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THERAPEUTIC APPROACHES

Perspective targeted diagnosis and therapy of mitochondrial bioenergetics across different diagnoses

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ABSTRACT

Important metabolic variables that lead to the development of many diseases, including "mitochondrial diseases," include increased oxidative stress and mitochondrial malfunction. Given that the clinical picture and metabolic alterations in individuals suspected of having mitochondrial illnesses lack distinct characteristics, the development of sensitive and specific diagnostic techniques to detect alterations in mitochondrial bioenergetics is imperative. High-resolution respirometry (HRR), is a minimally invasive technique that enables the analysis of mitochondrial function in platelets taken from peripheral blood. This method allows for the detection of even the most subtle changes prior to disease development. HRR can identify minute variations in mitochondrial bioenergetics. Determining mitochondrial function and endogenous levels of CoQ_{10} in platelets can aid in the early detection of pathobiochemical changes in mitochondria and assessment of treatment efficacy. When combined with the measurement of endogenous coenzyme Q_{10} levels, HRR may be an effective approach for early identification of compromised mitochondrial function along with monitoring the therapeutic outcomes. Supplementing with coenzyme Q_{10}, applying molecular hydrogen, transplanting mitochondria, and applying platelet-rich plasma (PRP) are some of the therapeutic strategies utilized to enhance mitochondrial function and reduce oxidative stress *(Tab. 1, Fig. 1, Ref. 62)*.
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KEY WORDS: mitochondrial dysfunction, oxidative stress, mitochondrial bioenergetics, high-resolution respirometry, therapeutic approaches.

Introduction

Mitochondrial malfunction and increased oxidative stress are essential metabolic factors that contribute to the development of many diseases, including "mitochondrial diseases". Based on their cause, mitochondrial illnesses can be classified into two categories. Primary mitochondrial diseases are caused by genetic abnormalities and are linked to mutations and deletions in nuclear (nDNA) or mitochondrial DNA (mtDNA). Secondary mitochondrial disorders are caused by external influences and frequently occur in conjunction with other systemic diseases. Neurodegenerative illnesses, cardiovascular diseases, renal diseases, immunodeficiencies, rheumatologic problems, obesity, and oncological diseases are a few examples of these systemic issues. Some known triggers for secondary mitochondrial disorders include mental stress, infections, use of statins, alcohol or tobacco use, aging, or living in an unhealthy environment.

Diagnosing mitochondrial illness is challenging due to the non-specific symptoms that often overlap with those of other conditions. Extensive research efforts are underway to recognize the necessity of developing treatments for mitochondrial diseases, and to identify an optimal diagnostic technique that is both sensitive and precise. Currently, the standard and most effective method for assessing mitochondrial function lies in skeletal muscle biopsy. Mitochondrial function is assessed using high-resolution respirometry (HRR) method, which involves analyzing permeabilized tissue or isolated mitochondria from the biopsy samples. Over the past decade, scientists have explored the feasibility of replacing tissue biopsy with blood cells, which can be collected with minimal invasiveness from the peripheral blood of patients. Current research assumes that even subtle changes in mitochondrial functions in blood cells may indicate alterations of mitochondrial bioenergetics in the target organ. Respirometry of blood cells represents a novel area of translational research in this field.

Importance of mitochondria in health and disease

In eukaryotic cells, mitochondria are double-membraned organelles consisting of four compartments: the matrix, intermembrane space, outer membrane, and inner membrane. The respiratory

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chain, crucial for ATP synthesis, is located within the inner membrane. This membrane is characterized by numerous protrusions, referred to as cristae, which significantly increase its surface area, ensuring a greater capacity for energy production. It is worth noting that the total surface area of the inner membrane is five times larger than that of the outer membrane (Cserép et al, 2018).

The proper function of the respiratory chain relies on five complexes (CI, CII, CIII, CIV, and CV) and two mobile components: cytochrome *c* and coenzyme Q_{10} (CoQ_{10}). Electrons are transferred from NADH through complex I (NADH ubiquinone reductase) and from succinate through complex II (succinate dehydrogenase) to CoQ_{10}. Subsequently, electrons from CoQ_{10} are transferred to complex III ($CoQH_2$-cytochrome *c* reductase) and to cytochrome *c*. Finally, complex IV (cytochrome *c* oxidase) transfers electrons from cytochrome *c* to oxygen. During this electron transfer process, protons are pumped out of the matrix by complexes CI, CIII and CIV, forming an electrochemical potential on the inner mitochondrial membrane. This proton motive force is then utilized by complex V (ATP-synthase) to phosphorylate ADP, resulting in ATP synthesis. The process is termed oxidative phosphorylation (OXPHOS). This arrangement of complexes I, III, and IV into a supercomplex enhances electron transport efficiency by reducing the distance travelled and increasing the stability of the complexes (Hidalgo-Gutiérrez et al, 2021, Kohler et al, 2023).

