

## **Mild Uncoupling of Oxidative Phosphorylation and Prospects of Its Use in Medicine (Review)**

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### **ABSTRACT**

The review summarizes and analyzes modern data about the structure and mechanism of action of endogenous and exogenous substances that have been able to uncouple softly oxidative phosphorylation from respiration in mitochondria of various body tissues. The data on the effect of mild uncouplers on the redistribution of hydrogen ions on both sides of the inner mitochondrial membrane and the value of the proton membrane potential are systematized. Evidence of a decrease in the level of reactive oxygen species under the action of these substances is given. Typical physiological effects that develop as a result of the action of mild uncouplers (thermogenesis, prevention of mito- and apoptosis) are described. Possible ways of using these substances as drugs for the prevention and treatment of obesity, cardiovascular, neurodegenerative, inflammatory and oncological diseases are discussed.

**Keywords:** uncoupling thermogenesis oxidative stress mild uncouplers use in medicine

### **INTRODUCTION. OXIDATIVE PHOSPHORYLATION. ROLE IN THE BIOENERGETICS OF THE BODY**

Oxidative phosphorylation (OP) is the main pathway for the synthesis of a universal high energy compound, adenosine triphosphate (ATP). The polenzyme system of this synthesis, including the respiratory (electron transport) chain and ATP synthase, is localized in the lipid bilayer of the inner mitochondrial membrane and carries out the synthesis of ATP from ADP, inorganic orthophosphate and protons pumped by the respiratory chain into the intermembrane space. These protons return to the matrix through the ATP-proton channel of ATP synthase. The gross contribution of oxidative phosphorylation to the bioenergetics of body cells under aerobic conditions is significantly greater than the total contribution of other pathways of ATP synthesis (substrate phosphorylation, creatine kinase and adenylate kinase reactions). OP is a coupled and interdependent process of two types of chemical transformations in mitochondria: electron transfer from the initial substrates (NADH and succinate) to oxygen and ATP synthesis due to the energy of electron transport. The P:O or ADP:O ratios are usually used as a quantitative measure of OP that express the number of high-energy phosphates (i.e. amount of ATP) synthesized per an atom of oxygen consumed, or per a mole of water produced. If the OP process is reproduced in vitro in an artificial incubation medium, this coefficient should be equal to 3 for NAD-dependent substrates or 2 for succinate. However, under real in vivo conditions, such "ideal" coupling does not take place, since the energy of electron transport in

mitochondria is used not only for ATP synthesis, but also for heat generation. The latter is a very important function of homoiothermic animals and humans. In addition to ATP synthesis and heat production mitochondrial respiration is involved in the production of reactive oxygen species (superoxide anion and hydrogen peroxide), which play an important signaling role in body cells and control the phase state of membranes and the rate of renewal of their structural components. [1, 2]. However, excessive production of free radicals in mitochondria leads to damage to mitochondrial membranes, opening of the pore of variable permeability (permeability transition pore), desorption of cytochrome c from the inner membrane, release and activation of proteolytic enzymes (procaspases), and triggering the process of mitoptosis (swelling and termination of all functions of mitochondria). Mitoptosis is a key event the final result of which is cell apoptosis. If a critical number of cells undergoes apoptosis, organoptosis (death of the organ) develops and, eventually, it results in phenoptosis (death of the body) [3,4,5].

### UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION.

#### Classification and mechanism of action

The mitochondrial respiratory chain not only transports electrons to oxygen, but also forms a proton concentration gradient ( $\Delta pH$ ) on both sides of the inner mitochondrial membrane (proton-motive force) which is an integral part of the proton membrane potential ( $\Delta \mu H^+$ ).

$$\Delta \mu H^+ = \Delta \psi + z \Delta pH,$$

where  $z$  is the number of millivolts of the proton membrane potential which corresponds to a change in the pH gradient per unit. The energy of this potential is used to perform a number of endergonic functions, such as heat generation, transport of substances across the inner membrane, production of reactive oxygen species (ROS), which are used either to perform signaling functions or to remove damaged mitochondria [6, 7]. Heat production in mitochondria is induced by physiological OP uncouplers. These substances activate mitochondrial respiration without directly affecting the activity of the respiratory chain enzyme complexes and without interacting with ATP synthase but reduce the rate of ATP synthesis. In this case the proportion of free energy of electron transport which is converted into heat increases.

The action of all OP uncouplers, both of endogenous and exogenous origin, has been well studied. It is directed not to any enzyme systems, but to the lipid bilayer of the inner mitochondrial membrane. Depending on the mechanism of their effect, uncouplers are divided into 3 classes:

- protonophores,
- ionophores,
- detergents.

Uncouplers of the first class, dissolving in the lipid bilayer of the inner mitochondrial membrane, make it permeable to protons and induce proton "leak" from the intermembrane space back into the matrix. This leads to a decrease or to the complete disappearance of the proton membrane potential. The latter makes impossible synthesis of ATP in the active center of ATP synthase. A classic example of this class of uncouplers is 2,4-dinitrophenol (DNP) which was the first to be discovered among protonophores. It dramatically stimulates mitochondrial respiration and completely blocks ATP synthesis. At present, much more effective uncouplers

are widely used in experimental biochemistry, for example, carbonyl cyanide derivatives of phenylhydrazine (FCCP, CCCP), fluorine-containing polycyclic structures (BAM15), and other substances that can cause a powerful uncoupling effect at micromolar concentrations [8]. Uncouplers of the second class induce not proton, but ionic conductivity for such ions as potassium, sodium, calcium, phosphonium, etc. In this case, the proton concentration gradient on both sides of the membrane is not changed, but the electric charge (proton-motive force) decreases or completely disappears. It becomes impossible for protons to enter the active center of ATP synthase, and, consequently, ATP synthesis decreases or completely stops. Uncouplers of the third class are surfactants that cause destruction of the lipid bilayer of the inner mitochondrial membrane, which leads to the loss of its electrical insulating properties, equalization of the concentrations of all low molecular weight substances, and inorganic ions on both sides, therefore the ATP synthesis disrupts completely. The uncouplers used in experimental biochemistry are, as a rule, very toxic compounds, and therefore they are either not used as medicines, or they are tried to be used at very low concentrations [9,10]. On the other hand, some medicinal compounds used in clinical practice are able to uncouple OP, and this is their toxic side effect [11,12].

