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The Effect of Taheri Consciousness Fields on ATP Production in HEK-293 Cell Line by Measuring Luciferase Activity

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Abstract

Various T-Consciousness Fields with different functions have been introduced by Mohammad Ali Taheri. Not only has the existence of these fields been investigated in the initial phase through various experiments, but also the empirical evidence of Mohammad Ali Taheri's theories, such as the existence of the "mind in the matter", has been observed. This study aimed to explore the effect of Taheri Consciousness Fields (TCFs) 1, 2, and 3 in the production process of ATP or biological energy in the human cell lines. In this experiment, a human HEK 293 cell line was used in a cell culture medium at the 24th hour of growth. The luciferase enzyme, the concentration of ATP in the sample under the influence of TCFs and control was measured and evaluated. Our findings showed that TCF 1, 2, and 3 resulted respectively in a 5, 11 and 7-fold increase in the number of ATP molecules, compared to the control samples. Due to the limited fuel cell resources and the brief treatment time (one hour) with TCFs, this increase in ATP levels cannot be attributed to the normal cytosolic and mitochondrial glucose oxidation pathways. Thus, there seems to be an alternative pathway. It is possible that TCFs influenced the ionization of water molecules in the mitochondrial intermembrane space and led to more ATP production by maintaining the proton gradient. The factor that makes this possible is nothing but the existence of a type of mind in the cells. According to Taheri's theory, transmitted information through TCFs has been received by the mind of the cells, and as a result, has led to the ionization of water molecules in the mitochondrial intermembrane space. In conclusion, it seems that regardless of the biological system, under the influence of TCFs, there is an alternative way to increase ATP at the immediate time, which is associated with an increase in information and a decrease in the entropy of the system.

Keywords: Taheri Consciousness Fields; ATP; Cell mind; Entropy; Information; Luciferase; Water ionization; Glucose oxidation

Introduction

The word consciousness is defined in different ways in science and there is no general agreement on a unified definition. One of the most popular and fundamental definitions is related to Descartes' "I think; therefore, I am" (Descartes 2006, p.73) in which animals were regarded as unconscious creatures. In the 19th century, biologists such as Darwin and Loeb studied the behavioral responses of plants to light or chemicals. Plants' behavior has been an interesting area of research. There are intelligent behavioral responses in plants; for instance, Chamovitz describes the complex capabilities of a plant to process information in "What a Plant Knows" (Chamovitz, 2017). Some do not consider plants and single-celled organisms' movements an act of intelligent behavior due to the lack of a brain and nervous system, while plants, for example, breathe without lungs, move, and digest nutrients without a stomach. Bacteria also sense and respond to their environment. Therefore, intelligent behavior capabilities in organisms should not be overlooked (LeDoux, 2020).

Living cells are responsible to do a wide variety of functions including growth, movement, reproduction, etc. and most of these functions require energy. This raises two main questions, first, how do cells receive this energy and second, how do they use this energy most efficiently? Cells, in a similar way to humans, cannot produce energy without using a source in their environment; just as humans look for materials such as fossil fuels for energy, cells also search for energy in the form of food molecules or sunlight. In fact, the sun is the ultimate source of energy for almost all cells, as photosynthetic prokaryotes, algae, and plant cells use solar energy to build complex organic food molecules that other cells rely on for energy needed to maintain growth, metabolism, and reproduction.

Finally, in the cellular mechanism formed during cell evolution, the production of usable bioenergy called ATP is a common pathway between eukaryotic cells which begins in the cytosol and ends in the mitochondria (more details are provided in the Appendix). There are several disorders in the human body associated with the improper activity of the enzyme involved in the production of ATP and in turn, its optimal production: For instance, after the presentation of various theories on the relationship between amyloidopathy and the role of Tau protein in Alzheimer's and Parkinson's disease (Tomiyama 2010). Studies have identified mitochondrial dysfunction and cellular energy deficits as the origins of these diseases (Winklhofer and Haass, 2010; Wellstead and Cloutier, 2011; Desler et al., 2018; Onyango et al., 2017; Swerdlow et al., 2017; Schägger et al., 2008; Ludtmann et al., 2018). The relationship between impaired ATP production and cardiovascular diseases, Alzheimer's disease, and Amyotrophic Lateral Sclerosis (ALS) has also been reviewed in a study (Galber et al., 2021).

In addition to the role of ATP in being an energy molecule, it is also viewed in the concept of biological information. This concept generally has different meanings. According to Shannon's definition of information, when information is increased in a system, entropy is decreased proportionally. Entropy is also a measure of the distribution and sharing of energy within a system (Shannon 1948). On the other hand, we know that in biological systems, the most useful and accessible form of bioenergy for the performance of work (quanta of useful energy) is the energy stored by glucose oxidation in ATP molecules. In fact, the increase in useful energy occurs along with the decrease in the energy available for sharing and, consequently, the decrease in the entropy of the system. Thus, according to information theory, the formation of more ATP molecules in biological systems is equivalent to an increase in useful energy, a decrease in the energy available for sharing, and in other words, a decrease in entropy. The thermodynamic calculation of the ATP production process in biological systems has also been empirically tested and confirms the reduction of entropy. The results are: (1) H^+/ATP = 4.0, independent of pH. (2) $\Delta rG'^0 = 31.3$ kJ mol^{-1} at 20 ° C, pH 8.0, pMg 2.5 and 0.08 M ion strength. (3) $\Delta rH'^0 = 28.1$ kJ mol^{-1} and $\Delta rS'^0 = -11$ JK^{-1} mol^{-1} (Pänke 1997 & Rumberg).

In addition to the thermodynamic and entropy-related interpretation of available bioenergy and in its extracellular form, ATP also acts as a neurotransmitter (information-containing molecule) in biological systems. The concept of the function of ATP and purine derivatives as extracellular signaling molecules was introduced in the late 1920s and early 1930s, when the physiological effects of adenine derivatives were discovered (Ralevic & Burnstock 1998). In the 1970s, this approach culminated in a study of purine neurotransmission mediated by specific plasma membrane receptors called purinoceptors (Burnstock 1978).

