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Psychopathology, mental disorders and mitochondrial disorders

Dissertation

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Abstract

Projekt je zaměřen poznání souvislosti mitochondrialních patofyziologických procesů s psychopatologickými příznaky při bipolární afektivní poruše (BPD). Změny aktivity vybraných složek dýchacího řetězce a celková respirační rychlost byly měřeny u pacientů s bipolární afektivní poruchou v porovnání s kontrolní skupinou. byly použity diagnostické dotazníky, respirometrie s vysokým rozlišením a metody radiochemické a spektroskopické. Analýzy provedeny u 21 zdravých kontrol a 37 osob s diagnózou bipolární afektivní poruchy (F31). Statistická analýza zahrnovala parametrické a neparametrické analýzy, faktorovou analýzu, jednocestnou analýzu rozptylu a lineární regresní analýzu. Získané výsledky ukázaly velkou roli buněčné energetiky v patofyziologii bipolární poruchy. Mírný rozdíl mezi různými aktivitami mitochondriálních enzymů byl získán u pacientů s manickou a depresivní epizodou onemocnění. Byly také prokázány změny mitochondriálního dýchání u pacientů s BPD ve srovnání se zdravými kontrolami. Mitochondriální respirační indexy u pacientů v remisi ve srovnání se zdravými kontrolními osobami byly změneny v souvislosti s předchozí fází onemocnění. Byla zjištěna souvislost mezi stavem onemocnění, psychopatologickými příznaky, klinickým zlepšením a mitochondriální patologií. Byla stanovena doba trvání mezi akutním manickým stavem a remisí a její závislost na indikátorech mitochondriální patologie.

Annotation

This study investigates the connection between different pathophysiological processes in mitochondria and psychopathological symptoms in patients with bipolar disorder. Changes in activity of selected components of the respiratory chain and overall respiratory rate of mitochondria were analyzed in patients with bipolar disorder when compared to healthy controls. Diagnostic scales and questionnaires, high-resolution respirometry, radiochemical and spectroscopic methods were used. 37 patients with a diagnosis of bipolar disorder (F31) and 21 healthy volunteers were involved in the study. Statistical analysis included the methods of parametric and nonparametric analysis, factor analysis, one-way analysis of variance and linear regression analysis. Obtained results revealed that cellular energetics plays a great role in the pathophysiology of bipolar disorder. There was a mild difference between different mitochondrial enzymes activity in patients within manic phases and depressive phases of the disease. Changes in mitochondrial respiration in patients with BD as compared to healthy controls were also shown. Mitochondrial respiration indexes for patients with BD in remission as compared to healthy controls were altered in accordance with the previous phase of the disease. Association between the state of the disease, psychopathological symptoms, clinical improvement and mitochondrial pathology was established. The duration period between the acute manic state and remission and its dependence on the mitochondrial pathology indicators was established.

Klíčová slova

afektivní poruchy
biomarker
bipolární porucha
deprese
krevní destičky
mánie
mitochondrie
mitochondriální dýchání
mitochondriální patologie
respirační řetězec

Keywords

biomarker
bipolar disorder
blood platelet
depression
mania
mitochondria
mitochondrial pathology
mitochondrial respiration
mood disorders
respiratory chain

1. Introduction

Mental disorders are a big group of complex and serious diseases affecting mainly the psychic sphere and are characterized by a high prevalence, difficulties with the diagnosis, high levels of disability and mortality, a significant societal cost and different serious risks for the patients.

During the last decades many publications revealed an increasing need for further research on this topic because of characteristics such as frequent life-threatening conditions, urgent intervention requirement, clinical pathomorphosis, prolonged duration and a delayed treatment response postulate a problem of mental disorders as one of the central problems of modern psychiatry and general medical practice. Psychopathological symptoms also often cause a significant impairment of social functioning which may have an irreversible affect on patient's life.

Although research is ongoing many important questions still remain open. Questions of early diagnosis and prevention, clinical assessment of the symptoms, therapeutic approaches and pathomorphological mechanisms undermining the disease continue to be unanswered. One of these questions is a comprehensive study of typical pathogenetic features associated with the psychopathological symptoms of the disease, including cell mechanisms.

Cell respiration in psychiatric disorders had been a subject of large research interest for many years as the nerve tissue is highly dependent on oxidative metabolism because of a high energy demand and thus the brain is extremely vulnerable to an insufficient ATP production. Many researchers found evidence for mitochondrial dysfunction and oxidative stress in different mental disorders, although most of the patients do not have any 'classical' mitochondrial disease.

Mood disorders are one of the main focuses in mitochondria-related research since 2000 when Dr. Kato offered a mitochondrial hypothesis based on the findings that patients with bipolar disorder have an abnormal energy metabolism and abnormal mitochondrial DNA in the brain.

Mood disorders (depressive, manic and bipolar disorders) are very common illnesses, often with recurrent or chronic courses. Their pathophysiology is not yet

well known. There is currently no reliable biochemical, genetic, physiological or other biological test to diagnose bipolar affective disorder or to predict the success of pharmacotherapy.

The etiology of mood disorders, including BPD, remains uncertain. Both genetic background and environmental factors, such as stressful life events or substance abuse, are related to the risk of development of BPD (Uher R, 2014). Insights into the processes underlying neuroprogression in BPD have been provided by studies examining genetic and epigenetic changes, structural and functional changes in the brain, damage in neuronal circuits, disturbed circadian rhythms, changes in immune and endocrine systems, impairment in neuronal plasticity and resilience, increased apoptosis, disturbances of synaptic transmission and signal transduction, activation of neurotoxic mechanisms, and changes in neurogenesis (Berk M et al, 2014). Pathways underlying neuroprogression in BPD include the dopaminergic system, inflammatory cytokines, oxidative and nitrosative stress, mitochondrial dysfunction and endoplasmic reticulum stress, alterations in cAMP response element-binding protein (CREB) and neurotrophic system, dysregulation of calcium signaling, neuroin- flammation, autoimmune processes, tryptophan and tryptophan metabolites, and hypothalamic–pituitary–adrenal (HPA) axis dysregulation. (Berk M et al, 2011; Anderson G, Maes M, 2015; Andreazza AC, Young LT, 2014)

Research for biological markers of bipolar affective disorder is based on a current mood hypothesis that the activity of monoaminergic neurotransmitter systems, energy cell metabolism, growth factor and other components affecting neuronal plasticity. Nerve cells need an extraordinarily large amount of cellular energy to provide for the synthesis of molecules that allow them to receive, process and transmit information, develop axonal and dendritic branches, and create new synaptic connections. Therefore, the hypothesis of mitochondrial dysfunction is a prospective hypothesis for a number of diseases including bipolar affective disorder.

The aim of the following research is to determine the connection between selected mitochondrial functions and psychopathological symptoms during the disease, i.e. in manic, depressive and remission episodes of the bipolar disorder.

2. Literature review

2.1. Mitochondria, its structure, role and functions

Mitochondria were discovered more than 100 years ago and were then viewed as a cytoplasmic element with undetermined functions. Between discovery and the 1950s, some observations of mitochondria in ultra thin sections were made via the development of the electron microscopy. In the following decades the mitochondria structure and biochemical processes taking part in it were thoroughly explored. Most research was reduced to the energy production and to different changes of mitochondrial structure during the respiration cycle.

During the last decades we have wintessed a change in the approach to mitochondria. An old idea from our textbooks, postulating mitochondria as an ‘energetic station‘ of the cell is now changing to a kind of ‘Pandora’s box‘ hypotheses, which gently suggests that mitochondria take part in various processes through various mechanisms. Some components of mitochondria and their impact on men’s health are still to be investigated (DiMauro S, Andreu AL, 2000).

According to the previous paradigm, the role of mitochondria in different pathologies and diseases was limited by the description of energy supply dysregulation of cell respiration, either in terms of hypoxic and toxic damage or in genetic impairment (DiMauro S, Hirano M, 2009). Now so-called ‘side processes‘ of mitochondria are coming to the stage. Many studies of mitochondria and its role in the pathogenesis of various diseases are being done. Such terms as ‘mitochondrial pathology‘ and ‘mitochondrial disease‘, having appeared not long ago (Luft R, 1994), are also changing their meaning to include a broader range of disturbances and lesions. One thing still seems unarguable - mitochondria dysfunction is causing diverse effects within the cell and in whole organism (Nunnari J, Suomalainen A, 2012).

Mitochondria are a small cell organelles with two membranes, mostly not contacting each other. The outer membrane of mitochondria (7 nm thickness) is

smooth, built of proteins and two layers of lipids. It separates mitochondria from the cell cytoplasm and provides the transport of small molecules and ions; it also has such enzymes as monoaminoxidase, monooxygenase, acyl-CoA (coenzyme A) synthase and phospholipase A.

The inner membrane is plicated and forms numerous folds (cristae), which significantly increases the surface area. It contains cardiolipine and proteins, and does not have any transport pores. ATP-synthase molecules are located on its matrix side. There are also some respiratory chain components in this membrane, filling the membrane space.

Between outer and inner membrane there is an intermembrane space (10-20 nm thickness). One protein is located in it is cytochrome *c*, a respiratory chain component.

The internal space of the mitochondria is called matrix. Located in matrix enzyme systems of pyruvate and fatty acids oxidation are enzymes of tricarboxylic oxid cycle (Krebs cycle), DNA, RNA and protein-synthetizing systems.

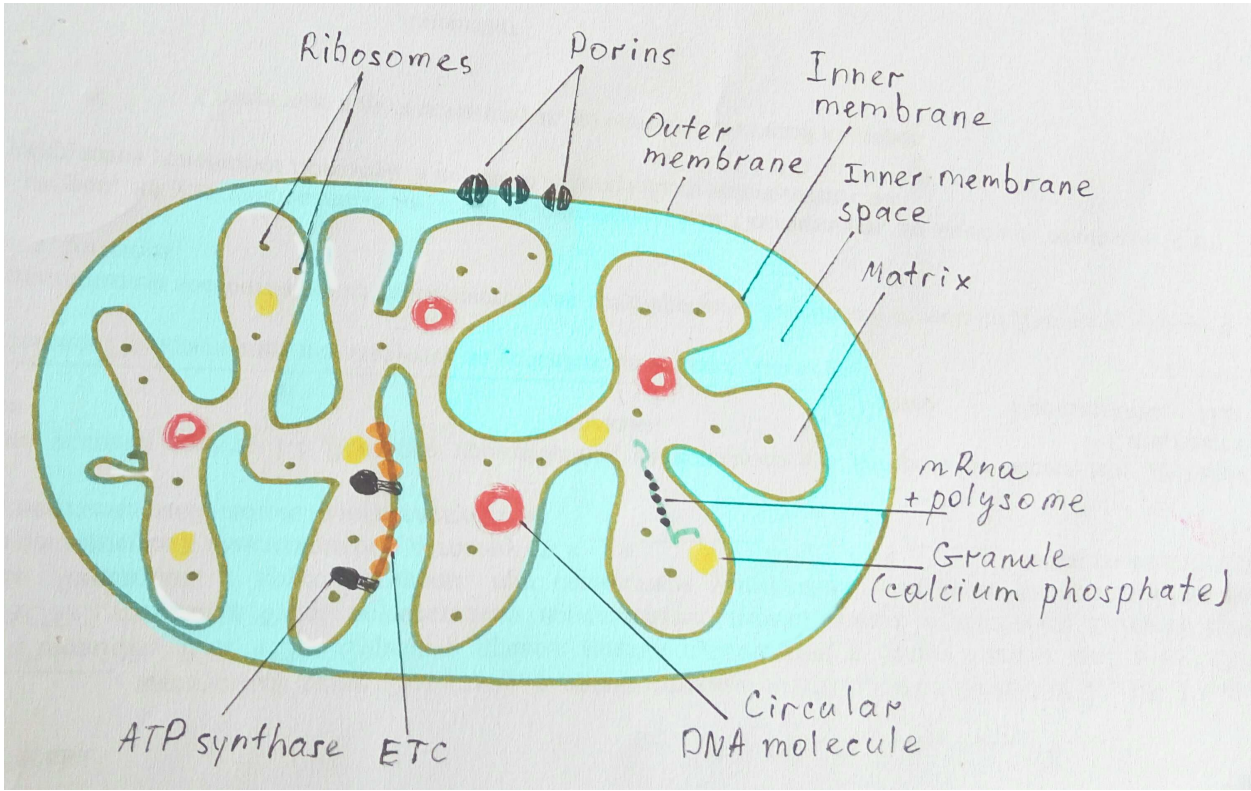

Fig. 1. Structure of the mitochondria

Mitochondria serve many functions, but their primary goal is participation in biochemical cycles of cell respiration. The main processes taking place in mitochondria are: the tricarboxylic acid cycle, fatty acids oxidation, carnitine cycle, electron transport in the respiratory chain (via I-IV enzyme complexes) and oxidative phosphorylation (V enzyme complex).

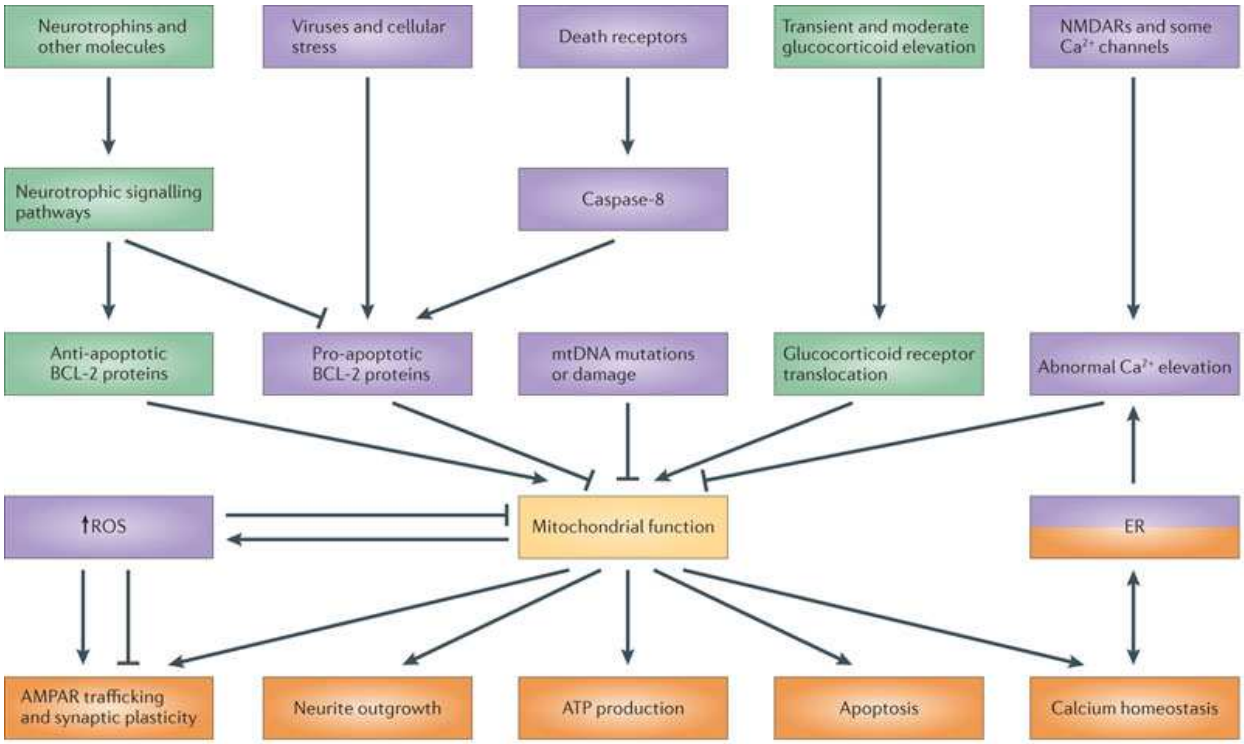

Fig. 2. Mitochondrial function is at the nexus of several pathways that regulate synaptic plasticity and cellular resilience. (Manji H.K. et al, 2012)

2.2. Tricarboxylic acid cycle

Tricarboxylic acid cycle (TCA, Krebs cycle) is the key stage of cell respiration; a crossway of several metabolic pathways and an intermediate point between glycolysis and respiratory chain. Its role is not only energetic, it is a significant source of the precursors' molecules for synthesis of many important compounds.

A cyclical biochemical process takes place in mitochondria, in which one molecule of acetyl-CoA is completely oxidized into two molecules of carbon

dioxide (CO_2) with reduced form of nicotinamide adenine dinucleotide (NADH) (from nicotinamide adenine dinucleotide, NAD+), reduced form of flavin adenine dinucleotide ($FADH_{2)}$ (from flavine adenine dinucleotide, FAD) and guanosine-5'-triphosphate (GTP) production (from guanosine diphosphate, GDP, and inorganic phosphate, P_i) (Krebs HA, Weitzman PDJ, 1987). Electrons from NADH and $FADH_2$ are transported to the respiratory chain where ATP is formed.

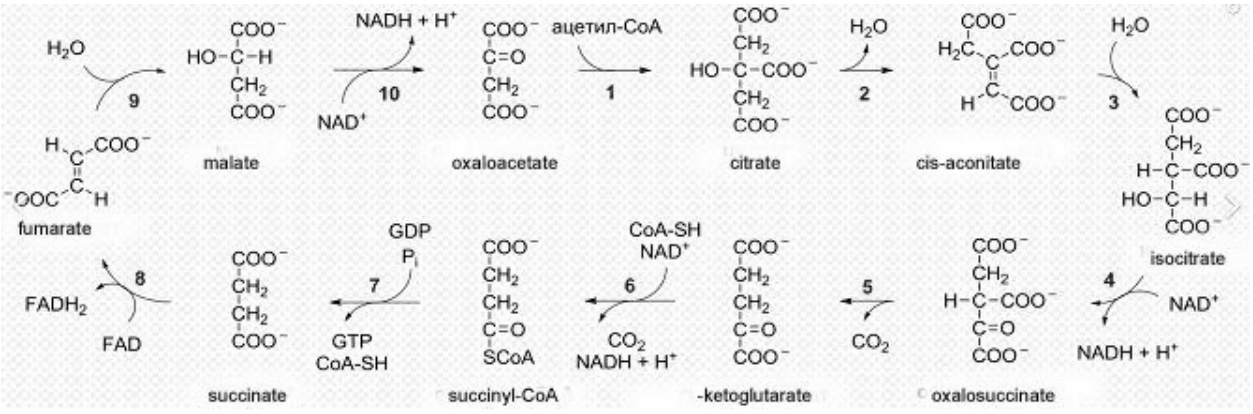

Fig. 3. Tricarboxylic acid cycle

Acetyl-CoA, a substrate for the Krebs cycle, is derived from carbohydrates, fats, and proteins; in the brain it comes mostly from the glycolysis reaction, where glucose is converted to pyruvate, followed by pyruvate decarboxylation by pyruvate dehydrogenase complex. The second resource of acetyl-CoA in the brain is fatty acids oxidation.

The cycle consists of several basic reactions. First, two-carbon acetyl group of the acetyl-CoA is transferred to a four-carbon acceptor (oxaloacetate) and a six-carbon compound (citrate) is formed. Two carboxyl groups from oxaloacetate are later being lost as CO_2, and the missing parts are being donated by acetyl-CoA. The loss of donated carbons requires several turns of the cycle, as it is continuously supplied by the new acetyl-CoA as a carbon donator (Jones RC et al, 2000). The energy of the reactions is transferred as electrons to form NADH and $FADH_2$, or to other metabolic processes.

Steps

1) Aldol condensation reaction catalyzed by a citrate synthase enzyme

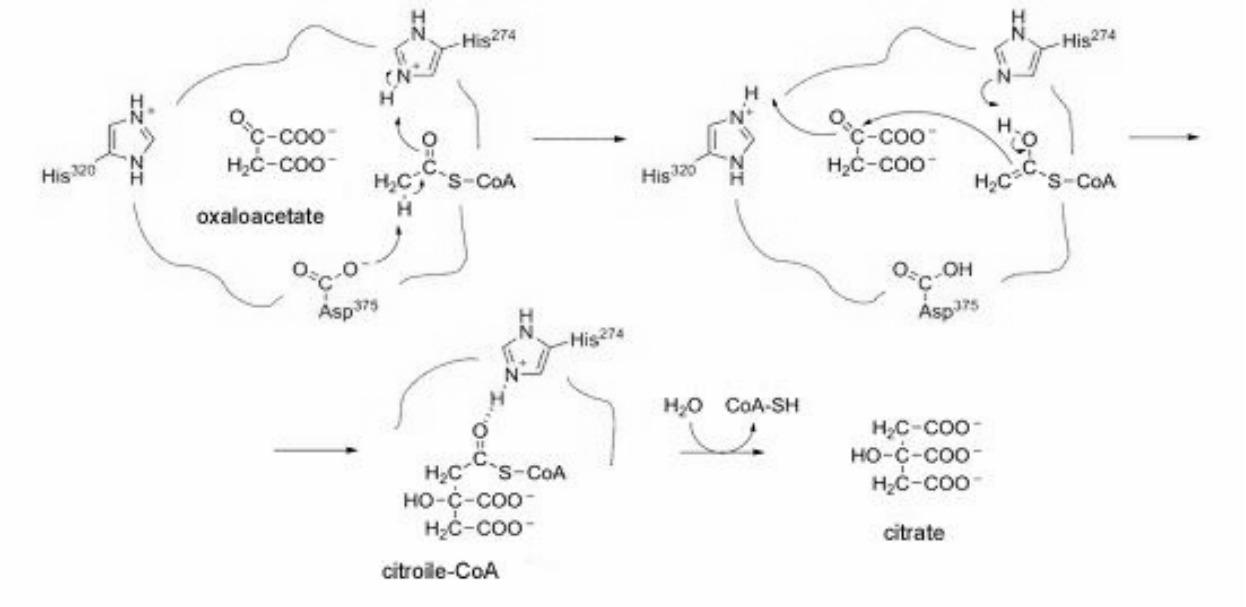

Fig. 4. Tricarboxylic acid cycle, step 1

A methyl group of the acetyl-CoA is attached to the carbonyl group of the oxaloacetate. During this reaction an intermediate compound is formed. It is quickly hydrolyzed and splits into a free CoA and citrate, which is eliminated from the enzyme active point. This reaction is highly exergonic because of hydrolysis of the thioether compound, and this negative energy change is necessary for the cycle management, because oxaloacetate concentration in the cell is quite low. CoA from this reaction takes part in the oxidative decarboxylation of the next pyruvate molecule.

2) Dehydration-hydration reaction catalyzed by aconitase

Aconitase is an enzyme that contains of FeS-claster and catalyzes a reversible dehydration of citrate into an intermediate compound, a tricarbozylic acid named cis-Aconitate, which does not leave the active point of the enzyme normally. The enzyme attaches a water molecule to the olefinic link of *cis*-Aconitate and the reaction goes in two different ways, with a citrate (app. 90%) and isocitrate (app. 10%) as its main products.

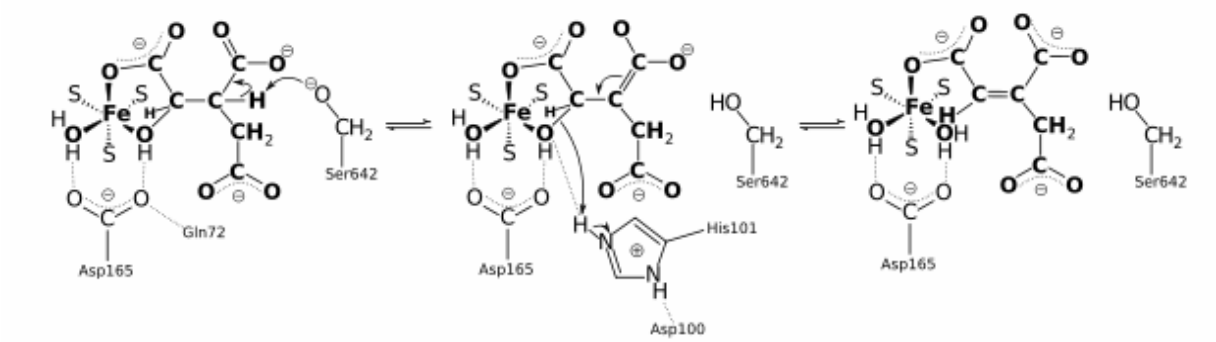

Fig. 5. Tricarboxylic acid cycle, step 2

3) Isocitrate oxidation and decarboxylation reaction

This step contains of two stages. The first stage is oxidative decarboxylation of isocitrate catalyzed by isocitrate dehydrogenase which requires proton transfer from NAD+ (or NADP+). At the end of this stage oxalosuccinate is formed. It is an intermediate compound and it does not leave active point of the enzyme. The second stage involves an interaction between carbonyl group of oxalosuccinate and Mn^{2+} (or Mg^{2+}) ion of the enzyme which pulls the electron density so that α-Ketoglutarate can be formed. Isocitrate dehydrogenase exists as two izoenzymes, eukaryotic cells that have mostly NAD+ dependent izoenzyme.

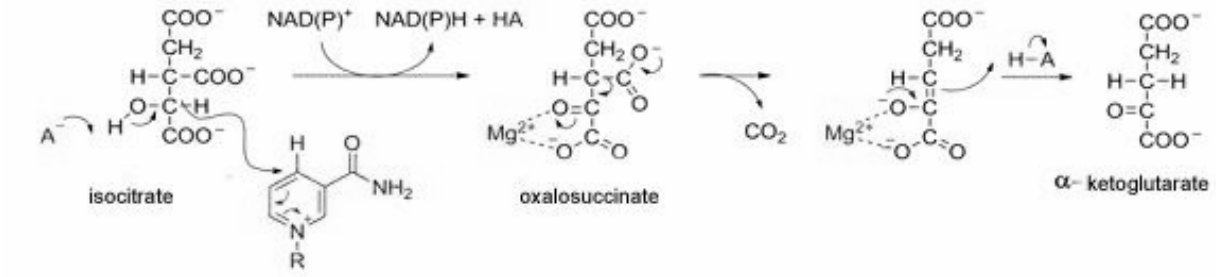

Fig. 5. Tricarboxylic acid cycle, step 3

4) α-Ketoglutarate oxidative decarboxylation reaction

This reaction is catalyzed by α-ketoglutarate dehydrogenase which is a complex enzyme containing 3 sub-enzymes with several co-factors. This enzyme is largely similar to pyruvate dehydrogenase and the whole reaction is almost identical to pyruvate oxidative decarboxylation.

During the oxidative decarboxylation succinyl-CoA is formed. NAD+ behaves as an electron acceptor and CoA transports the succinyl group.

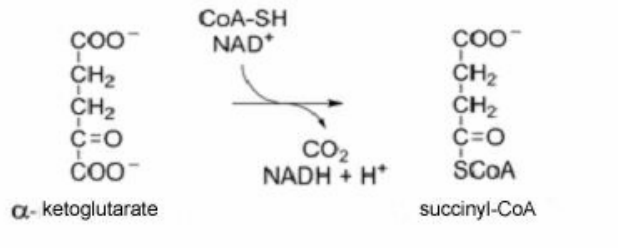

Fig. 6. Tricarboxylic acid cycle, step 4

5) Substrate-level phosphorylation reaction

Succinyl-CoA has a thioesher bond with a big negative energy which is released in hydrolysis reaction ($\Delta G'^{o} \approx -36$ KJ/mol). This energy is useful for phosphoanhydride bonding of GTP/ATP and thus succinate can be formed. The reaction is catalyzed by Succinyl-CoA synthetase which gets phosphorylated (by the histidine residue) itself during the intermediate steps of the reaction. It has two subunits, α-subunit carries a phposphoryl group with a high potential for transfer, and β-subunit determines the specificity of the izoenzyme (GDP/ADP). The active point of the enzyme is located between subunits.

ATP/GTP formation is a substrate-level phosphorylation because of energy saved during α-Ketoglutarate decarboxylation. If GDP is formed, it can donate its end phosphoryl group to ADP, so we can say the final point of every Succinyl-CoA synthetase izoenzyme activity is ATP formation.

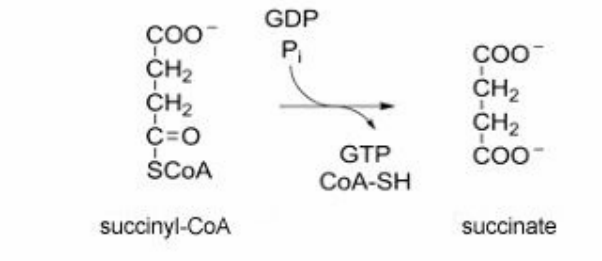

Fig. 7. Tricarboxylic acid cycle, step 5

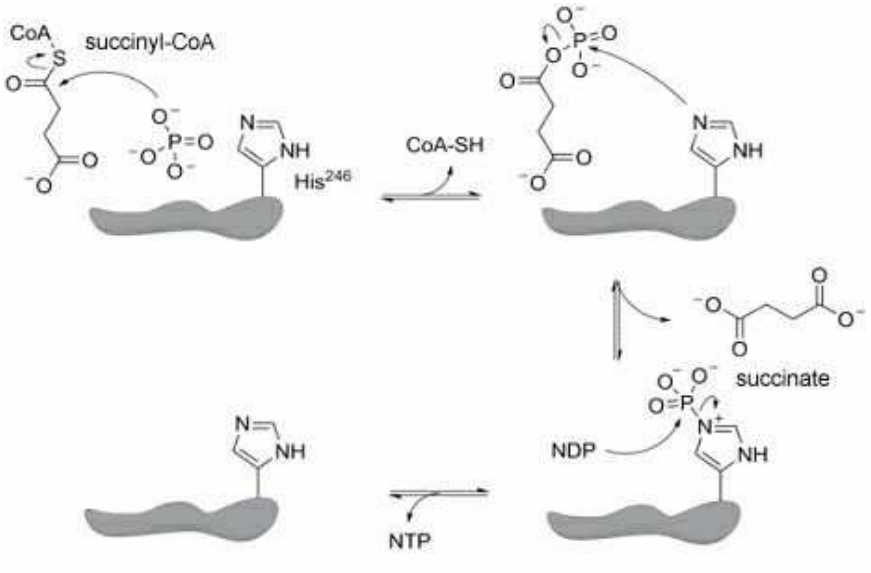

Fig. 7a. Tricarboxylic acid cycle, step 5

6) Succinate oxidation

An enzyme succinate dehydrogenase catalyses the succinate oxidation reaction in which fumarate is formed. This enzyme is located on the inner mitochondrial membrane and contains of three FeS subunits and one FAD molecule (a prosthetic group).