Physiological uncouplers of OP cause the so-called "mild" uncoupling, in which there is no complete stop of ATP synthesis. Therefore, they can only belong to the first or second class. The beneficial effect of mild uncoupling primarily involves thermogenesis, i.e., the production of heat to maintain a constant body temperature regardless of the ambient temperature. In addition, mild uncouplers can reduce the level of free radicals in mitochondria, preventing the development of mitoptosis as the initial stage of apoptotic cell death [13]. What substances can actually cause the effect of mild uncoupling?

First of all, they should be divided into endogenous and exogenous. The latter can be subdivided into substances of natural origin and artificially synthesized compounds.

### **ENDOGENOUS SOFT UNCOUPLERS OXIDATIVE PHOSPHORYLATION**

Complete uncoupling of OP is detrimental to mitochondria and cells due to a sharp drop in the level of ATP and disruption of all energy-dependent metabolic processes and functions. However, as it was found out relatively recently, the absolutely complete coupling of oxidative phosphorylation is also very unfavorable because heat production is disturbed and free oxygen radicals accumulate which can initiate cell apoptosis program. The respiration of isolated mitochondria in vitro is accompanied by the generation of an inner membrane proton potential ( $\mu\text{H}^+$ ) of the order of 200 mV. The energy of this potential is used for the synthesis of ATP [14, 15]. However, at the same time, the production of free radicals by respiring mitochondria progressively increases. If the proton membrane potential reaches values of 150 mV or more, then the production of ROS increases sharply and non-linearly, that is associated with the risk of mitoptosis and subsequent apoptosis [16]. Therefore, in vivo, intact cell mitochondria should contain regulatory factors that limit the degree of OP coupling and prevent the development of cell death. Such factors include mild uncouplers of oxidative phosphorylation [17–20]. These uncouplers reduce the production of free radicals in mitochondria, reducing the proportion of  $\mu\text{H}^+$  energy that is used for ATP synthesis. However, this is not accompanied by a violation of cell bioenergetics, since these substances activate mitochondrial biogenesis, which compensates for the decrease in ATP production by each mitochondrion [21]. The first substances thought to perform this function in the whole organism were free (non-esterified)

fatty acids (FFAs). It has been shown that, depending on the concentration and structure, FFA molecules can have a protonophoric and detergent-like effect, as well as affect the packaging of membrane phospholipids. It could be assumed that non-dissociated molecules of higher fatty acids, due to the high degree of hydrophobicity of their radicals, penetrate into the mitochondrial matrix along the concentration gradient. Inside the matrix, they dissociate with the release of the  $H^+$  ion, thus increasing their matrix concentration. As a result, the proton membrane potential decreases (Fig. 1).

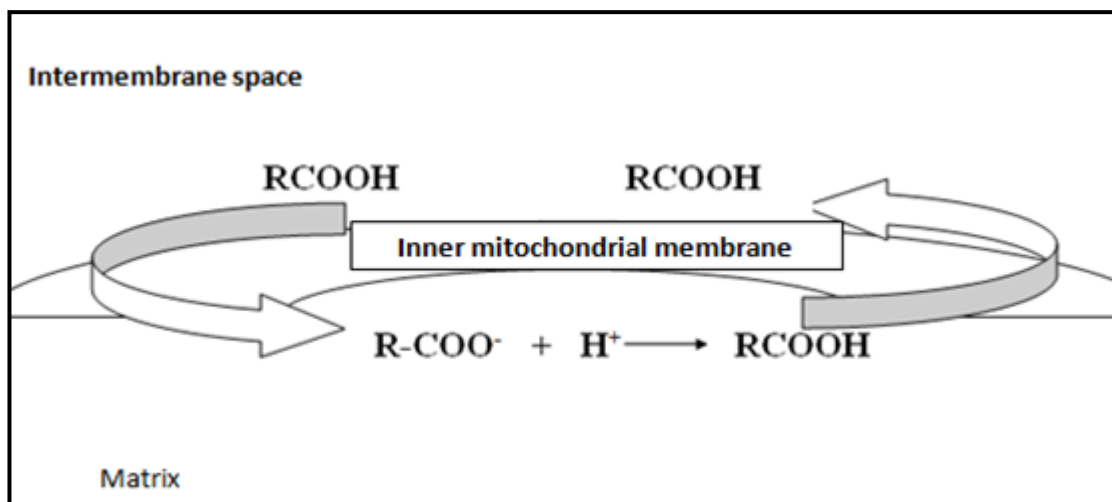


**Fig. 1: Diffusion mechanism of the uncoupling action of fatty acids**

However, such a simplified character of fatty acid action can not describe the real mechanism of their influence on the coupling of OP. For the transport of fatty acids into mitochondria, carrier molecules are certainly needed, for example, carnitine and related acyltransferases I and II. A carnitine-independent cyclic mechanism of fatty acid transport, accompanied by a decrease in the proton membrane potential, was proposed by V.P. Skulachev in 1991 [22]. This mechanism included the participation of thermogenin (UCP1) and ATP/ADP antiporter as proteins facilitating the movement of fatty acid anions across the inner mitochondrial membrane. It increases the nonspecific energy expenditure of the body and contributes to weight loss. There is still no unanimous idea about the details of the mechanism of participation of UCP(s) in the implementation of the uncoupling effect of fatty acids. The following facts are now firmly established: 1. Uncoupling proteins are not able to induce membrane proton conductivity in the absence of fatty acids, just as fatty acids cannot perform an uncoupling action without the participation of inner membrane transport proteins. 2. These proteins can carry out the transport of inorganic anions, for example, GDP-dependent transport of  $Cl^-$  ions. However, according to some authors, such a transfer does not play any physiological role (the case when there is a “dog that does not bark”) [23]. It is assumed that uncoupling proteins form a channel for the transfer of undissociated fatty acid molecules into the mitochondrial matrix along a concentration gradient (FA shuttling mechanism), as well as groups temporarily binding anionic forms of fatty acids for their electrophoretic return to the intermembrane space (FA cycling).