Thera are chemical and pharmacological studies in which changes in ATP production have been investigated. For example, several studies have been conducted on cancer cells and prevention of their replication (Wang et al., 2021) as well as chemical and physical methods to increase ATP production by regulating intracellular calcium (Distelmaier et al., 2009) or under the influence of magnetic fields during more than 6 hours without limitation in raw materials by about 2.5 times the enzymatic state (Buchachenko & Kuznetsov 2008). Using consciousness at the cellular level and by measuring energy production in ATP is completely novel and unprecedented.

The nature of consciousness and its place in science has received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the 1980s, Mohammad Ali Taheri introduced novel fields with non-material/non-energetic nature named Taheri Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. According to this theory, there are various TCFs with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN). The major difference between the theory of TCFs and other theoretical concepts about consciousness is related to the practical application of the TCFs. These fields can be applied to all living and nonliving creatures, including humans, plants, animals, microorganisms, materials, etc.

Mohammad Ali Taheri, the founder of Erfan Keyhani Halqeh, a school of thought, introduced a new science in 2020 as a branch of this school. He coined the term Sciencefact for this new science because it utilizes scientific investigations to prove the existence of T-Consciousness as an irrefutable phenomenon and a fact. Although science focuses solely on the study of matter and energy, Sciencefact, by contrast, explores the effects of the non-material/non-energetic TCFs; Sciencefact has provided a common ground between the two by conducting reproducible laboratory experiments in various scientific fields, and it has used the scientific approach in proving TCFs.

The influence of the TCFs begins with the connection between CCN as the Whole Consciousness of the universe and the subjects under study. This connection, called “Ettesal”, is established by Faradarmangar's mind (a certified and trained individual who has been entrusted with the TCFs). The human mind (the Announcer) has an intermediary role that initiates the Ettesal by imparting a swift and brief attention to the object under study. The observed effect is, therefore, solely the result of the TCFs affecting the system. These fields cannot be directly measured by scientific means, but it is possible to investigate their effects on various subjects through reproducible laboratory experiments (Taheri 2013).

The research methodology in the study of T-Consciousness has been founded on the process of Assumption, Argument, and Proof, in which the basic Assumption is: The Cosmos was formed by a third element called

T-Consciousness that is different from matter and energy.

The Argument: The existence of TCFs can be demonstrated by their effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, etc.)

The Proof is the scientific verification of the effects of TCFs on matter and energy (according to the Argument) through various reproducible scientific experiments.

Accordingly, to investigate and verify the existence, effects and mechanisms of TCFs, the following five research phases (Phases 0 through 4), and the aims of each phase are outlined below.

Phase-0 studies aim to prove the existence of TCFs by observing their effects. The nature of T-Consciousness and what it is will not be addressed in this phase. Phase-1 explores the varied effects of different TCFs. Phase-2 examines the reason behind the varied effects of these fields. Phase-3 investigates the mechanism of TCFs effects on matter and energy. Finally, Phase-4 draws significant conclusions, particularly with regard to the mind and memory of matter and their relation to the T-Consciousness, etc.

According to the theory of TCFs, the energy commonly consumed in life is ATP, the rate of production of which is constant and it is impossible to produce a large amount of ATP in short time; in other words, regardless of the synthesis of ATP in mitochondria, there is an alternative way to produce immediate energy. Therefore, this way is completely independent from metabolism.

The aim of this study is to investigate the effect of three different TCFs on HEK-293 cell lines in ATP production through reproducible experiments and to screen the effect of these fields on conventional bioenergy production.

Materials and Methods

In this study the following materials were used: ATP (Roche), D-luciferin potassium salt (Resem, The Netherlands), Fetal bovine serum (FBS) (BIO-IDEA), Dulbecco's modified Eagle's medium (DMEM) (BIO-IDEA), Penicillin/streptomycin (BIO-IDEA), Trypsin-EDTA 25% (BIO-IDEA), Tris-HCl (Merck), NaOH (Merck), $MgSO_4$ (Merck), PMSF.

Application of the TCFs: The TCFs were applied to the samples according to protocols developed by the COSMOintel research center (www.COSMOintel.com). A request for connection to CCN to utilize TCF1 can be submitted through the COSMOintel website in the “Announcement” section. This access is available to the public at no cost. To study and experience this connection, researchers can register on the website above at any time and report the experiment to the COSMOintel research center. It is necessary to provide the center with the design and details of the experiments; for example, the number and the type of cases need to be specified.

The current experiment was performed as a double-blind study where the lab technicians were completely unaware of the TCFs theory, and the Faradarmangar at the COSMOintel research center who established the Consciousness Bond was blinded to the details of the study. In this study, the TCFs were applied once in the last hour of the study.

Cell culture

Human embryonic kidney cells (HEK-293) were cultured in a controlled environment in a 6-well cell culture plate (4×10^5 cells) in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C with 5% CO_2. After one hour, cell treatment, the surface of each well was drained and washed with a PBS buffer and a certain amount of trypsin (1X 0.25% trypsin-EDTA) was added to each well.

Then, the plate was placed at 37 °C for 5 minutes, and following that the effect of trypsin was neutralized with a volume of 300 µl of culture medium, and cells were collected from the bottom of the wells and finally, centrifuged at 1200 rpm for 5 minutes. Precipitated cells were stored in a -80 freezer until performing the ATP assay. Luminometer (Berthold Technologies GmbH & Co.KG) was used to perform ATP assay and read the luciferase activity.

Cell lysis

In order to lyse the cells, 30 µl of CCLR buffer (Tris 50 mM, NaCl 150 mM, Triton x-100 1%, PMSF 0.1 mM - pH 6.9) was used.

The CCLR buffer was added to the cell sediments and was incubated on ice for about 20 minutes, and then centrifuged at 13000 rpm for 15 minutes at 4 °C before the supernatant was used for the ATP assay.

ATP assay

Before processing the samples, an ATP standard curve should be made. The serial dilution of ATP in the concentration range of 0.001-1 mM was prepared. The luciferase enzyme was then dialyzed in a 50 mM Tris buffer for 24 hours to remove ATP contamination. Finally, in order to measure the amount of ATP in the treated cells in a 1: 1: 1 ratio of the dialyzed luciferase enzyme, luciferin and cell lysis were mixed in a tube and luciferase count was read by a luminometer (Jouaville et al., 1999).