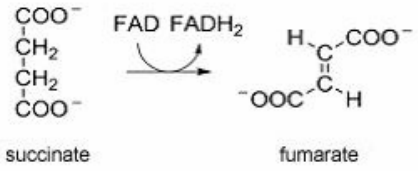

Fig. 8. Tricarboxylic acid cycle, step 6

Electrons are transferred from succinate through FAD and FeS subunits. Electrons then get into the respiratory chain, where FAD is reducted to $FADH_2$, and the acceptor role is taken by the ubuquinone. An electronic transfer through all these carriers to oxygen is connected with the ATP synthesis (1,5 molecule ATP per one electron pair).

7) Fumarate hydration

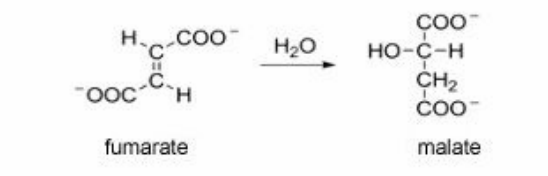

Fig. 9. Tricarboxylic acid cycle, step 7

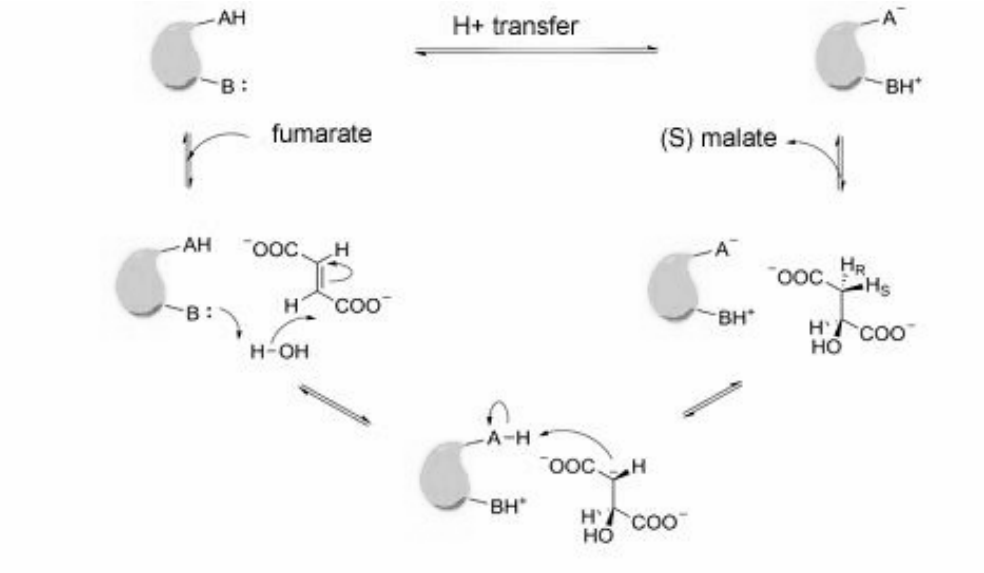

Fig. 9a. Tricarboxylic acid cycle, step 7

Fumarate is reversibly hydrated into L-Malate. During this reaction a carbanion (an intermediate compound of the hydration reaction) is formed. The enzyme engaged in this step is called fumarase. It is a stereospecific enzyme because it catalyzes a fumarate (trans-isomeride) olefinic linkage hydration. Maleate (cis-isomeride) hydration is not catalyzed by the enzyme.

8) L-Malate oxidation

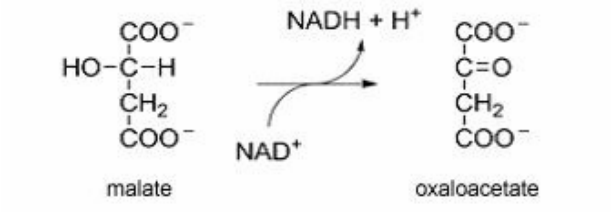

Fig. 10. Tricarboxylic acid cycle, step 8

This reaction is catalyzed by L-Malate dehydrogenase, which is a NAD-dependent enzyme. L-Malate is oxidized into oxaloacetate. In vitro this reaction

balance is displaced to the left, although in the cell oxaloacetate is constantly engaged in high-exergonic citrate-synthase reaction (step 1). That's why cincentration of the oxaloacetate in cell is quite low, and the reaction balance of the last step displaces it to the right.

All enzymes of the Krebs cycle exist within the cell as multienzyme systems, which provide an effective transfer of the rections compounds from one step to another. These systems are called metabolones.

The cycle is regulated by substrate availability, enzyme down-regulation and inhibition of the end products, which provides a stable concentration of all compounds. For instance, all exergonic reactions can be rate-limiting; substrate availability depends on the cell state; NADH/NAD^+ rate can inhibit or slow down dehydration reactions; end products also can inhibit reactions so that carbon flow is enough for optimal ATP and NADH concentrations. The glycolysis rate and TCA cycle rate are interconnected, and other biochemical reactions also regulate this rate in several ways.

2.3. Fatty acids oxidation and carnitine cycle

A hypothesis of β-oxidation was first introduced by F. Knoop in 1904. Modern view of fatty acids oxidation is widely based on this theory.

For now there are known at least 25 known enzymes and specific transport proteins in the β-oxidation pathway. 18 of them have been associated with human disease (Tein I, 2013). The oxidation contains of the following:

1) Fatty acids activation

Free fatty acids are non-reactive regardless the hydrocarbon chain length. They pass the cell membrane through specific transport proteins such as the SLC27 family fatty acid transport protein (Stahl A, 2004). For oxidation reaction they need to be activated. Activation takes place on the outer mitochondrial membrane and involves ATP, HS-CoA and. Mg^{2+}. It is catalyzed by long fatty acyl-CoA synthase and is driven to completion by inorganic pyrophosphatase. First ATP and

fatty acid form acyl adenilate, a fatty acid and AMP ether; then a sulfhydryl group attacks acyl adenilate; the end product of the reaction is acyl-CoA. This compound is an active form of fatty acid and can be oxidized.

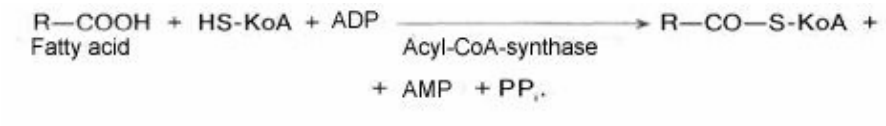

Fig. 11. Fatty acids activation

2) Fatty acids transport

Acyl-CoA is not able to reach the oxidation point which is located inside the mitochondria, so carnitine acts as a carrier for fatty acids with a long chain and transports it through the inner membrane. Acyl group is transferred from S atom to a hydroxyl group of carnitine. The cytoplasmic enzyme catalyzing the reaction is palmitoyl transferase. The end product of the reaction is acylcarnitine. This compound can diffuse through the inner membrane of the mitochondria with the help of carnitine-acylcarnitine translocase. If the fatty acyl-CoA contains a short chain (less than 10 carbons) it can simply diffuse through the inner mitochondrial membrane.

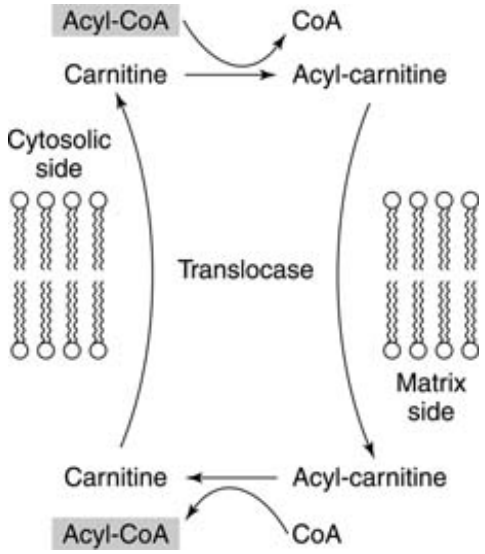

Fig. 12. Fatty acids transport

After the diffusion the acyl group is transferred back to CoA in a counter reaction of breakage of the acyl carnitine with a HS-CoA and mitochondrial palmitoyl transferase engaged.

3) Fatty acids oxidation

The oxidation itself contains of several consistent steps.

A. Dehydrogenation phase 1

In mitochondria acyl-KoA undergoes an enzymatic dehydration and loses two hydrogen atoms in α- and β-positions. The end product of the dehydration is a CoA ether of the unsaturated acid with a double bond between C2 and C3 (trans-delta2-enoyl CoA). The enzyme catalyzing this reaction (fatty acyl CoA dehydrogenase) has the specificity for the length of the hydrocarbon chain. FAD is an electron acceptor and it is reduced to $FADH_2$.

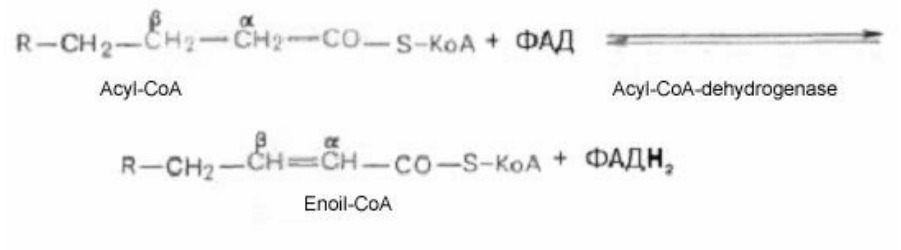

Fig. 13. Fatty acids oxidation, dehydrogenation phase 1

B. Hydration

The trans-delta2-enoyl CoA adds a molecule of water and forms L-β-hydroxyacyl-CoA (3-hydroxyacyl-CoA). This reaction is catalyzed by enoyl-CoA-hydratase which is stereospecific, similar to fumarate and aconitate hydration reactions.

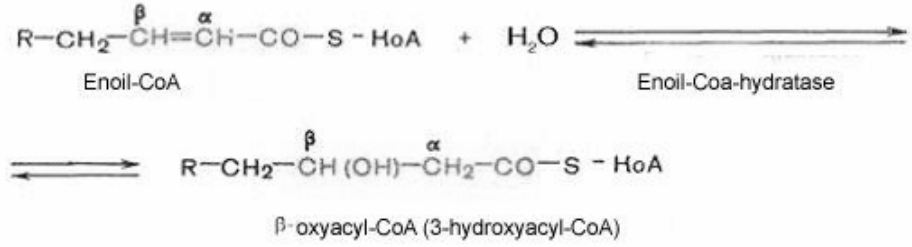

Fig. 14. Fatty acids oxidation, hydration

C. Dehydrogenation phase 2

In this step β-oxyacyl-CoA is dehydrogenated again to get β-ketoacyl-CoA. This reaction is catalyzed by NAD^+ dependent β-hydroxyacyl CoA dehydrogenase. NAD acts as an electron acceptor.

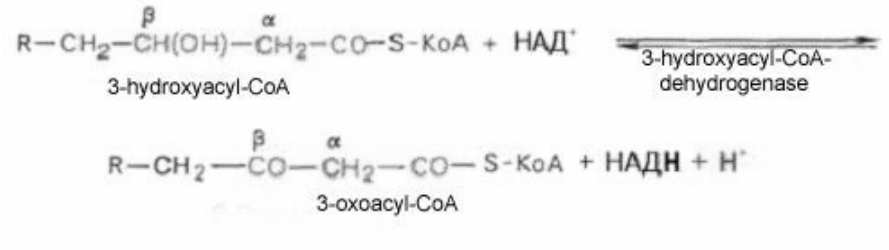

Fig. 15. Fatty acids oxidation, dehydrogenation phase 2

D. Thiolysis

This is a reaction of C2 and C3 carbons of the β-ketoacyl-CoA and is a degradation reaction. It is catalyzed by Acetyl CoA acyl transferase. The intermediate compounds are the acyl-CoA which is shorter by two carbon atoms and a dicarbone segment (acetyl-CoA).

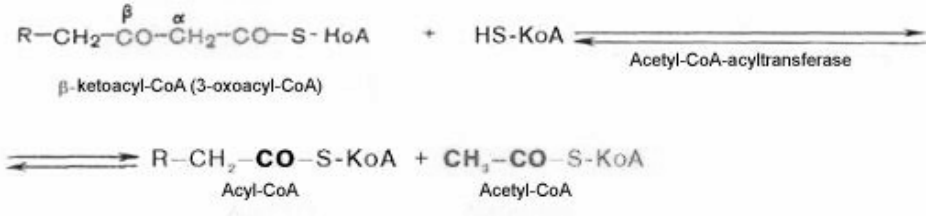

Fig. 16. Fatty acids oxidation, thiolysis

Acetyl-CoA goes into the TCA cycle for oxidation. Acyl-CoA goes further into the fatty acids β-oxidation cycle again and again until the tetracarbon compound (butyryl-CoA) is formed. Butyryl-CoA is also oxidized and 2 molecules of acetyl CoA are formed. So, we can see that an oxidation of the fatty acid containing of N carbon atoms requires N/2–1 β-oxidation cycles, and the end quantity of acetyl CoA is N/2.

Approximately 990 kcal or 42% of the reaction energy is used for the ATP resynthesis, and the rest of it is lost as heat. So the energy yield is about 40%, which is close to TCA energy yield, glycolysis energy yield and oxidative phosphorylation energy yield.

2.4. Electronic transport in the respiratory chain

During the pyruvate dehydrogenase reaction and Krebs cycle, substrates (pyruvate, isocitrate, α-ketoglutarate, succinate, malate) are being metabolized, and NADH and $FADH_2$ are formed. These coenzymes take part in the mitochondrial respiratory chain, which involves ATP synthesis and together it is called oxidative phosphorylation.

The respiratory chain is a pathway of enzymes, providing hydrogen ions and electron transport from the substrate to the molecular oxygen – the final hydrogen acceptor. Energy in these reactions releases gradually and can be accumulated as an ATP molecule. Enzymes of the respiratory chain are located on the inner mitochondrial membrane. The chain consists of five multi-enzyme complexes, two electron carriers, a quinone (coenzyme Q), and a small hem-containing protein (cytochrome *c*) (Kang D, Hamasaki N, 2006).

I. NADH-ubiquinone oxidoreductase (contains seven subunits which are encoded by the mtDNA and at least 39 nuclear-encoded subunits of complex I).

II. Succinate-ubiquinone oxidoreductase (composed of four subunits, all encoded by the nuclear genome).

III. Ubiquinol-ferricytochrome *c* oxidoreductase (holds one subunit, cytochrome b, encoded by the mitochondrial genome and 10 subunits encoded by the nuclear genome).

IV. Cytochrome *c* oxidase (COX) (composed of 13 subunits, three of which are encoded by mtDNA and the other 10 by nuclear DNA).

V. ATP synthase (composed of two mtDNA-encoded subunits, and at least 13 nuclear DNA-encoded subunits)

Coenzyme Q (a lipoidal quinone) and cytochrome *c* are also involved in mitochondrial respiration, serving as 'electron shuttles' between the complexes (Wallace DC, 1999).

Coenzyme Q is a fat-soluble vitamin-like molecule, able to easily diffuse in hydrophobic phase of inner mitochondrial membrane. Its biological role is

electronic transport in the respiratory chain, from flavoproteins (complexes I-II) to cytochromes (III).

Cytochrome c is a chromoprotein with a hem as a prosthetic group, which holds ferrum with variable valence (Fe3+ and Fe2+). It is water-soluble and located at the periphery of the inner mitochondrial membrane in hydrophilic phase. Its biological role is electron transport in the respiratory chain, from complex III to complex IV.

All intermediate electron carriers in the respiratory chain are arranged according to their redox potential. In this line the electron donating capacity (oxidating) decreases, and the electron attaching capacity (recovering) increases. The largest electron donating capacity carrier is NADH, and the largest electron attaching capacity is a molecular oxygen.

Electrons enter the respiratory chain in various ways. From the NADH+ complex I transports electrons through FMN and Fe/S-points to ubiquinone. From the succinate, electrons are transported to ubiquinone by complex II or by some other mitochondrial dehydrogenase through $FADH_2$ or flavoprotein linked to an enzyme. Oxidized coenzyme Q is reduced to an aromatic ubihydroquinone, which transports electrons to complex III through two haems b, one Fe/S point and one haem c1 – to the haem-containing protein, cytochrome *c*. Cytochrome *c* transports electrons to complex IV, cytochrome *c* oxidase.

Cytochrome *c* oxidase contains two cuprum-bearing points (CuA, CuB) and two haems (a, a3), which help with transporting electrons to the oxygen. During the O_2 reduction a strong anion O_2- takes two protons and forms molecule of water. The flow of electrons is connected to a proton gradient (complexes I, III, IV).

Thus, the respiratory chain is actually a proton pump generating a membrane potential of about 180 mV with a negative polarity at the matrix side of the membrane. ATP synthase uses this potential for matrix ADP phosphorylation, and causes proton gradient decrease and electron transport activation (Saraste M, 1999).

2.5. Oxidative phosphorylation and regulation of the ATP synthesis

Protons transfer provided by complexes I, III and IV happens in a vector way from the matrix to the intermembranous space. During the electron transport a concentration of H+ in respiratory chain increases, and pH decreases. The outer part of the inner membrane gets +, inner part gets -, which results in a proton gradient with acidic pH outside. This is called proton transmembranous potential (ΔμH+, a chemosmotic theory by P. Mitchell). In mitochondria, the ATP-synthase can provide reverse proton transport to the matrix. That's why the connection of electron transport and ATP production is a regulating connection.

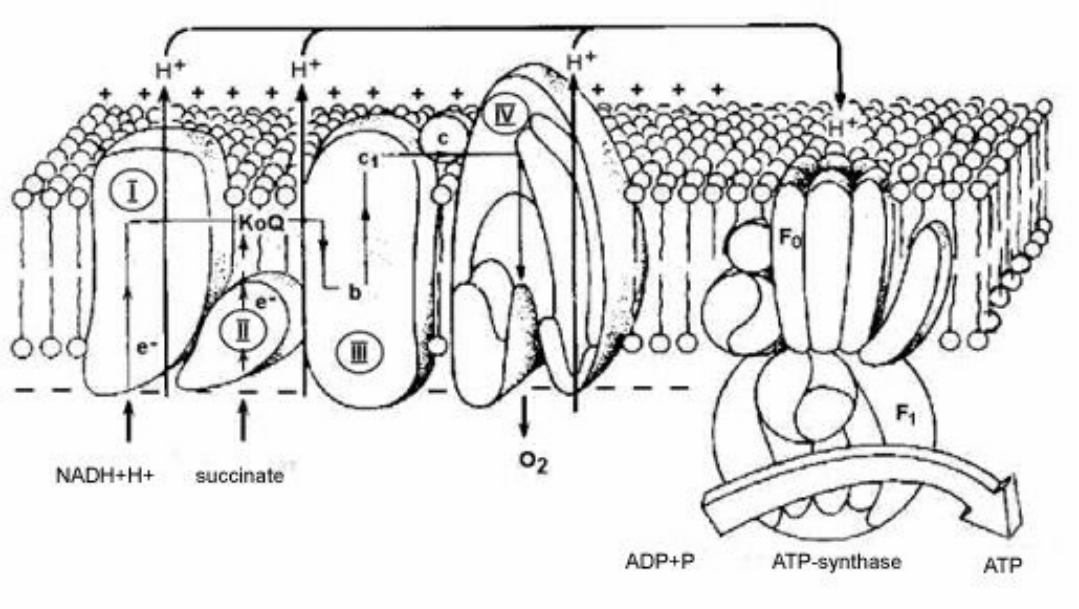

Fig. 17. ATP synthesis

All complexes are located in the inner mitochondrial membrane and they can form supercomplexes; electron transport is provided by cytochrome *c* and ubiquinone mainly. Ubiquinone has a nonpolar side chain and due to this chain it can move within the membrane.

Cytochrome *c* is located on the outer side of the inner membrane. NADH oxidation is happening on the inner side and in the matrix, where the citrate cycle and β-oxidation (sources of NADH) also occur (Fig. 17).

ATP synthesis is the fundamental reaction of the mitochondrial respiratory chain, in which complex V (ATP synthase) is involved. ATP is being transported

with an antiport mechanism (opposite to ADP) into the intermembranous space, and then through porins to go to the cytoplasm.

We can see that in the respiratory chain electrons are going from NADH or $FADH_2$ to O2. Released energy is used for proton gradient on an inner mitochondrial membrane. ATP synthesis is connected with a reverse proton flow – from the intermembranous space to the matrix: when H+ goes from an area with a high concentration to an area with a low concentration, there is an energy release, and this energy is used for ATP synthesis.

Electrons given by NADH are not going directly to oxygen. They take part in at least 10 redox systems, most of them are interlinked prosthetic groups of complexes I, III, IV. The quantity of coenzymes taking part in electron transport is outstanding. The total energy release is divided into small packages; each package size depends on the redox potential difference of the transitional products. This division provides decreased energy loss (app. 60% energy output).

H+-translocating ATP-synthase is composed of two parts: protons channel (F0) is inserted into the membrane and is composed of at least 13 subunits; and catalytic subunit (F1) protruding into the matrix. The head of the catalytic part is formed by three α-subunits and three β-subunits, holding three active points between them. Body of this structure is formed by polypeptides of F0-part and γ-, δ- and ε-subunits of the head.

Catalytic cycle is divided into three phases; each phase takes place in each active point. At first, ADP is being linked with Pi, then pyrophosphate link is formed and finally the end product is released. Three catalytic points catalyze the reaction phase during every proton transport through the protein channel F0 into the matrix. It is supposed that the energy of the proton transport is primarily spent to the γ-subunit rotation, which results in some cyclic transformation of the α- and β-subunits.

The biochemical process of ATP production always depends on the cell energy demand. This need of harmonizing ATP production and ATP consumption is connected with the small concentration of enzymes: every ATP/ADP molecule is

to be phosphorylated and dephosphorylated many times. A simple mechanism of ATP production/consumption is called respiratory control and based on the connection with processes mentioned above. If the cell does not consume ATP at the moment, it hardly has any ADP. In the absence of ADP no proton gradient on the inner membrane can be used by ATP-synthase, which leads to inhibition of the electron transfer in the respiratory chain, and thus NADH can't be re-oxidize into NAD+. A high ratio of NADH/NAD+ slows down the citrate cycle. This mechanism can work in a reverse way in case of high ATP consumption rate. If proton gradient creation is suppressed, substrate oxidation and electron transport go faster than usual, and end up with a heat, not ATP.

There are also substances functionally dividing oxidation and phosphorylation, the so-called 'dissociating agents' or 'uncouplers'. They contribute to the proton transfer from the intermembranous space to the matrix without any ATP-synthase involved. This kind of dissociation can occur as a result of mechanical damage of inner membrane, or as a result of exposure to some substances (uncoupling proteins), which transport protons through the membrane. For instance, a natural dissotiating agent is thermogenin, an uncoupling protein found in the mitochondria of brown adipose tissue.

The most important cycle regulation factor is NADH/NAD+ ratio. Along with the pyruvate dehydrogenase and oxoglutarate dehydrogenase, NADH also inhibits citrate synthase and isocitrate dehydrogenase. All of these enzymes (excl. isocitrate dehydrogenase) can also be inhibited by the end product of acetyl-CoA and succinyl-CoA. Enzyne activity is also regulated by the interconversion process.

2.6. Mitochondrial DNA and proteins

As mentioned above, energy production must be adjusted to the energy demand. This requires a bidirectional flow of information between the nuclear genome and the mitochondrial genome (Poyton RO, McEwen JE, 1996). Moreover, almost all subunits of complexes (e.g. complex II) are encoded by both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) (Calvo S et al, 2006). The 13 genes encode seven subunits of NADH-ubiquinone reductase, three subunits of COX (complex IV), subunits 6 and 8 of ATPsynthase (complex V), and apocytochrome *b*, which is part of ubiquinol-cytochrome *c* reductase (complex III) (Zeviani M, Di Donato S, 2004). Complex II is encoded by only nDNA.

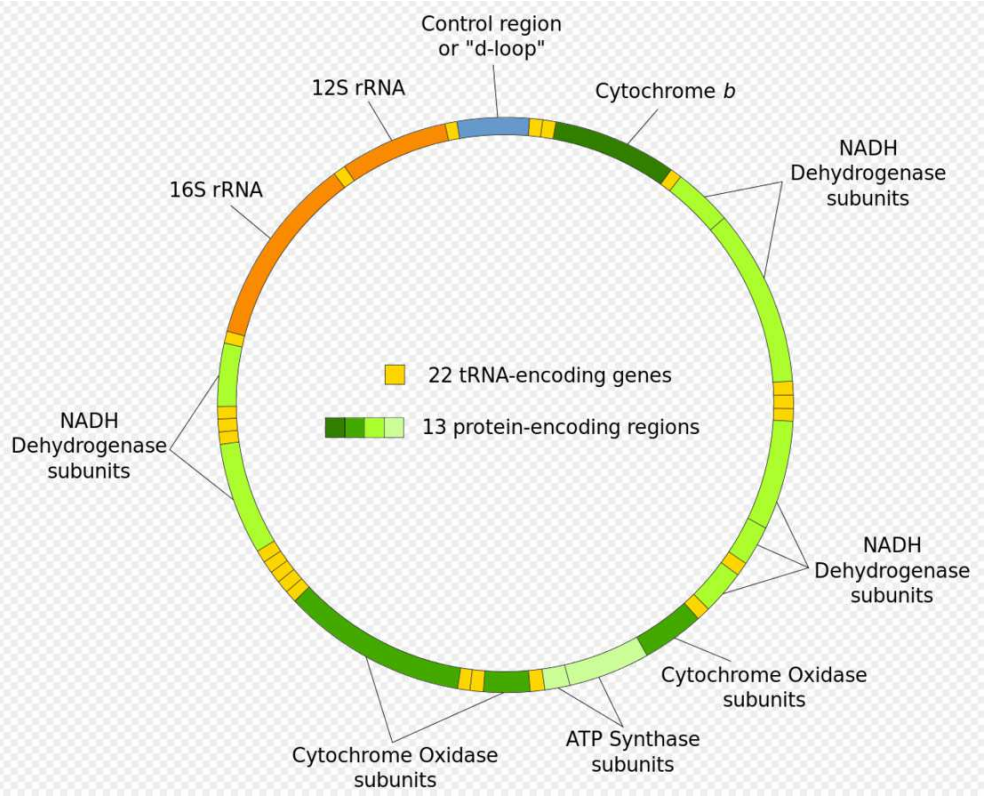

Fig. 18. Mitochondrial DNA (Wikipedia, 2017).

The following proteins and enzymes taking part in respiratory chain and are encoded by human mtDNA:

Encoded	Genes
NADH dehydrogenase (complex I)	MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6
coenzyme Q – cytochrome *c* reductase /cytochrome *b* (complex III)	MT-CYB
cytochrome *c* oxidase (complex IV)	MT-CO1, MT-CO2, MT-CO3
ATP synthase	MT-ATP6, MT-ATP8

MtDNA is a circular double-stranded DNA molecule 16569 bps long that encodes 37 genes, comprising 13 proteins, 22 mitochondrial tRNAs, and 2 rRNAs (Zeviani M, Di Donato S, 2004). All mtDNAs are presented as multiple copies collected in groups or clasters. The total quantity of the mtDNA in one cell is about 1%. mtDNA synthesis isn't coupled with nDNA synthesis. The inheritance of mtDNA is almost exclusively maternal, although some exceptions (a few sperm mitochondria entering the egg) have been reported (Schwartz M, Vissing J, 2003). Most of the information is encoded in the heavy (purine-rich) strand (two rRNAs, 14 tRNAs and 12 polypeptides) (Alexeyev MF et al, 2004). Both strands have no introns (Anderson S et al, 1981). mtDNA has a small region called displacement-loop (D-loop) which is non-coding and contains some promoters for two strand transcription (Zeviani M, Di Donato S, 2004).

Human mtDNA contains protein-encoding and protein synthesis genes. Two novel transcriptional factors (TFB1M and TFB2M) cooperate with mitochondrial RNA polymerase and mitochondrial transcription factor A to carry out basal transcription of mammalian mtDNA (Falkenberg M et al, 2002).

Replication of mtDNA continues throughout the lifespan of an organism in both proliferating and post-mitotic cells. It was believed that replication proceeds bidirectionally, asynchronously andasymmetrically, initiated at two spatially and

temporally distinct origins of replication, OH and OL, for the heavy and light strand origins of replication respectively (Clayton model) (Taanman JW, 1999). In recent decades an experimental evidence supporting the existence of conventional, strand-coupled replication of mammalian mtDNA has come out (Holt IJ et al, 2000; Yang MY et al, 2002).

As mtDNA is essential for the aerobic ATP synthesis system, alterations cause various effects on the respiratory chain itself and on the cells.

The mitochondrial genotype is composed of a single mtDNA species (homoplasmy). Recently it has been found that wild-type (normal) and mutated mtDNA may coexist in the same cell (so-called heteroplasmy) (Wallace DC, 1992). Due to mitochondrial polyploidy, the two mtDNA species are stochastically distributed to daughter cells during mitosis (Jenuth JP et al, 1996), which can contribute to mutation loads observed in different generations of families carrying heteroplasmic mtDNA. This results in a wide variety of phenotypes and diseases caused by mitochondrial pathology.

Phenotypic expression of this pathology depends on the threshold effect: at some point the accumulated quantity of mutated gene copies are no longer balanced by the wild-type mtDNA, a cellular dysfunction that expresses phenotypically (Thorburn DR, Dahl HH, 2001).

Despite the fact that human mtDNA is fully deciphered and many mutations had been discovered, the molecular mechanisms for maintenance and clinical presentation of mtDNA changes are much less elucidated (Zeviani M, Carelli V, 2003; Uehara N et al, 2014). There is an evidence that these clinical presentations are influenced by a diverse range of factors, i.e. the mutation itself, its point and pathogenicity, and the affected organ energy demands. Thus, the most sensitive organs to mitochondrial pathology are nervous system, muscles, liver, kidney and heart.

It is known that mitochondria contains >1500 different proteins in mammals and >1000 different proteins in yeast (Sickmann A et al, 2003; Sickmann, J et al, 2003; Reinders J et al, 2006; Pagliarini DJ et al, 2008).

Mitochondria have all the needments for protein synthesis, including ribosomes, tRNA and mRNA. But as the 13 proteins synthesized in mitochondria are mostly insoluble, they substantially form the subunits of respiratory chain complexes and the F_1F_o-ATPase synthase. Nearly all soluble mitochondrial proteins are coded in the nucleus and synthesized in cytoplasmic ribisomes, and are then transported into mitochondria (Fox TD, 2012). Some proteins including electron carriers, mitochondrial translocase, components of proteins transport in mitochondria and specific factors necessary for transcription, translation and replication of mDNA are synthesized this way.