In general, it can be concluded that the only statement has been proven - that UCP(s) cause a physiologically important mild uncoupling of OP and reduce the level of ROS in mitochondria

in the presence of fatty acids. In the genome of humans and animals, 5 types of genes have been found that are most highly expressed in the cells of the following tissues:

**Table 1: Distribution of UCP genes in body tissues**

| UCP gene | Tissue cells                                                                                                                                           |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| UCP1     | brown adipose tissue, beige adipocytes, T-lymphocytes                                                                                                  |
| UCP2     | ubiquitously: kidneys, liver, spleen, gastrointestinal tract, brain, stem cells, skeletal muscle, pancreas, adipose tissue, tumor cells, T-lymphocytes |
| UCP3     | skeletal and smooth muscles, heart, thymus                                                                                                             |
| UCP4     | brain, neuroblastoma, cartilage, stem cells                                                                                                            |
| UCP5     | brain                                                                                                                                                  |

The functions of different types of UCP(s) seem to be unequal. So the UCP1 protein serves to generate heat in brown adipocytes, but the role of the other proteins is rather not heat production, but protecting against excess of free radicals.

Some types of “classic” hormones have an uncoupling effect. First of all, these are catecholamines of the adrenal medulla and iodothyronines of the thyroid gland. Initially, it was assumed that these hormones are classical protonophores, since they, like 2,4-dinitrophenol, have a hydrophobic phenolic ring capable of weak dissociation with the release of a proton. Indeed, if any of these hormones is added to respiring mitochondria in vitro, the uncoupling effect can be registered as increasing the rate of respiration and reducing the P:O (ADP:O) ratio. However, this effect is observed only at concentrations several orders of magnitude higher than the physiological level of these hormones in the blood. As has now been clarified, their uncoupling effect observed in vivo is not direct but is mediated through several factors. Catecholamines, as well as thyroid hormones acting through the synthesis of catecholamines, activate hormone-sensitive adipocyte triglyceride lipase. This effect is accompanied by an increase in the concentration of fatty acids in the blood, which, in turn, have an indirect uncoupling effect mediated by UCP proteins. On the other hand, catecholamines are able to activate the expression of the UCP1 gene through a cascade mechanism that includes cAMP, protein kinase A, p38 kinase, and transcription factors of this gene [24,25]. Based on these data, it is possible to understand why chronic stress or hyperthyroidism result in body mass loss. Relatively recently, it was found that bile acids are the most important regulators of OP coupling [26–29]. The detergent uncoupling effect of bile acids observed in vitro cannot be manifested in vivo, since the concentration of bile acids in biological fluids and tissues is very low.

Moreover, bile acids circulating in the bloodstream are mainly associated with albumin. Primary bile acids (cholic and chenodeoxycholic) are synthesized in the liver from cholesterol, secreted into the biliary system and enter the small intestine. It is well known that bile acids are amphiphilic surfactants that act as dietary fat emulsifiers, pancreatic lipase activators, and stabilizers of complex micelles consisting of lipid digestion products, cholesterol, fat-soluble vitamins, and other substances. In addition, bile acids are involved in the elimination of toxic substances and xenobiotics from the body [30]. The gut microbiome transforms bile acids with chemical modification, mainly reduction with the formation of deoxy forms [31]. A part of the bile acids can be reabsorbed and circulate within the enterohepatic cycle. When returning to the liver, bile acids undergo conjugation with glycine and taurine [32]. In this case, a small

proportion of bile acids can enter the systemic circulation. As it turned out in recent years, these bile acids have the functions of steroid hormones that can bind to a number of specific tissue receptors [33, 34]. The secondary bile acids that are dehydroxylated by the enzymes of intestinal bacteria and are the strongest signaling molecules [35]. The main receptors for bile acids are:

- G protein-coupled membrane receptors (TGR5, also known as GPBAR1, M-BAR and BG37),
- nuclear receptors such as the farnesoid X receptor (FXR),
- vitamin D receptor,
- pregnane-X-receptor and a number of others [36, 37].

What responses arise as a result of the interaction of bile acids with these receptors? First of all, this is an increase in nonspecific energy expenditure of the body as a result of mild uncoupling of oxidative phosphorylation [38, 39]. The hormonal signal from bile acids leads to the induction of UCP1 in brown adipocytes. The consequence of this is an increase in energy expenditure and a decrease in fat reserves in white adipose tissue [40]. In general, the regulatory effects of bile acids, shown in Fig. 2 can be characterized as preventing the development of obesity, type 2 diabetes mellitus, metabolic syndrome, and inflammation of adipose tissue, usually recorded in obesity [26, 33].

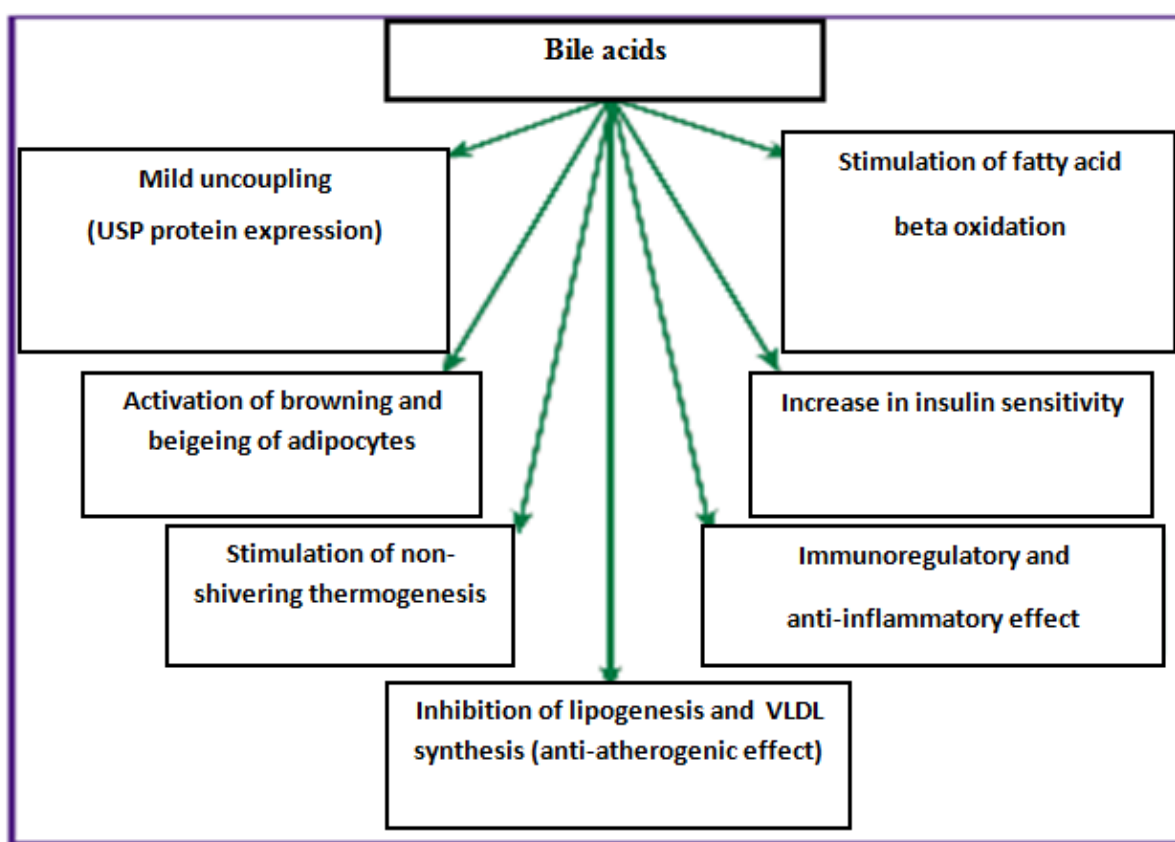
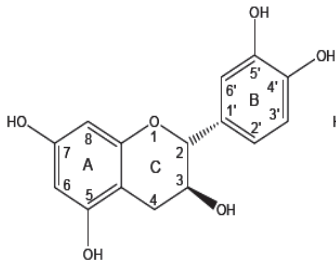
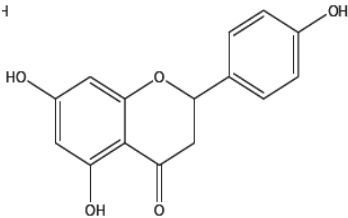
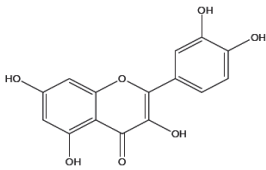
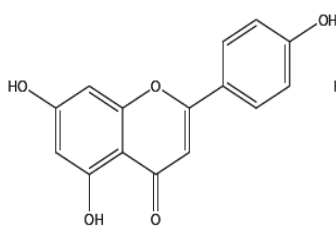
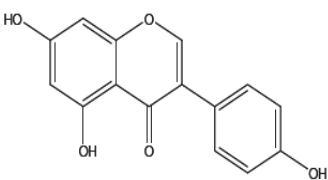