Statistical analysis

Data were analyzed using GraphPad Prism software version six. The values were expressed as mean ± standard error and analyzes were repeated at least three times. Then two-way variance analysis followed by multiple comparisons with 95% confidence intervals was performed and significant values less than 0.05 ($p < 0.05$) are presented.

Results

Measurement of the produced ATP in cells under the influence of the three TCFs

Due to the treatment of TCFs in the last hour of this study, the data related to the changes in the amount of ATP made in HEK-293 cells were calculated and normalized per the last hour of the present study. In this normalization, within 24 hours of this study, the number of cells has tripled (from 400,000 to 1,200,000), therefore, the average number of ATP produced per hour in the control in the last hour (time of comparison of samples with control) was considered three times the average (1.41E+17). Accordingly, as shown in Table 1, in comparison with the control samples, the concentration changes per final hour of treatment increased in the samples under TCFs 1, 2 and 3 by 5, 11, and 7-fold, respectively.

Table 1. Differences in cellular ATP levels in control and samples of this study

Sample	[ATP]/mM	No. of ATP	No. of ATP/ (last) hr	Production Rate (No. of ATP/cell/ last hr)	Relation to Basal Rate in 1 hour
Control	0.006	3.52E+18	4.40E+17	3.67E+11	1
TCF1	0.009	5.47E+18	2.39E+18	1.99E+12	5
TCF2	0.013	7.95E+18	4.87E+18	4.05E+12	11
TCF3	0.010	6.28E+18	3.20E+18	2.67E+12	7

*TCF: Taheri Consciousness Fields

Concentration of glucose required to produce the measured amount of ATP

The normal process of ATP production in living cells begins in the cytosol and is completed in the mitochondria and in the oxidative phosphorylation process. The glucose molecules have been considered as a limiting factor in this pathway, however, the same cannot be said for ATP precursors (e.g., ADP and Pi) because they are produced through the metabolic mechanism of the cell.

According to stoichiometry obtained from the process of cellular metabolism, assuming the conventional number of ATP produced by cells, per molecule of glucose (each molecule of glucose producing 32 molecules of ATP) and based on the data of produced ATP concentration measured in cells in culture medium, other values related to the number of glucoses required for oxidation and its theoretical oxidation rate are given in Table 2.

Table 2. Estimated glucose required to produce the amount of ATP produced in the control and samples of this study

Sample	Total ATP per total cells /last hr	Total required Glc/No.	Required Molarity of Glc/ mM	Required [Glc] (gr Glc/lit)	Required [Glc]/ Primary DMEM [Glc]	Theoretic Lysis rate/ µM/s
Control	4.40E+17	1.37E+16	5	0.86	0.19	1.32
TCF1	2.39E+18	7.46E+16	26	4.65	1.03	7.17
TCF2	4.87E+18	1.52E+17	53	9.48	2.11	14.62
TCF3	3.20E+18	1.00E+17	35	6.24	1.39	9.62

*TCF: Taheri Consciousness Fields

The DMEM culture medium with a high glucose type (4.5 g/l) was used in this study. The cells spent 24 hours in the culture medium at the time of calculating the values in Table 2, so the initial amount of glucose has decreased. As shown in Table 2, the control in this study, in order to make the measured amount of ATP, must have at least about 5 mM or 0.86 g/l intracellular glucose concentration, which according to the initial concentration of glucose in the environment (4.5 g/l), the amount of glucose required by the cell for this target is about 19% of the initial glucose concentration at the time of measuring. However, the amount of glucose required to provide the energy needed to produce the amount of target ATP in the case of samples affected by the TCF 1, 2 and 3 is 103%, 211% and 139% of the initial glucose content, respectively. it is important to note that this is a minimal estimate because all the glucose in the culture medium is considered in the cytosolic environment of the cell and the absorption efficiency is less than 1 and the energy loss due to natural and vital processes are not taken into account, an amount of 10-20% should be added at minimum to the glucose usage values. Moreover, considering stoichiometries of ATP production per one molecule of glucose (about 38), and other carbonaceous substances such as glutamine in the cell culture, and from the calculations in Table 1, the amount of glucose required for samples influenced by the TCF 1, 2 and 3 would be 87%, 177% and 117% of the amount of primary glucose, respectively. Again, given the amount of ATP needed for growth and proliferation and at the time of assay, these amounts of glucose are not available to the cells at the time of assay.

In fact, it is basically not possible to have more than the amount of primary glucose in the culture medium after 24 hours, to be used for production of ATP as a result of the influence of the TCFs. In particular, the required glucose for ATP production in TCF2-treated cells was more than double the primary glucose in the culture medium. This means that this amount of glucose did not exist from the beginning in the experimental conditions in samples affected by the TCFs.

In addition, the last column of Table 2 shows the theoretical glycolysis rate calculated for the controls and samples in this study. According to the methods of measuring the rate of glycolysis in cellular dimensions, in muscle and nerve cells (which have the highest rate of glycolysis among somatic cells), the rate of glycolysis is about 8 and 2.5 micromolar per second, respectively. In Hela cancer cells, it is about 6 micromolar per second (Bittner et al., 2010). This number is about 1.32 micromolar per second for control; and the renal somatic cell line under the TCFs during one hour of treatment, showed between about 7 to 15 micromolar per second glycolysis rate which is close to nerve cells and more than twice of Hela cancer cells. Due to the basic and evolutionary features of the cell line of this study, this change is practically not possible during the short time of the application of the TCFs.

Energetics calculation of ATP production process at the cell level and ATP synthase microenvironment (in mitochondrial):

According to the classic biochemistry textbooks, the amount of energy required to produce ATP is 30.8 kJ per mole (Prat 2021). This amount of energy is reported in some studies in natural conditions of cells in the range between 50-60 kJ per mole and in a study in mitochondria of rat liver is estimated at 64 kJ per mole (Slater et al., 1973). On the other hand, its theoretical value is estimated between 69 to 81 kJ/mol (for electromotive force -180 to -210 mV) (Müller & Hess 2017). The energy required to produce ATP is attributed to the force due to the difference in the concentration of hydrogen ions (protons).[1] The energy generated by the transfer of each hydrogen ion across the inner mitochondrial membrane and the resulting membrane potential difference (measured only for microorganisms is estimated in the range of -150 to -210 mV) is equal to a minimum of -14.5 kJ per mol to a maximum of -20.2 kJ per mol (Müller & Hess 2017).