The matrix of mitochondria houses a variety of metabolic pathways' enzymes, including those involved in Krebs cycle, beta-oxidation of fatty acids, oxidation of amino acids, and others. Notably, approximately two thirds of all mitochondrial proteins are located within the matrix (van der Bliek et al, 2017). Mitochondria play a crucial role in the oxidation of proteins, lipids, and carbohydrates. Via oxidative phosphorylation (OXPHOS), they generate over 90% of the cell's required energy in the form of adenosine triphosphate (ATP). Depending on the type of cell, mitochondria exhibit variations in size, shape, location within cells, and metabolic activities (Kuznetsov et al, 2009). Another significant function of mitochondria lies in the production of reactive oxygen species (ROS) as byproducts of the respiratory chain. The most important ROS include hydroxyl radicals, superoxide, and hydrogen peroxide. Mitochondria possess antioxidant mechanisms that intercept and convert potentially harmful ROS into less reactive molecules. Key antioxidants involved in this process include superoxide dismutase, catalase, glutathione peroxidase, cytochrome *c*, and coenzyme Q_{10} (Clayton et al, 2021). In addition to their role in ROS management, mitochondria play essential roles in maintaining Ca^{2+} homeostasis, synthesizing heme, and regulating apoptosis (Picard et al, 2016). Moreover, mitochondria serve as dynamic signaling hubs within cells, directly influencing the immune system and participating in intracellular and intercellular signaling process via proteins, DNA, lipids, metabolites, and reactive oxygen species (Clayton et al, 2021). Recent research has revealed the existence of extracellular mitochondria, which are assumed to play a significant role in some pathological processes, including those associated with SARS-CoV-2 infection (Milliotis et al, 2019; Singh et al, 2020).

The nuclear genome (nDNA) and mitochondrial genome (mtDNA) collaborate in regulating mitochondrial activity. Mutations in either genome can lead to mitochondrial malfunction, including diminished OXPHOS capacity and increased ROS production capacity (Hahn et al, 2019).

Mitochondrial dysfunction and an imbalance between oxidative stress and antioxidant mechanisms play significant roles in numerous diseases. These include autoimmune disorders, neuromuscular conditions, diabetes mellitus, obesity, metabolic dysfunction linked to liver steatosis, sepsis, cancer, and cardiovascular disorders. The dysregulation of mitochondrial function and the preferential formation of ROS contribute to the pathogenesis of these diverse diseases (Koopman et al, 2012).

Coenzyme $Q_{10,}$ an endogenous component of biological membranes, plays an essential part in the mitochondrial respiratory chain. Its reduced form, ubiquinol, shields cells from nitrative and oxidative damage (Gvozdjáková et al, 2018). The concentration of CoQ_{10} varies across tissue types; it is abundant in the heart, kidney, liver, and skeletal muscle, while less abundant in the brain and lungs (Turunen et al, 2004). When CoQ_{10} is deficient in blood, tissues and mitochondria, the condition is referred to as "CoQ_{10} disease". Primary CoQ_{10} shortage arises from mutations in the genes responsible for its production and is often associated with mitochondrial diseases. These can present clinically with a variety of conditions, including encephalomyopathies, cerebellar ataxia, myopathy, and nephropathy. Secondary CoQ_{10} deficiency is associated with mitochondrial disorders (Hidalgo-Gutierrez et al, 2021) and implicated in diseases such as Parkinson's disease, Alzheimer's disease, Down syndrome, autism (Hazirah et al, 2020; Gvozdjáková et al, 2014), cardiovascular diseases, type 2 diabetes mellitus, oncological diseases (Palacka et al, 2022; Chowdhury et al, 2017), rheumatoid arthritis (Gvozdjáková et al, 2021; Kucharská et al, 2021), chronic kidney disease (Gvozdjáková et al, 2019; Gvozdjáková et al, 2020), bronchial asthma (Gvozdjáková et al, 2005), infertility (Gvozdjáková et al, 2015), viral infections (Sumbalová et al, 2022), and aging (Gvozdjaková et al, 2018). Additionally, the seasonal variation may influence endogenous concentration of CoQ_{10} (Gvozdjaková et al, 2019).