**Fig. 2: Main regulatory effects of bile acids**

## **EXOGENOUS SUBSTANCES THAT INDUCE MILD UNCOUPLING OF OXIDATIVE PHOSPHORYLATION**

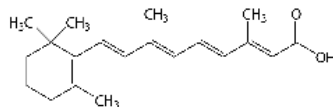
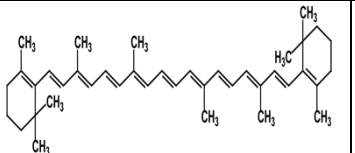
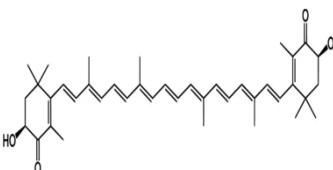
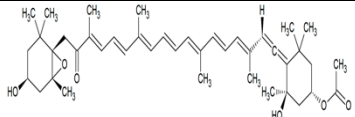
Natural food products contain a number of substances that can cause the activation of thermogenesis and a decrease in the level of free radicals as a result of the mild uncoupling of OP. These substances include long chain polyunsaturated fatty acids. The metabolic effects and mechanism of the regulatory effect of dietary fatty acids are no different from those of endogenous fatty acids presented in Section 3 above. Other food components, mainly of plant origin, include a group of polyphenols called bioflavonoids (Table 2). These substances have several phenolic rings in their structure; therefore, it could be assumed that their thermogenic and antiradical action is a consequence of a direct uncoupling effect on mitochondria [41, 42]. However, it has been clearly shown in many scientific publications that their uncoupling effect is still mostly not direct, but includes either the induction of uncoupling proteins (UCP) or is aimed at changing the nature of differentiation of preadipocytes towards brown forms of adipose tissue cells [44, 45, 46, 47].

**Table 2: The most common bioflavonoids, inducing mild uncoupling OP**

| Substance              | Structure                                                                           | Foodstuffs                                     | Reference |
|------------------------|-------------------------------------------------------------------------------------|------------------------------------------------|-----------|
| Flavenol (catechin)    |    | cocoa beans, apples, grape seeds, red wines    | [43]      |
| Flavanone (naringenin) |    | citrus fruits                                  | [47]      |
| Flavonol (Quercetin)   |   | Flavonol (Quercetin)                           | [48]      |
| Flavon (apigenin)      |  | Chamomile, parsley, onion, grapefruit, oranges | [49]      |
| Isoflavone (genistein) |  | Soya beans                                     | [45]      |

Of the group of non-flavonoid polyphenols with a mild uncoupling effect, resveratrol should be noted. It is present in the roots of white hellebore (*Veratrum grandiflorum*) and in various food products such as grapes, berries, red wine and nuts [51]. Retinoids (vitamin A derivatives) and carotenoids ( $\beta$ -carotene and its analogues) have a similar effect (Table 3). Due to their mild uncoupling effect, these substances and foodstuffs containing them can be used to prevent obesity and cardiovascular diseases [55].

**Table 3: Vitamin A derivatives inducing mild OP uncoupling**

| Substance                 | Structure                                                                          | Foodstuffs                                         | Reference |
|---------------------------|------------------------------------------------------------------------------------|----------------------------------------------------|-----------|
| Retinol and Retinoic acid |   | Fish oil, liver, dairies                           | [51,52]   |
| $\beta$ -carotene         |   | Vegetables and fruits that are orange-red in color | [54]      |
| Astaxanthin               |   | Vegetables and fruits that are orange-red in color | [53]      |
| Fucoxanthin               |  | brown algae                                        | [54]      |

In 2014, a group of scientists from the Fukushima Medical University (Janonia) isolated a new effective uncoupling agent from slime mold (*Dictyostelium discoideum*) cells [55]. This substance with the abbreviation Ppc-1 is a substituted nitrogen-containing heterocycle with the following structure (Fig. 3):