In all the calculations of this study and based on the thermodynamic data extracted from the experiment and under standard conditions, the Gibbs energy of the ATP production process is 31.3 kJ / mol. In order to achieve an enthalpy of 28.1 kJ per mol of ATP made in proportion to the selected Gibbs energy, the required amount of proton free energy is about 19.8 kJ per mol with a stoichiometry of 3:1 which is within the range of empirical and theoretical data and is acceptable. The entropy under these conditions will be -10.7 joules per mole per Kelvin. Also, the measured enthalpy of formation of each molecule of ATP from the breakdown of one glucose molecule is equal to about 90 kJ per mole (the relevant information is given in Table 1 and Table 6).

The baseline rate of ATP production per last hour of the study (time of treatment by the TCFs) was calculated based on the energy of ATP molecule production, proton transfer, heat released at the cell and mitochondria level in the control and is presented in Table 3 and this amount in differential mode for samples, in comparison with control is illustrated in Table 4.

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[1] Proton Motivation Force

Table 3. Baseline ATP production rate calculated based on control data for one final hour with the energetics of the process of making ATP molecules, the heat released in exchange at the level of mitochondria and cells of this study and its equivalent temperature.

	Mole of ATP	**Mole of H^+**	**Q-mitochondria (kJ)**	**ΔT-mitochondria/K**	**Q-Cell (kJ)**	**ΔT-Cell/K**
Control (basal rate)	7.31E-07	2.19E-06	3.54E-02	8	6.58E-05	3

Table 4. The rate of ATP production calculated in a differential from the control data (Table 3) per final hour with the energetics of the process of making ATP molecules, the heat released in return at the level of the mitochondria and cells of this study and their equivalent temperature

Sample	**Δ Mole of ATP**	**Δ Mole of H^+**	**Δ Q-mitochondria (kJ)***	**ΔT-mitochondria/K**	**Q-Cell (kJ)***	**ΔT-Cell/K**
TCF1	3.24E-06	9.71E-06	9.09E-05	37	2.91E-04	13
TCF2	7.35E-06	2.21E-05	2.07E-04	85	6.62E-04	29
TCF3	4.59E-06	1.38E-05	1.29E-04	53	4.13E-04	18

*TCF: Taheri Consciousness Fields

* The total number of cells at the end of the study is about 1,200,000 and the amount of intracellular water in eukaryotic cells based on literature is 0.0000000044 ml, with a density of 1.008 g / ml, and the total estimated amount of mitochondrial water is at about 12% of the total cell water (Bolender & Weibel 1973).

As can be seen from the data in the previous sections, the results confirm the impossibility of producing high amounts of ATP in the samples as a result of cell and mitochondrial proliferation and promotion of enzymatic function as well as glucose oxidation as "cell fuel". Also, in completing the data in the previous sections, as shown in Table 4, by calculating the enthalpy of the general pathway of glucose oxidation in cells and considering the difference between the energy stored in ATP molecules and obtained from the transfer of H^+ ions, which is released in the environment as heat, we expect temperature changes in cells and microenvironment of ATP synthase to be about 3 °C in the case of control and 8 °C per cell population and mitochondrial space. These changes are within the temperature tolerance range of cell and mitochondrial (Chrétien et al., 2018).

However, in the samples, the temperature is expected to increase between 13 °C and 29 °C for the cells and 37 °C to 85 °C for the mitochondria during the one hour of treatment with the TCFs. Obviously, such a rise in temperature, cell death, enzyme denaturation, and mitochondrial destruction must occur in the last hour of the study instead of a surprising increase in energy of the biological system outside the basic nature of the biological system. In fact, according to the data, it can be concluded that the conditions of bioenergy production in control are in accordance with normal conditions, but in the case of samples, it is undoubtedly impossible to produce these amounts of ATP according to conventional biological mechanisms.

Discussion

The results of investigating ATP production showed that TCF 1, 2 and 3 causes a 5, 11 and 7 times increase in ATP compared to the control. It has been reported that the increase in cellular ATP levels is possible under the influence of metabolic shocks (Mookerjee et al., 2017) and environmental conditions (including the application of magnetic fields (Buchachenko & Kuznetsov 2008)) which is about 2.5 times in at least several hours after the relevant treatment, and in some cases, depending on the energy of the magnetic field, we face a decrease of more than this amount. The observation of an increase of 5-11 times, particularly, in a period of 1 hour without applying material and energetic changes in the cell culture medium, seems completely mechanistically and materially unachievable. There are possibilities that may cross our mind for this observation, including an increase in the number of cells treated with the TCFs; but considering that in this study, before the cell lysis stage (24 hours), the number of cells in the sample and control was approximately 1,200,000 (tripled in 24 hours), an increase in the number of ATP in the samples, which is 5-11 times higher, could not be due to an increase in the number of cells and, consequently, an increase in the number of total mitochondria. In addition, previous studies have shown that the effect of the TCFs on increasing the proliferation of healthy and cancer cells was less than 20% (Taheri et al., 2022a; Taheri et al., 2022) and the observed change in ATP levels in this study is very different. About a possible increase in the number of mitochondria, it is to be noted that the time required for mitochondrial proliferation is more than the one hour of the TCFs treatment. Similarly, an increase in the number of ATP synthase in mitochondria cannot be the reason for this increase, because the formation of this enzymatic complex with 17 subunits requires the participation of nuclear and mitochondrial genes, which take more than an hour. There are different amounts of ATP synthase in the mitochondrial inner membrane depending on the cellular metabolic requirements, and some of them are active. In a certain condition, most of them can be activated by about 2 to 3 times.