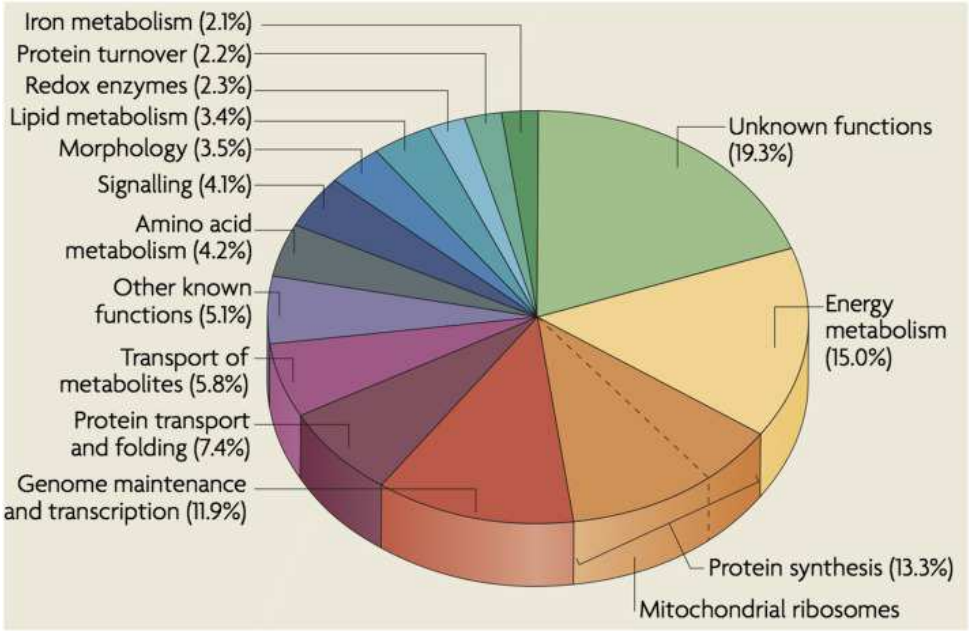

Fig. 19. Classification of identified mitochondrial proteins according to function (Schmidt O et al, 2010).

These proteins have signal peptides on their ends ranging in size from 12 to 80 aminoacid residues. Peptides form amphiphilic curls and provide a special contact of proteins and binding domains of mitochondrial receptors which are localized on the outer membrane. Proteins are transported to the outer membrane in a partially unfolded state connected to chaperones (i.e., hsp70). After being transported through the outer and inner membrane, the protein binds to a new chaperone of mitochondrial origin, which picks up the protein, is drawn into the

mitochondria and controls the folding process of the polypeptide chain. Most chaperones have ATPase activity, so that mitochondrial proteins are transported into mitochondria and the formation of their functionally active forms are energy-dependent (Neupert W, Herrmann JM et al, 2007; Schmidt O et al, 2010).

Mitochondrial ribosomes are of smaller size than 80S cytoplasmic ribosomes, and the initiating aminoacid for mitochondrial protein synthesis is not a free methionine like in cytoplasm but a bonded methionine, like N-formyl-methionine. This suggests that mitochondrial protein synthesis mechanism is close to a prokaryotic one.

2.7. Reactive oxygen species, apoptosis and mitochondrial theory of aging

One of the causes for the mutations is that mtDNA is more vulnerable to various factors than nuclear DNA. For instance, about 95% of the all oxygen consumption in mitocnondria is reducted to water during the oxidative phosphorylation. The obligatory stage of this phosphorylation is a formation of two OH-groups with the participation of the cytochrome *c* oxidase. The remaining 5% of oxygen undergoes various reactions (mostly enzymatic) and becomes a reactive oxygen species (ROS), an unevitable byproducts of the oxidative phosphorylation.

ROS include oxygen ions, free radicals and peroxides both of organic and non-organic origin. Free radicals are molecules of a small or medium size with an outstanding reactivity due to a presence of the unpaired electron on the outer electron level (Turrens JF, 2003). As we said previously, ROS are constantly formed in the cell as byproducts of normal oxygen metabolism. Some of them can appear after an ionizing radiation exposure.

ROS carry various functions including mediation of some important intercellular processes, immune system induction, ionic transport mobilization and contribution to the programmed cell death (apoptosis). An increased ROS production leads to the so-known oxidative stress.

Oxidative stress is a balance disruption between the free radicals production and the mechanisms of antioxidant control in the cell. This disruption is accompanied by the increased free radicals formation and the antioxidant system failure, which leads to the cell death (Richter C., 1998; Orrenius S et al, 2006). The main cause of the oxidative stress is not considered to be ROS production itself but rather the imbalance between their generation and removal.

Standard inhibitory analysis has shown that the generation of ROS is basically located within complexes I and III. Studies revealed that ROS-generating center must be located between flavine and rotenone-binding site, and that there can be more than one ROS-generating domain (Herrero A, Baria G, 2000). Within complex I a flavine or its union with NAD can be ROS-generating and this releases ROS into matrix; within complex III it is considered that ROS-generating is an unstable compound of semiquinone (Ku HH et al, 1993) and this releases ROS to both sides of the inner membrane.

There are a few antioxidant protection systems in the mitochondria:

1) enzymes – glutathione peroxidase, superoxide dismutase, cytochrome *c*, coenzyme Q;

2) natural antioxidants in food – ascorbate, alpha-tocopherol, rutin, cerupoplasmin, etc.;

3) low-molecular antioxidants which are synthesized in the organism itself – glutathione, uric acid, melatonin, lipoic acid, etc.

During the last few decades, there has been a large amount of research data proving the role of mitochondria in the intracellular signal pathways leading to apoptosis (Zoratti M, Szabo J, 1995; Skulachev VP, 2000). There are several interrelated pathways for this.

There are several mitochondrial proteins located in the intermembranous space such as caspase activators, cytochrome *c*, AIF, endonuclease G, etc. After they are released into the cytoplasm, they launch apoptosis and induce the cell death (Liu X et al, 1996; DU C et al, 2000). For instance, cytochrome *c* is one of the key elements of the respiratory chain in mitochondria and at the same time, it is

one of the most important proteins in the mitochondrial apoptosis pathway. Normally it is held in mitochondria by cardiolipine. After the free-radical oxidation in mitochondria is activated, acardiolipine is also oxidized, cytochrome *c* is freed and can move to the cytoplasm. There it formes a complex with apoptosis activation factor Apaf-1 and procaspase 9 (apoptosome), which causes a caspase activation downstream and launches the apoptotic cell death (Susin SA et al, 1999; Diehl NL, 2000).

Increased ROS production in mitochondria and lack of antioxidants can lead to the damage of the electron transport chain and to a decrease of both ATP synthesis and activity of the ATP-dependent enzymes, including Na, K-ATPase, which is responsible for the cell membrane potential maintenance. As a result, a depolarization of the cell membrane occurs and ions flow intensivity through membrane increases; Ca2+ massively enters mitochondria, triggering a cascade of intracellular catabolic reactions (Aronis A et al, 2003). Because of the lack of ATP, the capacities of Ca-pump are also broken and Ca2+ cannot be removed from the cell (Ichas F et al, 1997; Boudreault F, Grygorczyk R, 2004). Intracellular Ca2+ accumulation uncouples oxidative phosphorylation and leads to a swelling of the mitochondria. ROS and Ca2+ leads to phospholipases activation, which cleave fatty acids from phospholipids.

Fatty acids also contribute to the oxidative phosphorylation uncoupling and the mitochondria swelling with a change in membrane permeability known as the mitochondrial permeability transition (MPT) (Marchetti P et al, 1996). As MPT allows the entrance of the substances with molecular weight <1,5kDa, it allows the nucleotides to leave the matrix (Crompton M, 1999). When that happens, NAD-dependent substrates breathing is inhibited and the mitochondria start swelling. We can see that MPT is enhanced by a variety of conditions, causing the elimination of the proton electrochemical potential and the membrane potential decrease, which is often used as a cell death indicator (Vercesi J et al, 2007).

In 1972 Denham Harman, the author of the free radical theory of aging (FRTA), modified the theory into a mitochondrial one. It was known that there is a

chemical mechanism for ROS production in the mitochondria and the modified theory proposed that ROS cause damage to proteins, other molecules and mtDNA, because it is not as well protected as nuclear DNA. This in turn causes mutations and increases ROS production in mitochondria so that free radicals are accumulated in the cell, which in the long-term leads to the deterioration of cells and organs in the entire body (Harman D, 1972; Harman D, 1983).

The theory has been widely debated (Poovathingal SK et al, 2009) and there were several hypotheses suggesting how can ROS induce mtDNA mutations (Conte D et al, 1996; Perez VI et al, 2009; Afanas'ev I, 2010). There were also some contra arguments to the association between ROS damage and aging (Yee S et al, 2014), suggesting that this evidence of this association is not cause-and-effect, but may only be an indicator of some changes in the existing signal transduction pathways as a part of a cellular response to the aging process.

2.8. Mitochondrial pathology and mitochondrial disease

Mitochondrial diseases are a heterogeneous group of maladies associated with various disorders in the functioning of mitochondria which lead to energy metabolism violation. They can be caused by mutations in either mtDNA or nuclear genes (Koopman WI et al, 2012) or by structural or biochemical defects of mitochondria, resulting in a disruption of various units of the Krebs cycle, respiratory chain, beta-oxidation processes, etc.

There are two main groups of these diseases:

- a group of well-known mitochondrial syndromes, caused by mutations (Leber hereditary optic neuropathy (LHON), Leigh syndrome (LS), myoclonic epilepsy with ragged-red fibers (MERRF) syndrome, mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS) syndrome, neuropathy, ataxia, and retinitis pigmentosa (NAPR) syndrome, Kearns-Sayre syndrome, Pearson syndrome, etc.);

- a group of diseases or syndromes which include mitochondrial pathology as an important pathogenetic element (Poulton J, Tumbull DM, 2000) (diseases of the connective tissue, chronic fatigue syndrome, cardiomyopathy, migraine, liver failure, pancytopenia, different genetic disorders, including ethylmalonic aciduria (Rimoldi M et al, 2009), Friedreich ataxia (Rötig A et al, 1997), hereditary spastic paraplegia (Zeviani M et al, 1998), and Wilson disease (Lutsenko S, Cooper MJ, 1998)). These are not strictly mitochondrial diseases.

The concept of 'mitochondrial disease' was first postulated in the twentieth century, after several studies of the normal mitochondrial structure and function and the development of dye and staining techniques were published. In 1959-62 Luft et al. studied the enzymic activities of human skeletal muscle mitochondria, and found an association of metabolic dysfunction with a defect in the maintenance of mitochondrial respiratory control and loose coupling between respiration and phosphorylation of ADP (Ernster L et al, 1959; Luft R et al, 1962). Later only one more patient with this pathology was described (Luft R, 1992). In 1964 Shy and Gonatas described ultrastructural changes in muscle mitochondria in children with myopathies (Shy GM, Gonatas NK, 1964). In 1958-68 Drachman, Kearns and Sayre described a syndrome of chronic progressive external ophtalmoplegia (CPEO) accompanied by mitochondrial changes (Kearns TP, Sayre GP, 1958; Drachman DB, 1968). In 1972 Olson used a modified three-color stain for muscle fibers of patients with CPEO and found several with an abnormally increased number of mitochondria of an unusual shape with crystalloid inclusions (the so-called 'ragged-red' fibers) (Olson et al, 1972; Engel WK, Cinningham GG, 1963). Later it was evident that all these syndromes varied widely and included many signs and symptoms (Shapira et al, 1977).

In 1970s-1980s, two research groups (DiMauro group, Columbia University; Morgan-Hughes group, UK) independently identified defects of different units in the respiratory chain (cytochrome *c* oxidase deficiency) which provided new knowledge about the mitochondrial pathology (Byrne E et al, 1985). Various mitochondrial enzyme deficiencies were described (Blass JP et al, 1970; DiMauro

S, DiMauro PM, 1973; Engel AG, Angelini C, 1973) and the first classification of the mitochondrial disorders appeared (substrate transport defects into the mitochondrial matrix; substrate utilization defect in the mitochondrial matrix; Kreb's cycle defects; electron transport system (ETS) defects; oxidation/phosphorylation coupling defects) (Lombes A1 et al, 1989).

Modern laboratory techniques allowed researchers to know more about the disease allowing, for instance, protein levels to be determined with the use of special antibodies for respiratory chain complexes, etc. In some cases a general suppression of all units can be detected, but sometimes only a few subunits of deficiency are discovered. The cause of this variability remained unclear until 1963 when Nass and Nass had discovered a mammalian mitochondrial DNA (a full primary structure of human mtDNA was published in 1981 by Anderson) (Anderson et al, 1981) and described the phenomenon of heteroplasmy. In the following decade there was a breakthrough in mitochondrial pathology studies: Egger and Wilson postulated mitochondrial genetic inheritance (Egger J, Wilson J, 1983; Hutchison CA, 1974); the mitochondrial genome and its abnormalities had been recognized; a specific point mutation of mtDNA in LHON (Shuster RC et al, 1988) and large-scale deletions in muscle mtDNA in patients with myopathies (Holt IJ et al, 1988) were found; mtDNA deletions were associated with the phenotype of CPEO (Moraes CT et al, 1989) and genotype-phenotype correlation of many syndromes followed.

We already know that during the division process of the mitochondria DNA copies are randomly distributed among their offspring and each cell contains thousands of copies. They can be all identical (homoplasmy) or they can be mixed, mutated copies with normal ones (heteroplasmy). If only one DNA molecule contains a mutated fragment, during this random distribution they can accumulate in different mitochondria (Wonnapinij P et al, 2008). When a certain amount of mitochondria in many cells of the tissue get these mutated copies, talk of mitochondrial disease (a threshold effect) begins (Schon EA et al, 1997).

As the distribution of mutated DNA in different organs and tissues differs, two patients with the same mutation can have different organs affected; a level of function impairment can also differ and change within years. In general, mitochondrial diseases affect organs and tissues with the highest energy demand most: muscles, brain, nervous tissue, etc. (Finsterer J, 2007)

As stated previously, mtDNA mutates approximately 17 times more often than nDNA (Wallace DC et al, 1987) due to many different reasons, meaning that mitochondrial diseases can appear ‘de novo’ (Thorburn DR, 2004). Sometimes mtDNA mutation rate increases because of nuclear genes mutations encoding enzymes that control mtDNA replication.

The way from the mutation to the clinical symptom of the disease is still not clearly understood. There are several hypotheses on this: mutations lead to the ROS accumulation, Ca2+ metabolism change and MPT activation, which cause apoptosis in summary (this scenario is probably more common for the neurodegenerative diseases) (Abramov A.Y. et al, 2010; Turnbull HE et al, 2010).

The classification of the mitochondrial diseases is challenging because a purely clinical classification can not be applied, as many individuals do not fall into a specific category. Moreover, there is a poor correlation between phenotype and genotype: several individuals with the same clinical features can have different genetic variants (mtDNA mutation, mtDNA deletion, nuclear gene mutation etc.); and in addition, the research is still ongoing with changes being made all the time (Mancuso M et al, 2015). The only applicable classification is based on the molecular genetic basis of mitochondrial disease and consists of two groups: mtDNA mutations and nuclear DNA mutations. It is also arguable that the pathogenic allelic variant in a significant number of affected patients is not identified; moreover, there is still no comprehensive list of all genes known to impair mitochondrial function.

A classification of mtDNA mutations is based on the mutated domain of the mtDNA and consists of the three groups:

- Mutations of the structural genes.

These mutations alter a nucleotide sequence of the structural mtDNA genes. There are several subgroups of syndromes depending on the oxidative phosphorylation (OXPHOS) complex involved.

1) Complex I genes mutations group is the largest group (LS, Leber syndrome, etc.) (Rotig A, 2010).
2) Complex III genes mutations group consists of over 30 mutations so far, which cause myopathy as a rule (Wong LJ, 2007). They are associated with encephalopathy, cardiomyopathy, tubulopathy and LHON (Johns DR, Neufeld MJ, 1991; Valnot I. et al, 1999; Abu-Amero KK, Bosley TM, 2006)
3) Complex IV genes mutations group is a large group of mutations, which is mostly associated with neuromuscular syndromes (Facts about genetics and neuromuscular diseases. Genetic and neuromuscular diseases, MDA, 2011).
4) The Complex V genes mutations group consists of several mutations associated with different symptoms, for instance, maternally inherited diabetes and deafness (MIDD) (Desnuelle C et al, 2000).

- Mutations of the rRNA and tRNA genes.

Some mutations in the ribosome RNA and transport RNA that take part in mitochondrial protein synthesis can cause mitochondrial syndromes. Approximately 2/3 of the all mtDNA mutations are located in tRNA genes. For instance, a mutation A3243G from this group is diagnosed in 80% of MELAS syndrome patients, chronic progressive external ophtalmoplegia (CPEO) patients, Kearns–Sayre syndrome (KSS) patients, sensorineural hearing loss (SNHL) patients and in some MERRF syndrome patients (Silvestri G. et al, 1993; Sue CM et al, 1998; Finsterer J, 2007; Ma Y. et al, 2009), though it is essential to have high levels of heteroplasmy for the disease to appear in a clinical picture (Shoffner JM et al, 1990).

- Structural changes of the large DNA segments

mtDNA deletions undermine many mitochondrial diseases and may play a key role in the aging process. There are two models of mtDNA deletions: an asynchronous mtDNA replication mechanism model and the double-strand mtDNA ruptures reparation model (Krishnan KJ et al, 2008). Most deletions are sporadic and are not transferred to the offspring (Chinnery PF et al, 2004). A big deletion of the domain 8488-13460 is known to be the most common cause of KSS syndrome (Maceluch JA, Niedziela M, 2006); the most common cause of CPEO is one or several deletions (van Goethem G et al, 2003; López-Gallardo E et al, 2009); Pearson syndrome is also attributed to multiple mtDNA deletions (Rötig A et al, 1990).

Nuclear DNA mutations also can cause mitochondrial disease because of the many genes encoding mitochondrial protein synthesis (Dimmer KS, Rapaport D, 2008). Mitochondrial dysfunctions associated with these gene mutations are studied to a lesser extent. In 1989 Moraes showed dominant inheritance of multiple mtDNA deletions (Moraes CT et al, 1989)). In 1995 Bourgeron described a mutation of the flavoprotein subunit of the complex II (nucleus encoded) (Bourgeron T et al, 1995). In 1995 Suomalainen showed linkage to the chromosome 10q in autosomal dominant CPEO pedigrees with multiple mtDNA deletions (Suomalainen A et al, 1995). In 1996 Kaukonen showed that patients with autosomal dominant progressive external ophthalmoplegia had multiple deletions of mtDNA and a mild deficiency of one or more respiratory-chain enzymes carrying mtDNA-encoded subunits (Kaukonen JA et al, 1996).

Nuclear DNA defects are more highly variable than mtDNA defects. The mutations of the genes can affect the oxidative phosphorylation system, protein synthesis, protein transport system, mitochondria movement and division, mtDNA transcription and replication and different enzymatic cycles and pathways in mitochondria (Zhu X et al, 2009). Symptoms of the mitochondrial diseases may vary considerably and this variety is mediated by threshold effect (Kmiec B et al, 2006), heteroplasmy (Gilkerson RW, 2009) and the bottle-neck effect (Lightowlers R.N et al, 1997; Shoubridge EA, Wai T, 2007).

A classification of nuclear DNA mutations associated with mitochondrial diseases is also based on the mutated domain and consists of several groups:

1. Mutations causing disorders of the mitochondrial respiratory chain

A. Mutated genes encoding structural subunits (LS with complex I deficiency, leukodystrophy with complex II deficiency, etc.)

B. Mutated genes encoding assembly factors (LS, hepatopathy and ketoacidosis, etc.)

C. Mutated genes encoding translation factors (lactic acidosis, developmental failure, and dysmorphism; leukodystrophy and polymicrogyria, etc.)

2. Disorders associated with multiple mtDNA deletions or mtDNA depletion (autosomal progressive external ophthalmoplegia, mitochondrial neurogastrointestinal encephalomyopathy, Alpers-Huttenlocher syndrome, etc.)

3. Other disorders (Coenzyme Q_{10} deficiency, Barth syndrome, mitochondrial phosphate carrier deficiency) (Chinnery PF, 2000).

It can be assumed that the number of the syndromes of this group should be much higher as genes encoding 98% of mitochondrial proteins are located in the nucleus (Scarpulla RC, 2006). Elucidation of the molecular basis for these disorders is limited because only half of the estimated 1,500 mitochondrial proteins have been identified.

We can see that a leading role of mtDNA mutations or nuclear genes mutations responsible for mitochondrial protein synthesis in many diseases has become evident. The number of known syndromes is increasing year by year. It was believed before that the cumulative incidence of all hereditary diseases associated with mitochondrial pathology is 1:5000 – 1:8000 in the total population (Chinnery PF, 2000), but some later studies proposed that at least one in 200 healthy humans harbors a pathogenic mutation can potentially cause a mitochondrial disease in the offspring (Elliott et al, 2008).

Because of the remarkable expansion of knowledge on the molecular characterization of human disorders associated with the energy pathways of mitochondria, the term ‘mitochondrial disorders’ is currently restricted to indicate the clinical syndromes associated with abnormalities of OXPHOS only (Calvo S et al, 2006). OXPHOS disorders can be regarded as the most common group of inborn errors of metabolism and perhaps the most challenging to diagnose and manage because of their clinical and genetic variability. Initial symptoms are often nonspecic as respiratory-chain deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age and with any mode of inheritance (Munnich A et al, 1996). A family history suggesting maternal inheritance is not common for children with OXPHOS disorders caused by mtDNA mutations; many of them have de novo mutations and many are likely to have nuclear-encoded disorders (Thorburn DR, 2004).

Establishing the diagnosis of a mitochondrial disease can be difficult in many cases because some patients display a cluster of clinical features that fall into a discrete clinical syndrome (LHON, NARP, maternally inherited LS), but many do not. A diagnosis can be confirmed by molecular genetic testing of DNA extracted from a blood or muscle sample (a serial testing of single genes, a multi-gene panel testing, and a genomic testing). A collection of the family history (3-generation) can be useful in the detection of the inheritance mode, though many diseases appear as de novo mutations. There are also other methods of clinical investigation including plasma and/or CSF lactate concentration, ketone bodies, neuroimaging, cardiac evaluation, urinary organic acids, muscle biopsy, etc. They can be applied when the clinical picture is non-specific but there is a strong suspicion of the mitochondrial origin of the disorder.

There is no effective treatment for the mitochondrial diseases so far, though many researchers work on this problem (Kanabus M et al, 2014). Some therapies are effective in animal disease models. There are also generalist (applicable to a wide spectrum of different disease conditions) and tailored (applicable to a single

condition) therapies emerging at the pre-clinical level, however, most of the approaches warrant more work (Zeviani M et al, 2015).

Modern clinical practice for treatment of the various mitochondrial syndromes include early diagnosis and a prevention of the morbidity and mortality in certain conditions. Supportive pharmacological methods are being applied, special diets, physical exercises, surgery for some pathology, etc (Chinnery P et al, 2006; DiMauro S, Rustin P, 2009; Finsterer J, 2010). Pharmacological treatment is focused on the mitochondrial functions reparation: increasing the respiratory chain enzymes activity, coenzymes predictors, electron carriers substitutes, antioxidants, etc., however not all patients respond to this treatment (Lopez LC et al, 2014; Nouws J et al, 2014). There are also some experimental methods based on interaction with the genetic system of mitochondria. Recently genetic engineering was employed into the clinical practice of reproductive medicine in UK, allowing women with mitochondrial diseases to have healthy children after their DNA was transplantated to the egg cell of the healthy woman with normal mitochondria (a three-person IVF procedure) (Knapton S, 2014).

All these studies have made a revolution in the understanding of medical aspects of human energy metabolism. In addition to contributing to the theoretical pathology and medical taxonomy, one of the main achievements of these studies will be a set of effective diagnostic tools (clinical, biochemical, genetic, etc.) and maybe a treatment for mitochondrial pathology and mitochondrial disease.

2.9. Mental disorders and mitochondrial pathology

A hypothesis of a ‘lack of psychic energy as a result of the cell energy metabolism impairment’ in patients with mental disorders such as schizophrenia (SZ) was first postulated in the early 1950s (Easterday OD et al, 1952). This hypothesis seemed too revolutionary for the time period and did not accrue many followers even though further studies revealed a wide range of evidence for energy metabolism violation in different mental illnesses.

These lines of evidence can be divided into two groups. First group includes psychiatric signs and symptoms of mitochondrial diseases and syndromes.

As neurons spend 40 to 60% ATP energy on maintaining the ion gradient on the membrane and transmitting the impulse, the OXPHOS impairment in mitochondrial diseases takes a key place in the clinical picture of 'mitochondrial encephalomyopathy' (Sukhorukov VS, 2008). Main neurological symptoms are mental retardation, seizures and stroke-like episodes. The high intensity of their severity often causes psychiatric symptoms to fall outside of the doctors' attention.

There are four main psychiatric symptoms in mitochondrial diseases: cognitive impairment, affective symptoms and anxiety, psychotic symptoms, personality disorders. Sometimes they manifest before the other classical symptoms of the mitochondrial disease appear and patients initiate treatment for psychosomatic condition (Norby S et al, 1994). The main difficulty of the differential diagnosis in those cases is the origin of the psychiatric symptoms: whether they act as the reaction to the disease (for instance, a lifetime major depressive disorder, MDD) or they can be assigned in the structure of the general mitochondrial condition.

In 2000 Dr. Kato proposed a mitochondrial hypothesis of the mood disorders (Kato T, Kato N, 2000), which based on the findings that patients with BPD have an abnormal energy metabolism and abnormal mitochondrial DNA in the brain (Deicken RF et al, 1995; Kato T et al, 1997).

The hypothesis suggests an impairment of cell metabolism associated with the mood disorders pathology, especially in the function of mitochondrial respiratory chain complexes (Rezin GT et al, 2009). Today there are several lines of evidence for different mitochondrial dysfunctions being an important component of BPD (Kato T, 2007; Kato T, 2008; Frey BN et al, 2007) and MDD (Erkan OM et al, 2004; Sarandol A et al, 2007). The evidence includes the results of magnetic resonance spectroscopy, electron microscopy, co-morbidity with mitochondrial diseases, the effects of psychotropics on mitochondrial functions, increased mtDNA deletion in the brain, and association with mtDNA

mutations/polymorphisms or nuclear-encoded mitochondrial genes (Jou SH et al, 2009).

Dr. Kato and his research team found an altered brain energy metabolism resembling that of CPEO (Kato T, 2005) and discovered a decrease of intracellular pH and phosphocreatinine in frontal and temporal lobe in non-treated and lithium-resistant patients with BPD. This data was then confirmed by other researches. Van Goethem discovered a causative gene for CPEO, comorbid with depression (Van Goethem G et al, 2001). Siciliano reported a linkage between CPEO and BPD (Siciliano G et al, 2003). Konradi discovered a specific mtDNA alteration in postmortem brains of patients with BPD (Konradi C et al, 2004) and Dager et al revealed a lactate accumulation in those brains (Dager SR et al, 2004). Gardner detected an ATP production decrease in frontal lobe, basal ganglia and skeleton muscles of patients with MDD and showed a correlation between ATP production decrease and clinical symptoms of the disorder (Gardner A et al, 2003). Stork and Renshaw proposed the existence of a mitochondrial dysfunction in the pathology of the BPD that involves impaired OXPHOS, a resultant shift toward glycolytic energy production, a decrease in total energy production and/or substrate availability, and an altered phospholipid metabolism (Stork C, Renshaw PF, 2005).

Second group includes mitochondria dysfunction in different mental disorders.

2.10. Mitochondria dysfunction in mood disorders

Data from multiple studies covering the mitochondria function alteration in mood disorders can be accumulated in the following groups:

1) Glycolytic shift: increased lactate levels and decreased potential of hydrogen (pH)

There is data providing information of the shift away from oxidative phosphorylation toward glycolysis. Regarding the altered pH, it was confirmed that patients with BPD have an increased/decreased pH in all three states of the disease:

euthymic, depressed and manic state. There is evidence for the pH decrease in the euthymic state and in the manic or depressed state it is increased (Kato T et al, 1998; Hamakawa H et al, 2004).

Some of these research indicates not only a pH increase but a lactate elevation in the brains of the patients with BPD (Dager SR et al, 2004) and in other sources (Regenold WT et al, 2009). The only energy production pathway in the cell with altered mitochondrial function that doesn't meet the energy demand is anaerobic glycolysis in which pyruvate is converted into lactate. That's why lactate levels are used worldwide to confirm a diagnosis of mitochondrial disorder. Research suggests that decreased pH observed in previous studies may somehow act as a result of the increased lactate levels (Clausen T et al, 2001).

The glutamate-glutamine cycle alteration is also known to take part in the pathophysiology of the glycolytic shift in bipolar disorder (Castillo M et al, 2000; Michael N et al, 2003; Dager SR et al, 2004) through increasing the brain cells energy demand (which normally elevates along with the levels of glutamate/glutamine). As mentioned above if the cell energy production doesn't meet the current demand the only energetic pathway is to increase the glycolysis rate, which manifestly increases lactate levels and decreases the pH (Rudkin TM, Arnold DL, 1999).

Phosphocreatine levels also appear to be altered in patients with BPD (Kato T et al, 1995). This compound acts as a reservoir of ATP and, in case of high neuronal activity, its level decreases to maintain the ATP concentration (Erecinska M, Silver IA, 1989; Sauter A, Rudin M, 1993). A massive and long-term decrease can be a sign of insufficient energy supply in the cell (Rothman DL, 1994; Kato T et al, 1996) and was found in a number of studies investigating the pathophysiology of mitochondrial diseases (Eleff SM et al, 1990; Barbioli B et al, 1992; Barbioli B et al, 1993; Welch KM et al, 1993).

These findings contribute to the hypotheses of a glycolytic shift in the pathophysiological mechanism of mood disorders.

2) Disrupted phospholipid metabolism in the cell

Normal cell metabolism includes cell membrane synthesis and maintenance which takes 10 to 15% of the verall ATP produced in the cell. Consequently when cell energy supply is reduced due to mitochondrial dysfunction or other reasons it is likely that phospholipid metabolism also gets broken. There are several studies, indicating this process in patients with BPD, for instance, investigating total choline levels (Sharma R et al, 1992), myo-inositol levels (Allison JH, Stewart MA, 1971; Manji HK et al, 1996; Silverstone PH et al, 2002), phosphoethanolamine and other phosphomonoethers levels (Kato T et al, 1993; Yildiz A et al, 2001) and proposing other hypotheses regarding the phospholipid metabolism that can potentially bring understanding to a complex pathogenetic mechanism of mood disorders.