Importance of mitochondria in platelets

Thrombocytes are anucleated fragments of megakaryocytes that circulate in the peripheral bloodstream, making them a conveniently available source of mitochondria. Each platelet contains 5 to 8 mitochondria and has an average lifespan of 8-9 days (Harker et al, 2000; Ravera and Panfoli, 2019). Platelet mitochondrial function was found to be lower in winter than in spring (Gvozdjaková et al, 2019), and lower in the elderly compared to young, healthy individuals (Gvozdjaková et al, 2018). Although the invasive biopsy of skeletal muscle is the gold standard for studying mitochondrial activity in humans, cells from peripheral blood such as platelets and peripheral blood mononuclear cells (PBMC) can serve as surrogates for the respirometric determination of mitochondrial bioenergetics in a minimally invasive manner. Using high-resolution respirometry, even minor changes in mitochondrial respiration and ATP production can be detected.

High-resolution respirometry method

High-resolution respirometry is a precise method used to measure respiration in various types of mitochondrial preparations and living cells. This technique allows for the assessment of mitochondrial function by analyzing oxygen consumption rates with high sensitivity and resolution (Gnaiger 2020). The use of comprehensive protocols with stepwise titration of substrates, uncoupler and inhibitors (SUIT protocols) facilitates the assessment of metabolic activity across different mitochondrial pathways within a single measurement, using a minimal amount of sample (Pesta and Gnaiger 2012). For blood cell respirometry, the purity of cells isolated from whole blood is ensured by developed methods and verified by counting on a hematology analyzer. Typically, $200x10^6$ PLT or $4x10^6$ PBMC are used for one respirometric measurement (Sumbalova et al, 2020).

Coenzyme Q_{10} determination

One of the most commonly used current methods for determining the concentrations of CoQ_{10} in human biological materials (such as blood, plasma, blood particles, tissues, mitochondria) is High Performance Liquid Chromatography (HPLC). This analytical method is designed for the separation and analysis of mixtures of substances. The separation of substances occurs in the chromatographic column and is based on the interaction between the stationary and mobile phases. CoQ_{10} is detected at 275 nm in the spectrophotometric detector based on its characteristic maximum absorbance wavelength. Since CoQ_{10} occurs in both oxidized and reduced forms, total CoQ_{10} (ubiquinol + ubiquinone) can be measured after the oxidation of ubiquinol (Kucharska et al, 2023).

The determination of coenzyme Q_{10} is important for the early detection of its deficiency in the body, prevention and treatment of diseases, and monitoring therapeutic levels during supplementation to ensure that an adequate plasma level of CoQ_{10} s is reached. The levels of CoQ_{10} in plasma do not always reflect its concentration in tissues. Therefore, methods have been developed for determining CoQ_{10} in various accessible human tissues, isolated blood particles (platelets, leukocytes), skin fibroblasts, skeletal and cardiac muscle biopsy samples, and seminal fluid (sperm) (Mantle et al, 2023) (Tab. 1).

Lower endogenous levels of $CoQ_{10\text{-TOTAL}}$ in isolated platelets and modest alterations of mitochondrial respiration in platelets were observed in patients with rheumatoid arthritis (RA) patients. The concentration of endogenous $CoQ_{10\text{-TOTAL}}$ positively correlated with parameters of platelet mitochondrial respiration (Gvozdjáková et al, 2021). Patients with urothelial carcinoma were found to exhibit a disruption of the glycerophosphate pathway and a significantly decreased CI-linked OXPHOS capacity (reduced to 81.4% of control data; p=0.020) (Palacka et al, 2022). Additionally, evidence indicated decreased levels of endogenous CoQ_{10} in patients suffering from chronic kidney disease. Although platelet mitochondrial function was not affected, researchers speculate that a deficiency in the body's synthesis of CoQ_{10} may be linked to impaired kidney function in CKD patients (Gvozdjaková et al, 2020). The CoQ_{10}/tChol ratio may be used to predict the course of chronic kidney disease (CKD) in individuals with lifestyle illnesses such as dyslipidemia, type 2 diabetes mellitus, or arterial hypertension (Gvozdjaková et al, 2020b). In individuals with nonalcoholic fatty liver disease (NAFLD), complex I-linked LEAK respiration was elevated, while complex I-linked OXPHOS capacity and complex II-linked electron transfer capacity were diminished, leading to lower efficiency of oxidative phosphorylation (Sumbalová et al, 2023). The activity of, citrate synthase, complex II in isolated platelets, and mitochondrial respiration in intact platelets markedly declined with age in individuals with neurological illnesses (such as Huntington's, Parkinson's, and Alzheimer's diseases) as well as in healthy individuals (Fišar et al, 2023). Therefore, mitochondrial respiration in platelets could be a potential biomarker of aging, regardless of the degree of cognitive impairment (Fišar et al, 2023).