A significant increase of 5-11 times of ATP with this probability is also not justified. In previous studies on enzymatic activity, an increase in catalytic capacity between 5-20% under the influence of the TCFs has been observed (Taheri et al, 2022b and c). In this experiment, the rate of increase in ATP as a final product is about 500-1100% and cannot be attributed to the increase in the catalytic capacity of the enzyme under influence of the TCFs.

According to the stoichiometric calculation of mitochondrial function efficiency, it was determined that the amount of energy sources and glucose in the culture medium is much less than the amount required to increase ATP. In addition, the theoretical glycolysis rate obtained for the samples under influence of the TCFs shows a 5-11-fold increase compared to the control, which may not be possible due to the short period of time of treatment as well as the type of cell line. Therefore, this significant increase in ATP cannot be attributed to glucose oxidation as a cellular fuel. By calculating the energy of the ATP production process, we expect temperature changes in the cell and mitochondria for the samples to increase between 13°C and 29 °C for the cells and 37 °C to 85 °C for the mitochondria. Obviously, this is not possible because it may cause enzyme denaturation while we observed an increase in ATP production under the influence of the TCFs.

In general, based on the obtained data, ATP production influenced by the TCFs cannot be achieved through normal biological processes; The four limitations for this purpose in biological system are as follows: (1) Lack of sufficient amount of glucose to provide the required fuel for the cell and consequently insufficient number of NADH and FADH2 to create and maintain a proton gradient in the mitochondria; (2) renal somatic cell was used in the present study, however, the obtained glycolysis rate was higher than expected for cancer cell and around the nerve cell, which is not possible; (3) the influence period of the TCFs was less than an hour, which is not enough to provide the biological needs of this process, and (4) the impossibility of the release of a lot of energy in a short time (immediate energy) in a limited cellular space (energy limitation), meaning that in biological systems, the catabolic pathways of glucose oxidation have evolved in way that energy is released from glucose breakdown in a phased process and at specific intervals in the space of the cell cytoplasm and mitochondrial matrix during more than 20 reactions; Otherwise, the fuel of each glucose molecule at a shorter and smaller time and place will cause overheating and cell death. The question that arises with respect to the above limitations is how the sample cells, which have the same conditions as the control, produce such a significant increase in ATP under influence of the TCFs without sufficient time and resources (matter and energy)? In response to this key question, based on the theoretical concept of TCFs, the proposed mechanism for increasing ATP is related to a kind of interaction between the TCFs and the mind (software) of the cells. In this way, according to Taheri and previous observations, our interpretation is as follows:

1. Ionization of water molecules under influence of the TCFs:

In a previous study, we observed that the effect of the TCFs on water molecules can lead to an increase in H^+ or a decrease in pH (up to 1 unit less than control). As it has been mentioned in the introduction section, proton gradient in the intermembrane space of mitochondria provides required energy for ATP production by ATP synthase molecules in the inner mitochondrial membrane, which normally oxidizes electron carrier molecules including NADH and FADH2 (formed in the anaerobic and aerobic pathways of glucose oxidation) and the entry of H^+ into the space between these two membranes.

Due to the four limitations mentioned, the proposed pathway for increasing H^+ under influence of TCFs in the conditions of this study is the ionization of water molecules in the intermembrane space of mitochondria. By applying the TCFs, this alternative pathway can maintain the proton motive force across the inner mitochondrial membrane through water ionization instead of the usual pathway (oxidation of NADH and FADH2 as electron-carrier molecules and entry of H^+ into the intermembrane space of mitochondria). In this case, the proton concentration difference between the space between the membrane and the mitochondrial matrix must be kept constant: “Three molecules of water need to be decomposed to make up for the three protons consumed in one ATP production”.

Table 5 presents the reactions and thermodynamics of the water ionization process and ATP production in the alternative pathway. In Table 6, for a better comparison, using the data in Tables 1 and 5, the thermodynamic parameters of the total biological pathway reaction and the alternative pathway under the influence of TCFs for the production of each ATP molecule are provided.

Table 5. Reactions related to the proposed pathway of ATP production under the influence of TCFs in this study and its thermodynamic parameters

Reaction name	Chemical Reaction/Stoichiometry	$\Delta G°$/kJmole^{-1}	$\Delta H°$/kJmol^{-1}	$\Delta S°$/ Jmol-1K^{-1}
Water ionization	H_2O (liq.) → H^+ (aq.) + OH^-(aq.)	-79.9	-55.8	+80.8
ATP production	ADP + Pi → ATP	+31.3	+28.1	-10.7

Table 6. Comparison of thermodynamic parameters of alternative pathway of bioenergy production (water ionization model) with thermodynamic parameters of general reaction and oxidation of electron carriers of normal biological pathway; All parameters are in the normalized state for the production of one ATP molecule.

Pathway	Reaction	Stoichiometry	ΔG*/ kJmol^{-1} per ATP	ΔH/ kJmol^{-1} per ATP	ΔS/ JK^{-1}.mol^{-1} per ATP
Conventional biologic pathway	Glucose oxidation	1/32 Glc:1 ATP	–89.69	+90	+602
Alternative pathway under influence of TCFs	Water ionization	3Water:1ATP	-239.7	-167.4	+242

As can be seen in Table 5, ionization of water is a spontaneous process (Gibbs free energy is negative) and endothermic. Basically, this reaction is equilibrium. However, according to the hypothesis presented in this study, water ionization is responsible for providing an H^+ gradient across the inner mitochondrial membrane and it provides the energy needed to produce the amount of ATP obtained in this study. According to the data in Table 6, by comparing the two energy supply paths required to create a proton gradient between the two mitochondrial membranes and the production of ATP, we reach the following conclusions:

A. The amount of free energy required to form each ATP molecule, during normal biological reactions (oxidation of each molecule of glucose - about 90 kJ/mol), is approximately 1/3 of the required energy in the proposed alternative pathway (water ionization - approx. 240 kJ /mol); Therefore, from the perspective of energy efficiency, this alternative path does not occur in the normal state, since the biological evolutionary path of energy production is optimal.

B. Conventional biological reactions are extremely exothermic in general, and its biological staging over more than 20 reactions, from the cellular cytoplasm to the mitochondrial matrix and the inner membrane of the mitochondria, has been a way to energetically make it feasible. In contrast, the alternative pathway under the influence of the TCFs is endothermic and prevents the rise of cell heat.