3) Oxidative stress

As said previously, oxidative stress leads to various consequences within the cell, especially in mitochondria and DNA, which has now been established beyond reasonable doubt (Morris G, Berk M, 2015). Some of the consequences were found to be associated with symptom severity in mood disorders. They include decreased antioxidant response, increased calcium influx, lipid peroxidation, enzymes imbalance, nuclear DNA and mtDNA damage and others (Erkan OM et al, 2004; Forlenza M, Miller E, 2006; Andreazza AC et al, 2007; Machado-Vieira R et al, 2007; Sarandol A et al, 2007; Soeiro-de-Souza MG et al, 2013; Brown MC et al, 2014). Some of these findings were studied in further detail and were found responsive to mood stabilizers such as lithium and others (Machado-Vieira R et al, 2014).

4) Structural and morphological changes of the mitochondria

Mitochondrial shape and intracellular distribution of mitochondria are believed to be closely linked to the normal energy metabolism in the cell (Escobar-Henriques M, Langer T, 2006; Logan DC, 2006; McBride HM et al, 2006) and consequently any change in the size or shape or quantity of mitochondria or mitochondrial reticulum in different cell types can cause diverse effects including normal aging and apoptosis (Bossy-Wetzel E et al, 2003), ROS production and cell

respiration (Picard M et al, 2013) and also some pathological events (Hermann GJ et al, 1998; Koopman WJ et al, 2005; Mannella CA, 2006). There are several mitochondrial structural abnormalities reported in patients with BPD and MDD. They include abnormal size, increased/decreased quantity in the cell or distribution within cell regions, abnormal shape, location/movement, and different changes in the morphology of mitochondria (Cataldo AM et al, 2010).

5) Impairment of the OXPHOS and respiratory chain activity

Research data confirms an impaired activity of different respiratory chain complexes and OXPHOS components. For instance, a decreased complex I activity and increased protein oxidation and nitration was found in the prefrontal cortex of patients with BPD (Andreazza AC et al, 2010). In animal models of mania a decreased activity of Krebs cycle enzymes, mitochondrial respiratory chain complexes, and creatine kinase in different brain structures were observed (Valvassori SS et al, 2010; Rezin OT et al, 2014) and all these dysfunctions were later reversed by mood stabilizers (Feier G et al, 2013). There are also studies linking OXPHOS of tubulin and actin, mitochondria movement, and synaptic function in bipolar disorder, suggesting that mood stabilizers may have different influences on OXPHOS and ETS and consequently on the direction and extent of mitochondrial movement (Corena-McLeod M et al, 2013). Impaired activity of ETS complexes of different cell types of patients with mood disorders is also reported by many researchers (Rezin GT et al, 2009; Gubert C et al, 2013; Andreazza AC et al, 2013). Vulnerability to depression in animal models is also linked to the impaired oxidative metabolism in mitochondria (Harro J et al, 2014). There are also studies suggesting alterations in levels of protein oxidation and nitration in dopamine-rich regions of the prefrontal cortex (Kim NK et al, 2014) and mitochondrial dysfunction and decreased expression of genes of the electron transport chain, particularly that of complex I (Andreazza AC et al, 2010; Scola G et al, 2012) in patients with BPD.

6) Impaired metabolic activity in general

Different energy metabolism abnormalities are widespread in the brains of patients with mood disorders. For instance, lower metabolic rates were observed in different brain regions of patients with BPD and MDD (prefrontal cortex, anterior cingulate cortex, caudate nucleus) (Videbech P, 2000). In patients with BPD this rate is reported to be increased during manic episode (frontal lobe and medial temporal lobe, thalamus, occipital lobe) (Strakowski SM et al, 2008). There is also research reporting a decrease of various metabolic compounds in the brain involved in different processes in mitochondria as well as an altered mitochondrial response to stimulation in patients with SZ, BPD and MDD (Frey BN et al, 2007; Naydenov AV et al, 2007; Shao I et al, 2008).

A decrease of cerebral N-acetyl-aspartate, a compound strongly connected with the mitochondria metabolism, was also found in the brain of patients with BPD. Normally N-acetyl-aspartate is present at concentrations of 8-10 mmol/l (Urenjak J et al, 1993) and is synthesized in mitochondria. This synthesis is energy dependent and stimulated by ADP (Patel TB, Clark JB, 1979). Though the exact function of this substance remains unclear, it is hypothesized to play an integral role in the energetics of neuronal mitochondria (Stork C, Renshaw PF, 2005), and its rates are strongly related to mitochondrial energy metabolism (Truckenmiller ME et al, 1985; Clark JB, 1998). Several researchers revealed a decreased N-acetyl-aspartate levels in patients with BPD, with a correlation of illness duration (Winsberg ME et al, 2000; Cecil KM et al, 2002; Bertolino A et al, 2003; Deicken RF et al, 2003) or the effect of mood stabilizers on N-acetyl-aspartate levels. Other studies did not find any alteration on these levels (Ohara K et al, 1998; Hamakawa H et al, 1999; Castillo M et al, 2000) perhaps because of the specific treatment.

7) Other findings

There are several studies proposing other causes of mitochondrial pathology associated with mental disorders: Ca 2+ homeostasis (Dubovsky SL et al, 1992; Kusumi I et al, 1992; Kato T et al, 2003); dopamine and other neurotransmitters dysregulation (Brenner-Lavie H et al, 2009; Chen S et al, 2008); environmental factors (Kyle UG et al, 2006; Kroll JL, 2007) and other impacts and risk factors

known to affect mitochondria and/or cell respiration. There is a strong research trend regarding the studies for the genetic cause of mood disorders among these impacts.

2.11. Genetic research for a mitochondrial-associated cause of mood disorders

Searches for a genetic cause of mood disorders have yielded massive but inconclusive results. There is a suggestion that mitochondrial genetic variations and mtDNA mutations may play an important role in psychopathological symptoms of mood disorders, but the evidence is still limited and inconsistent (Anglin RE et al, 2012).

As mentioned above, Dr. Kato and colleagues found an increased ratio of deletions in the postmortem brains of the patients with BPD (Kato T, Kato N, 2000) and later reported a significantly higher rate of the 5178C mtDNA genotype accompanied by a lowered pH levels in similar groups of patients (Kato T et al, 2000). In 2004 Konradi and colleagues found 'a pronounced and extensive decrease in the expression of genes regulating oxidative phosphorylation and the ATP-dependent process of proteasome degradation' in patients with BPD (Konradi et al, 2004). Iwamoto and colleagues have reported a differential expression of mitochondria-related genes between controls and subjects with BPD (Iwamoto et al, 2005). Sun et al found that expression of 23 mitochondria-related genes, including downregulated components of the mitochondrial ETS, were altered in subjects with BPD (Sun X et al, 2006). Washizuka et al reported a downregulation of a complex I gene in lymphoblastoid cell lines (Washizuka S et al, 2009) and Naydenov found similar abnormalities in peripheral blood mononuclear cells of patients with BPD (Naydenov et al, 2007). MacDonald et al found a downregulation of creatine kinase mRNA levels in the hippocampus and prefrontal cortex of patients with BPD (MacDonald ML et al, 2006).

McMahon demonstrated that there is a disproportion in several mtDNA positions (incl. 10398A) between family members affected by maternally transmitted bipolar disorder and healthy ones (McMahon FJ, 2000). Kato et al found an increased level of mtDNA deletions in the brains of patients with BPD (Kato T et al, 1997; Kato T et al, 1997a) and also suggested that mtDNA 10398A position mutation is a risk factor for bipolar disorder (Kato T, 2001). Turecki et al studied families of patients with a good response to lithium treatment and found some locuses on different chromosomes possibly implicated in the pathogenesis of BPD (Turecki G et al, 2001).

Altered mitochondrial genome expression has also been suggested, particularly on the gene encoding complex I (Iwamoto K et al, 2005). Later a mitochondrial DNA 3644 mutation was associated with bipolar disorder (Munakata K et al, 2004), and an accumulation of mtDNA 3243 mutation in the brains of bipolar patients was found (Munakata T et al, 2005). Munakata also discovered a linkage between the polymorphism in the mt-ND1 gene 3644T>C (associated with BPD) and a decreased mitochondrial membrane potential and complex I activity (Munakata K et al, 2004). Later 10398A>G polymorphism has been linked to decreased mitochondrial matrix pH, and higher baseline and post-stimulation mitochondrial Ca^{2+} levels in patients with BPD and those effects observed mostly in patients who did not respond to lithium treatment were modulated by valproic acid (Kazuno AA et al, 2008). Konradi suggests that observed mtDNA mutations in BPD 'might be somatic rather than inherited, indicating either an overall increased vulnerability of mtDNA, or a higher exposure to DNA-damaging factors' (Konradi et al, 2004).

There is also increased incidences of maternal inheritance in some mood disorders which can also be an indirect implication of genetic mitochondrial pathology involved in the pathogenesis of the disease.

Several researchers did not find any evidence for mtDNA deletions or mutations taking part in the development of BPD (Stine OC et al, 1993; Iwamoto K et al, 2005) or, in contrast to the previously mentioned studies, discovered the

increased mRNA levels of complex I genes in BPD (Ben-Shachar D, Karry R, 2008). Other researchers discovered mutations typical for patients with BPD compared with healthy individuals (5178, 10398 positions in complex I zone) and the difference in mtDNA haplotypes between these two groups (Kirk R et al, 1999).

There is also data confirming not only the complex I mtDNA genes mutation but also nDNA (Washizuka S et al, 2003; Munakata K et al, 2004; Iwamoto K et al, 2005). As mentioned above, Konradi et al explored respiratory chain genes and found molecular-genetic pathology in both the prefrontal cortex and gyppocampus of the patients with BPD (Konradi C, 2004). Later this and other research found affected by the pH (an observed downregulation can be an artifact of sample pH rather than indicative of disease characteristics) (Vawter MP et al, 2006). But later there was a repeated data regarding the decreased expression of a cluster of genes in components of the mitochondrial ETS in postmortem BPD samples even with contriolled pH (Iwamoto K et al, 2005).

Research on the apoptosis as the possible pathogenetic mechanism of affective disorders revealed an upregulation of 19 apoptotic genes (out of 44) in postmortem brains of patients with BPD although antioxidant genes were found to be markedly downregulated (Benes FM et al, 2006). Other research showed a significant downregulation of ubiquitin cycle genes and intracellular transport in patients with BPD (Ryan MM et al, 2006); an amount of shared suicide candidate genes in patients with MDD, BPD and SZ (Kim S et al, 2007); genes of the ETS, phosphatidylinositol-signalling system (Harwood AJ, 2005) and glycolysis/ gluconeogenesis (Sun X et al, 2006); and a global down-regulation of mitochondrial genes, such as those encoding respiratory chain components, in BPD patients (though the first result is supposed to be affected by drug treatment) (Bezchlibnyk YB, 2001; Iwamoto K et al, 2004).

Symptoms of mood disorders may also be the first psychiatric manifestation of the MD or are somehow comorbid. Fattal reported 19 cases of mitochondrial diseases accompanied by different psychopathological symptoms (Fattal O et al,

2006). Di Mauro provided a lot of data on depression as a common symptom of the well-known mitochondrial disease CPEO (DiMauro S, Moraes CT, 1993). Siciliano suggested an association between MELAS (another mitochondrial disease) and BPD (Siciliano et al, 2003) and Grover reported a case of MELAS misdiagnosed as mania (Groover S et al, 2006).

There are several other studies indicating that kind of comorbidity, including animal models (Fattal O et al, 2006; Kato T, 2006; Anglin RE et al, 2012). Moreover, genealogical studies have shown an increased prevalence of mood disorders symptoms among the maternal relatives of the patient with mitochondrial disease. For instance, Boles revealed a high predisposition to affective disorders in mothers and other relatives on maternal line of patients with mitochondrial diseases (Boles RG, 2005). Shoffner measured depression levels in relatives of the proband with mitochondrial disease on the maternal line and found an increase of three fold in comparison with healthy relatives (Shoffner JM, Wallace DC, 1995). Burnet conducted an anonymous survey in the group of patients with mitochondrial diseases and their family members and detected an increased frequency of depression symptoms in relatives on the maternal side (Burnet BB et al, 2005).

This research data contributes to the hypothesis of mitochondria dysfunction playing a significant role in the pathogenesis of depression and bipolar disorder although we still can't conclude that certain symptoms of mood disorders have a causal relationship with certain mitochondrial pathology.

2.12. Other biological hypotheses of the bipolar disorder

Current knowledge provides the background for formulation of several biological hypotheses of BPD based on the identification of biomarkers for vulnerability, disease expression and course, and treatment response. Biomarkers for BPD are still being researched: structural brain changes are searched for using neuroimaging methods; polymorphisms in a number of susceptibility genes have

been discovered in genetic studies; and neurochemical biomarkers are studied in periphery above all. (Doung A et al, 2015; Scola G, Andreazza AC, 2014)

Neuroimaging

Structural imaging studies in patients with BPD have identified anatomical and neuropathological abnormalities, which are associated with a neuroprogression. Moreover, previous mild traumatic brain injury has been identified as a risk factor for development of heterogeneous neuropsychiatric diseases, including BPD. (Perry DC et al, 2016)

Disruptions to cortico–striatal–limbic circuits are the most straightforward method of describing the pathophysiology and symptomatology of affective disorders. (Mayberg HS, 2003; Savitz J, Drevets WC, 2009; Licznerski P, Duman RS, 2013) Morphometric measurements discover in BD patients enlargement of lateral and third ventricles after several manic episodes (Strakowski SM et al, 2002)), progressive decline in hippocampal, fusiform, and cerebellar gray matter density after repeated episodes (Moorhead TW et al, 2007), subregion-specific gray matter volume reductions in the prefrontal cortex (López-Larson MP et al, 2002), and increased rates of deep white matter hyperintensities (Kempton MJ et al, 2008). Neuroimaging studies have consistently demonstrated association of grey matter reduction in left rostral anterior cingulate cortex and right frontoinsular cortex thickness; that is, the most robust grey matter reductions in BPD occur in anterior limbic regions, which may be related to executive control and emotional processing abnormalities (Bora E et al, 2010). In a large study of 1710 BPD patients and 2594 healthy controls, the volumes of the nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen, thalamus, and lateral ventricles were measured. BPD patients have been found to have volumetric reductions for the mean hippocampus and thalamus and enlarged lateral ventricles (Hibar DP et al, 2016). However, brain volume may be altered by environmental factors, including medication.

Imaging data suggest that decreased activity in prefrontal cortical areas may result in inadequate modulation of limbic/subcortical areas, which contributes to

depressed mood and inadequate cognitive coping. Both regional gray-matter and white-matter changes appear to be present relatively early in BPD development (Maletic V, Raison C, 2014). Functional magnetic resonance imaging (fMRI) confirmed that excessive activation in brain regions associated with emotional regulation may contribute to the affective symptoms of BPD. Altered brain activation was identified in various regions in the cortico-limbic pathways; the most consistent findings were overactivation of the amygdala, striatum, and thalamus (Cerullo MA et al, 2009). All studies using fMRI in BPD supported the cortico-limbic hypothesis and suggest that connectivity can be more complex and that intra-regional disturbances should also be studied (Vargas C et al, 2013).

Neurotransmitters

The biological hypotheses of mood disorders are under development (Fišar Z, 2013). The monoamine hypothesis was initially formulated as catecholamine (Schildkraut JJ, 1965) and/or indolamine (Coppen A, 1967) deficiencies in the brain; that is, as the neurotransmitter hypothesis. Later, the monoamine hypothesis was revised to include the role of neurotransmitter receptors, transporters, catabolizing enzymes (monoamine oxidase [MAO], and COMT), and other brain neurobiological systems (Savitz J, Drevets WC, 2009; Heninger GR et al, 1996; aan het Rot M et al, 2009; Hamon M, Blier P, 2013).

The monoamine hypothesis of mood disorder posits that an imbalance in monoaminergic neurotransmission in the central nervous system is causally related to the clinical features of depression or mania. This hypothesis has been supported by mechanisms of action of antidepressants (Fišar Z, 2013; Hillhouse TM, Porter JH, 2015; Fišar Z, 2016). Moreover, many candidate genes associated with BPD encode compounds influencing directly the monoamine neurotransmitter systems, for example, SLC6A4 (encoding serotonin transporter), TPH2, DRD4, SLC6A3 (encoding dopamine transporter), MAO-A (encoding MAO type A, MAO-A), and COMT (Craddock N et al, 2001; Serretti A, Mandelli L, 2008; Rivera M, 2009).

The advanced monoamine hypothesis46 supposes that serotonin or norepinephrine concentrations in the brain are regulated by MAO-A activity, and

that the severity of symptoms of depression is linked to changes in the activity of monoamine transporters in specific brain regions. The hypothesis has been supported by observation of elevated MAO-A density and reduced 5-HTT density during depressive episode. (Meyer JH et al, 2006; Meyer JH et al, 2009; Selvaraj S et al, 2011)

Hyperdopaminergic function has been reported in BPD. A dopamine hypothesis of BPD was formulated that suggests a role of increased dopaminergic transmission in mania and the converse in depression (Berk M et al, 2007; Dunpol BW, Nemeroff CB, 2007). The hypothesis is supported by the fact that altered availability of dopamine transporter has been accepted as a biomarker for BD (Anand A et al, 2011).

Monoamine depletion studies, genetic association studies, PET studies, and mechanism of action of antidepressants supported an important role of disturbed monoamine neurotransmission in the pathophysiology of mood disorders but have not evidenced the primary role of monoaminergic system in development of the disorder. The molecular changes underlying imbalances of neurotransmission in BD are not agreed upon; it is hypothesized that alterations in excitatory amino acid transporters, 5-HTT, and dopamine transporter contribute to altered glutamatergic and monoaminergic function in BD patients (Rao JS et al, 2012).

The hypothesis that the dysfunctional muscarinic acetylcholine system is involved in the pathophysiology of BPD has been supported by the finding that there is reduced muscarinic acetylcholine M2 receptor binding in subjects with BPD (Cannon DM et al, 2006), which could be accounted for by a reduction in M2 receptor affinity caused by genetic variation in the gene for M2 receptor (Cannon DM et al, 2011).

Glutamate and GABA systems are posited in the pathophysiology of major depression and BD that extends beyond monoaminergic dysfunction (Lener MS et al, 2016). Glutamate levels were increased in the post-mortem brains of subjects with BD, while the glutamate/glutamine ratio was decreased following valproate treatment, and GABA levels were increased after lithium treatment. The balance of

excitatory/inhibitory neurotransmission seems to be central to the BPD (Lan MJ et al, 2009). A meta-analysis confirmed that brain glutamate + glutamine levels are elevated in BPD patients (Gigante AD et al, 2012), which supported an important role of glutamate in the pathophysiology of BD. Due to the role of glutamate in neurotransmission, brain energy metabolism, astrocyte function, neurotoxicity, neuroplasticity, and learning, the glutamate hypothesis of mood disorders is expected to complement and improve the prevailing monoamine hypothesis (Sanacora G et al, 2008; Jun C et al, 2014). The hypothesis is supported by the observation that antagonists of glutamate N-methyl-D-aspartate (NMDA) receptor produce rapid antidepressant effect (Gerhard DM et al, 2016; Machado-Vieira R et al, 2015).

Neurotrophic factors

Neurotrophic factors are growth factors that promote neuroplasticity, neurogenesis, survival, differentiation, and maintenance in healthy and regenerating brain cells. They include nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), glial cell line-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF). They are two classes of neurotrophic receptors: low-affinity nerve growth factor receptor (LNGFR, p75 neurotrophin receptor) and a family of high-affinity tropomyosin receptor kinase (Trk, also tyrosine receptor kinase; types TrkA, TrkB, and TrkC). Activation of LNGFR by neurotrophins (NGF, BDNF, NT-3, and NT-4) may induce apoptosis; activation of Trk receptors support cell growth and may be antiapoptotic (pro-survival). TrkA binds NGF, TrkB binds BDNF, NT-3, and NT-4, and TrkC binds NT-3.

Intracellular signaling pathways of growth factors include: (i) Ras/mitogen activated protein kinase (MAPK) pathway; (ii) phosphatidylinositol-4,5-bisphosphate 3-kinase (PI-3K)/protein kinase B (Akt)/glycogen synthase kinase 3 (GSK-3) pathway; and (iii) phosphoinositide pathway (phospholipase Cγ [PLCγ]/protein kinase C [PKC]) linked to MAPK, Akt, and/or Ca2+/calmodulin-dependent protein kinase (CaMK) (Kaplan DR, Miller FD, 2000; Jones DM et al,

2003; Miller FD, Kaplan DR, 2003; Skaper SD, 2008; Fišar Z, Hroudová J, 2010). An important intracellular regulator of apoptosis and cell survival in the developing, adult, and injured nerve cells is the p53 protein family (Jacobs WB et al, 2006). Neurotrophin signaling and vesicular transport is linked; disturbances of vesicular transport lead to disturbed neurotrophin signaling and to diseases of the nervous system (Bronfman FC et al, 2014).

Disturbances in activities of neurotrophic factors and related impairment in plasticity and resilience in brain cells has been reported in BPD. Attention is paid to neurotrophins and other growth factors that regulate function of brain cells, such as BDNF, GDNF, insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF). (Einat H, Manji HK, 2006; Scola G, Andreazza AC, 2015)

Brain-derived neurotrophic factor

Post-mortem brains of BPD patients have shown significantly decreased protein and mRNA levels of BDNF in the frontal cortex, which indicates that decreased BDNF is part of the pathophysiology of BPD (Kim HW et al, 2010). Several meta-analyses have shown that there may be a correlation between low BDNF levels and the emergence of BPD (Fernandes BS et al, 2011; Fernandes BS et al, 2014; Lin PY, 2009). A systematic review and meta-analysis confirmed that peripheral BDNF levels in BPD are consistently reduced during manic and depressive episodes and plasma levels of BDNF are recovered after treatment for acute mania, that is, the BDNF plasma or serum levels are not different in euthymia when compared to controls (Fernandes BS et al, 2015; Polyakova M et al, 2015; Fernandes BS et al, 2011).

Thus, reduction in serum BDNF may be a potential biomarker of acute episodes and could differentiate patients in a manic or depressive episode from those in the euthymic phase. Moreover, serum BDNF may distinguish BPD from unipolar depression (Fernandes BS et al, 2009), may be a biomarker of MD progression and severity (Fernandes BS et al, 2011; Fernandes BS et al, 2015; Grande I et al, 2010), may discriminate initial and advanced BD episodes (Karamustafalioglu N, 2015), and may reflect response to treatment (Tramontina

JF et al, 2009; Fernandes BS et al, 2015). The role of BDNF in the pathophysiology of BPD has been supported by findings that BDNF mRNA is decreased in cingulate and temporal cortices and in the hippocampus (Thompson Ray M et al, 2011; Ray MT et al, 2014) and that precursor protein proBDNF encoded by the BDNF gene has been suggested as a biomarker of mood disorders (Hashimoto K, 2010).

Note that BDNF levels in the blood could be derived from its production by both brain and peripheral tissues, such as skeletal muscle, liver, and the cardiovascular system, and BDNF in the blood is stored predominantly in platelets (Pláteník J et al, 2014). Nevertheless, intact BDNF in the peripheral circulation crosses the blood–brain barrier (BBB) by a high-capacity saturable transport system (Pan W et al, 1998) and blood and plasma BDNF concentrations reflect brain-tissue BDNF levels (Klein AB et al, 2011).

BDNF plays a key role in the pathophysiology of stress-related mood disorders. Acute stress, such as partial sleep deprivation, induced a fast increase in BDNF serum levels, whereas long-term stress led to sleep disturbance and depression as well as decreased BDNF levels (Schmitt K et al, 2016). The role of BDNF in the pathophysiology of BD has been evidenced by elevation of serum and brain BDNF levels by antidepressant, mood stabilizers, and antipsychotics (Sen S et al, 2008; Björkholm C, Monteggia LM, 2016), including lithium (Rybakowski JK, 2014).

The Val66Met polymorphism (refSNP Cluster Report: rs6265) of the BDNF gene is a functionally relevant SNP affecting the secretion of BDNF and is implicated in differences in hippocampal volumes. The hippocampal volumes were reduced signifi- cantly in association with the presence of the BDNF met allele and with BPD diagnosis (Chepenik LG et al, 2009). However, a systematic meta-analytical review of findings on the impact of the rs6265 SNP on hippocampal volumes in patients with BD suggested that there is no association between this BDNF polymorphism and hippocampal volumes (Harrisberger F et al, 2015). Another meta-analysis was performed to determine the overall strength of

associations between BDNF genetic polymorphism Val66Met and susceptibility to BD. It was concluded that there is no compelling evidence to support Val66Met polymorphism in the BDNF gene playing an important role in the susceptibility to BPD (Wang Z et al, 2014). However, plasma BDNF levels were significantly negatively correlated with depression scores in patients with BD. It was supposed that plasma BDNF in BD is not affected by BDNF Val66Met gene variants, but by progression of the illness itself (Chen SL et al, 2014).

Other growth factors

There are few studies that evaluate the role of NGF in the pathophysiology of BPD and the results are inconsistent (Scola G, Andreazza AC, 2015). For example, decreased plasma NGF was reported in BPD in the manic episode (Barbosa IG et al, 2011), but another study stated significantly higher IGF-1 in patients with bipolar I disorder and no changes in NGF (Kim YK et al, 2013).

Effects of NT-3 and NT-4 also require more research. Serum NT-3 levels in drug-free and medicated patients were found to be increased when compared with controls and serum NT-3 levels did not differ between drug-free and medicated patients (Fernandes BS et al, 2010). Serum concentrations of NT-4 were reported to be significantly higher in BD patients than in controls (Walz JC et al, 2009). At baseline, in bipolar depressed patients, there were no differences between responders and non-responders to treatment with ketamine in serum BDNF, NGF, NT-3, NT-4, or GDNF (Rybakowski JK et al, 2013).

A study of GDNF in BPD revealed that different stages of the disorder and drug treatment can alter activity of GDNF (Scola G, Andreazza AC, 2015). Lower GDNF concentrations might be involved in the pathophysiology of BD and drug treatment increases the GDNF (Zhang X et al, 2010).

The evidence has been provided that IGF-1 plays a role in the pathophysiology of BPD (Scola G, Andreazza AC, 2015). The IGF1 gene has been identified as a candidate gene for susceptibility to BPD (Pereira AC et al, 2011). Peripheral levels of IGF-1 were found to be unchanged in patients with BPD compared to controls (Palomino A et al, 2013); however, it seems that lithium may

regulate IGF-1 levels (Squassina A et al, 2013). Low expression of IGF1 has been suggested as a putative biomarker for lithium unresponsiveness (Milanesi E et al, 2015).

Plasma VEGF levels were elevated in patients with a manic episode of BD, which may be associated with a neuroprotective role for VEGF (Lee BH, Kim YK, 2012). Increased expression of the VEGF gene was also found in a depressive episode of BD compared to healthy control subjects (Shibata T et al, 2013). Lithium treatment led to decrease of VEGF mRNA levels (Kikuchi K et al, 2011). These results indicate that VEGF may vary with the episode of the disease and may be associated with the pathophysiology of BPD (Scola G, Andreazza AC, 2015).

It was reported that some members of the neurotrophin family, such as NGF, NT-3, and NT-4, can cross the BBB; NGF had the fastest influx rate and NT-3 the slowest (Pan W et al, 1998b). CNTF is saturably transported across the BBB from blood to brain (Pan W et al, 1999). IGF-1 enters the CNS by a saturable transport system at the BBB, which functions in synchrony with IGF-binding proteins in the periphery to regulate the availability of IGF-1 to the CNS (Pan W et al, 2000). It indicates that peripheral administration of some neurotrophins could have therapeutic effects within the CNS. In contrast, GDNF and VEGF do not cross the BBB (Boado RJ, Pardridge WM, 2009); VEGF enhances angiogenesis and promotes BBB leakage in the ischemic brain (Zhang ZG et al, 2000).

Neurotrophic hypothesis

Neurotrophic, neuroplasticity, and neurogenesis hypotheses were formulated, supposing a key role of post-receptor and intracellular processes (regulating intercellular and intracellular signaling) in the development of mood disorders. These hypotheses emphasize the role of stress, growth factors and neurogenesis in the pathophysiology of BPD (Einat H, Manji HK, 2006; Duric V, Duman RS, 2013; Zarate CA et al, 2006; Duman RS, 2014; Duman RS et al, 1997) and they are supported by neurotrophic effects of various antidepressants and mood stabilizers. Wnt signaling pathway and GSK-3 activity have been implicated

in both regulating neuroplasticity and neuroprotection (Gould TD et al, 2002; Gould TD et al, 2006).

The neurogenesis hypothesis of depression proposes that depression can arise from impaired hippocampal neurogenesis and that antidepressants stimulate neurogenesis. Coupling of hippocampal neurogenesis to pathophysiology of depression requires further research to be confirmed (Berk M et al, 2014; Santarelli L et al, 2003; Sapolsky RM, 2004; Gass P, Riva MA, 2007).

Neuroprogression is an important mechanism of BPD, and neurotrophins are key regulators of neurogenesis and neuroplasticity (Berk M et al, 2014; Scola G, Andreazza AC, 2015; Pittenger C, Duman RS, 2008). The neurotrophic hypothesis (Einat H, Manji HK, 2006; Duman RS et al, 1997; Duman RS, 2002; Zarate CA et al, 2006) postulates that mood disorders, such as BPD, are associated with a lower activity of neurotrophic factors, such as BDNF. Vulnerability to depression can arise as a result of neuronal damage (e.g., after chronic stress, long-term increased levels of glucocorticoids, ischemia, hypoglycemia, viral infections, and action of neurotoxins) and therapeutic effects of antidepressants may consist in activation of higher expression of neurotrophin BDNF and its receptor TrkB (e.g., through activation signaling pathways linked to monoaminergic systems) leading to increased neuronal plasticity, reactivation of cortical plasticity, and resumption of neuronal functions (Castrén E, Rantamäki T, 2010).

2.13. Mood disorders treatment and its effect on mitochondrial function

The mood stabilizing effect of drugs was discovered by chance in many cases and so consequently the mechanism responsible for stabilizing moods is still not clearly understood. It is known that several of the drugs are metabolized in mitochondria and take part in various mitochondrial processes, pointing to why it is very important to study their effects on mitochondrial metabolism. Moreover, the research data regarding the effects of different psychotropic drugs on mitochondria function is also for mitochondria dysfunction involved in the pathogenesis of the psychiatric diseases.