Tab. 1. OXPHOS function and CoQ_{10} levels.

Study	Disease	P(n)	C(n)	Sample	Diagnostic tool	Sig.
Palacka et al, 2022	UCa	15	15	PLT	HRR	*
Gvozdjaková et al, 2021	RA	21	19	PLT	HRR	**
Gvozdjáková et al, 2020	CKD, NCD	58	19	PLT, plasma	HPLC	*
Sumbalová et al, 2023	NAFLD	30	15	PLT	HRR, HPLC	*
Fišar et al, 2023	Age-dependent alterations, Neurodegeneration			PLT	HRR	n.r.
Westerlund et al, 2017	MD	113	25	PLT	HRR	n.r.
Artuch et al, 2000	MD	24	87	LCT	HRR	**
Pecina et al, 2014	MD	7	6	LCT	HRR	n.r.
Hazirah et al, 2020	Autism	n.r.	n.r.	LCLs	HRR	**
Hsu et al, 2019	Stroke	15	15	PLT	HRR	*
Shirakawa et al, 2019	Heart failure	31	n.r.	PBMC	HRR	*
Bellar et al, 2023	AH	12	12	PBMC	HRR	*
Sumbalova et al, 2022	C19	36	15	PLT	HRR	*
Nedel et al, 2021	Sepsis	90	n.r.	LCLs	HRR	*
Chowdhury et al, 2017	CLL	20	n.r.	CLL cells	HRR	n.r.

UCa – urothelial carcinoma; RA – rheumatoid arthritis; CKD – chronic kidney disease; NCD – non-communicable disease; NAFLD – nonalcoholic fatty liver disease; MD – mitochondrial disease; MM – mitochondrial myopathy; P – patients; C – control; PLT – platelets; HRR – high-resolution respirometry; HPLC – high-performance liquid chromatography; LCT – lymphocyte; LCLs – lymphoblastoid cell lines; AH – alcohol- associated hepatitis; C19 – Covid 19; CLL – chronic lymphocytic leukemia; Sig. – significance; *= $p < 0.05$; **= $p < 0.01$; n.r. – not reported

Additionally, Westerlund et al (2018) examined oxygen consumption of platelets in juvenile patients suffering from mitochondrial illness and demonstrated that the probability of mitochondrial disease is higher when aberrant platelet respirometry is present. Similarly, Artuch et al (2000) and Pecina et al (2014) observed and validated the same finding, albeit using isolated lymphocytes rather than platelets.

The pathophysiology of autism has also been linked to mitochondrial dysfunction. Using high-resolution respirometry, Hazirah et al (2020) investigated the mitochondrial respiration and membrane potential of a lymphoblastoid cell line from an individual on autistic spectrum and a control cell line from a developmentally normal non-autistic sibling. Compared to the healthy control, the cell line from autistic individual exhibited considerably enhanced capacities for oxidative phosphorylation, electron transfer of the NADH- and succinate-linked pathways, and complex IV activity ($p<0.01$). Moreover, during LEAK respiration and oxidative phosphorylation, the mitochondrial membrane potential was higher also in the succinate pathway ($p<0.05$). The conjunction of these findings indicates aberrant mitochondrial function in autism. Understanding the link between autism and mitochondrial dysfunction is crucial for establishing timely and efficient therapies.

The study conducted by Hsu et al. (2019) aimed to elucidate the impact of exercise training (ET) on the aerobic capacity and platelet mitochondrial bioenergetics (MTB) of stroke patients. The findings revealed that while reducing the ventilation vs CO_2 production slope (−7.65%), ET led to a significant increase in VO_2peak (17.7%) and O_2 uptake efficiency slope (31.9%). By triggering the complex II-dependent pathway, patients undergoing ET also exhibited significantly increased platelet mitochondrial OXPHOS and electron transfer capacity, while reducing their plasma myeloperoxidase (−28.4%) and interleukin-6 levels (−29.9%). Furthermore, a favorable correlation was found between variations in platelet OXPHOS and electron transfer capacities and variations in VO_2 peak levels. In conclusion, ET improves Complex II activity in stroke patients, stimulating platelet MTB. Furthermore, stroke patients benefit from the exercise program by enhancing their aerobic fitness and reducing oxidative stress and pro-inflammatory state. Exercise training may impact physical fitness by affecting mitochondrial functions.