C. Increasing entropy in the alternative pathway (242 joules per mole Kelvin) is about 60% lower than biological pathway (602 joules per mole Kelvin); this implies the negantropic effect of the TCFs compared to the normal biological conditions. This conclusion is in concurrence with the previous study in which the effect of the TCFs on water molecules was investigated (Current Issue, pp. 6-32).

Based on these results, in order to produce high amounts of ATP, the hypothesis of substitution of water ionization (in the space between two mitochondrial membranes) in the role of the provider of the required proton gradient (equivalent to glucose) is proposed. This reaction, with a 1:3 stoichiometric reaction of water ionization and ATP production reaction under influence of the TCFs, produces very high amounts of bioenergy (5-11 times normal) in a short time (immediate energy) that is beyond the evolutionary potential of biological cells.

It is to be noted that the proposed alternative pathway is based on the results of the current study. The authors are going to complete the data by designing further experiments. Such as calculating the amount of glucose in the cell culture medium before and after applying TCFs, measuring pH changes in the culture medium and even mitochondria, as well as ATP production in bacterial cells.

2. Existence and function of cell management software (mind):

Previously, the effect of TCFs on materials led to changes in their behavior that required very high temperatures (or very high energies) (Taheri et al., 2022d) in laboratory. According to Taheri's theories, this observation has been attributed to the existence of the mind body in matter. In other words, this behavior, with the presence and function of mind and memory in matter and the functional mechanism of state change of the mind of matter has been proved and interpreted under the influence of the TCFs (Taheri et al., 2022e).

According to Taheri's theory, the mind has different levels, including the basic mind (common) (the mind of matter, which is the common basis in the universe and includes the pure matter software program), the intrinsic mind (biological) (including the human, animal, plant, microorganism) and the perceptual mind (specific to humans, which provides the ability to ask questions, be aware of its own existence and perceptual concepts). During the experiments, we have observed behaviors at the level of matter as well as in water molecules that are not possible without the existence of mind body (matter software program) and the transfer of information through it; especially since under the influence of the TCFs, there is no material and energetic intervention, and it begins only through the mind of the *announcer* and through a swift and brief attention to the TCFs.

What is observed in the present study, similar to the material, is at the level of living cells and in the process of ATP production in the mitochondria of the HEK-293 cell line, which suggests the existence of mind in living cells. In fact, the TCFs in interaction with the mind or the cell management software cause a new behavior that in this experiment, advances the ionization reaction of water molecules and maintains the necessary proton gradient under the influence of TCFs. This treatment led to enhanced ATP production (between 5-11 times the normal biological states) under the exact same conditions as the control. Detailed explanations of the type of energy required for this process and the role of cell management software and the provision of empirical evidence from the studies will be published in a review article by the authors of the present study.

3. Decrease in entropy and increase in cellular information:

According to Taheri's theory, T-Consciousness influences the world of matter and energy through information. In other words, under the influence of the TCFs, data and information (from the position of Whole Consciousness or CCN) transfer to the object under study and lead to an increase in energy (and consequently an increase in ATP). To illustrate, the law of conservation of matter and energy is presented by Taheri as conservation of "information, matter and energy". Since there is currently no direct measurement of information and the study of the relationship between the new law of conservation of information, matter and energy, using information theory accepted by the world of science, the information changes in cells under the influence of the TCFs will be discussed and analyzed by the authors of the present study in a separate review article.

Conclusion

1. The increase in the amount of cellular ATP in the samples compared to the control indicates a significant effect of the TCFs on the bioenergy production.

2. Considering the multiple biological limitations mentioned in the discussion section, it is not possible to produce bioenergy according to the normal biological pathway of ATP production in sample cells under the influence of the TCFs.

3. Considering the thermodynamics of the alternative pathway reaction of bioenergy production under the influence of the TCFs (ionization of 3 water molecules) compared to the set of normal biological pathway reactions (more than 20 reactions in different cell regions from cytoplasm to mitochondria), which are generally exothermic and entropy-increasing, the alternative pathway is the only available and suitable option for supplying energy between 5 and 11 times in short time (immediate energy). Interestingly, this amount of energy is released at the optimal temperature of the cell and without disturbing the cell mechanism. Moreover, a decrease in the entropy occurred in an alternative pathway by about 60% compared to the usual biological pathway. This amount of entropy reduction, considering the general conditions similar to the sample and control in this study, seems to play a significant role in maintaining homeostasis (steady state) of the living system.

4. Changes in the cellular metabolic behavior necessarily require energy supply. What can be seen in this study is that the treatment of the TCFs provides this energy under the management of the mind (or software) of the cell through a different way from the evolutionary path. This alternative pathway occurs as a result of information received under the TCFs and by changing the behavior of cells, to advance the ionization reaction of water and maintain the proton gradient in the mitochondrial intermembrane space to produce bio-immediate energy. According to Taheri's theories, there is software behind the cell's visible physical body (hardware). Therefore, this change in cell behavior occurs by transferring information received through the interaction between TCFs and the mind (or software) of the cells. This observation is consistent with previous findings related to the existence of mind in matters (Taheri et al., 2022e).

5. The enhancing effect of TCFs on ATP production in the cell associated with entropy reduction confirms the information received from the TCFs, which lead to an increase in system information (according to law of conservation of information, matter and energy presented by Taheri).

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Conflict of interest

The authors report no conflict of interest.

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Appendix: Basics of ATP Bioenergy Production[1]

Cellular nutrients come in many forms, including sugar and fat. To provide power to a cell, these molecules must pass through the cell membrane, a membrane that acts as a barrier - but not an impenetrable barrier. The plasma membrane is like the outer walls of a semi-permeable house.