The observed effects cover different aspects of the possible impairment. Most patients with mood disorders receive drug treatment with mood stabilizers, antidepressants and/or antipsychotics. There is data reflecting different effects of psychotropic drugs on mitochondria, and they are reported to normalize the number of mitochondria in the brain (Iniwa IM et al, 2005), reduce the oxidative stress (Moylan S et al, 2014), modulate the expression of the antiapoptotic genes (Zarate CA et al, 2006)), increase energy metabolism and decrease oxidative damage under specific pathological conditions such as excitotoxicity and oxidative stress (Nonaka S et al, 1998; Wang JF et al, 2004; Shao L et al, 2005; Cui J et al, 2007; Machado-Vieira R et al, 2007; Khairova R et al, 2012), inhibite some enzymes pathologically involved in mitochondrial energy metabolic processes (Chen G et al, 1999; Jope RS, Roh MS, 2006) and processes themselves (Burkhardt C et al, 1993; Prince JA et al, 1997; Prince JA et al, 1998; Banerjee U et al, 2012), down- or up-regulate gene expression (Ozaki N, Chuang DM, 1997; Bosetti F et al, 2002; Sun X et al, 2006), inhibit mitochondrial functions and expression of mitochonria-related genes in the brain (Weinbach BC et al, 1986; Keller BJ et al, 1992; Curti C et al, 1999; Modica-Napolitano JS et al, 2003), modulate calcium levels (Wasserman MJ et al, 2004) and some other activities (Moretti AI et al, 2003) though some of the first-generation antipsychotic drugs might have negative effects on mitochondrial respiration (Maurer I, Moller HJ, 1997; Sagara Y, 1998).

Lithium is known to enhance mitochondrial function in many ways including increase in cell respiration rate (Quiroz JA et al, 2008), modulation of the apoptosis through increasing the Bcl-2 level and reduction of the levels of some pre-apoptotic genes (Chen G et al, 1999; Michaelis M et al, 2006); protection of some genes involved in the functional damage to mitochondria against the alteration (Bachmann RF et al, 2009) and enhancement of their expression (Washizuka S et al, 2003b); desensitizing brain mitochondria to calcium, antagonizing permeability transition, diminishing cytochrome *c* release (Shalbuyeva N et al, 2007); increasing the activity of ETS complexes I, II and III

(Maurer IC et al, 2009); regulation of the intracellular Ca2+ (Kazuno AA et al, 2008), raising the expression of glutathione-S-transferase (Wang JF et al, 2004); increasing *N*-acetylaspartate (NAA) levels (Hajek T et al, 2012) etc.

Valproate effects are the inhibition of the substrate-specific oxygen consumption and mitochondrial ATP synthesis (Chiu CT et al, 2013), an interference with mitochondrial β-oxidation (Silva MF et al, 2008), neuroprotective effects on different types of cells in nervous tissue (Lai JS et al, 2006), a histone deacetylase inhibition (Gavin DP et al, 2009) and some are also the same as lithium effects, although there are differences between these two drugs (Gupta A et al, 2012; Kostrouchova M et al, 2007; Bielecka AM, Obuchowicz E, 2008). Its metabolites also inhibit dihydrolipoyl dehydrogenase activity and stabilize the oxidative phosphorylation (Luis PB et al, 2007).

Antipsychotic drugs of the first generation are known to affect the electronic transport chain (Prince JA et al, 1997a) and inhibit complex I activity or have neurotoxic effects (Ukai W et al, 2004) even though atypical antipsychotics do not. Both of the groups increase complex IV activity in different brain regions (Shao L et al, 2008). Thioridazine is reported to interact with the inner membrane of mitochondria and show a significant antioxidant activity (Rodrigues T et al, 2002). Antipsychotics also have an anti-apoptotic activity, for instance, they modulate the antiapoptosis genes Bcl-2 and Bcl-xL expression, inhibit the release of proapoptotic compounds (Saldona M et al, 2007), inhibit MPT (Kowaltovski AJ et al, 2001) and attenuate neurotoxicity (Wei Z et al, 2003). Some research suggests that these effects are a part of complex effects of antipsychotic drugs on the cell (Dean CE, 2006).

Evidence for the antidepressant-induced positive effects in mitochondrial functions is relatively low. In general, antidepressants were known to impair mitochondrial function and induce toxicity (Mattson MP et al, 2008; Dykens JA et al, 2008). Fluvoxamine is reported to alter energy metabolism by decreasing the activities of citrate synthase, malate dehydrogenase, and complexes I, II-III, and IV; and increasing the activities of complex II, succinate dehydrogenase, and

creatine kinase in different dose and different brain regions (Ferreira GK et al, 2014). Goncalves showed that citalopram and escitalopram decreased the activity of respiratory chain complexes (Goncalves CL et al, 2012). Hwang discovered an indirect modulation of mitochondrial function by tricyclic antidepressants through a decrease in nitric oxide production (Hwang J et al, 2008). Hroudova and Fisar showed that amitriptyline, fluoxetine, and tianeptine might be potent partial inhibitors of energized mitochondrial respiration (Hroudova J, Fisar Z, 2012). Ferreira suggested that antidepressant-induced mitochondrial dysfunction could be involved in early biochemical processes leading to changes in neuroplasticity (Ferreira GK et al, 2014) and Abdel-Razaq suggested that 'the weak antimitochondrial actions of antidepressants could provide a potentially protective preconditioning effect, in which antidepressant-induced mitochondrial dysfunction below the threshold of injury results in subsequent protection' (Abdel-RazaqW et al, 2011).

In some research reports, the effect of the so-called metabolic drugs on different psychiatric symptoms of mitochondrial diseases. Researchers report the positive effect on mutism, hallucinations, psychotic symptoms and aggressive behavior in patients with MELAS after coenzyme Q treatment (Suzuki T el al, 1990; Inagaki T et al, 1997; Kiejna A et al, 2002). Another case report describes a positive effect of dichloroacetat (a medicine which is used to reduce the lactate levels) on delirium and productive symptoms in patient with MELAS (Thomeer EC et al, 1998). These effects of coenzyme Q and other medications treatment such as carnitine and nicotinic acid were observed also in patients with the diagnosed mental disorder only (without any mitochondrial disease) (Onishi H et al, 1997; Gardner A et al, 2003; Filipek PA et al, 2003).

3. Statement of purpose and hypotheses

The review was focused on the data revealing multiple connections between different signs and symptoms of mental disorders and various mitochondrial pathology. We can now see that the interpretation of all these data requires careful attention because it is partially controversial and some effects were observed in certain brain regions which can possibly indicate a result of other influences and not necessarily indicate a direct cause-and-effect relationship. Obviously the role of mitochondria in the pathogenesis of mental disorders is very complicated and might be different in different brain regions with maximum observed effects in the most vulnerable domains for each disease.

Some promising results were obtained leading to perspective studies addressing deeper connections between mitochondrial functions and the pathology of mood disorders. A complex view of the pathology of mood disorders and the role of mitochondria in them is crucially important to develop new diagnostic tools and various therapeutic strategies for this group of devastating diseases. Mitochondrial parameters can be also evaluated as biological markers of bipolar disorder, one of the mood disorders.

Summarizing various connections between pathophysiological processes in bipolar disorder and mitochondrial dysfunctions, we state a purpose for the study: to explore how energy metabolism in mitochondria corresponds to clinical evaluation of psychopathological symptoms in patients with bipolar disorder.

Hypotheses of the study:

Hypothesis 1. There is a set of mitochondrial functional impairment indexes specific for the current phase of the disorder.

Hypothesis 2. The severity of the symptoms of bipolar disorder is associated with the severity of the alteration of the mitochondrial function.

Hypothesis 3. There is a difference in the levels of mitochondrial respiration and enzyme activity in manic state and depressive state.

Hypothesis 4. There is a difference in the levels of mitochondrial respiration and enzyme activity in patients with BPD and healthy controls in both the acute state and remission.

4 . Material and methods

4.1. Study design and participants

37 patients with diagnosis of bipolar disorder (F31) according to ICD-10 were recruited from acute wards of the Department of Psychiatry of the First Faculty of Medicine, Charles University and General University Hospital in Prague and repeatedly tested using different psychopathology scales and blood platelets analysis methods (measurement 1 – acute phase, measurement 2 – remission). The control group consisted of 21 healthy volunteers matched by age and gender tested once using blood platelets analysis methods. Demographic data was collected for each person.

The included criteria were as follows:

- all in-patients and out-patients are already treated for BPD (at least second current episode);
- acute state;
- within one week upon hospitalization;
- diagnosis of the BPD – F31 (phase manic, depressive, remission);
- age 18-65.

The excluded criteria were as follows:

- additional diagnosis of any listed in F10-F19, F20-F29, F70-F79;
- psychoactive substance abuse;
- organic brain damage;
- significant cognitive impairment;
- history of medication abuse of any kind;
- diagnosis of cancer or any neoplastic disease within the last 3 years;
- a diagnosed mitochondrial disorder;
- constantly taking medicines such as coenzyme Q, L-carnitine, vitamin E, chloramphenicol, doxycycline, ofloxacin, ciprofloxacin, perofloxacin, azathioprine, cyclosporine, tacrolimus, everolimus,

monoclonal antibodies, amiodarone, statins, levomepromazine, haloperidol;

- participation in any study involving investigational drug within the last 3 months.

The study was carried out according to the principles expressed in the Declaration of Helsinki and the study protocol was approved by the Ethical Review Board of the First Faculty of Medicine and General University Hospital in Prague, Czech Republic. Written informed consent was obtained from all participants.

4.2. Questionnaires and scales

All patients included were screened for bipolar disorder using the mood disorder questionnaire (MDQ) (Hirschfeld RM et al, 2000).

The Mood Disorder Questionnaire is a brief, self-report screening instrument for bipolar disorder with both good sensitivity and very good specificity which includes 13 questions plus items assessing clustering of symptoms and functional impairment.

Severity of current depression was tested using the MADRS (Montgomery–Åsberg Depression Rating Scale).

MADRS is a ten-item diagnostic questionnaire which psychiatrists use to measure the severity of depressive episodes in patients with mood disorders. It was designed in 1979 by British and Swedish researchers as an adjunct to the Hamilton Rating Scale for Depression to be more sensitive to the changes brought on by antidepressants and other forms of treatment than the Hamilton Scale.

A higher MADRS score indicates more severe depression, and each item yields a score of 0 to 6. The overall score ranges from 0 to 60. The questionnaire includes questions on the following symptoms 1. Apparent sadness 2. Reported sadness 3. Inner tension 4. Reduced sleep 5. Reduced appetite 6. Concentration

difficulties 7. Lassitude 8. Inability to feel 9. Pessimistic thoughts 10. Suicidal thoughts.

Severity of current mania was tested using the Young Scale of Mania (YMRS).

YMRS is one of the most frequently utilized rating scales to assess manic symptoms. The scale has 11 items and is based on the patient's subjective report of his or her clinical condition over the previous 48 hours. There are four items that are graded on a 0 to 8 scale (irritability, speech, thought content, and disruptive/aggressive behavior), while the remaining seven items are graded on a 0 to 4 scale. These four items are given twice the weight of the others to compensate for poor cooperation from severely ill patients. The scale is generally done by a clinician or trained rater with expertise of manic patients and takes 15–30 minutes to complete.

Severity of illness

General severity of illness was assessed using Clinical Global Impression - Improvement scale (CGI-I). CGI-I is a 7-point scale that requires the clinician to rate the improvement of the patient's mental illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis.

Psychopathology symptoms were evaluated twice during the illness (before treatment, in acute state, and during treatment, in remission).

4.3. Laboratory methods

Peripheral blood samples were taken from the antecubital vein of each participant between 7:00 and 8:00 am, when all subjects were nicotine- and and coffee-free, before their morning medications. 24 milliliters of blood were drawn into BD Vacutainer® blood collection tubes with anticoagulant. Platelet rich plasma was separated by centrifugation at 200×g for 10 min at 25 °C. Platelets

were counted by microscopy using a counting chamber and immediately used for measuring of mitochondrial parameters.

The energy metabolism related to mitochondrial dysfunctions was analyzed in biochemical laboratories (First Faculty of Medicine). Selected mitochondrial parameters (citrate synthase and electron transport chain complexes activities, ATP production and mitochondrial respiratory rate) and functional changes in monoaminergic system (MAO activity, serotonin uptake) were measured in peripheral blood components. High-resolution respirometry, fluorescence, radiochemical and spectrophotometric methods were used.

Complexes of ETS – complex I, II, II+III and IV and citrate synthase were measured spectrophotometrically (Hroudová and Fišar, 2010). The relative activities of mitochondrial complexes were expressed as a ratio between specific enzyme activities and citrate synthase serving as the control mitochondrial matrix enzyme.

Mitochondrial respiration was evaluated by both respiratory rate and respiratory control ratios (RCRs) using high resolution respirometry using (oxygraph) with Clark type oxygen electrodes (Fišar et al., 2016). Respiratory rate was determined as time derivation of oxygen concentration in the sample and RCRs was calculated as ratios of respiratory rates measured before and after substrates and/or inhibitors of OXPHOS.

4.4. Statistical methods

The study materials were statistically processed using the methods of parametric and nonparametric analysis in accordance with the results of testing the compared populations for normal distribution. Accumulation, corrections and systematization of the initial information and results visualization were performed in Microsoft Office Excel 2010. The statistical analysis was performed using the IBM SPSS Statistics v.20 program.

Each of the comparable sets of quantitative data was evaluated for compliance with the standard normal distribution law using the Shapiro-Wilk test which is recommended when a number of subjects are less than 60. The data distribution histogram, asymmetry and kurtosis parameters were also taken into account.

If a normal distribution of quantitative data was confirmed, the obtained data was combined into a variation series, in which the arithmetic mean values (M) and the standard deviations (σ) were calculated. The analysis was performed using the parametrical statistics method.

If the quantitative data distribution was non normal, the obtained data was described using the median (Me) and the lower and upper quartiles (Q1 and Q3). The analysis was performed using the nonparametric statistics method.

To assess the statistical significance of the differences in the mean values of normally distributed populations, the Student t-test was calculated (1):

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}, \qquad (1)$$

where: M_1 and M_2 – compared averages, m_1 and m_2 – standard errors of the average values.

When comparing the average values calculated for dependent populations (for example, before treatment and after treatment values), the paired Student t-test was calculated (2):

$$t = \frac{\overline{X}_D - \mu_0}{\frac{s_D}{\sqrt{n}}}, \qquad (2)$$

where: X_D - the average, s_D - standard deviation of those differences, μ_0 - non-zero. The degree of freedom used is $n - 1$, where n represents the number of pairs.

The obtained values of Student t-test were compared with critical values. Differences were considered statistically significant at a significance level of $p<0.05$.

To compare independent sets of quantitative data with a non normal distribution the Mann-Whitney U test was used. First a single ranked series from both of the compared samples were formed, where elements were sorted according to the value increase. A smaller rank was attributed to a smaller value. Then a single ranked series was divided into two, consisting, respectively, of the first and second samples units. The rank amounts were counted separately for each of the series. The Mann-Whitney U test was calculated (3):

$$U = n_1 \cdot n_2 + \frac{n_x \cdot (n_x + 1)}{2} - T_x,$$

(3)

where n_1 – number of elements in sample 1, n_2 – number of elements in sample 2, n_x – number of elements in the bigger sample, and T_x – ranks sum for the bigger sample.

The calculated Mann-Whitney U test values were assessed by comparing them with the critical values: whether the calculated value was less or equal to the critical one, the statistical significance of the differences was accepted.

To assess the differences between two compared pairs of samples with a non normal distribution the Wilcoxon W-test was used. The change value was calculated for each patient. All the changes were ordered according to the absolute value. Then the signs of change ("+" or "-") were assigned to ranks and the ranks were summed up for each sign. The smaller rank amount (W) was compared to the W test critical value: whether the calculated value was less or equal to the critical one, the statistical significance of the differences was accepted.

To compare several groups of the patients (more than 2), a one-way analysis of variance was used. To assess the statistical significance of the differences the Fisher F test was calculated (4):

$$F = \frac{Q_1 /(m-1)}{Q_2 /(n-m)} \qquad (4)$$

where Q_1 – sum of the sample means to overall average squared deviations, Q_2 – sum of the observed values squared deviations, n – number of the elements, and m – number of the samples.

If the calculated value of Fisher's F test was less than critical, we made the conclusion that there was no statistically significant effect of the studied factor on the mean values of the trait. If the calculated value of Fisher's F test was larger than critical, the significant influence of the independent factor on the mean values for a certain level of statistical significance was recognized.

If statistically significant differences between groups existed, an additional pair comparison of the populations using the a posteriori criterion of Scheffe was carried out. To check the tightness of the relationship of the quantitative indicators the linear correlation coefficient rxy of Pearson was calculated (5):

$$r_{xy} = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \cdot \sum (y - \bar{y})^2}} \quad (5)$$

To evaluate the quality of the linear function selection, the square of the linear correlation coefficient R^2 (the coefficient of determination) was calculated. The coefficient of determination shows the percent of factors considered in the model.

For the evaluation of the statistical significance of the correlation coefficient t-test was calculated (6):

$$t_r = \frac{r_{xy}}{\sqrt{1 - r_{xy}^2}} \cdot \sqrt{n - 2} \quad (6)$$

The obtained value was compared with the critical value for a certain level of significance and the number of degrees of freedom n-2. If the calculated value of t_r was larger than $t_{crit,}$ a certain level of statistical significance was recognized.

The values of the correlation coefficient were interpreted in accordance with the Chaddock scale (Tab. 1)

Tab. 1. The determination of the closeness of the correlation relationships, Chaddock scale

Coefficient	Quality characteristic
<0,1	No relationships
0,1 – 0,3	weak
0,3 – 0,5	moderate
0,5 – 0,7	salient
0,7 – 0,9	high
0,9 – 0,99	Very high

To assess the dependence of one quantitative parameter to others, the linear regression method was used, and the reduced equation of the following kind was given (7):

$$y = a_0 + a_1x_1 + \ldots + a_nx_n, \quad (7)$$

where y – quantitative trait, $x_1 \ldots x_n$ – factor traits, a_0 – constant, $a_1 \ldots a_n$– regression coefficients, showing the average change in the result y with a change in the factor x by one unit.

The obtained regression model allows us to calculate the theoretical values of the effective sign y from the given values of the factor x.

To compare the nominal scale values Pearson χ^2 test was used. It allows us to assess the significance of the differences between the actual number of outcomes or qualitative characteristics of a sample falling into each category, and the theoretical amount that can be expected in the study groups when a null hypothesis is valid.

First, the expected number of observations in each of the cells of the conjugacy table was calculated, provided that the null hypothesis of the absence of an interrelation was valid. For this purpose, the sums of rows and columns (marginal totals) were multiplied with the subsequent division of the obtained product by the total number of observations.

Then the value of the χ^2 was calculated (8):

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \quad (8)$$

where i – the row number (from 1 to r), j – the column number (from 1 to c) O_{ij} – actual number of observations in the cell ij, and E_{ij} – the expected number of observations in the cell ij.

Then the value of the Pearson χ^2 test was compared to the critical values for (r-1) × (c-1) number of degrees of freedom. If the obtained value was larger than critical, a certain level of statistical significance was recognized and a statistical relationship between the studied risk factor and the outcome was confirmed.

For the four-field table analysis, when the number of expected observations in any of the cells of the four-field table was less than 10, the χ^2 test with the Yates correction was calculated. It reduces the risk of the first type error, i.e., detection of non-existent differences. The Yeats correction includes subtracting 0.5 from the absolute value of the difference between the actual and expected number of observations in each cell, which leads to a decrease in the χ^2 test value (9):.

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(\left|O_{ij} - E_{ij}\right| - 0{,}5)^2}{E_{ij}} \qquad (9)$$

To estimate the significance of the differences when the number of expected observations in any of the cells of the four-field table was less than 5, an accurate Fisher P test was calculated (10):

$$P = \frac{(A+B)!(C+D)!(A+C)!(B+D)!}{A!\,B!\,C!\,D!\,N!}, \qquad (10)$$

where A, B, C, D – actual numbers of observations in the cells of the contingency table, N – total number of the participants, and ! – a factorial, equal to the multiplication of a number by a sequence of numbers, each of which is less than previous by 1.

An obtained value of Fisher's exact P test more than 0.05 indicated the absence of statistically significant differences. An obtained value of Fisher's exact P test less than 0.05 indicated their presence.

To compare the relative values characterizing the associated populations (at the beginning and at the end of the observation) the McNemar test was used. It is

used to determine whether any changes in the distribution structure values of two dependent variables occur (11):

$$Q = \frac{(b-c)^2}{b+c} \tag{11}$$

where Q – McNemar test, b – the number of patients with a negative result in the first observation and positive result in the second, and c – the number of patients with a positive result in the first observation and negative result in the second.

The McNemar test values were interpreted by comparison with critical values.

To identify factors that characterize the relationships between groups of characteristics and to reduce the number of analyzed variables, a four-stages factor analysis was used:

1) calculation of the correlation matrix for all variables participating in the analysis;

2) extraction of factors by the principal component method;

3) the rotation of factors to create a simplified structure using Varimax method;

4) analysis of factor loads matrix and the interpretation of factors.

5. Results

5.1. Clinical evaluation of the BPD patients in manic or depressive episode

All the subjects were divided into 2 groups: experimental group (37 patients with BPD) and control group (21 healthy individuals). The experimental group, in turn, consisted of 24 patients in manic episode of the disease (subgroup A) and 13 patients in depressive episode of the disease (subgroup B). Clinical evaluation and biochemical measurement of BAD in-patients were done both at the beginning of treatment and when released from hospital treatment (in remission or partial remission). The average age within groups is shown in Table 2.

Tab. 2. Age structure in the experimental group (patients with bipolar disorder) and control group

Group	Subgroup	Age (years)		N
		min-max	M±SD	
Experimental	- All	21 – 65	42.2±12.2	37
	- Subgroup A	21 – 65	39.5±13.2	24
	- Subgroup B	30 – 59	46.9±8.7	13
Control		25 – 61	40.3±10.3	21

Subgroup A = manic episode; Subgroup B = depressive episode

A one-way analysis of variance (ANOVA) did not show a statistically significant difference in age between control group and experimental groups (all p=0.565, subgroup A p=0.156, and subgroup B p=0.147); it proves that groups are age-matched and no correction for age is necessary in data analysis.

First stage of the research included the assessment of the mental state of the patients with BPD. The following tests were used for patients in the manic episode: Brief Psychiatric Rating Scale (BPRS), Young Mania Rating Scale (YMRS), Mood Disorder Questionnaire (MDQ). Tests for patients in the depressive episode

included: BPRS, Montgomery-Asberg Depression Rating Scale (MADRS), MDQ. For patients in remission we added Clinical Global Impression – Improvement scale (CGI-I) to measure the clinical improvement. (Tab. 3)

Tab. 3. Mental state assessment in the experimental group at the beginning of treatment and when released from treatment (measurements 1 and 2)

Test method	Episode	State of the patient		p
		Disease (acute state), measurement 1	Remission, measurement 2	
BPRS	Depressive	55 (44.5-68.5)	30 (27-33)	<0.001
	Manic	51.5 (41.5-66.5)	33 (29-41.5)	0.018
	p	0.952	0.177	-
MADRS	Depressive	26.5 (19-31)	4 (0-4)	<0.001
YMRS	Manic	20 (13.5-26)	1.5 (0-5)	<0.001
CGI-I		-	xx	

Mean (range); p – significance level

Data obtained from the Wilcoxon-Mann-Whitney test shows that the difference between the BPRS test scores in the acute state of the disease and in remission was significant within both A ($p<0.001$) and B ($p=0.018$) subgroups. In patients with mania the median BPRS score decreased from 51.5 to 33 and in patients with depression it decreased from 55 to 30. The decrease was comparable in both the subgroups. The difference in BPRS test scores between patients with mania and patients with depression were not significant in either the acute phase of the disease ($p=0.952$) or in remission ($p=0.177$) (Fig. 20).

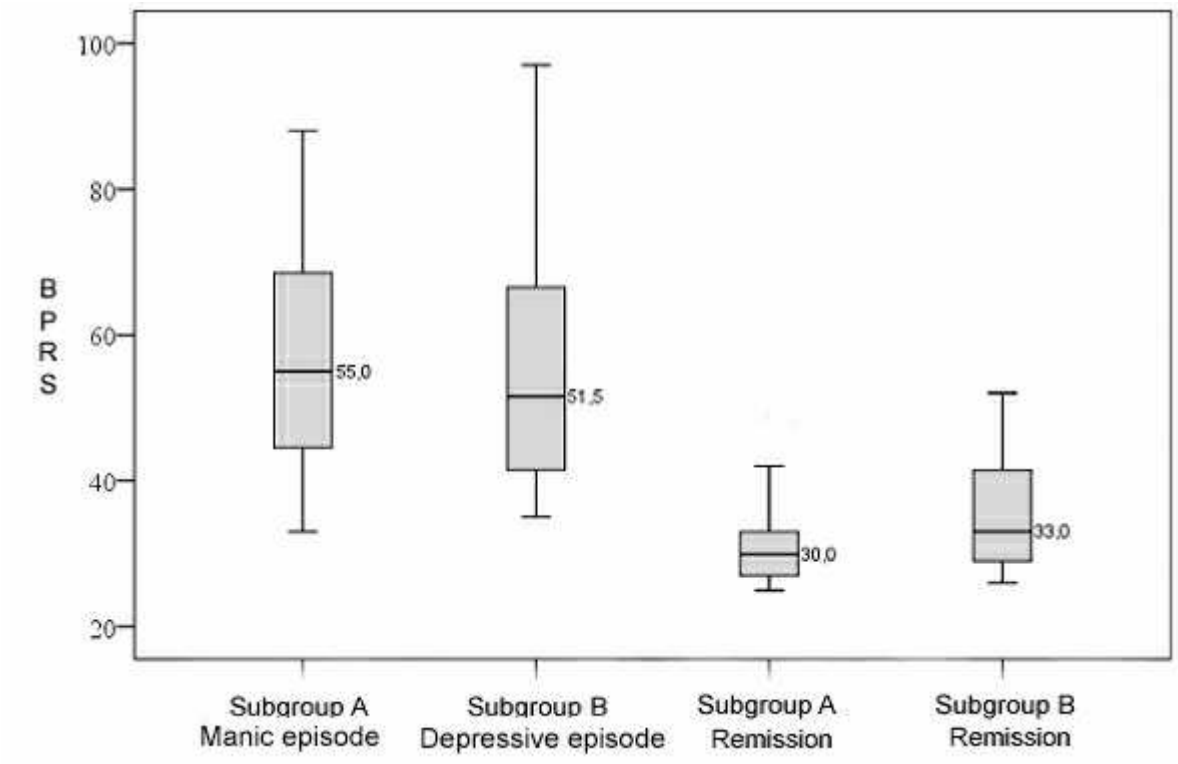

Fig. 20. BPRS test score in patients with mania and depression at the beginning and at the end of the study. Subgroup A = manic episode, Subgroup B = depressive episode. BPRS – Brief Psychiatric Rating Scale

The Wilcoxon-Mann-Whitney test was also applied to establish the difference between the MADRS test score in the acute state of the disease and in remission. The decrease after treatment was significant ($p<0.001$). The median MADRS assessment decreased from 26.5 to 4 (Fig. 21).