In 2019, Shirakawa et al. investigated the hypothesis that the reactive oxygen species (ROS) production by circulating peripheral blood mononuclear cells (PBMCs) contributes to the progression of chronic heart failure. Their study revealed a significant rise in ROS levels in PBMC mitochondria, along with a decrease in respiratory efficiency of PBMC in CHF patients with NYHA functional class III compared to those with NYHA functional class I–II. Urinary 8-hydroxydeoxyguanosine, a marker of systemic oxidative stress, was positively correlated with ROS production in PBMC mitochondria in patients with congestive heart failure. Notably, mitochondrial ROS generation in PBMC was inversely associated with peak oxygen uptake, a measure of exercise capability in CHF patients, and positively correlated with plasma levels of B-type natriuretic peptide, a biomarker for HF severity. The results of the study demonstrated that individuals with advanced congestive heart failure exhibited higher levels of ROS generation in their PBMC mitochondria, which was linked to both disease severity and exercise intolerance.

The study of Bellar et al (2023) found that individuals with alcohol-associated hepatitis (AH) had shorter telomeres in non-survivors, increased plasma TCA cycle intermediates, and decreased mitochondrial oxidative capacity. At baseline and in response to lipopolysaccharide (LPS), there was a cell type-specific differential expression of mitochondrial intermediary metabolites, and senescence-regulatory genes in PBMC from AH patients and healthy controls (HC). Maximum respiration and function of complexes I and II in permeabilized PBMC were lower in AH patients than in HCs (Bellar et al, 2023).

Sumbalova et al (2022) examined the effects of a unique mountain spa rehabilitation program on patients with post-COVID-19 lung function, clinical symptoms, endogenous CoQ_{10} levels, and platelet mitochondrial bioenergetics. The rehabilitation program was combined with supplementation of ubiquinol, a reduced form of coenzyme Q_{10}. Both patient groups exhibited significantly lower platelet mitochondrial complex I (CI)-linked oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacities. The study suggests that supplemental ubiquinol plays a major role in accelerating the recovery of mitochondrial health in individuals with post-COVID-19 symptoms. The mountain spa rehabilitation along with coenzyme Q_{10} supplementation may be beneficial to these patients.

Chronic lymphocytic leukemia (CLL), the most common blood malignancy among adults in Western countries, is likewise associated with impairments in mitochondrial physiology. According to Chowdhury et al (2017), selectively targeting the mitochondrial bioenergetics of leukemia cells could be a useful treatment strategy for CLL. The study determined the bioenergetic profile of CLL patients and examined the impact of major treatment medications (FK866 and idelalisib) on this profile, as well as on reactive oxygen species (ROS), cell viability, mitochondrial membrane potential (MMP), and protein levels of mitochondrial complexes and pPI3Kδ. The results of this study indicate that a therapeutic combination of these drugs may result in decreased toxicity and greater drug efficacy in CLL by targeting mitochondrial metabolism.

A potentially fatal illness known as septic shock forces immune cells to alter their mitochondrial metabolism in order to produce more ATP and mount an effective defense. Nedel et al (2021) conducted a single-center prospective cohort study in the intensive care unit (ICU) of a tertiary referral hospital in Brazil. The study evaluated mitochondrial respiration related to complexes I, II, and V, as well as biochemical coupling efficiency (BCE) in isolated lymphocytes at days 1 and 3 (D1 and D3) after admission. In patients who did not survive, there was a drop in ΔCII and in respiratory rates associated with CII at D1 and D3. The ΔBCE signature in lymphocytes enabled early identification of ICU patients in septic shock who were at risk of their health state deteriorating over time.

The most recent guidelines of the European Association for the Study of the Liver (EASL) on Drug-induced Liver Injury

(DILI) emphasize the importance of screening for stress in cell systems and isolated mitochondria as predictor of severity of DILI (Andrade, 2019). When glutathione is severely depleted, particularly in mitochondria, toxic metabolites covalently bind to mitochondrial proteins and induce increased ROS production. Idiosyncratic DILI is a serious condition associated with the risk of life-threatening liver failure, death, or the need for liver transplantation. Early prediction of its occurrence should be a preventive strategy in indication and treatment management, employing new immunotherapeutic agents including biologics or immune checkpoint inhibitors used frequently in oncology.