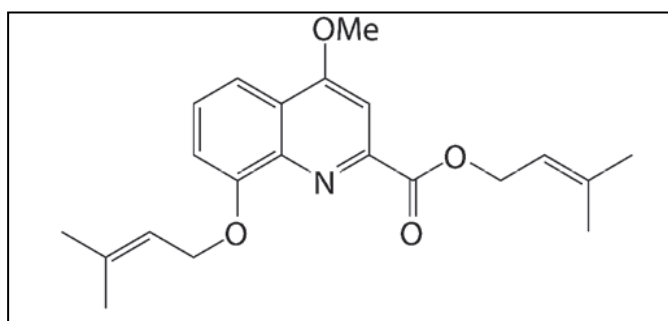


**Fig. 3: Structure of Ppc-1**

According to the results of the authors' studies, Ppc-1 stimulated oxygen consumption in mitochondria without an inhibitory effect on ATP production. Administration of Ppc-1 to mice inhibited weight gain with no apparent side effects. The level of free fatty acids in the serum of animals treated with Ppc-1 increased significantly while the body fat content was relatively low. Ppc-1 stimulated cultured adipocytes by releasing fatty acids into the incubation medium, which, according to the authors, may be due to an increase in serum fatty acid levels in mice

treated with Ppc-1. It is proposed that Ppc-1 is a unique uncoupling agent useful as a molecular probe for research and possibly a promising agent for the treatment of obesity.

### ARTIFICIALLY SYNTHESIZED SOFT UNCOUPLERS

Attempts to create mild uncouplers for the prevention and treatment of obesity, cardiovascular, endocrine and oncological diseases by artificial synthesis have been repeatedly undertaken by many researchers over the past 20 years. They were based on the principle of covalent binding of some lipophilic cation penetrating into mitochondria with a substance with protonophor properties. Rhodamine, natural and synthetic mitochondria-targeted peptides, and nanoparticles with desired characteristics were used as penetrating cations. However, alkylated triphenylphosphonium became the most popular as a lipophilic penetrating cation [56, 57, 58]. It was not always possible to obtain the desired effect of mild uncoupling. So in the laboratory of Michael Murphy, 2,4-DNP was combined with the triphenylphosphonium cation. However, the attempt was unsuccessful, since the uncoupling effect of DNP was completely lost due to the impossibility of removing the dissociated form of DNP from the mitochondrial matrix [59]. The most effective mild uncouplers based on triphenylphosphonium were obtained in the laboratory of V.P. Skulachev. They received the official name "Skulachev ions" (SkQ). For example, the SkQ1 ion was synthesized based on decyltriphenylphosphonium and plastoquinone [60]. The structure of this penetrating ion is shown in Fig. 4. Triphenylphosphonium imparts the properties of a penetrating cation to this ion, and plastoquinone (a plant analogue of the animal ubiquinone) determines the high degree of specificity of SkQ1 binding with the mitochondria of human and animal tissue cells.

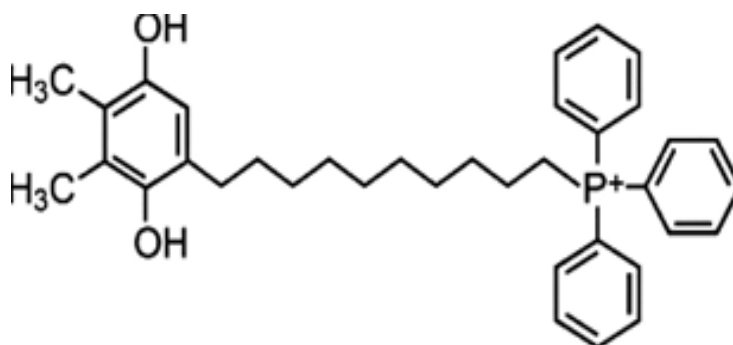


Fig. 4: Structure of the SkQ1 ion

The property of Skulachev ions to soft uncoupling OPs and reduce the level of ROS in mitochondria determines the ability of these ions to prolong the life of experimental animals, plants, fungi, insects, and mammals, as well as to inhibit the development of aging manifestations. and pathological conditions that have a causal relationship with the aging of the body [ 61]. Skulachev ions have a pronounced nephro- and neuroprotective effect [62, 63]. In recent years, the first drugs based on Skulachev ions have been developed, clinically tested and introduced into practical healthcare. These drugs are used to treat eye diseases [64], wounds, and skin burns [65].

### PHYSIOLOGICAL ACTIVATORS OF MILD UNCOUPLING

Mild uncoupling of OP is effectively induced by mechanical loading of animal and human muscle tissue. The uncoupling effect is observed in both white and brown adipose tissue. Therefore, in

accordance with modern concepts, the effect of physical training on weight loss is associated not only with the energy consumption of the muscle tissue itself, but also with the restructuring of adipose tissue. Systematic muscle training activates the differentiation of preadipocytes towards brown adipocytes (adipocyte browning), and thus increases the breakdown of fat with the expenditure of energy for heat production in the body [66, 67, 68]. Adipocyte browning activation can also be increased by exposure to cold [69]. It has been established that thermogenesis not associated with shivering can be mediated by the recently identified transcriptional activator Zfp516, which stimulates the transcription of the UCP1 gene in adipocytes. It has been suggested that this activator can be used therapeutically for the treatment of obesity [70].

## CONCLUSION

This review summarizes information about the chemical structure of substances that can either directly change the OP coupling by different mechanisms of mild uncoupling or induce this effect through appropriate mediating mechanisms. Mild uncouplers increase the body's nonspecific energy expenditure (thermogenesis) and reduce the production of free radicals in the mitochondria of body tissues. Excessive production of free radicals, the inadequately high level of lipid peroxidation, the development of oxidative stress are the most important elements in the pathogenesis of a number of very common forms of pathology. Therefore it can be assumed that mild uncouplers will be used as drugs for the prevention and treatment of many pathological states in the very near future (Fig. 5) [71-76].