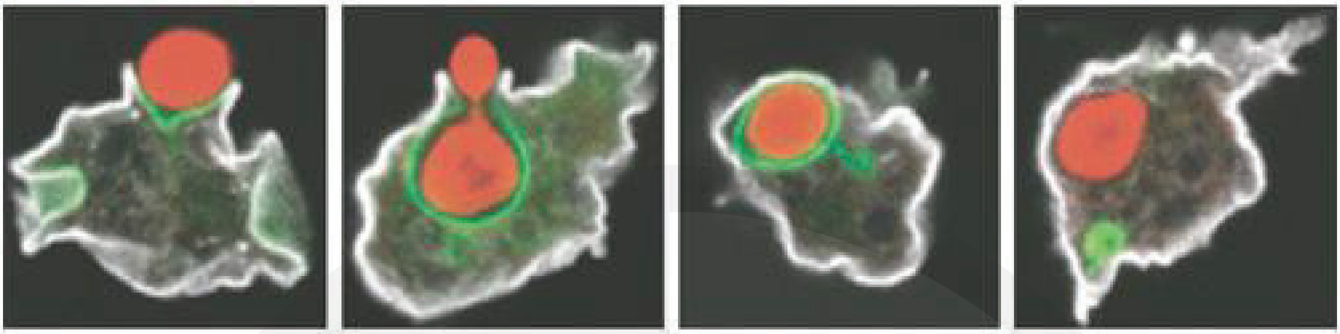

Figure 1: Cells can receive nutrients through phagocytosis. An amoeba, a single-celled organism, obtains energy by absorbing nutrients in the form of a (red) yeast cell. Through a process called phagocytosis, the amoeba surrounds the yeast cell with its membrane and pulls it in.

Complex molecules of organic foods, such as sugars, fats, and proteins, are rich sources of energy for cells because most of the energy used to form these molecules is literally stored in the chemical bonds that hold them together. Instead of burning all their energy in a large reaction, cells release energy stored in their food molecules through a series of oxidation reactions. Oxidation describes a chemical reaction in which electrons are transferred from one molecule to another, altering the composition and energy content of the donor and receptor molecules. Food molecules act as electron donors. During any oxidation reaction involved in the decomposition of food, the reaction product has lower energy content than the electron molecule that previously moved in the path. At the same time, electron-acceptor molecules capture some of the energy lost from the food molecule during each oxidation reaction and store it for later use. Finally, when the carbon atoms of a complex organic food molecule are completely oxidized at the end of the reaction chain, they are released as waste and in the form of carbon dioxide (Albert et al., 2002).

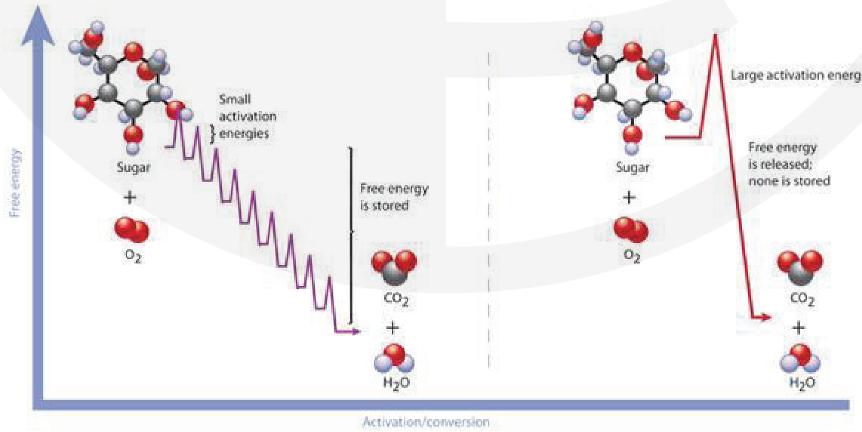

Figure 2. Step-by-step oxidation (left) versus direct burning of sugar (right). In small steps, Gibbs energy is released from sugar and stored in carrier molecules in the cell (ATP and NADH). Direct burning of sugar requires more activation energy. In this reaction, the same total free energy is released as in stepwise oxidation, but none is stored in the carrier molecules, so most of it is lost as heat. Direct combustion is therefore very inefficient because it does not inhibit energy for later use (details of reactions are given in Table 1).

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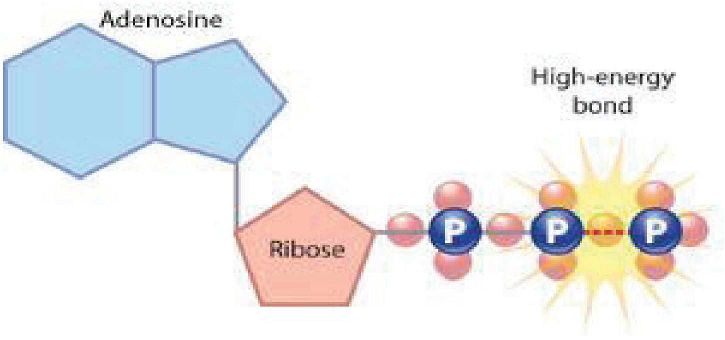
[1] All figures in this section are taken from multiple free educational resources.

Figure 3. ATP molecule; It consists of an adenosine (blue) base, a ribose sugar (pink) and a phosphate chain. High-energy phosphate bonding in this phosphate chain is the key to ATP energy storage potential.



Cells do not consume energy from oxidation reactions as soon as they are released. Instead, they convert it into small, energy-rich molecules such as ATP and nicotinamide adenine dinucleotide (NADH), which can be used throughout the cell to boost metabolism and build new cellular components. Adenosine 5'-triphosphate, or ATP, is the most abundant energy-carrying molecule in cells. This molecule is made up of a nitrogen base (adenine), a ribose sugar and three phosphate groups.

Mitochondria: A key organ of cellular energy production

Mitochondria are key organelles that make up about 12% of the mass of each cell in liver cells. It is described as a cell power plant which is surrounded by two membranes and has its own genome. They also divide independently of the cell. This means that mitochondrial proliferation is not associated with cell division. Some of these features of this cellular organ belong to the ancient ancestors of mitochondria. Mitochondria are thought to have originated from an ancient coexistence; when an early nucleated cell swallows an aerobic prokaryote (bacterium). The ingested bacteria depend on the host cell's protective environment, and the host cell relies on the prokaryotic cell to produce energy. Over time, the ingested prokaryotes became mitochondria. Today's mitochondria bear significant similarities to some modern prokaryotes; For example, the inner mitochondrial membrane contains electron transfer proteins such as the plasma membrane of prokaryotes, and mitochondria have the prokaryotic cyclic genome.