According to the findings of multiple clinical trials, the bioenergetics of blood cells evaluated by HRR may serve as a measure of mitochondrial health of individuals with suspected mitochondrial disorders.

Targeted therapy strategies for dysfunctional mitochondria

Coenzyme Q_{10}-targeted support treatment

Coenzyme Q_{10} (CoQ_{10}) supplementation is one of the most extensively researched targeted therapies for mitochondrial illnesses. CoQ_{10} is an endogenous component of biological membranes playing an important role in the respiratory system of the inner mitochondrial membrane. All eukaryotic cells contain CoQ_{10} in their cell membranes, as well as high- and low-density lipoproteins. Ubiquinol (the reduced form of CoQ_{10}) protects cells against oxidative and nitrative damage. In addition to its bioenergetic and antioxidant properties, coenzyme Q_{10} has recently been shown to play roles in mitochondrial metabolism, gene expression regulation, and apoptotic signaling. CoQ_{10} is essential for cell energy synthesis under both normal and pathological conditions. CoQ_{10} levels in organs vary, with endogenous concentrations determined by energy requirements of individual tissues. CoQ_{10} is a lipophilic antioxidant capable of recycling and regenerating other antioxidants, including vitamin E and ascorbic acid. Ubiquinol is a potent antioxidant capable of inhibiting free radical processes. Ubiquinol accounts for approximately 90% of all CoQ_{10} in the cell. The remaining 10% is in the form of ubiquinone (oxidized form). A partially reduced version of CoQ_{10} known as "semiquinone" is a generator of superoxide radicals and thus acts as a pro-oxidant. Interconversion of these 3 forms takes place continuously in the organism. Eighty percent of the cell's total CoQ_{10} content is found in the mitochondria; the remaining quantity is distributed throughout the nucleus, endoplasmic reticulum, plasma membrane, Golgi apparatus, peroxisomes, and lysosomes (Mantle et al, 2023).

The usual daily consumption of CoQ_{10} from food is between 3 and 5 milligrams. If endogenous production is diminished due to various disorders, ATP generation in the mitochondria decreases. CoQ_{10} supplementation heightens its concentration in the body and boosts ATP production in mitochondria via the OXPHOS pathway. Following oral ingestion, CoQ_{10} enters the stomach, then the duodenum, where it is absorbed by enterocytes, integrated into chylomicrons, and transported to the bloodstream via lymphatic capillaries. CoQ_{10} travels through the blood to the liver, where it is absorbed into LDL and HDL particles at rates of roughly 58% and 28%, respectively (Mantle and Dybring, 2020). CoQ_{10} is thus a key antioxidant that protects LDL particles, prevents protein, lipid, and DNA damage, and exerts an anti-atherosclerotic effect. An imbalance between the body's protective antioxidant system and excessive ROS generation can harm biomolecules and mitochondrial respiratory chain function. Linnane and Eastwood (2004) proposed that the redox balance of the ubiquinone-to-ubiquinol ratio ($CoQ_{10\text{-}OX}/CoQ_{10\text{-}RED}$) regulates gene expression and plays an important metabolic role in energy generation.

Primary CoQ_{10} deficiency, commonly referred to as "CoQ_{10} disease" is linked to mitochondrial disorders with a variety of clinical conditions, such as encephalomyopathies (including recurrent myoglobinuria, encephalomyopathy, and mitochondrial myopathy), cerebellar ataxia (cerebellar atrophy combined with other neurological conditions), myopathy, and nephropathy. This condition is caused by mutations in the COQ genes, which are involved in the biosynthesis of CoQ_{10}. Secondary CoQ_{10} depletion, on the other hand, is caused by illnesses not related to COQ gene mutations (Salviati et al, 2017). It is driven by mutations in the aprataxin gene, which causes ataxia, and by mutations in the flavoprotein dehydrogenase gene, which causes myopathy. CoQ_{10} insufficiency is associated with mtDNA depletion, deletions, and point mutations. Secondary CoQ_{10} depletion is linked to mitochondrial dysfunction (Hidalgo-Gutiérrez et al, 2021). CoQ_{10} deficiency in the body (in blood, plasma, tissues, platelets, and sperm) has been linked to the onset and progression of a variety of diseases associated with mitochondrial disorders. These include neurological diseases (including Parkinson's disease, Alzheimer's disease, Huntington's chorea, Friedreich's ataxia), Down's syndrome, autism, cardiovascular diseases, diabetes mellitus, oncological diseases, nephropathies, immunodeficiencies, bronchial asthma, rheumatoid arthritis, infertility, periodontal disease, viral infections, aging and others.