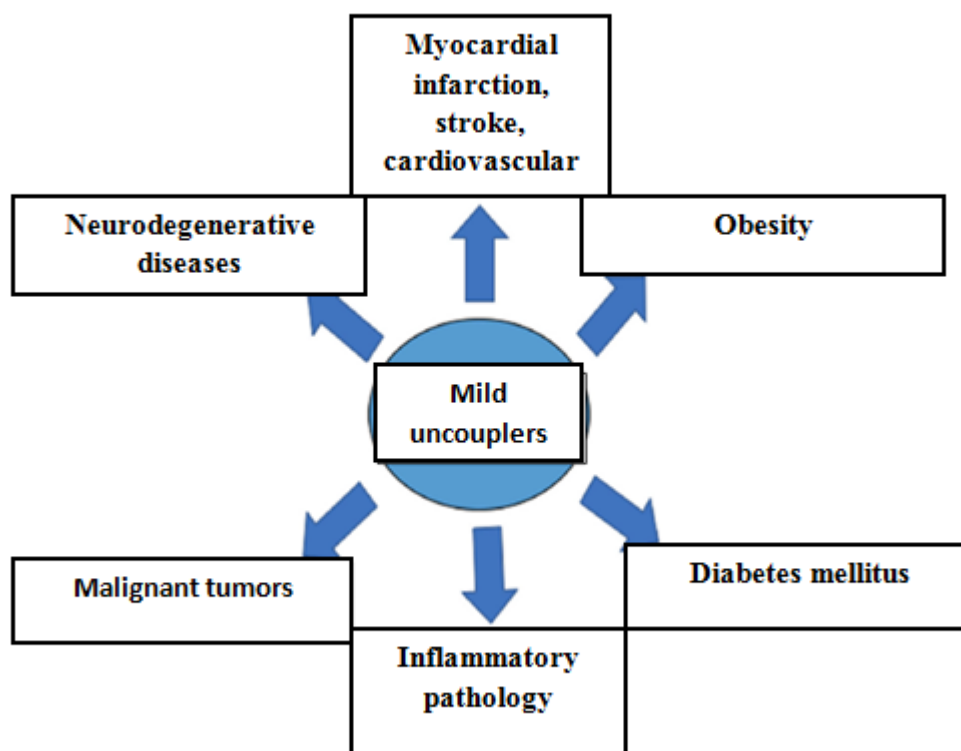


**Fig. 5: Prospects on use mild uncouplers as medicines**

The prospect of using soft uncouplers seems to be especially important for the prevention and treatment of cardiac pathology, since the heart consumes more oxygen than any other organ of the body per unit mass. Mitochondria of cardiomyocytes occupy 25% of the volume of these cells, and OF is practically the only way of ATP synthesis. Myocardial ischemia, which develops with heart attack and angina pectoris, sharply increases the level of free radicals in the mitochondria of cardiomyocytes. Therefore, gentle uncoupling contributes to the elimination of oxidative stress. The catchphrase “uncoupling to survive” that has become popular reflects the essence of this approach to the treatment of heart diseases [77].

## References

1. Ježek P., Holendová B., Plecitá-Hlavatá L. Redox Signaling from Mitochondria: Signal Propagation and Its Targets. *Biomolecules*. 2020; 10(1): 93. Published online 2020 Jan 6. doi: 10.3390/biom10010093
2. Venditti P., and Di Meo S. The Role of Reactive Oxygen Species in the Life Cycle of the Mitochondrion. *Int J Mol Sci*. 2020; 21(6): 2173. Published online 2020 Mar 21. doi: 10.3390/ijms21062173
3. Swerdlow N.S., and Wilkins H.M. Mitophagy and the Brain. *Int J Mol Sci*. 2020; 21(24): 9661. Published online 2020 Dec 18. doi: 10.3390/ijms21249661
4. Venditti P., and Di Meo S. The Role of Reactive Oxygen Species in the Life Cycle of the Mitochondrion. *Int. J. Mol. Sci*. 2020; 21:2173 doi: 10.3390/ijms21062173
5. Lomonosova, E., Ryerse J., and Chinnadurai G. BAX/BAK-Independent Mitoptosis during Cell Death Induced by Proteasome Inhibition? *Mol Cancer Res*. 2009; 7(8): 1268–84 doi: 10.1158/1541-7786.MCR-08-0183
6. Berrya, B.J., et al., Use the protonmotive force: mitochondrial uncoupling and reactive oxygen species. *J Mol Biol*. 2018 October 19; 430(21): 3873–3891 doi: 10.1016/j.jmb.2018.03.025
7. Zorova, L.D., et al., Mitochondrial membrane potential. *Anal Biochem*. 2018; 1,552:50-59 doi: 10.1016/j.ab.2017.07.009
8. Khailova, L.S., et al., Interaction of Potent Mitochondrial Uncouplers with Thiol-Containing Antioxidants. *Antioxidants (Basel)* 2019 Jun; 8(6): 194. Published online 2019 Jun 23. doi: 10.3390/antiox8060194
9. Geisler, J.G., et al., DNP, Mitochondrial Uncoupling and Neuroprotection: A Little Dab'll Do Ya. *Alzheimers Dement*. 2017; 13(5): 582–591 doi: 10.1016/j.jalz.2016.08.001
10. Geisler, J.G. 2,4 Dinitrophenol as Medicine. *Cells* 2019, 8, 280; 1-36 doi: 10.3390/cells8030280
11. Pozdnyakov D.I. 4-Hydroxy-3,5-di-tret-butyl cinnamic acid restores the activity of the hippocampal mitochondria in rats under permanent focal cerebral ischemia. *Iran J Basic Med Sci*. 2021 Nov; 24(11): 1590–1601. doi: 10.22038/IJBMS.2021.58435.12979
12. Zholobenko, A.V., et al., On the causes and consequences of the uncoupler-like effects of quercetin and dehydrosilybin in H9c2 cells. *PLOS ONE*. 2017; <https://doi.org/10.1371/journal.pone.0185691> October 4, 1-22
13. Skulachev, V.P. Cationic antioxidants as a powerful tool against mitochondrial oxidative stress. *Biochem Biophys Res Commun*. 2013; 15;441(2):275-9
14. Severina F.P., et al., Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. *PNAS* January 12, 2010; 107, 2, 663–668 doi: 10.1073/pnas.0910216107
15. Ardalan, A., Smith, M.D., Jelokhani-Niaraki M. Uncoupling Proteins and Regulated Proton Leak in Mitochondria. *Int. J. Mol. Sci*. 2022; 23, 1528. <https://doi.org/10.3390/ijms23031528>
16. Plotnikov, E.Y., et al., A new generation of Skulachev ions possessing apparent nephro- and neuroprotective properties. *Biochemistry (Mosc)*. 2010; 75 (2):177-85
17. Tejerina1, S., et al., Mild mitochondrial uncoupling induces 3T3-L1 adipocyte de-differentiation by a PPARγ-independent mechanism, whereas TNFα-induced de-differentiation is PPARγ dependent. *J Cell Sci* 2009; 122:, 145-155