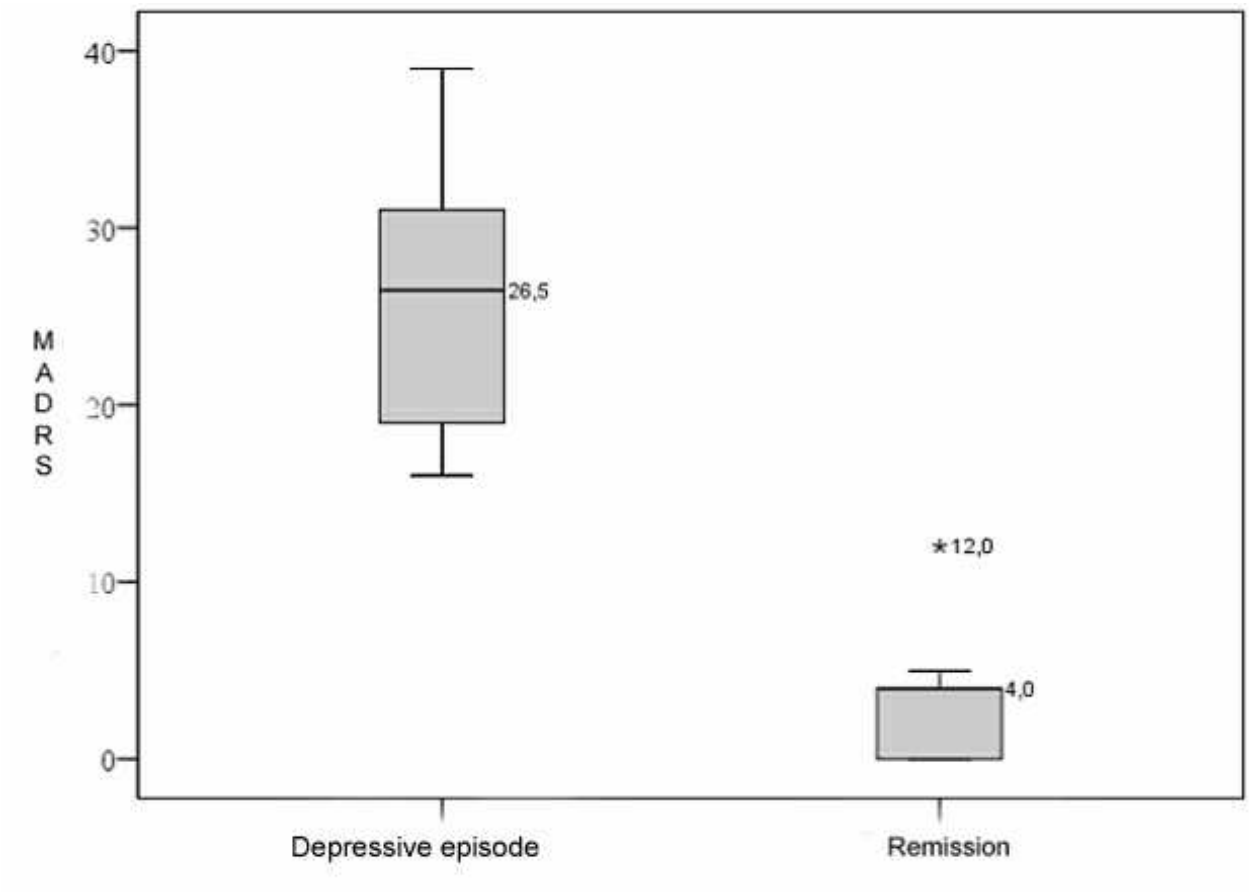

Fig. 21. MADRS test results in patients with depression at the beginning and at the end of the study

A significant decrease in the YMRS test score between patients in the manic episode and patients in remission was also established ($p<0.001$). The median in the acute phase was 20, in remission it decreased to 1.5. (Fig. 22)

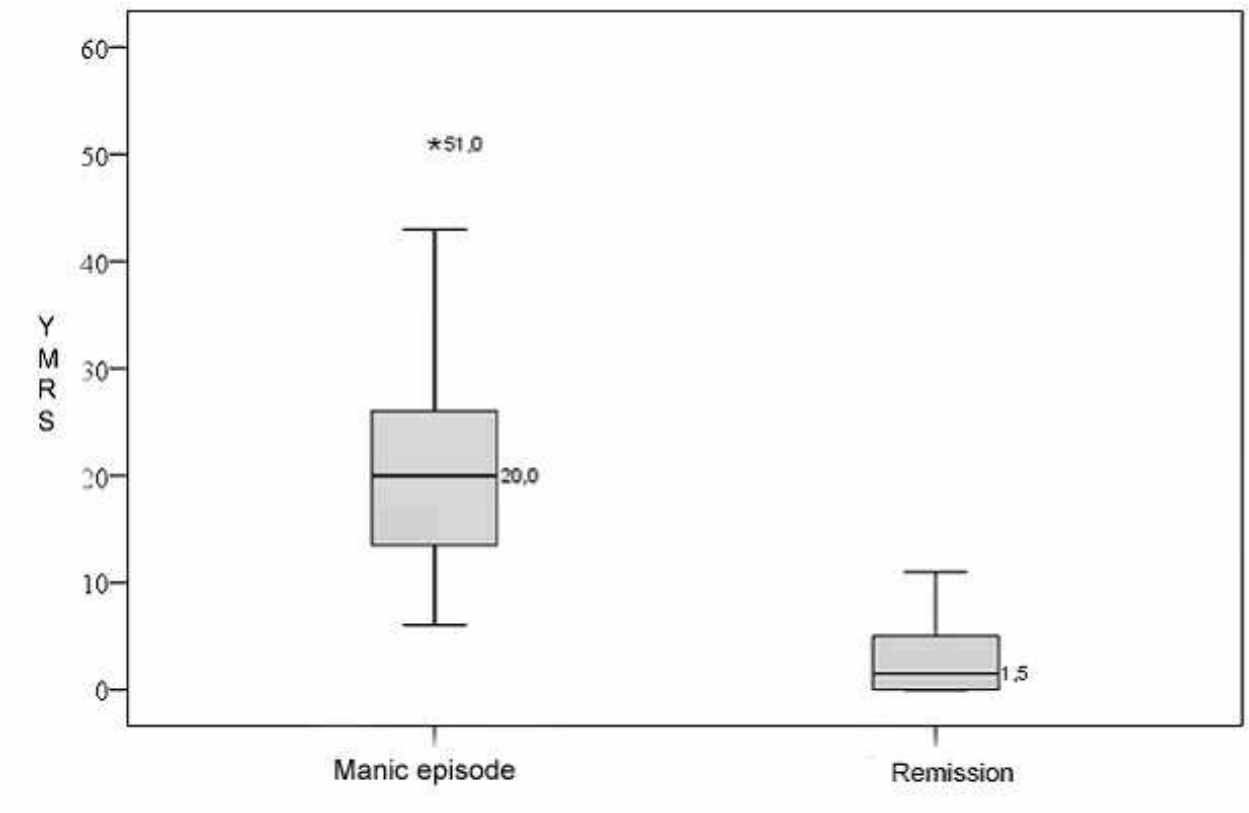

Fig. 22. YMRS test results in patients with mania and depression at the beginning and at the end of the study

The results of the CGI-I test were examined only at the end of the research as it is used to assess the quality of remission in mentally ill patients. (Tab. 4)

Tab. 4. CGI-I test results in patients with bipolar disorder in manic and depressive episode at the end of the study (remission)

CGI test	Subgroups				p
	A (mania)		B (depression)		
	Me	Q_1-Q_3	Me	Q_1-Q_3	
Scale I	2	1-2	2	1-3	0.694
Scale II	2	1-2	2	1-2	0.885
Scale III	41	41-42	41	31,5-41,5	0.462

p – significance level; Me = mean range; Q_1- Quartile 1, Q_3 - Quartile 3.

The Wilcoxon-Mann-Whitney test did not reveal a significant difference between the CGI-I test results in either A or B subgroups ($p>0.05$ for all CGI-I scales). Median assessments for the scales I, II, III were 2, 2, 41 respectively. We can summarize that there was no difference in the quality of clinical improvement between patients with bipolar disorder in a manic episode and patients with bipolar disorder in a depressive episode.

5.2. Mitochondrial functions in BPD patients and healthy controls

The second stage of the research consisted of the comparison of the mitochondrial function in patients with BPD (in acute manic or depressive episodes) and healthy controls.

Activities of mitochondrial enzymes, citrate synthase (CS), complexes I (CI), II (CII) and IV (COX) in patients with BPD are graphically presented in Fig. 23 and 24.

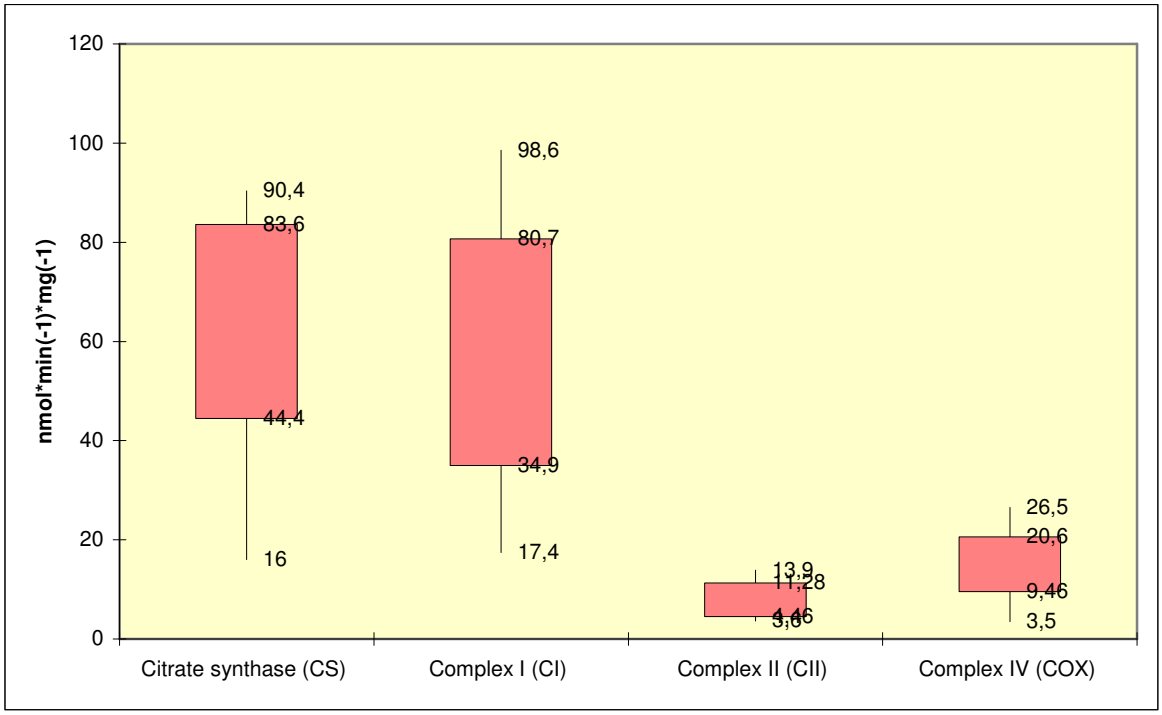

Fig. 23. Mitochondrial enzymes activity in the experimental group (patients with bipolar disorder in depressive episode, N=13). Min, Mean-SD, Mean+SD, Max, where SD – standard deviation

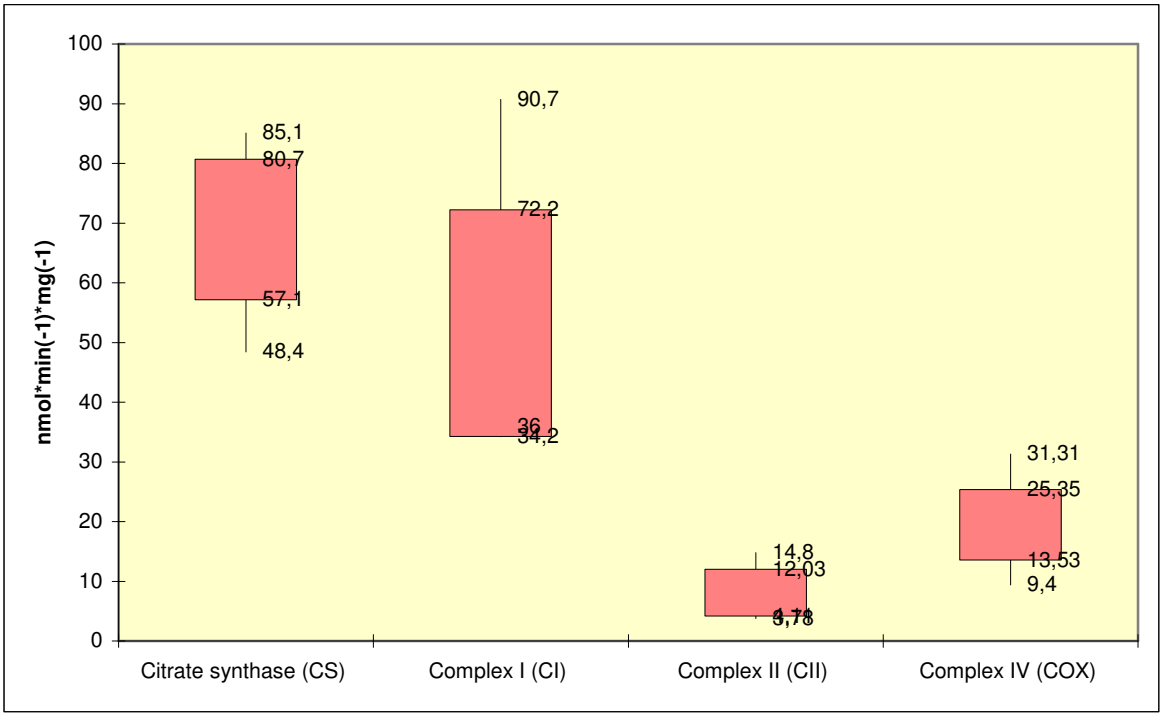

Fig. 24. Mitochondrial enzymes activity in the experimental group (patients with bipolar disorder in manic episode, N=24). Min, Mean-SD, Mean+SD, Max, where SD – standard deviation

After the post-hoc Scheffe test was performed, significant differences were not found between any of mitochondrial enzymes activity in patients with mania and depression (Tab. 5). The mitochondrial enzymes activity data from the group of healthy controls was not available; reference ranges of mitochondrial enzyme activities were obtained from mitochondrial laboratory of the Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague.

Tab. 5. Mitochondrial enzymes in patients with bipolar disorder in a manic or depressive episode

Mitochondrial enzyme	Group		p	Reference range
	Mania	Depression		
CS (nmol·min^{-1}·mg^{-1})	68.9±11.8	64.0±19.6	0.397	60-92
CI (nmol·min^{-1}·mg^{-1})	53.2±19.0	57.8±22.9	0.561	21-55
CII (nmol·min^{-1}·mg^{-1})	8.07±3.96	7.87±3.41	0.89	5-15

COX (nmol·min^{-1}·mg^{-1})	19.44±5.91	15.03±5.57	**0.054**	16-40

CS = citrate synthase; CI = Complex I; CII = Complex II; COX = Complex IV

Mean ± SD; p – significance level

Complex I (CI) activity in BPD patients with mania was lower than in patients with depression; Complex II (CII) activity in BPD patients with mania was higher than in patients with depression; citrate synthase (CS) activity in BPD patients with mania was higher than in patients with depression, though none of the above had reached statistical significance. When comparing a decrease in Complex IV (COX) activity in BPD patients with depression with BPD patients with mania, the significance level was close to 0.05 (Fig. 25). Complex IV activity in BPD patients in depressive episode was slightly below reference range.

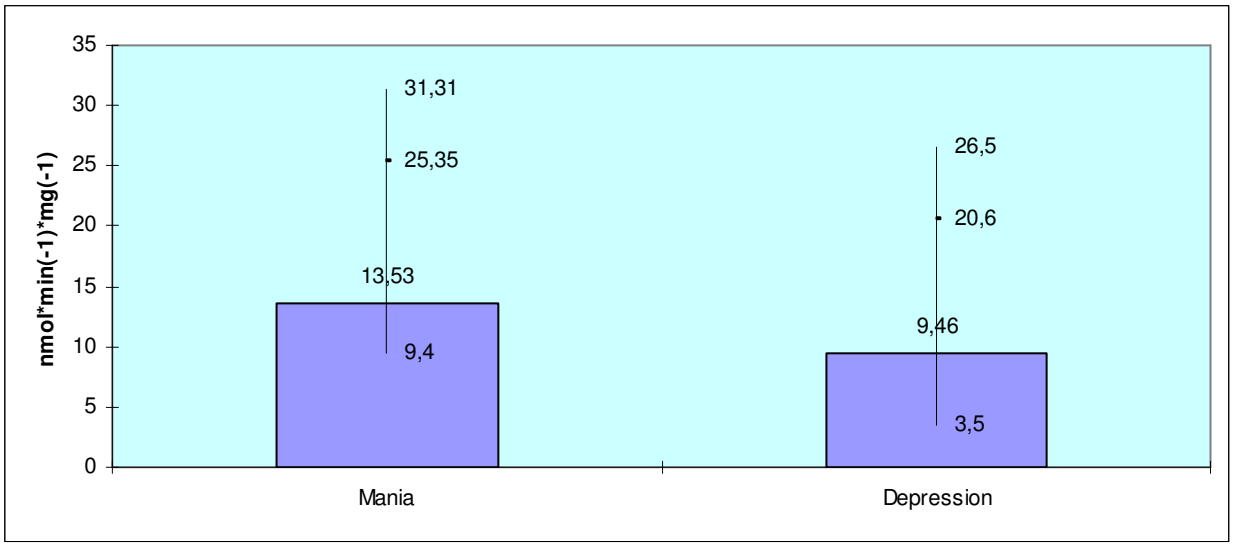

Fig. 25. Complex IV (COX) activity in patients with bipolar disorder in manic episode and depressive episode, p=0.054. Min, Mean-SD, Mean+SD, Max, where SD – standard deviation

Changes in mitochondrial respiration in the blood platelets isolated from patients with BPD and healthy controls were examined through the general linear model, one-way analysis of variance and post-hoc Scheffé test. The results are summarized in Tab. 6.

Tab 6. Mitochondrial respiration in the blood platelets from patients with bipolar disorder (measurement 1, manic or depressive episode) and healthy controls

Platelets	Respi-ratory state	Groups					
		Mania	P (Mania vs Controls)	Depres-sion	P (Depres-sion vs Controls)	Controls	P (Mania vs Depression)
Intact	PR	0.105±0.017	0.752	0.101±0.014	0.463	0.106±0.023	0.343
	LEAK	0.00656±0.00483	**0.005**	0.00534±0.00241	0.267	0.00169±0.00123	0.568
	ETSC	0.124±0.022	0.256	0.117±0.02	0.164	0.132±0.03	0.233
	Rotenone	0.00044±0.00036	0.148	-0.00151±0.00109	0.64	0.00164±0.00119	0.451
Permeabilized	IR (p)	0.087±0.021	0.32	0.082±0.026	0.24	0.094±0.021	0.678
	DMP (p)	0.046±0.028	0.188	0.034±0.014	0.564	0.038±0.014	0.355
	ADP (p)	0.108±0.031	0.418	0.097±0.032	0.873	0.112±0.03	0.823
	Glutamate (p)	0.115±0.036	0.817	0.107±0.044	0.114	0.115±0.03	0.913
	Succinate (p)	0.183±0.042	0.485	0.166±0.059	0.424	0.186±0.047	0.418
	LEAK (p)	0.03042±0.00825	**0.034**	0.02643±0.0104	0.093	0.02339±0.00745	0.6872
	ETSC (p)	0.177±0.054	0.453	0.162±0.056	0.111	0.188±0.06	0.462
	Rotenone (p)	0.073±0.026	0.941	0.075±0.025	0.723	0.076±0.031	0.338

Mean ± SD; p – significance level in reference to controls.

PR – physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity measured after titration with uncoupler (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, FCCP), IR – initial respiration in washed platelets before permeabilization with digitonin, DMP – respiration measured after the addition of digitonin+malate+pyruvate, ADP – stage 3 respiration supported through Complex I measured after the addition of ADP, Glutamate – stage 3 respiration measured after the addition of glutamate, Succinate – state 3 respiration supported through both Complex I and Complex II measured after the addition of the succinate, Rotenone – respiration after Complex I inhibition measured after the addition of rotenone. (p) indicate permeabilized platelets.

In intact platelets, the nonphosphorylating respiration measured after the addition of oligomycin (LEAK) was significantly higher in BPD patients with mania than in controls (p=0.005). LEAK was also higher in patients with depression than in controls, and higher in patients with mania than in patients with depression, though the difference did not reach statistical validity. Other indexes such as electron transport system capacity (ETSC) and respiration after inhibiting complex I with rotenone (Rotenone) were lower in patients in both phases of BPD than in healthy controls, and lower in depression than in mania, though these differences were not significant. Physiological respiration (PR) appeared to be similar in all the subgroups (Fig 26, 27, 28).

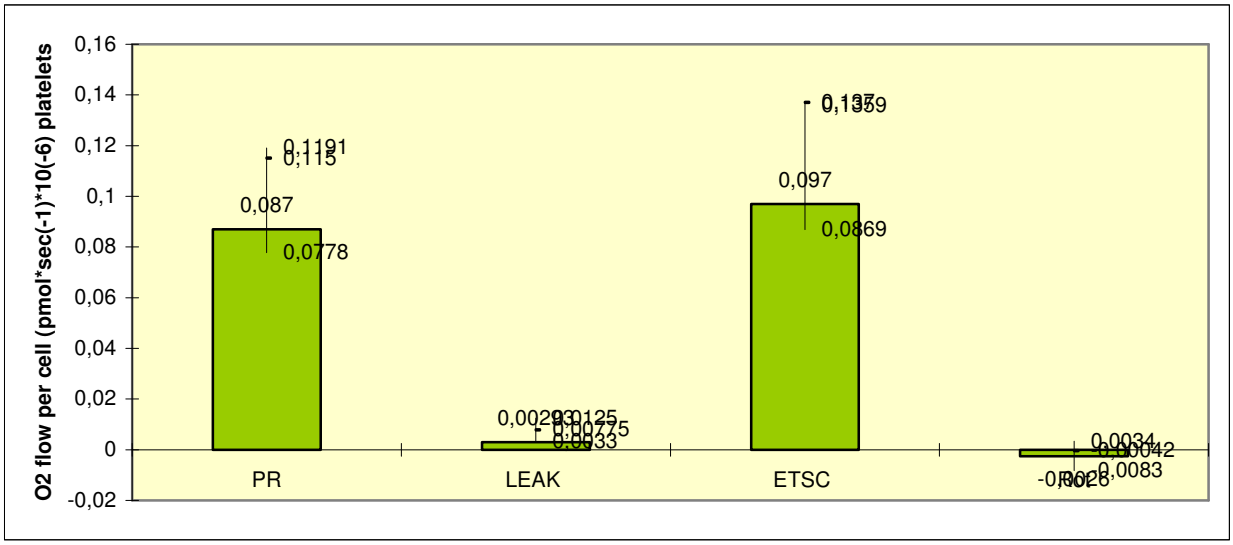

Fig. 26. Mitochondrial respiration normalized for platelet concentration in intact platelets of patients with BPD in depressive episode. PR - physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity, Rot – respiration after complex I inhibition, measured after the addition of rotenone.

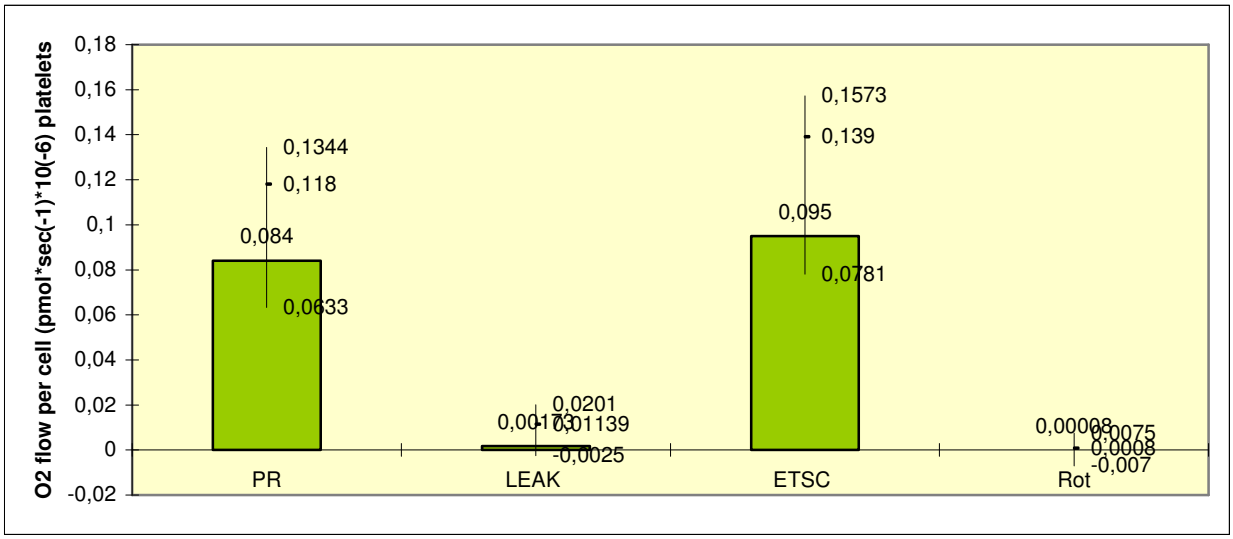

Fig. 27. Mitochondrial respiration normalized for platelet concentration in intact platelets of patients with BPD in manic episode. PR - physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity, Rot – respiration after complex I inhibition, measured after the addition of rotenone.

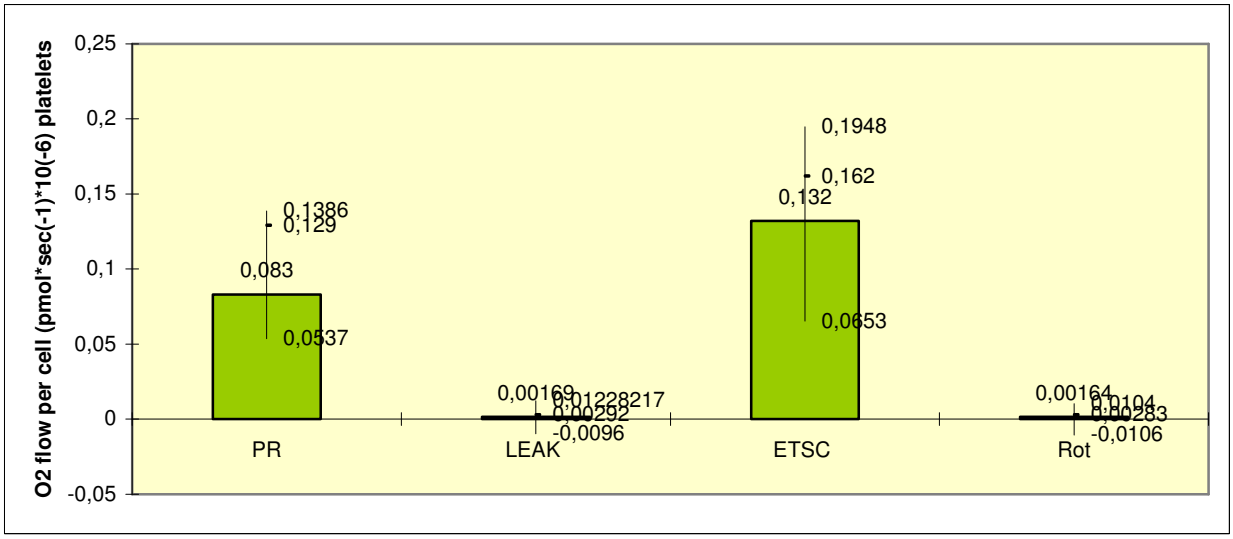

Fig. 28. Mitochondrial respiration normalized for platelet concentration in intact platelets of healthy controls. PR - physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity, Rot – respiration after complex I inhibition, measured after the addition of rotenone.

After the normalization for CS activity, mitochondrial respiratory rate did not show any significant difference between the group of patients with BPD in a manic episode and control group or between the group of patients with BPD in a manic or depressive episode.

We also measured a flux control ratio (the ratio of a respiratory rate at a specific respiratory state divided by ETSC); e.g. LEAK/ETSC ratio was significantly higher in patients with BPD in a manic episode than in healthy controls (p=0.03) and in patients with BPD in a depressive episode than in healthy controls (p=0.042) (Fig. 29, 30)

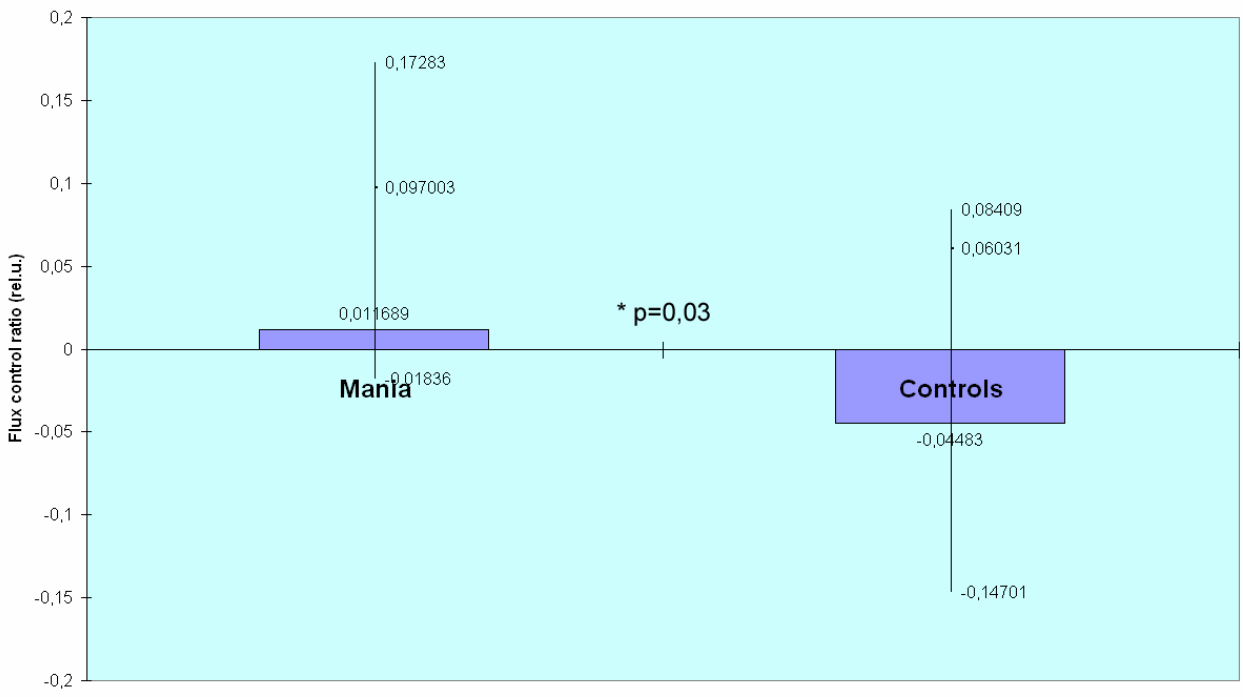

Fig. 29. The LEAK/ETSC index (flux control ratio) in intact platelets of patients with BPD with mania and healthy controls, p=0.03

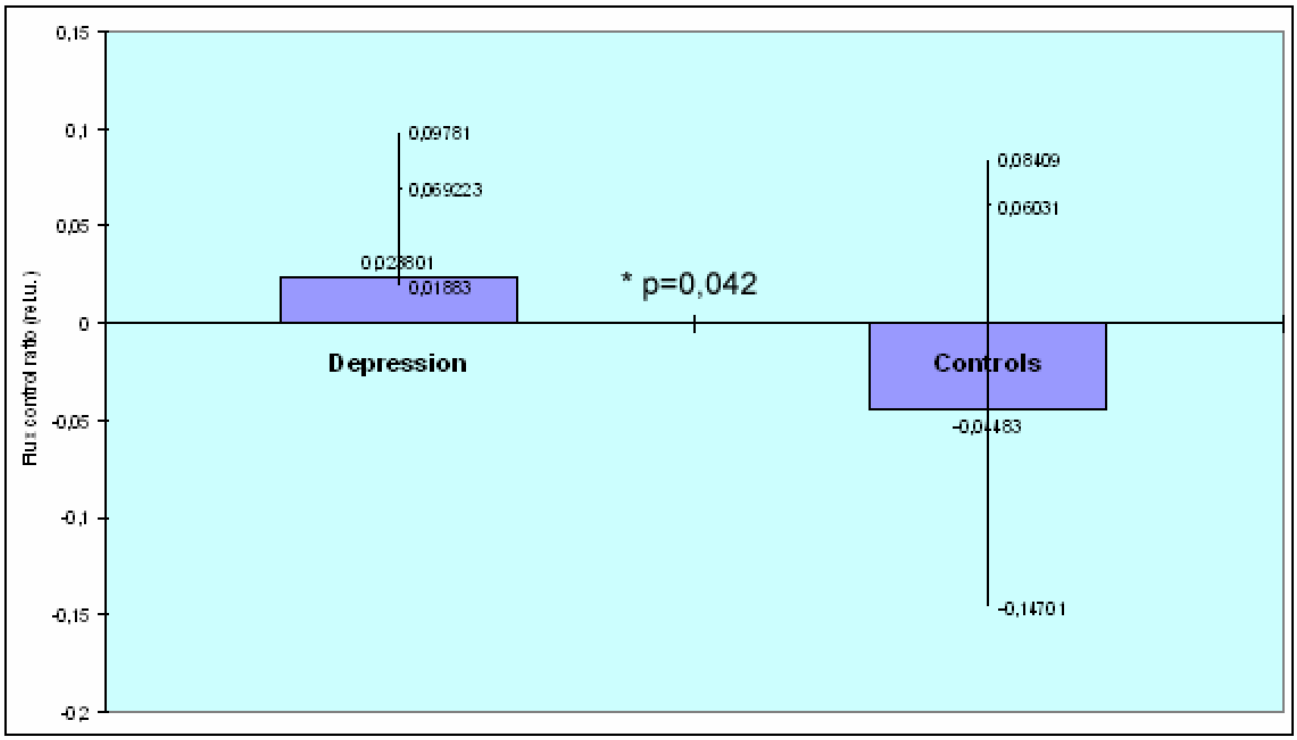

Fig. 30. The LEAK/ETSC index (flux control ratio) in intact platelets of patients with BPD with depression and healthy controls, p=0.042

The mean LEAK/ETSC in intact platelets of patients with BPD episode was slightly higher in BPD patients in both manic and depressive episodes compared to controls, which may indicate a disturbance in the mitochondria coupling process and/or functional integrity in the inner mitochondrial membrane in BPD.