According to recent studies, the importance of CoQ_{10} is irreplaceable. For example, arterial-related cardiovascular disorders, such as coronary heart disease, have shown positive clinical response to CoQ_{10} treatment. (Yu Liu et al, 2022) When compared to placebo, CoQ_{10} provided significant long-term therapeutic benefits (Mortensen et al, 2019). Furthermore, a large body of clinical research indicates that CoQ_{10} supplementation ($\geq$200 mg/day) improves cardiac function in individuals with heart failure and coronary heart disease (Martelli et al, 2020), as well as in patients on statin therapy.

Mitochondrial transplantation

Mitochondrial transplantation offers a potential new approach to the targeted treatment of defective mitochondria. It involves an invasive therapeutic procedure of introducing healthy autologous mitochondria into the organism (McCully et al, 2023). These healthy mitochondria can fuse with endogenous malfunctioning mitochondria in recipient cells, leading to regeneration, improved performance, increased ATP production, and replacement of damaged mtDNA in recipient cells. Mitochondrial transplantation expands therapeutic options for dysfunctional mitochondria in

various organs. A review of Gvozdjáková et al. (2024) summarizes the latest insights into the prospective strategy of targeted therapy for mitochondrial disorders through mitochondrial transplantation across multiple medical fields, including cardiology, hepatology, neurology, nephrology, rheumatology, and oncology, as well as in individuals with post-COVID-19 syndrome.

The employment of platelet-rich plasma (PRP) has been widespread across distinct clinical specializations, including sports medicine, orthopedics, cancer, dentistry, and cosmetics. PRP typically includes 150,000–450,000 platelets per microliter of plasma, each of which contains 5-8 mitochondria. Due to the abundance of platelets containing mitochondria in PRP, this therapeutic approach is considered a form of mitochondrial transplantation (Gvozdjaková et al, 2023).

Molecular hydrogen – a novel treatment strategy

The first study on the effects of molecular hydrogen (H_2) as a natural antioxidant was published in 2007 (Ohsawa et al, 2007). H_2 is a tasteless, odorless, and colorless minute molecule known for its ability to penetrate deep into cells and quickly cross the blood-brain barrier to remove free radicals in the brain. Specifically, H_2 neutralizes the hydroxyl radical (·OH) (Slezák et al, 2016), as well as the peroxynitrite radical ($ONOO^-$).

H_2 is a nonpolar diatomic chemical composed of two protons and two electrons, making it the smallest antioxidant with a molecular weight of two. Figure 1 illustrates the molecular weights of H_2 (2), vitamin C (176), vitamin E (431), and coenzyme Q_{10} (863) (Gvozdjaková et al, 2024).

Widely used in medicine, molecular hydrogen was demonstrated to have beneficial effects in the prevention and supportive

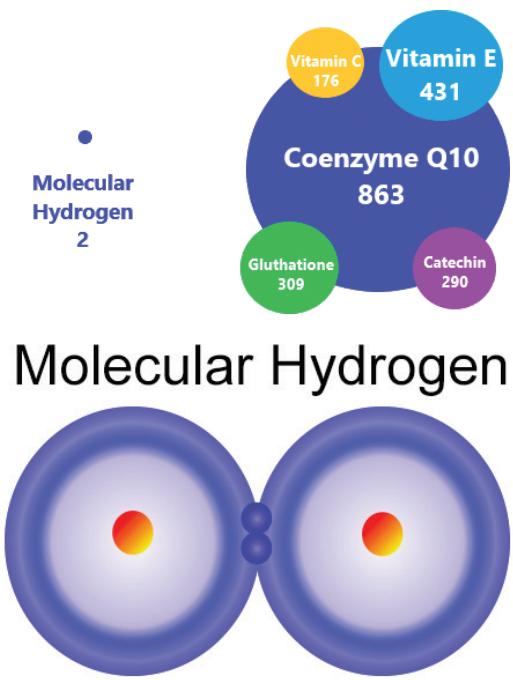

Fig. 1. Molecular weight of H_2 in comparison with other antioxidants.

therapy of patients with a variety of diseases, including cardiovascular disease, Parkinson's disease, metabolic syndrome, respiratory system disease, radiation-treated oncology patients, cerebral infarction, diabetes mellitus, rheumatoid arthritis and metabolically linked steatotic liver disease. All these disorders are related to mitochondrial dysfunction (Slezák et al, 2020).

