotides (ATP, ADP and AMP) according to metabolic needs.

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Comments

If some of these parameters are suboptimal it may be useful to perform appropriate biochemical tests, such as investigating what may need to be addressed, e.g. heavy metals/contaminants (Hg, Al, glyphosate, Bisphenol A, PCBs, etc.), infections (such as *Borrelia*, viruses), deficiencies in mitochondrial substrates (B vitamins, CoQ10, magnesium, etc.). It may also be helpful to measure fatty acid composition, and redox status.

The ATP profile determines the above parameters in a cell at rest. If we consider your individual values of the ATP generation, we come to the following assessment of your energy supply:

The cellular energy (ATP) in resting cells is in the normal range. A lack of energy when energy is required is not to be expected. Should there be any suspicion of insufficient energy reserves, further analyzes are advisable.

The "Mitochondrial Health Index" (MHI) identifies the changes to these parameters and others when the cell is under stress or energy demand. If the ATP Profile suggests compromised mitochondrial function, it can be helpful to perform an MHI to obtain further information on where the issues lie. If the ATP profile is in normal range, it is still possible that mitochondrial weakness becomes evident when the cells are put under stress (energy demand). If there is a clinical suspicion of mitochondrial weakness but a normal ATP profile in a basal state, performing the MHI is recommended.

Various supplementary markers may also be useful, such as the ratio of mitochondrial DNA to nuclear DNA (mtDNA:nDNA, which also indicates the mitochondrial mass), PGC-1 α , Nrf-2, the 4977 deletion mutant, and the lactate/pyruvate ratio. For further details on these, please see the AONM "Mitochondrial testing" website.

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