18. Urrea, F.A., et al., FR58P1a; a new uncoupler of OXPHOS that inhibits migration in triple-negative breast cancer cells via Sirt1/AMPK/ $\beta$ 1-integrin pathway. *Scientific Reports*. 2018; 8:13190 | doi:10.1038/s41598-018-31367-9
19. Jaburek, M., et al., Antioxidant Synergy of Mitochondrial Phospholipase PNPLA8/iPLA2 $\gamma$  with Fatty Acid-Conducting SLC25 Gene Family Transporters. *Antioxidants*. 2021; 10: 678. <https://doi.org/10.3390/antiox10050678>
20. Ruiz-Ramírez, A., et al., Cell Death and Heart Failure in Obesity: Role of Uncoupling Proteins. *Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity* Volume 2016, Article ID 9340654, 11 pages <http://dx.doi.org/10.1155/2016/9340654>
21. Zorov, D.B., et al., Neuroprotective Potential of Mild Uncoupling in Mitochondria. *Pros and Cons. Brain Sci*. 2021, 11, 1050. <https://doi.org/10.3390/brainsci11081050>
22. Skulachev, V.P., Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. *FEBS Lett*. 1991; 294: 158–162
23. Jezek, P., et al., Mitochondrial Uncoupling Proteins: Subtle Regulators of Cellular Redox Signaling. *Antioxidants & Redox signaling*. 2017; 29, 7: 667-714 doi: 10.1089/ars.2017.7225
24. Maslov, L.N., Vychuzhanina, E.A., Role of sympatho-adrenomedullar system in adaptation to cold [Article in Russian]. *Russ Fiziol Zh Im I M Sechenova*. 2015; 101(2):145-62.
25. Demine, S., Renard, P., and Arnould, T., Mitochondrial Uncoupling: A Key Controller of Biological Processes in Physiology and Diseases. *Mitochondrial Uncoupling: A Key Controller of Biological Processes in Physiology and Diseases. Cells*. 2019; 8, 795 doi: 10.3390/cells8080795
26. Teodoro, J.S., et al., J. Enhancement of brown fat thermogenesis using chenodeoxycholic acid in mice. *Int J of Obesity*. 2014; 1–8
27. Zietak, M., and Kozak L.P., Bile acids induce uncoupling protein 1-dependent thermogenesis and stimulate energy expenditure at thermoneutrality in mice. *Am J Physiol Endocrinol Metab*. 2016; 310(5): 346–54 doi: 10.1152/ajpendo.00485.2015
28. Chiang, J.Y.A., Bile Acid Metabolism and Signaling. *Compr Physiol*. 2013; 3(3): 1191–1212
29. Tiangang, Li, Chiang J.Y.A., Bile acids as metabolic regulators. *Curr Opin Gastroenterol*. 2015; 31(2): 159–165 doi: 10.1097/MOG.0000000000000156
30. Chiang, J. Y. L., Bile acid metabolism and signaling. *Compr Physiol*. 2013 July ; 3(3): 1191–1212
31. Jones, M.L., et al., The human microbiome and bile acid metabolism: dysbiosis, dysmetabolism, disease and intervention. *Expert Opin. Biol. Ther*. 2014; 14(4): 467-82
32. Li, T., Chiang, J. Y. L., Bile acids as metabolic regulators. *Curr Opin Gastroenterol*. 2015; 31(2): 159–65
33. Zagoskin, P.P., and Erlykina, E.I., Bile Acids as a New Type of Steroid Hormones Regulating Nonspecific Energy Expenditure of the Body (Review). *Sovrem Tekhnologii Med*. 2021; 12, 5: 114-28. doi: 10.17691/stm2020.12.5.13
34. Quad de Aguiar, T.V., and Edwards, P.A., Bile Acids Have the Gall to Function as Hormones. *Cell Metabolism*. 2009; 10, 2: 162-64
35. Joyce, S.A., Gahan, C.G.M., Disease-Associated Changes in Bile Acid Profiles and Links to Altered Gut Microbiota. *Dig Dis* 2017; 35:169–177
36. D'Amore, C., et al., Design, Synthesis, and Biological Evaluation of Potent Dual Agonists of Nuclear and Membrane Bile Acid Receptors *J. Med. Chem*. 2014; 57: 937–54
37. Krasowskia, M.D., Nib, A., Hageyc, L.R., and Ekins, S., Evolution of promiscuous nuclear hormone receptors: LXR, FXR, VDR, PXR, and CAR. *Mol Cell Endocrinol*. 2011; 334(1-2): 39–48.
38. Cong, Xie,, et al., Role of Bile Acids in the Regulation of Food Intake, and Their Dysregulation in Metabolic Disease. *Nutrients* 2021, 13, 1104. <https://doi.org/10.3390/nu13041104>