In permeabilized platelets LEAK index (nonphoshorylating respiration after the addition of oligomycin) was also significantly higher in BPD patients with mania (p=0.034) than in healthy controls. LEAK was also lower in patients with depression than in patients with mania, though these changes did not reach statistical validity (p=0.058). Other respiratory rates such as ADP, Succinate did not reveal a significant difference between the groups. The results are summarized in Table 6 and Fig. 31.

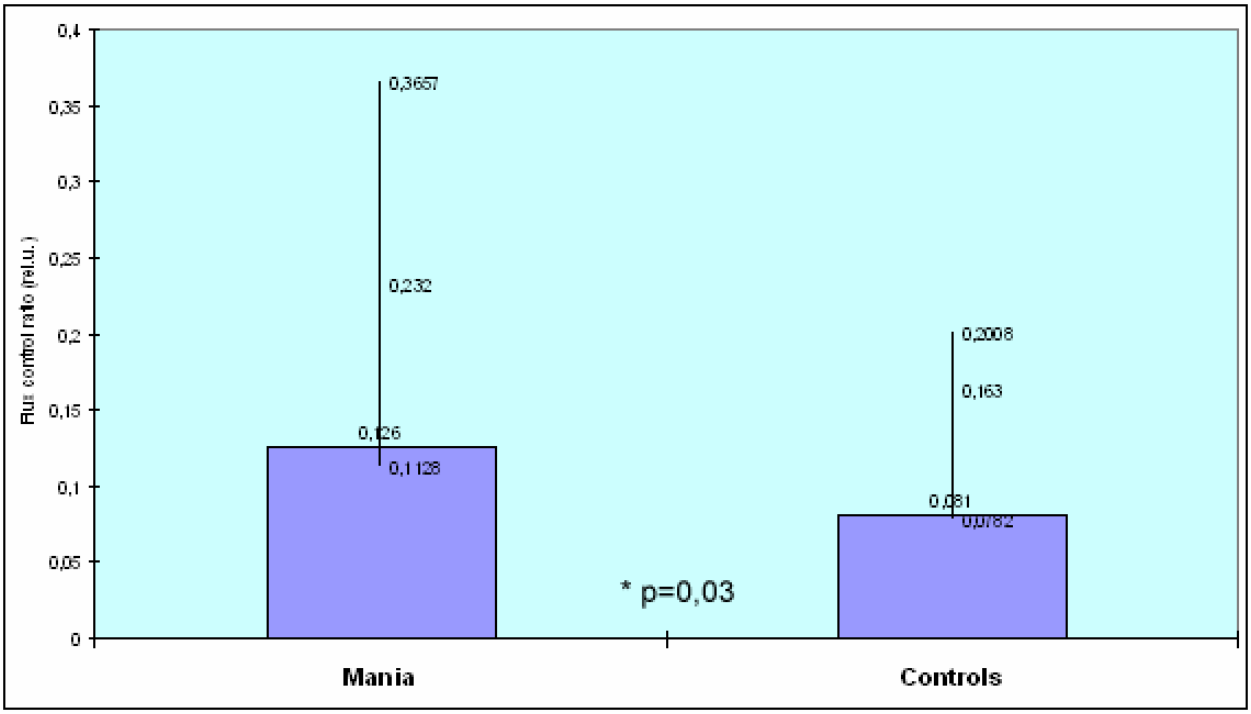

Fig. 31. The LEAK/ETSC index (a part of the flux control ratio) in permeabilized platelets of patients with BPD with mania and healthy controls, p=0.042

A comparable analysis for the mitochondrial enzymes activity and mitochondrial respiration in the group of BPD patients in remission and healthy controls was also performed. The comparability of the indexes in subgroups A and B was estimated through Student t-test. The results are summarized in Tab. 7.

Tab. 7. Mitochondrial respiration in the blood platelets from patients with bipolar disorder (measurement 2, remission) and healthy controls

	Mitochon-drial function	Groups					
		Mania	P (Mania vs Controls)	Depression	P (Depression vs Controls)	Controls	P (Mania vs Depression)
Intact platelets	PR	0.106± 0.029	0.982	0.106± 0.024	0.362	0.106± 0.023	0.533
	LEAK	0.00466±	**0.049**	0.00356± 0.00101	0.573	0.00169± 0.00123	0.174

		0.00088					
	ETSC	0.127± 0.039	0.64	0.129± 0.031	0.677	0.132± 0.03	0.462
	Rotenone	-0.0007± 0.00045	0.079	0.00075± 0.0006	0.185	0.00164± 0.00119	0.788
Permeabilized platelets	IR (p)	0.084± 0.028	0.158	0.089± 0.025	0.663	0.094± 0.021	0.211
	DMP (p)	0.037± 0.013	0.856	0.037± 0.013	0.56	0.038± 0.014	0.33
	ADP (p)	0.107± 0.042	0.649	0.0109± 0.035	0.267	0.112± 0.03	0.583
	Glutamate (p)	0.118± 0.049	0.788	0.116± 0.041	0.145	0.115± 0.03	0.672
	Succinate (p)	0.188± 0.065	0.913	0.187± 0.053	0.989	0.186± 0.047	0.699
	LEAK (p)	0.0283± 0.01152	0.068	0.0256± 0.00984	0.164	0.02339± 0.00745	0.13
	ETSC (p)	0.185± 0.071	0.892	0.186± 0.065	0.463	0.188± 0.06	0.462
	Rotenone (p)	0.081± 0.033	0.601	0.079± 0.032	0.555	0.076± 0.031	0.54

Mean ± SD; p – significance level in reference to controls . PR – physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity measured after titration with uncoupler (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, FCCP), IR – initial respiration in washed platelets before permeabilization with digitonin, DMP –respiration measured after the addition of digitonin+malate+pyruvate, ADP – stage 3 respiration supported through Complex I measured after the addition of ADP, Glutamate – stage 3 respiration measured after the addition of glutamate, Succinate – state 3 respiration supported through both Complex I and Complex II measured after the addition of the succinate, , Rotenone – respiration after Complex I inhibition measured after the addition of rotenone. (p) indicate permeabilized platelets.

In intact platelets, the LEAK was significantly higher in patients with bipolar disorder in remission after a manic episode (0.00466 $pmol \cdot sec^{-1} \cdot 10^{-6}$ platelets) than in controls (0.00169 $pmol \cdot sec^{-1} \cdot 10^{-6}$ platelets, $p<0.05$) (Fig. 32). Other indexes such as ETSC and respiration after inhibiting complex I with

rotenone (Rotenone) were lower in patients with patients with bipolar disorder in remission after a manic episode than in healthy controls, however these differences were not significant. PR index was similar in both groups. After the normalization for CS activity, the mitochondrial respiratory rate had shown no significant difference between the group of patients with bipolar disorder in remission after a manic episode or a depressive episode and control group.

In permeabilized platelets there was no significant difference in the mitochondrial respiration for all the respiratory states.

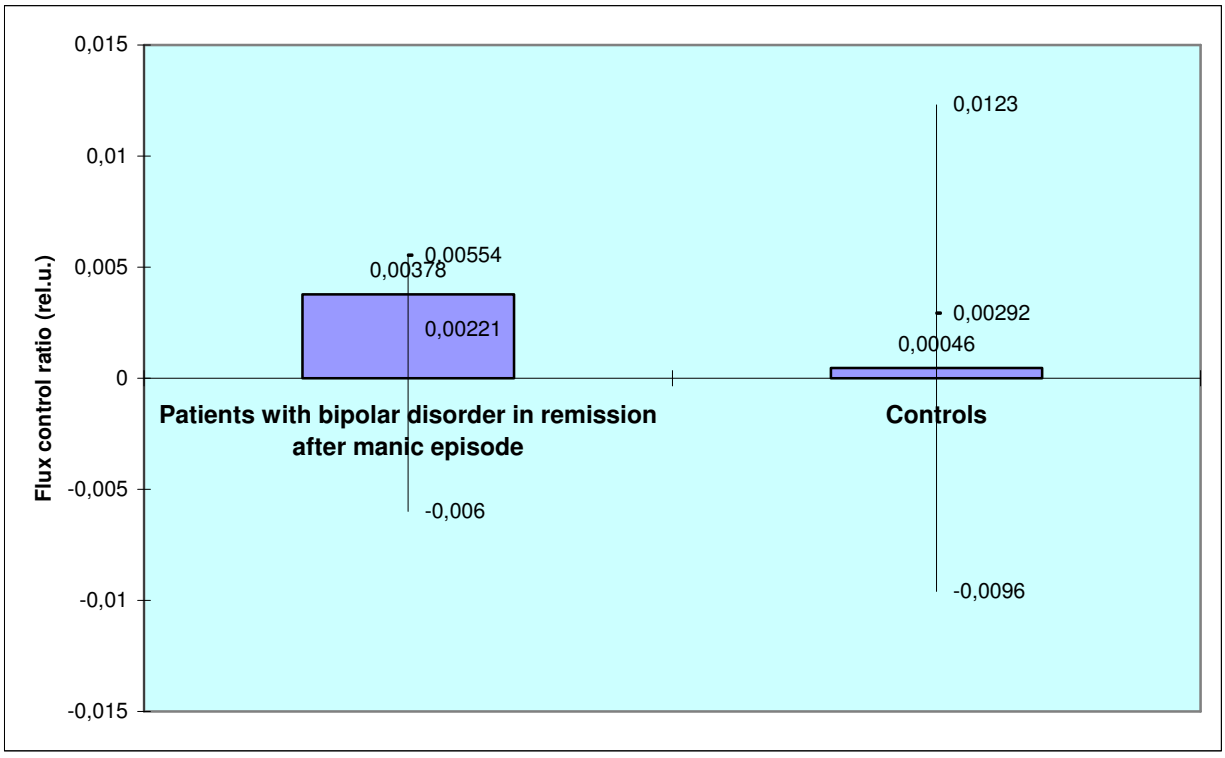

Fig. 32. The LEAK index in intact platelets of patients with bipolar disorder in remission after manic episode and healthy controls, p=0,042

5.3. Changes in mitochondrial function of BPD patients during the research period

An assessment of the changes in mitochondrial enzymes activity and mitochondrial respiration of BPD patients during the research period was also performed, i.e. values in the acute phase before treatment (manic or depressive episode) and after treatment (in remission) were compared (Tab. 8).

Tab. 8. Activities of mitochondrial enzymes in platelets of BPD patients in acute phase of the disease compared with remission

Mitochondrial enzymes	Phase of the disease	Disease state		p
		Acute	Remission	
CS, nmol·min^{-1}·mg^{-1}	Mania	68.9±11.8	63.9±9.7	0.063
	Depression	64.0±19.6	65.3±19.3	0.687
CI, nmol·min^{-1}·mg^{-1}	Mania	53.2±19.0	59.3±27.8	0.526
	Depression	57.8±22.9	80.2±19.3	0.352
CII, nmol·min^{-1}·mg^{-1}	Mania	8.07±3.96	7.62±3.33	0.467
	Depression	7.87±3.41	8.09±3.41	0.799
COX, nmol·min^{-1}·mg^{-1}	Mania	19.44±5.91	19.39±4.29	0.985
	Depression	15.03±5.57	15.57±5.43	0.72

Mean ± SD; p – significance level. CS – citrate synthase, CI – Complex I, CII – Complex II, COX – Complex IV.

The CS activity in BPD patients with mania was higher than in healthy controls though the difference did not reach statistical validity (p=0,063). The difference between other enzymes activity such as CI, CII and COX for the groups of BPD patients and healthy controls also did not reach statistical validity. The results are summarized in Fig. 33.

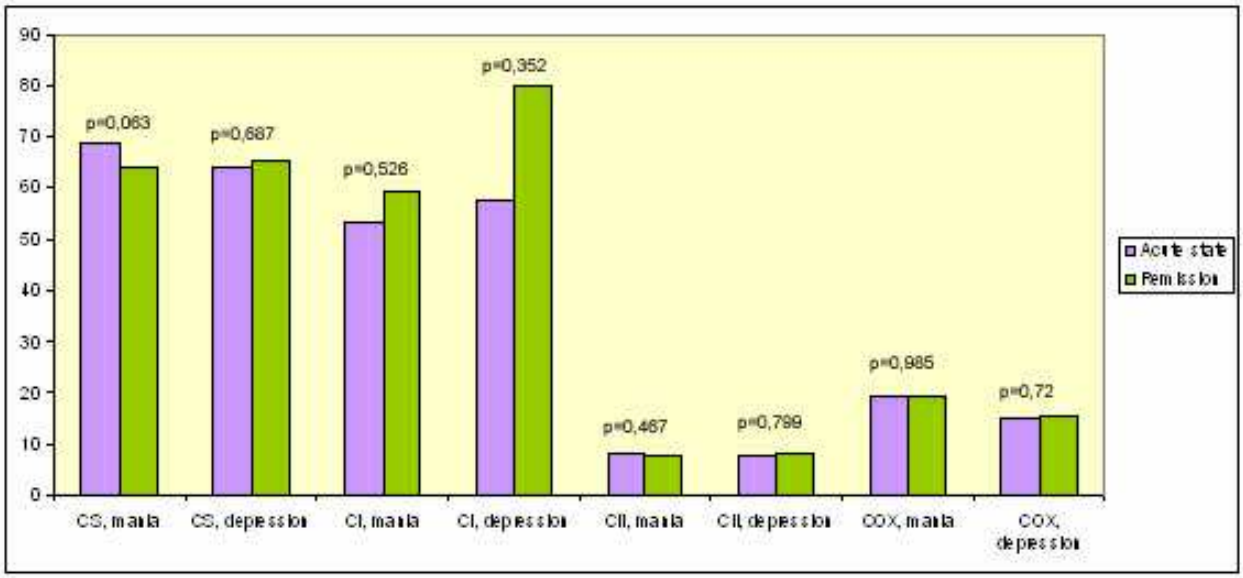

Fig. 33. Activities of mitochondrial enzymes in patients with BPD in an acute phase of the disease (manic or depressive episode) compared with remission

We have also compared the mitochondrial enzymes activity and respiration rates in the subgroups A and B: patients with mania vs patients with depression. The results obtained through paired test calculation are summarized in Tab. 9.

Tab. 9. Mitochondrial respiration in blood platelets from patients with bipolar disorder in manic or depressive episodes before and after treatment

	Respiratory state	Episode of the disease	Disease state		p
			Acute	Remission	
Intact platelets	PR	Mania	0.105±0.017	0.106±0.033	0.885
		Depression	0.101±0.014	0.106±0.022	0.538
	LEAK	Mania	0.00656±0.00483	0.00548±0.00121	0.431
		Depression	0.00534±0.00241	0.00301±0.00095	0.079
	ETSC	Mania	0.124±0.022	0.129±0.044	0.604
		Depression	0.117±0.02	0.123±0.025	0.559
	Rotenone	Mania	0.00044± 0.00036	-0.00044± 0.00026	0.207

		Depression	-0.00151± 0.00109	-0.00121± 0.00074	0.989
Permeabilized platelets, pmol*sec^{-1}*10^{-6}	IR (p)	Mania	0.087±0.021	0.083±0.028	0.349
		Depression	0.082±0.026	0.088±0.029	0.629
	DMP (p)	Mania	0.046±0.028	0.038±0.014	0.215
		Depression	0.034±0.014	0.036±0.012	0.555
	ADP (p)	Mania	0.108±0.031	0.107±0.049	0.964
		Depression	0.097±0.032	0.108±0.029	0.547
	Glutamate (p)	Mania	0.115±0.036	0.114±0.055	0.925
		Depression	0.107±0.044	0.125±0.039	0.478
	Succinate (p)	Mania	0.183±0.042	0.186±0.072	0.945
		Depression	0.166±0.059	0.192±0.055	0.429
	LEAK (p)	Mania	0.03042±0.00825	0.02939±0.01298	0.552
		Depression	0.02643±0.0104	0.02647±0.00858	0.66
	ETSC (p)	Mania	0.177±0.054	0.183±0.082	0.775
		Depression	0.162±0.056	0.186±0.049	0.49
	Rotenone (p)	Mania	0.073±0.026	0.077±0.031	0.799
		Depression	0.075±0.025	0.089±0.039	0.448

Mean ± SD; significance level. PR – physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity measured after titration with uncoupler (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, FCCP), IR – initial respiration in washed platelets before permeabilization with digitonin, DMP –respiration measured after the addition of digitonin+malate+pyruvate, ADP – stage 3 respiration supported through Complex I measured after the addition of ADP, Glutamate – stage 3 respiration measured after the addition of glutamate, Succinate – state 3 respiration supported through both Complex I and Complex II measured after the addition of the succinate, Rotenone – respiration after Complex I inhibition measured after the addition of rotenone. (p) indicate permeabilized platelets.

There were no significant differences between an acute stage of illness and remission in BPD patients ($p>0.05$ for all the measurements).

5.4. Connections between mitochondrial function and psychopathological symptoms in BPD patients

We also calculated correlation coefficients between the BPRS, YMRS, MADRS, MDQ and CGI-I tests and mitochondrial complexes activity to establish the association between the state of the disease, psychopathological symptoms, clinical improvement and mitochondrial pathology. A significant correlation was observed between Complex I and the BPRS score in the subgroup A (patients with mania, acute state – measurement 1) (p=0.001). The Pearson coefficient showed a high closeness of relationships according to Chaddock scale (r_{xy} = 0.747), which is the evidence of the correlation validity. The paired linear regression equation shows the Complex I value dependence of BPRS score (1):

$$BPRS = 18.66 + 0.7*CI \quad (1)$$

where BPRS – Brief Psychiatric Rating Scale, mental state assessment scale in patients with BPD, manic episode, acute state (points), CI – Complex I activity ($nmol \cdot min^{-1} \cdot mg^{-1}$).

Based on the regression coefficient value, with the CI increase of 1 $nmol \cdot min^{-1} \cdot mg^{-1}$ we expect a BPRS score increase of 0.7 points. The coefficient of determination R^2 was 0.558 which indicates that 55.8% factors are taken into account in the regression model (1).

The regression function diagram (1) is shown on the Fig. 34.

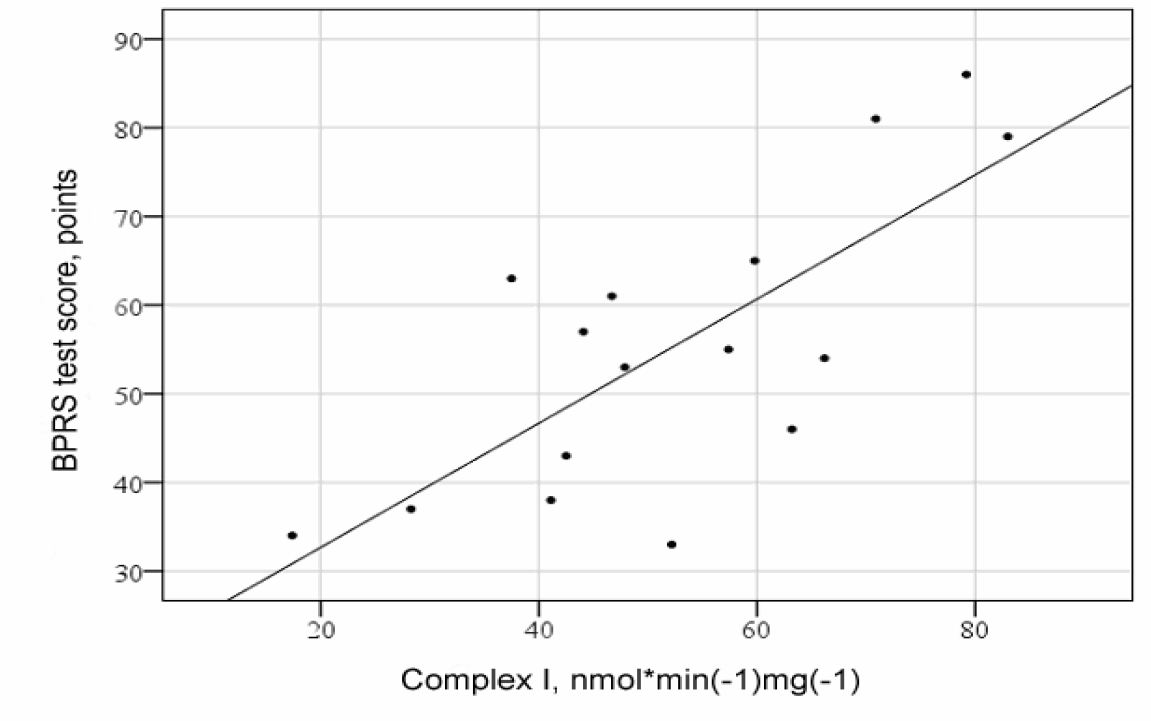

Fig. 34. Linear dependence diagram for the mental state of BPD patients with mania and Complex I activity

There were no significant correlation coefficients between other mental state assessment tests and mitochondrial pathology indicators in the other subgroups of the patients and in healthy controls.

In order to identify relationships between a large numbers of mitochondrial pathology indicators in the research, a factor analysis was performed. We could distinguish four factors through principal component analysis with a Varimax rotation method. The characteristics of these factors are summarized in Tab. 10.

Tab. 10. The characteristics of the mitochondrial pathology assessment factors in patients with bipolar disorder

Factor No.	Meaning	Total variance explained, %	Cumulative % of the explained variance
1	3.62	30.17	30.17
2	2.66	22.19	52.36
3	2.27	18.87	71.23
4	1.28	10.67	81.9

The eigenvalues of all the factors were >1. The factor load for each of the mitochondrial pathology indicators allowing the evaluation of the correlation between picked factors and other indicators is shown as a factor loadings matrix where the highest values are shown in bold (Tab. 11).

Tab. 11. Factor loadings matrix

Mitochondrial function	Factor 1	Factor 2	Factor 3	Factor 4
Rotenone (p)	**.876**	.015	-.063	.299
Succinate (p)	**.860**	.352	.288	.039
ETSC (p)	**.821**	.400	.237	.091
ADP (p)	**.647**	.507	.473	-.108
Glutamate (p)	**.635**	.380	.509	-.110
ETSC	.165	**.938**	.090	.068
PR	.228	**.866**	.175	.190
DMP (p)	.085	.175	**.863**	.141
LEAK (p)	.568	-.054	**.602**	.207
IR (p)	.389	.485	**.586**	.042
Rotenone	.238	.277	-.104	**.730**
LEAK	-.011	-.043	.373	**.721**

PR – physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity measured after titration with uncoupler (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, FCCP), IR – initial respiration in washed platelets before permeabilization with digitonin, DMP –respiration measured after the addition of digitonin+malate+pyruvate, ADP – stage 3 respiration supported through Complex I measured after the addition of ADP, Glutamate – stage 3 respiration measured after the addition of glutamate, Succinate – state 3 respiration supported through both Complex I and Complex II measured after the addition of the succinate, Rotenone – respiration after Complex I inhibition measured after the addition of rotenone. (p) indicate permeabilized platelets.

According to the components distribution, Factor 1 is characterized by high values of: respiration after Complex I inhibition, stage 3 respiration supported through both Complex I and II, electron transport system capacity and stage 3

respiration supported through Complex I, all in permeabilized platelets. Factor 2 is characterized by high values of: electron transport system capacity and physiological respiration in intact platelets. Factor 3 is characterized by high values of: initial respiration, respiration after addition of malate and pyruvate, and nonphosphorylating respiration, all in permeabilized platelets. Factor 4 is characterized by high values of: respiration after Complex I inhibition and nonphosphorylating respiration in intact platelets.

We made the assessment of the differences between the experimental and control group based on the calculated values for each of the identified factors. The values of the four combined factors in the BPD patients and control groups were compared for that purpose (Tab. 12).

Tab. 12. The comparison of combined factors in patients with bipolar disorders and control group

Combined factors	Groups				p
	Bipolar disorder		Controls		
	Me	Q_1; Q_3	Me	Q_1; Q_3	
Factor 1 (Rotenone (p), Succinate (p), ETSC (p), ADP (p), Glutamate (p))	-0.2	-0.95; 0.81	0.19	-0.56; 0.52	0.543
Factor 2 (ETSC, PR)	-0.13	-0.89; 0.28	0.34	-0.34; 1.19	**0.024**
Factor 3 (DMP (p), Oligomycin (p), IR (p))	0.16	-0.59; 0.63	-0.32	-0.83; -0.06	**0.023**
Factor 4 (Rotenone, LEAK)	0.15	-0.6; 1.05	-0.15	-0.7; 0.64	0.325

Me – Mean; SD – Standard deviation; ; Q_1 – Quartile 1; Q_3 – Quartile 3; p – significance level . PR – physiological respiration, LEAK – nonphosphorylating respiration measured after the addition of oligomycin, ETSC – electron transport system capacity measured after titration with uncoupler (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, FCCP), IR – initial respiration in washed platelets before permeabilization with digitonin, DMP –respiration measured after the addition of digitonin+malate+pyruvate, ADP – stage 3 respiration supported through Complex I measured after the addition of ADP, Glutamate – stage 3 respiration measured after the addition of glutamate, Succinate – state 3 respiration supported through both Complex I and Complex II measured after the addition of the succinate, Rotenone – respiration after Complex I inhibition measured after the addition of rotenone. (p) indicate permeabilized platelets

The Mann-Whitney test shows that patients with bipolar disorder had significantly lower Factor 2 values than healthy controls (p=0.024) and significantly higher Factor 3 values than healthy controls (p=0.023). For patients with bipolar disorder we can expect a decrease in ETSC and physiological respiration in intact platelets, and a decrease in DMP, nonphosphorylation respiration and initial respiration in permeabilized platelets.

We also explored the duration period between the acute state and remission and its dependence on the mitochondrial pathology indicators in patients within different phases of bipolar disorder.

We calculated multiple linear regression equation for the patients in manic state (2):

$$T_{rem} = -56{,}3 + 2{,}1*X_{CS} - 4{,}8*X_{CIV} + 1745{,}1*X_{PR} - 1475{,}4*X_{ETSC} + 386{,}5*X_{Glu} \quad (2)$$

where

T_{rem} – time period between the measurements (days),

X_{CS} – citrate synthase ($nmol \cdot min^{-1} \cdot mg^{-1}$),

X_{CIV} – Complex IV ($nmol \cdot min^{-1} \cdot mg^{-1}$),

X_{PR} – physiological respiration ($pmol \cdot sec^{-1} \cdot 10^{-6}$ platelets),

X_{ETSC} – electron transport system capacity ($pmol \cdot sec^{-1} \cdot 10^{-6}$ platelets),

X_{Glu} – respiration after the addition of glutamate ($pmol \cdot sec^{-1} \cdot 10^{-6}$ platelets).

The function was statistically valid (p=0.025), the Pearson correlation coefficient for the relationship between the mitochondrial function indicators and remission due date was $r_{xy} = 0.769$, which shows a high closeness of relationships according to Chaddock scale. The regression model (2) explains 59.1% of the variance for the remission due date in patients with manic episode of the bipolar disorder.

There was no valid model showing the remission due date dependence of the mitochondrial function indicators for patients with a depressive episode of the bipolar disorder.

6. Discussion

The current study contributes to the research on the connection between pathophysiological processes in mitochondria and psychopathological symptoms in different mental disorders.

One study was focused on finding biological markers of mitochondrial dysfunction measurable in peripheral blood (Fisar Z, Raboch J, 2008). Elements isolated from the peripheral blood, especially platelets and lymphocytes, are used to study changes in biochemical processes caused by mental disorders. Though mitochondrial pathology may not be similar across all brain regions and cell types, nor a number of neurochemical parameters, this is an acceptable model reflecting changes in the CNS because isolating blood platelets doesn't require a complicated and invasive procedure. Affected mechanisms of the cellular compensation can lead to an increased ETS activity in lymphocytes as they provide the energy for the cell, and, in turn, a low platelet sensitivity may be expected (Feldhaus P et al, 2011).

We found that CI, CII and CS activity in BPD patients with mania and depression were not statistically different. These findings are in conjunction with results of previous investigations. Gubert made study where the activities of ETS complexes of mononuclear blood cells were examined in BPD patients in euthymic mood (Gubert C et al, 2013). No significant changes were found in complex I, complex II and complex II + III activities. The obtained results are also consistent with the data received by deSouza in 2014 which stated that mitochondrial complexes I-IV activity was not changed during the depressive episodes of BPD (deSouza RT et al, 2015).

A decrease in COX activity was observed in BPD patients with depression and when compared with BPD patients with mania, the significance level was close to critical (Fig. 6). This data partially corresponds with the previous research made by Valla (Valla J et al, 2006) on the groups of patients with mild cognitive deficits (Alzheimer disease and other diseases), and data discovered by Fisar (Fisar

Z et al, 2016) for the group of patients with Alzheimer's disease, where COX activity was decreased and negatively correlated with the Mini Mental State Examination (MMSE) score. This may lead to a suggestion that a decreased complex IV activity indicates cognitive impairment which is more evident during a depressive phase of the disease. Prince found a decrease in COX activity in the frontal cortex and caudate nucleus and linked it to an increased emotional and cognitive impairment in patients with SZ. In general (Prince JA et al, 2000), neurocognitive deficits are commonly associated with BPD (Aydemir O et al, 2014) and they are often present in the very first episode (Bora E, Pantelis C, 2015).