Mitochondrial oxidative phosphorylation and H_2 effect

Molecular hydrogen has emerged as a novel medical gas with antioxidant properties, showing significant promise in the treatment of mitochondrial diseases and other medical conditions (Ohta, 2012). Studies have demonstrated its ability to scavenge hydroxyl radicals in cultured cells, protect against myocardial and brain injury in rat models of ischemia-reperfusion, prevent and treat Parkinson disease, mitigate the adverse effects of cis-platin as an anti-cancer drug, and protect against kidney transplant-related injury in rat models (Dixon et al, 2013).

Through a variety of mechanisms, including modulation of mitochondrial dynamics and the inhibition of mitochondrial permeability transition pore opening, H_2 can prevent mitochondrial oxidative stress. By stimulating the mitochondrial electron transport system, and enhancing the concentration of mitochondrial coenzyme Q, as well as boosting antioxidant defense mechanisms, H_2 can exert specific targeting of mitochondria. Recent research has also demonstrated that consuming H_2-rich water (HRW) can stimulate the respiratory chain of the rat heart and enhance ATP synthesis of linked to CI and CII substrates (Gvozdjáková et al, 2019).

In human medicine, H_2 has been shown to reduce oxidative stress in individuals with type 2 diabetes mellitus, metabolic syndrome, while also decreasing blood lactate and pyruvate in patients with MELAS (mitochondrial disorders) (Ohta, 2011). Furthermore, H_2 was also shown to have protective effects against cognitive decline, atherosclerosis, allergic reactions, inflammation, oxidation, radiation injury, and other organ injuries (brain, liver, kidney, heart, lung, pancreas, intestine, eye) (Ge et al, 2017).

The application of H2 represents a novel treatment method for targeted therapy of mitochondrial illnesses, as evidenced by multiple investigations highlighting its positive effects on damaged mitochondrial respiratory systems of different bodily organs (Gvozdjáková et al, 2019).

Conclusion

Evidence suggests that systemic mitochondrial health is reflected in platelet mitochondrial function. Prospective targeted method, such as HRR technique, for evaluating mitochondrial bioenergetics and ATP formation via oxidative phosphorylation in blood cells, along with the HPLC (High-Performance Liquid Chromatography) method for determining endogenous levels of CoQ_{10}, contribute to clarifying the pathobiochemical mechanisms of mitochondrial bioenergetic dysfunction in patients with various diseases Platelet mitochondrial function and coenzyme Q_{10} levels may serve as diagnostic and therapeutic markers of mitochondrial health in patients with mitochondrial dysfunction.

HRR of blood cells offers a quick and non-invasive method to assess mitochondrial dysfunction. While abnormal results from HRR testing increase the likelihood of a diagnosis and may support the decision to conduct further invasive studies, it is important to note that normal platelet respirometry results cannot definitely rule out illnesses. Given that severe symptoms cannot be reversed once established, early detection and treatment of these conditions characterized by reduced mitochondrial activity could potentially arrest disease progression and improve prognosis.

Given the diseases are often detected at the stage of their full symptomatology, the determination of the most appropriate time to measure mitochondrial function and commence treatment is an intriguing predicament for clinical medicine Screening for mitochondrial dysfunction, as one of the initial signals of disease onset, could represent a promising direction in modern mitochondrial medicine. Furthermore, early detection offers the possibility of evaluating optimal treatment combinations to effectively manage these conditions.

In general, supplementing with CoQ_{10} has few adverse effects and is considered safe and well-tolerated. H_2 application offers promise as a novel approach for targeted treatment of mitochondrial diseases. Among the most innovative methods is the transplantation of healthy mitochondria to cure various diseases; although the precise process of transplanting isolated, healthy external mitochondria requires further thorough investigation. The noninvasive HRR method in blood cells holds promise for diagnostic purposes, while approaches such as dietary interventions, antioxidants, coenzyme Q_{10} supplementation, and mitochondrial transplantation to target damaged mitochondria offer fresh perspectives on developing adjuvant medications for patients with mitochondrial dysfunction.

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