39. Zagoskin, P.P., Nonspecific Energy Expenditure of the Body and Body Mass Regulation. *J Nutri Bio.* 2019; 5(1): 328-49
40. Pournaras, D. J., and le Roux, C. W., Are Bile Acids the New Gut Hormones? Lessons From Weight Loss Surgery Models. *Endocrinology.* 2013; 154(7): 2255–56
41. de Pascual-Teresa, S., Moreno, D.A., and García-Viguera, C., Flavanols and Anthocyanins in Cardiovascular Health: A Review of Current Evidence. *Int. J. Mol. Sci.* 2010; 11: 1679-1703
42. Surai, P.F., Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. *Antioxidants.* 2015; 4: 204-247
43. Ishii, Y., et al., Repeated Oral Administration of Flavan-3-ols Induces Browning in Mice Adipose Tissues through Sympathetic Nerve Activation. *Nutrients* 2021; 13, 4214. doi.org/10.3390/nu13124214
44. Xuejun, Zhang., et al., Flavonoids as inducers of white adipose tissue browning and thermogenesis: signalling pathways and molecular triggers. Zhang et al. *Nutrition & Metabolism* 2019; 16:47 doi.org/10.1186/s12986-019-0370-7
45. Aziz, S.A., et al., Metabolic programming of a beige adipocyte phenotype by genistein. *Mol. Nutr. Food Res.* 2017; 61, 2: 1600574
46. Hye Won Kang, Sang Gil Lee, Otieno D., and Kyoungsoo Ha., Flavonoids, Potential Bioactive Compounds, and Non-Shivering Thermogenesis. *Nutrients* 2018; 10: 1168
47. Rebello, C., et al., Naringenin Promotes Thermogenic Gene Expression in Human White Adipose Tissue. *Obesity (Silver Spring).* 2019; 27(1): 103–11
48. Jing Dong., et al., Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPK 1/SIRT1. *Journal of Lipid Research.* 2014; 55: 363-74
49. Un Ju Jung., Yun-Young Cho., and Myung-Sook Choi., Apigenin Ameliorates Dyslipidemia, Hepatic Steatosis and Insulin Resistance by Modulating Metabolic and Transcriptional Profiles in the Liver of High-Fat Diet-Induced Obese Mice. *Nutrients.* 2016; 8(5): 305.
50. Aguirre, L., et al., Resveratrol: Anti-Obesity Mechanisms of Action. *Mol.* 2014; 19: 18632-55
51. Rai, S.N., et al., The Role of Vitamins in Neurodegenerative Disease: An Update. *Biomedicines.* 2021; 9, 1284. doi.org/10.3390/biomedicines9101284
52. Blaner, W.S., Vitamin A Signaling and Homeostasis in Obesity, Diabetes, and Metabolic Disorders. *Pharmacol Ther.* 2019; 197: 153–78.
53. Nishida, Y., et al., Astaxanthin as a Novel Mitochondrial Regulator: A New Aspect of Carotenoids, beyond Antioxidants. *Nutrients* 2022; 14, 107. doi.org/10.3390/nu14010107
54. Gammone, M.A., Riccioni, G., and D’Orazio, N., Carotenoids: potential allies of cardiovascular health? *Food & Nutrition Research.* 2015; 59: 26762 - doi.org/10.3402/fnr.v59.26762
55. Toshiyuki Suzuki, et al., Weight Loss by Ppc-1, a Novel Small Molecule Mitochondrial Uncoupler Derived from Slime Mold. *PLOS ONE.* 2015; February 10| DOI:10.1371/journal.pone.0117088
56. Zielonka, J., et al., Mitochondria-Targeted Triphenylphosphonium-Based Compounds: Syntheses, Mechanisms of Action, and Therapeutic and Diagnostic Applications. *Chem Rev.* 2017 August 09; 117(15): 10043–10120
57. Apostolova, N., and Victor, V.M., Molecular Strategies for Targeting Antioxidants to Mitochondria: Therapeutic Implications. *Antiox Redox Signal.* 2015 Mar 10; 22(8): 686–729
58. Sharma, A., et al., Targeting Mitochondrial Dysfunction and Oxidative Stress in Activated Microglia using Dendrimer-Based Therapeutics. 2018; 8(20): 5529-5547. doi: 10.7150/thno.29039
59. Blaikie Frances, H., et al., Targeting Dinitrophenol to Mitochondria: Limitations to the Development of a Self-limiting Mitochondrial Protonophore. *Biosci Rep* (2006) 26:231–43

60. Severin, F.F., et al., Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. *Proc Natl Acad Sci U S A*. 2010; 107(2): 663–68
61. Anisimov, V.N., et al., Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. *Biochemistry (Mosc)*. 2008; 73,12: 1655 –70
62. Plotnikov, E.Y., et al., A new generation of Skulachev ions possessing apparent nephro- and neuroprotective properties. *Biochemistry (Mosc)*. 2010; 75 (2):177-85
63. Isaev, N.K., et al., Alzheimer's Disease: An Exacerbation of Senile Phenoptosis. *Biochemistry (Mosc)*. 2015;;80(12):1578-81. doi: 10.1134/S0006297915120056.
64. Korshunova, G.A., Shishkina, A.V., and Skulachev, M.V., Design, Synthesis, and Some Aspects of the Biological Activity of Mitochondria-Targeted Antioxidants. *Biochemistry (Mosc)*. 2017; Jul;82(7):760-777. doi: 10.1134/S0006297917070021.
65. Stanford, K.I., and Goodyear, L.J., Exercise regulation of adipose tissue. *Adipocyte*. 2016; 5(2): 153–62
66. Dewal R.S., Stanford K.I. Effects of exercise on brown and beige adipocytes. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2019; 1864(1): 71–78
67. Jae Ho Le., et al., The Role of adipose tissue mitochondria: regulation of mitochondrial function for the treatment of metabolic diseases. *Int. J. Mol. Sci*. 2019, 20, 4924; doi:10.3390/ijms20194924
68. van der Lans, A.A.J.J., et al., Cold acclimation recruits brown fat and increases nonshivering thermogenesis. *The Journal of Clinical Investigation* .Volume 123 Number 8 August 2013
69. Dempersmier, J., et al., Cold-inducible Zfp516 Activates UCP1 Transcription to Promote Browning of White Fat and Development of Brown Fat. *Mol Cell*. 2015; 22, 57(2): 235–46
70. Chen, K.Y., et al., Opportunities and challenges in the therapeutic activation of human energy expenditure and thermogenesis to manage obesity. *J. Biol. Chem*. (2020) 295(7) 1926 –42
71. Maiese, K., New Insights for Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev*. 2015; 2015: 875961. Published online 2015 May 12. doi: 10.1155/2015/875961
72. Dang, C.P., et al., BAM15, a Mitochondrial Uncoupling Agent, Attenuates Inflammation in the LPS Injection Mouse Model: An Adjunctive Anti-Inflammation on Macrophages and Hepatocytes. *Innate Immun*. 2021; 13(6): 359–75. Published online 2021 Jun 1. doi: 10.1159/000516348
73. Jurcau, A., and Ardelean, A.I., Oxidative Stress in Ischemia/Reperfusion Injuries following Acute Ischemic Stroke. *Biomedicines*. 2022; 10(3): 574. Published online 2022 Mar 1. doi: 10.3390/biomedicines10030574
74. Shrestha, R., Johnson, E., and Byrne F.L., Exploring the therapeutic potential of mitochondrial uncouplers in cancer. *Molecular metabolism*. 2021; 51: 101222 \_ 2021 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).
75. Perez-Pinzon, M.A., Stetler, R.A., and Fiskum G., Novel mitochondrial targets for neuroprotection. *J Cereb Blood Flow Metab*. 2012; 32(7): 1362–76.
76. Seyyed Hossein Hassanpour, Mohammad Amin Dehghani, and Seyyedeh Zeinab Karami., Study of respiratory chain dysfunction in heart disease. *J Cardiovasc Thorac Res*. 2018; 10(1): 1–13. Published online 2018 Mar 17. doi: 10.15171/jcvtr.2018.01