Continuing the discussion of the changes in mitochondrial respiration in depressive phase of the disease we need to mention Gardner, who performed a research on mitochondrial enzymes activity and ATP production rate in patients with MDD and found an overall decrease in Complex I-IV in comparison with controls which correlated with the vulnerability to psychopathology in the following scales: 'Somatic Anxiety'. 'Psychasthenia' and 'Suspition' (Gardner A et al, 2003).

Correa found a decreased level of ETS complexes in an animal model of mania associated with manic symptoms (Correa et al, 2007). Freitas discovered an association between manic-like hyperactivity in a rat brain and a decrease in the activity of CS (Freitas TP et al, 2010).

As seen from the results of the conducted analyses, in our research we didn't find any significant correlation between certain psychometric scales and mitochondrial respiration indexes except for the correlation between Complex I and BPRS score in patients with mania. Based on the regression coefficient value, with the CI increase of 1 $nmol*min^{-1}mg^{-1}$ we expect a BPRS score increase of 0,7 points. A low quantity of obtained correlations may be the result of the small amount of participants and further research in this area will provide us with the necessary data.

Research covering the association of mitochondrial enzymes activity and psychopathological symptoms of the BPD are limited while research exploring those connections in patients with other psychiatric diseases are widely present. Ben-Shakhar repeatedly obtained results indicating the connection between the severity of the SZ symptoms and mitochondrial impairment (Ben-Shachar D et al, 1999, Ben-Shachar D et al, 2008) though there were no significant changes in the activity of complexes I and IV in mitochondria isolated from blood platelets of BPD patients in the same study. Dror et al. (2002) also performed a study exploring Complex I activity in schizophrenic and BPD patients and found that a degree of increase in complex I activity correlated directly with the severity of positive symptoms in patients with SZ (a tendency towards a negative correlation between complex I activity and negative symptoms did not reach statistical significance) (Dror N et al, 2002).

As many psychopathological symptoms and mitochondrial pathology found in patients with SZ and BPD overlap (Clay H et al, 2011), those findings may highlight a connection between the severity of psychopathological symptoms and a specific and selective alteration in mitochondrial respiration in both diseases. Those alterations in energy metabolism may partially define or underlay psychopathology in a manic state or during the psychotic episode of the disease. Alterations may also vary according to the state of the disease, with the positive peak in manic states, which can be measured and proved statistically and negative peak in depressive states which is downplayed. Further studies are needed to verify this suggestion.

Since Complexes I-IV play a key role in mitochondrial OXPHOS, their altered activity may reflect a mitochondrial dysfunction which, in turn, can result in impaired neuronal metabolism and neuronal plasticity expressed in certain psychopathological symptoms. Still there is not enough evidence whether this alteration is a causal or consequential effect of the disease.

We found that there was no statistical difference in physiological respiration in all the subgroups (BPD patients with mania, BPD patients with depression, BPD

patients in remission, healthy controls). Therefore PR index cannot be used as biological marker sensitive to BPD.

In the respiration rates there was a significant increase of LEAK index (nonphosphorylating respiration measured after the addition of oligomycin) both in intact and permeabilized platelets in the subgroup of BPD patients with mania compared to controls, though normalization for CS activity eliminated the difference. The LEAK respirations, as well as the flux control ratio LEAK/ETSC, are parameters characterizing mitochondrial damage. The flux control ratio LEAK/ETSC (i.e., oligomycin-inhibited respiration divided by uncoupled respiration at optimum FCCP concentration) in intact platelets remained very low, which indicated well-coupled mitochondria and the functional integrity of the inner mitochondrial membrane.

Flux control ratio for the intact platelets (the ratio of a respiratory rate at a specific respiratory state divided to ETS capacity) was also significantly higher both in patients with BPD in a manic state and in a depressive state than in healthy controls. This may indicate an increased intrinsic uncoupling in the platelets of BPD patients and the availability of these parameters as indicators of the platelet respiration.

Morris et al. (2017) postulates that symptomatically BPD is a biphasic disorder of energy ability; increased in mania and decreased in depression; and mitochondrial dysfunction may serve as a state dependent marker of the disorder with an increased mitochondrial function during mania and a decreased mitochondrial function during depression. The author offers a model explaining the biphasic nature of the disorder (Morris G et al, 2017). Our data partially corresponds with this postulate as the obtained data for the LEAK index increase and flux control ratio increase in patients with BPD seems to be mania-specific, though we did not obtain any data confirming a decrease of the same indexes during the depressive phase.

Factor analysis in our study showed that patients with BPD had significantly lower Factor 2 values than healthy controls (ETS capacity and physiological

respiration in intact platelets) and significantly higher Factor 3 values than healthy controls (stage 3 respiration, nonphosphorylation respiration and initial respiration in permeabilized platelets).

We speculate that a combination of those indexes with LEAK index and flux control ratio may serve as a clinical set of biological markers specific for the diagnosis of the bipolar disorder regardless of the phase of the disease.

The current study also explores the duration period between the acute state and remission and its dependence on the mitochondrial pathology indicators in blood platelets of the patients with different phases of BPD. Indicators sensitive for the period length turned out to be: CS (positive values), COX (negative values), PR (positive values), ETS capacity (negative values), and respiration after the addition of glutamate (positive values).

A possible connection between the illness duration and mitochondrial dysfunction in patients with BPD was also studied by Chang, who found a negative correlation between NAA/Creatine + Phosphocreatine or NAA levels and illness duration. However, later studies found that decreased NAA levels was restricted to the basal ganglia of the brain (Chang K et al, 2003). Berk proposed a general role of mitochondrial dysfunction in the disease progression (Berk M et al, 2011). Discemibly there is no suggested clinical test for a combination of the mitochondrial impairment indicators for the BPD, and therefore the data obtained from the current research may serve as an easily-accessible set of predictors for the episode duration in clinical practice.

There are few research findings confirming the role of mitochondrial respiration in the severity of the clinical symptoms of BPD (Scaini G et al, 2016). A body of evidence for the increased mitochondrial respiration and ATP production in a manic phase and decreased mitochondrial function in patients in the euthymic or depressive phase of the BPD was found, though the research data are partially controversial (Hroudova J, Fisar Z, 2011). It has yet to be discovered whether the impairment in mitochondrial function contributes to the disease process or is an independent process.

Various mitochondrial function alterations in patients with BPD do not indicate the same behavioral changes or psychopathological symptoms regardless of the tissue type or brain area. We suggest that the same mitochondrial impairment needs to be present in certain brain areas involved in certain clinical symptoms of the disease. Bioenergetic demand of the brain cells may vary in different brain areas and this demand is sensitive to different factors, which means that there is a certain threshold value of damaged mitochondria causing a symptom available for clinical measurement, and this value may be different for different neurons. These differences might enable some psychopathological symptoms to manifest while other symptoms remain hidden.

Explored abnormalities in mitochondrial function may reduce the cell ability for the appropriate stress response to such stimuli as emotional outbursts (an increased glutamate release), starvation (decreased glucose levels) and other risk factors known for psychotic episodes in affective disorders such as in-utero and infant malnutrition, substance abuse, and traumatic experiences (Kroll JL, 2007).

If we suggest that the obtained abnormalities in platelet mitochondrial respiration are similar to the abnormalities in brain mitochondrial respiration, it may further confirm the contribution of energy metabolism impairment to the pathophysiology of BPD. Given the lack of a reliable and clinically relevant biological markers for BPD and other mood disorders, a set of mitochondrial enzymes activity and respiration rates easily obtained from peripheral blood platelets might become a useful clinical tool in the diagnostic process.

A unique combination of the factors above in further studies may help to understand the effect of the certain mitochondrial function alteration on specific behaviors and psychopathological symptoms. Regardless of the rank of the certain index in the sequence of disease-causing events, an overall mitochondrial pathology is an important factor in the manifestation of clinical symptoms of BPD.

7. Conclusion

BPD is a complex disease that involves several biological pathways. Mitochondrial dysfunction was included when the mitochondrial hypothesis of BPD was firstly proposed by Kato in 2000. Since then it was supported by various data including decreased ATP production, upregulation of genes involved in apoptosis, downregulation of mitochondrial genes regulating OXPHOS, decreased antioxidant defences, abnormalities in the structure, and distribution of mitochondria and others. Some of the pathophysiological processes in BPD were discovered to be associated with certain clinical symptoms of the disease such as cognitive impairment, hyperactivity and others.

The main research question in the conducted study was whether energy metabolism in mitochondria corresponds to clinical evaluation of the psychopathological symptoms in patients with bipolar disorder.

The results obtained by the current study:

- support Hypothesis 1 that there is a set of mitochondrial functional impairment indexes specific for the current phase of the disorder. For patients with BPD we can expect a decrease in ETSC and physiological respiration in intact platelets, and an increase in DMP, nonphosphorylation respiration and initial respiration in permeabilized platelets. Healthy controls do not show this type of mitochondrial alteration. Obtaining peripheral blood platelets from patients with mental disorders is an easy and quick procedure which may be useful for *in vivo* studies of mitochondrial respiration in psychiatric diseases;

- support Hypothesis 2 that the severity of the symptoms of BPD is associated with the severity of the alteration of the mitochondrial function. A significant correlation was observed between Complex I and BPRS score in patients with manic symptoms;

- do not support Hypothesis 3 that there is a difference in the levels of mitochondrial respiration and enzyme activity in manic state and depressive state.

There was no significant difference in mitochondrial respiration and enzymes activity between subgroups of BPD patients in mania and depression.

- support Hypothesis 4 that there is a difference in the levels of mitochondrial respiration and enzyme activity in patients with BPD and healthy controls both in acute state and remission. LEAK index both in intact and permeabilized platelets was significantly higher in BPD patients with mania than in controls; flux control ratio (the ratio of a respiratory rate at a specific respiratory state divided to ETSC) was significantly higher in patients with mania than in controls and in patients with depression than in controls; in intact platelets, the LEAK index was significantly higher in BPD patients with remission than in healthy controls.

Additional results of the study include the exploration of the duration period between the acute state and remission and its dependence on the mitochondrial pathology indicators in patients with different phases of BPD. Indicators sensitive for the period length turned out to be: CS (positive values), COX (negative values), PR (positive values), ETS capacity (negative values), and respiration after the addition of glutamate (positive values).

Taken together, the obtained data provide evidence for the connection between psychopathological symptoms and mitochondrial function in mental disorders through cellular mechanisms involved in the pathology of BPD explored in the current study.

Results from this study provide information for clinicians and other researchers. This study also portrays mitochondria as a promising targets for the therapeutic modulation of cellular resilience and synapses in neuronal pathways involved in high-order functions of the brain in different mental disorders, including BPD.

Further research focused on treatment of this disorder, therapeutic strategies and diagnostic tools is needed to acquire a better understanding of BPD pathophysiology.

Abbreviations

ADP - adenosine diphosphate

AIF – apoptose inducing factor

AMP - adenosine monophosphate

ANOVA - one-way analysis of variance

ATP – adenosine triphosphate

BBB - blood–brain barrier

BDNF – brain-derived neurotrophic factor

BPD – bipolar disorder

BPRS – Brief Psychiatric Rating Scale

CGI-I - Clinical Global Impression - Improvement scale

CNS – central nervous system

CNTF - ciliary neurotrophic factor

CoA – coenzyme A

COX – cytochrome *c* oxidase or complex IV

CPEO - chronic progressive external ophtalmoplegia

CREB – cyclic AMP response element-binding protein

CS – citrate synthase

CSF - cerebrospinal fluid

DMP – digitonin+malate+pyruvate

DNA - deoxyribonucleic acid

ER - endoplasmic reticulum

ETS – electron transport system

ETSC – electron transport system capacity

FAD - flavin adenine dinucleotide

$FADH_2$ - flavin adenine dinucleotide, reduced form

FCCP - carbonylcyanide p-triflouromethoxyphenylhydrazone

FMN - flavinmononukleoktid

fMRI - Functional magnetic resonance imaging

FRTA - free radical theory of aging

GDNF - glial cell line-derived neurotrophic factor (

GTP - guanosine-5'-triphosphate

HPA - hypothalamic–pituitary–adrenal

IGF-1 - insulin-like growth factor 1

IR – initial respiration

IVF – *in vitro* fertilization

KSS - Kearns–Sayre syndrome

LHON - Leber hereditary optic neuropathy

LNGFR - low-affinity nerve growth factor receptor

LS - Leigh syndrome

MADRS - Montgomery–Åsberg Depression Rating Scale

MAO – monoamine oxidase

MD - mitochondrial disease

MDD – major depressive disorder

MDQ – Mood Disorder Questionnaire

MELAS - mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes

MERRF - myoclonic epilepsy with ragged-red fibers

MIDD - maternally inherited diabetes and deafness

MMSE – Mini Mental State Examination

MPT - mitochondria permeability transition

mtDNA - mitochondrial DNA

NAA - *N*-acetylaspartate

NAD+ - nicotinamide adenine dinucleotide, oxidized form

NADH - nicotinamide adenine dinucleotide, reduced form

NADP+ - nicotinamide adenine dinucleotide phosphate

NAPR - neuropathy, ataxia, and retinitis pigmentosa

nDNA - nuclear DNA

NGF - nerve growth factor

NT-3 - neurotrophin-3

NT-4 - neurotrophin-4

OXPHOS - oxidative phosphorylation

pH - potential of hydrogen

PR – physiological respiration

RCR - respiratory control ratio

RNA - ribonucleic acid

ROS – reactive oxygen species

SNHL - sensorineural hearing loss

SZ - schizophrenia

TCA – tricarboxylic acid cycle

Trk - tropomyosin receptor kinase

UK – United Kingdom

VEGF - vascular endothelial growth factor

YMRS - Young Scale of Mania

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Annexes

Annex 1. Mood Disorder Questionnaire (MDQ).

THE MOOD DISORDER QUESTIONNAIRE

Instructions: Please answer each question to the best of your ability.

	YES	NO
1. Has there ever been a period of time when you were not your usual self and...		
...you felt so good or so hyper that other people thought you were not your normal self or you were so hyper that you got into trouble?	❍	❍
...you were so irritable that you shouted at people or started fights or arguments?	❍	❍
...you felt much more self-confident than usual?	❍	❍
...you got much less sleep than usual and found you didn't really miss it?	❍	❍
...you were much more talkative or spoke much faster than usual?	❍	❍
...thoughts raced through your head or you couldn't slow your mind down?	❍	❍
...you were so easily distracted by things around you that you had trouble concentrating or staying on track?	❍	❍
...you had much more energy than usual?	❍	❍
...you were much more active or did many more things than usual?	❍	❍
...you were much more social or outgoing than usual, for example, you telephoned friends in the middle of the night?	❍	❍
...you were much more interested in sex than usual?	❍	❍
...you did things that were unusual for you or that other people might have thought were excessive, foolish, or risky?	❍	❍
...spending money got you or your family into trouble?	❍	❍
2. If you checked YES to more than one of the above, have several of these ever happened during the same period of time?	❍	❍
3. How much of a problem did any of these cause you – like being unable to work; having family, money or legal troubles; getting into arguments or fights? *Please circle one response only.* No Problem Minor Problem Moderate Problem Serious Problem		
4. Have any of your blood relatives (i.e. children, siblings, parents, grandparents, aunts, uncles) had manic-depressive illness or bipolar disorder?	❍	❍
5. Has a health professional ever told you that you have manic-depressive illness or bipolar disorder?	❍	❍

SCORING THE MOOD DISORDER QUESTIONNAIRE (MDQ)

The MDQ was developed by a team of psychiatrists, researchers and consumer advocates to address a critical need for timely and accurate diagnosis of bipolar disorder, which can be fatal if left untreated. The questionnaire takes about five minutes to complete, and can provide important insights into diagnosis and treatment. Clinical trials have indicated that the MDQ has a high rate of accuracy; it is able to identify seven out of ten people who have bipolar disorder and screen out nine out of ten people who do not.[1]

A recent National DMDA survey revealed that nearly 70% of people with bipolar disorder had received at least one misdiagnosis and many had waited more than 10 years from the onset of their symptoms before receiving a correct diagnosis. National DMDA hopes that the MDQ will shorten this delay and help more people to get the treatment they need, when they need it.

The MDQ screens for Bipolar Spectrum Disorder, (which includes Bipolar I, Bipolar II and Bipolar NOS).

If the patient answers:

1. "**Yes**" to seven or more of the 13 items in question number 1;

AND

2. "**Yes**" to question number 2;

AND

3. "**Moderate**" or "**Serious**" to question number 3;

you have a positive screen. All three of the criteria above should be met. A positive screen should be followed by a comprehensive medical evaluation for Bipolar Spectrum Disorder.

ACKNOWLEDGEMENT: This instrument was developed by a committee composed of the following individuals: Chairman, Robert M.A. Hirschfeld, MD – University of Texas Medical Branch; Joseph R. Calabrese, MD – Case Western Reserve School of Medicine; Laurie Flynn – National Alliance for the Mentally Ill; Paul E. Keck, Jr., MD – University of Cincinnati College of Medicine; Lydia Lewis – National Depressive and Manic-Depressive Association; Robert M. Post, MD – National Institute of Mental Health; Gary S. Sachs, MD – Harvard University School of Medicine; Robert L. Spitzer, MD – Columbia University; Janet Williams, DSW – Columbia University and John M. Zajecka, MD – Rush Presbyterian-St. Luke's Medical Center.

[1] Hirschfeld, Robert M.A., M.D., Janet B.W. Williams, D.S.W., Robert L. Spitzer, M.D., Joseph R. Calabrese, M.D., Laurie Flynn, Paul E. Keck, Jr., M.D., Lydia Lewis, Susan L. McElroy, M.D., Robert M. Post, M.D., Daniel J. Rapport, M.D., James M. Russell, M.D., Gary S. Sachs, M.D., John Zajecka, M.D., "Development and Validation of a Screening Instrument for Bipolar Spectrum Disorder: The Mood Disorder Questionnaire." *American Journal of Psychiatry* 157:11 (November 2000) 1873-1875.

Annex 2. Montgomery–Åsberg Depression Rating Scale (MADRS)

Name:_____ Date:_____

Montgomery-Asberg Depression Scale (MADRS)

Instructions: The ratings should be based on a clinical interview moving from broadly phrased questions about symptoms to more detailed ones which allow a precise rating of severity. The rater must decide whether the rating lies on the defined scale steps (0, 2, 4, 6) or between them (1, 3, 5). It is important to remember that it is only rare occasions that a depressed patient is encountered who cannot be rated on the items in the scale. If definite answers cannot be elicited from the patients, all relevant clues as well as information from other sources should be used as a basis for the rating in line with customary clinical practice. This scale may be used for any time interval between ratings, be it weekly or otherwise, but this must be recorded.

1. ***Apparent Sadness***
Representing despondency, gloom and despair, (more than just ordinary transient low spirits) reflected in speech, facial expression, and posture. Rate on depth and inability to brighten up.
0 No sadness
1
2 Looks dispirited but does brighten up without difficulty.
3
4 Appears sad and unhappy most of the time.
5
6 Looks miserable all the time. Extremely despondent.

2. ***Reported Sadness***
Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency or feeling of being beyond help without hope. Rate according to intensity, duration and the extent to which the mood is reported to be influenced by events.
0 Occasional sadness in keeping with the circumstances.
1
2 Sad or low but brightens up without difficulty.
3
4 Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances.
5
6 Continuous or unvarying sadness, misery or despondency.

3. ***Inner Tension***
Representing feelings of ill-defined discomfort, edginess, inner turmoil mounting to either panic, dread or anguish. Rate according to intensity, frequency, duration and the extent of reassurance called for.
0 Placid. Only reflecting inner tension.
1
2 Occasional feelings of edginess and ill-defined discomfort.
3
4 Continuous feelings of inner tension or intermittent panic which the patient can only master with some difficulty.
5
6 Unrelenting dread or anguish. Overwhelming panic.

4. ***Reduced Sleep***
Representing the experience of reduced duration or depth of sleep compared to the subject's own normal pattern when well.
0 Sleeps as usual.
1
2 Slight difficulty dropping off to sleep or slightly reduced light or fitful sleep.
3
4 Sleep reduced or broken by at least two hours.
5
6 Less than two or three hours sleep.

5. ***Reduced Appetite***
Representing the feeling of loss of appetite compared with when well. Rate by loss of desire for food or the need to force oneself to eat.
0 Normal or increased appetite.
1
2 Slightly reduced appetite.
3
4 No appetite. Food is tasteless.
5
6 Needs persuasion to eat.

6. ***Concentration Difficulties***
Representing difficulties in collecting one's thoughts mounting to incapacitating lack of concentration. Rate according to intensity, frequency, and degree of incapacity produced.
0 No difficulties in concentrating.
1
2 Occasional difficulties in collecting one's thoughts.
3
4 Difficulties in concentrating and sustaining thought which reduces ability to read or hold a conversation.
5
6 Unable to read or converse without great initiative.

7. ***Lassitude***
Representing a difficulty getting started or slowness initiating and performing everyday activities.
0 Hardly no difficulty in getting started. No sluggishness.
1
2 Difficulties in starting activities.
3
4 Difficulties in starting simple routine activities which are carried out with effort.
5
6 Complete lassitude. Unable to do anything without help.

8. ***Inability to Feel***
Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.
0 Normal interest in the surroundings and in other people.
1
2 Reduced ability to enjoy usual interest.
3
4 Loss of interest in surroundings. Loss of feelings for friends and acquaintances.
5
6 The experience of being emotionally paralyzed, inability to feel anger, grief or pleasure and a complete or even painful failure to feel for close relatives and friends.

9. ***Pessimistic Thoughts***
Representing thoughts of guilt. Inferiority, self-reproach, sinfulness, remorse and ruin.
0 No pessimistic thoughts.
1
2 Fluctuating ideas of failure, self-reproach or self-depreciation.
3
4 Persistent self-accusations, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future.
5
6 Delusions of ruin, remorse or unredeemable sin. Self-accusations which are absurd and unshakable.

10. ***Suicidal Thoughts***
Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and the preparations for suicide. Suicidal attempts should not in themselves influence the rating.
0 Enjoys life or takes it as it comes.
1
2 Weary of life. Only fleeting suicidal thoughts.
3
4 Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intention.
5
6 Explicit plans for suicide when there is an opportunity. Active preparations for suicide.

Total Score:_____

Citation: Montgomery SA, Asberg M: A new depression scale designed to be sensitive to change. British Journal of Psychiatry 134:382-389, 1979

Annex 3. Young Mania Rating Scale (YMRS)

Young Mania Rating Scale (YMRS)

OVERVIEW

The Young Mania Rating Scale (YMRS) is one of the most frequently utilized rating scales to assess manic symptoms. The scale has 11 items and is based on the patient's subjective report of his or her clinical condition over the previous 48 hours. Additional information is based upon clinical observations made during the course of the clinical interview. The items are selected based upon published descriptions of the core symptoms of mania. The YMRS follows the style of the Hamilton Rating Scale for Depression (HAM-D) with each item given a severity rating. There are four items that are graded on a 0 to 8 scale (irritability, speech, thought content, and disruptive/aggressive behavior), while the remaining seven items are graded on a 0 to 4 scale. These four items are given twice the weight of the others to compensate for poor cooperation from severely ill patients. There are well described anchor points for each grade of severity. The authors encourage the use of whole or half point ratings once experience with the scale is acquired. Typical YMRS baseline scores can vary a lot. They depend on the patients' clinical features such as mania (YMRS = 12), depression (YMRS = 3), or euthymia (YMRS = 2). Sometimes a clinical study entry requirement of YMRS $\geq$ 20 generates a mean YMRS baseline of about 30. Strengths of the YMRS include its brevity, widely accepted use, and ease of administration. The usefulness of the scale is limited in populations with diagnoses other than mania.

The YMRS is a rating scale used to evaluate manic symptoms at baseline and over time in individuals with mania.

The scale is generally done by a clinician or other trained rater with expertise with manic patients and takes 15–30 minutes to complete.

Young Mania Rating Scale (YMRS)

GUIDE FOR SCORING ITEMS:

The purpose of each item is to rate the severity of that abnormality in the patient. When several keys are given for a particular grade of severity, the presence of only one is required to qualify for that rating.

The keys provided are guides. One can ignore the keys if that is necessary to indicate severity, although this should be the exception rather than the rule.

Scoring between the points given (whole or half points) is possible and encouraged after experience with the scale is acquired. This is particularly useful when severity of a particular item in a patient does not follow the progression indicated by the keys.

1. **Elevated Mood**
   - 0 Absent
   - 1 Mildly or possibly increased on questioning
   - 2 Definite subjective elevation; optimistic, self-confident; cheerful; appropriate to content
   - 3 Elevated; inappropriate to content; humorous
   - 4 Euphoric; inappropriate laughter; singing

2. **Increased Motor Activity-Energy**
   - 0 Absent
   - 1 Subjectively increased
   - 2 Animated; gestures increased
   - 3 Excessive energy; hyperactive at times; restless (can be calmed)
   - 4 Motor excitement; continuous hyperactivity (cannot be calmed)

3. **Sexual Interest**
   - 0 Normal; not increased
   - 1 Mildly or possibly increased
   - 2 Definite subjective increase on questioning
   - 3 Spontaneous sexual content; elaborates on sexual matters; hypersexual by self-report
   - 4 Overt sexual acts (toward patients, staff, or interviewer)

4. **Sleep**
   - 0 Reports no decrease in sleep
   - 1 Sleeping less than normal amount by up to one hour
   - 2 Sleeping less than normal by more than one hour
   - 3 Reports decreased need for sleep
   - 4 Denies need for sleep

5. **Irritability**
   - 0 Absent
   - 2 Subjectively increased
   - 4 Irritable at times during interview; recent episodes of anger or annoyance on ward
   - 6 Frequently irritable during interview; short, curt throughout
   - 8 Hostile, uncooperative; interview impossible

6. Speech (Rate and Amount)

0 No increase
2 Feels talkative
4 Increased rate or amount at times, verbose at times
6 Push; consistently increased rate and amount; difficult to interrupt
8 Pressured; uninterruptible, continuous speech

7. Language-Thought Disorder

0 Absent
1 Circumstantial; mild distractibility; quick thoughts
2 Distractible, loses goal of thought; changes topics frequently; racing thoughts
3 Flight of ideas; tangentiality; difficult to follow; rhyming, echolalia
4 Incoherent; communication impossible

8. Content

0 Normal
2 Questionable plans, new interests
4 Special project(s); hyper-religious
6 Grandiose or paranoid ideas; ideas of reference
8 Delusions; hallucinations

9. Disruptive-Aggressive Behavior

0 Absent, cooperative
2 Sarcastic; loud at times, guarded
4 Demanding; threats on ward
6 Threatens interviewer; shouting; interview difficult
8 Assaultive; destructive; interview impossible

10. Appearance

0 Appropriate dress and grooming
1 Minimally unkempt
2 Poorly groomed; moderately disheveled; overdressed
3 Disheveled; partly clothed; garish make-up
4 Completely unkempt; decorated; bizarre garb

11. Insight

0 Present; admits illness; agrees with need for treatment
1 Possibly ill
2 Admits behavior change, but denies illness
3 Admits possible change in behavior, but denies illness
4 Denies any behavior change

REFERENCES
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Clinical Global Impression (CGI)

Reference: **Guy W, editor. ECDEU Assessment Manual for Psychopharmacology. 1976. Rockville, MD, U.S. Department of Health, Education, and Welfare**

Rating Clinician-rated

Administration time Varies with familiarity with patient

Main purpose To provide a global rating of illness severity, improvement and response to treatment

Population Adults

Commentary

Amongst the most widely used of extant brief assessment tools in psychiatry, the CGI is a 3-item observer-rated scale that measures illness severity (CGIS), global improvement or change (CGIC) and therapeutic response. The illness severity and improvement sections of the instrument are used more frequently than the therapeutic response section in both clinical and research settings. The Early Clinical Drug Evaluation Program (ECDEU) version of the CGI (reproduced here) is the most widely used format, and asks that the clinician rate the patient relative to their past experience with other patients with the same diagnosis, with or without collateral information. Several alternative versions of the CGI have been developed, however, such as the FDA Clinicians' Interview-Based Impression of Change (CIBIC), which uses only information collected during the interview, not collateral. The CGI has proved to be a robust measure of efficacy in many clinical drug trials, and is easy and quick to administer, provided that the clinician knows the patient well.

Scoring

The CGI is rated on a 7-point scale, with the severity of illness scale using a range of responses from 1 (normal) through to 7 (amongst the most severely ill patients). CGI-C scores range from 1 (very much improved) through to 7 (very much worse). Treatment response ratings should take account of both therapeutic efficacy and treatment-related adverse events and range from 0 (marked improvement and no side-effects) and 4 (unchanged or worse and side-effects outweigh the therapeutic effects). Each component of the CGI is rated separately; the instrument does not yield a global score.

Versions

CGI for bipolar disorder (CGI-BD), FDA Clinicians' Interview-Based Impression of Change (CIBIC), Clinicians' Interview-Based Impression of Change-Plus (CIBIC+), NYU CIBIC+, Parke-Davis Pharmaceuticals Clinical Interview-Based Impression (CIBI); the CGI has been translated into most languages.

Clinical Global Impression (CGI)

1. **Severity of illness**
   Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?
   0 = Not assessed
   1 = Normal, not at all ill
   2 = Borderline mentally ill
   3 = Mildly ill
   4 = Moderately ill
   5 = Markedly ill
   6 = Severely ill
   7 = Among the most extremely ill patients

2. **Global improvement:** Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment.
   Compared to his condition at admission to the project, how much has he changed?
   0 = Not assessed
   1 = Very much improved
   2 = Much improved
   3 = Minimally improved
   4 = No change
   5 = Minimally worse
   6 = Much worse
   7 = Very much worse

3. **Efficacy index:** Rate this item on the basis of **drug effect only**.
   Select the terms which best describe the degrees of therapeutic effect and side effects and record the number in the box where the two items intersect.
   EXAMPLE: Therapeutic effect is rated as 'Moderate' and side effects are judged 'Do not significantly interfere with patient's functioning'.

Therapeutic effect		**Side effects**			
		None	*Do not significantly interfere with patient's functioning*	*Significantly interferes with patient's functioning*	*Outweighs therapeutic effect*
Marked	Vast improvement. Complete or nearly complete remission of all symptoms	01	02	03	04
Moderate	Decided improvement. Partial remission of symptoms	05	06	07	08
Minimal	Slight improvement which doesn't alter status of care of patient	09	10	11	12
Unchanged or worse		13	14	15	16

Not assessed = 00

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