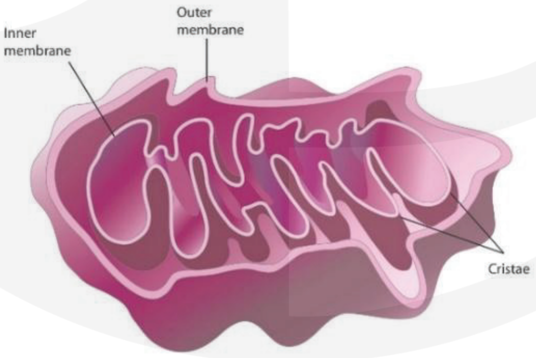


Figure 4. Membrane organization of a mitochondria

Energy production processes from materials in biological systems

Eukaryotic cells use several main processes to convert the energy contained in the chemical bonds of food molecules into more usable forms (details of all reactions are given in Table 1). Glycolysis: The first process in the release of energy in eukaryotic cells, glycolysis involves about 10 reactions, which literally means "breaking down sugar". During glycolysis, each molecule of glucose is broken down and eventually converted into two pyruvate molecules. Glycolysis is actually a series of ten chemical reactions that require two molecules of ATP. This input is used to produce four new ATP molecules, meaning that glycolysis leads to a net increase in two ATPs. It also produces two NADH molecules that act as electron carriers for other biochemical reactions in the cell.

Glycolysis is the old and main pathway of ATP production that occurs in almost all cells (eukaryotes and prokaryotes) equally. This process takes place in the cytoplasm of the cell and does not require oxygen. But the fate of pyruvate produced during glycolysis depends entirely on the presence of oxygen. In the absence of oxygen, pyruvate cannot be completely oxidized to carbon dioxide, so different intermediates are created. For example, when oxygen levels are low, skeletal muscle cells rely on glycolysis to meet their intense energy needs. This reliance on glycolysis results in the formation of an intermediate called lactic acid, which can cause a person’s muscles to feel “on fire.” Similarly, yeast, a single-celled eukaryote, produces alcohol (instead of carbon dioxide) in the absence of oxygen.

Krebs cycle: When oxygen is available, the pyruvates produced by glycolysis become the entrance to the next section of the eukaryotic energy pathway. During this stage, each pyruvate molecule enters the mitochondria from the cytoplasm, where it is converted to acetyl CoA, a two-carbon energy carrier, and its third carbon combines with oxygen and is released as carbon dioxide; At the same time, a NADH carrier is being produced. Acetyl CoA then enters a pathway called the Krebs cycle (citric acid), which is the second major energy process used by cells. The eight-phase cycle of citric acid produces three more NADH molecules and two more carrier molecules (FADH2 and GTP). This step is about 8 reactions in total and is repeated twice per glucose.

Oxidative phosphorylation: Another process in the eukaryotic energy pathway involves an electron transfer chain that is catalyzed by several protein complexes located inside the mitochondrial inner membrane. This process, called oxidative phosphorylation, takes electrons from NADH and FADH2 and transports them through membrane proteins, eventually to oxygen, where they combine to form water. As electrons move through protein complexes in the electron transport chain, a gradient of hydrogen ions or protons forms across the mitochondrial membrane. The cells harness the energy of this proton gradient to create three more ATP molecules for each electron moving along the chain. The reactions of this step are the three main reactions of the oxidation of electron carriers.

In general, the combination of the citric acid cycle and oxidative phosphorylation produces much more energy than glycolysis; Something close to 15 times the energy per molecule of glucose. In general, the processes that take place inside the mitochondria, the citric acid cycle, and oxidative phosphorylation are called respiration, a term used for processes that combine the absorption of oxygen and the production of carbon dioxide.

The electron transfer chain in the mitochondrial membrane is not the only chain that produces energy in living cells. In plants and other photosynthetic cells, chloroplasts have an electron transfer chain that collects solar energy. Although prokaryotes do not contain mitochondria or chloroplasts, prokaryotes have other types of energy-producing electron transfer chains in their plasma membranes that produce energy.

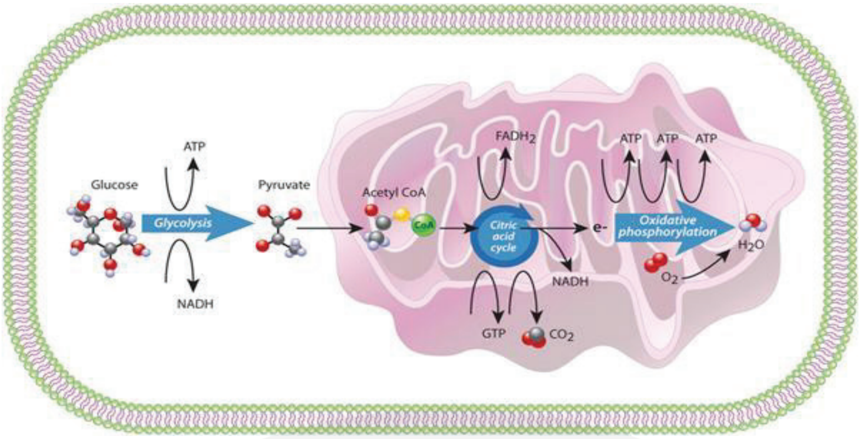


Figure 5. Metabolism in a eukaryotic cell: glycolysis, Krebs cycle (citric acid) and oxidative phosphorylation: Glycolysis takes place in the cytoplasm. The citric acid cycle occurs within the mitochondria and in its matrix and oxidative metabolism in the folded inner membrane of the mitochondria (cristae).

ATP production process in the inner mitochondrial membrane:

Mitochondria contain two main membranes. The outer membrane of the mitochondria completely surrounds the inner membrane and there is a small space between the membranes. The outer membrane has many protein pores that are large enough for ions and molecules the size of a small protein to pass through. In contrast, just like the plasma membrane, an inner membrane cell has much more permeability. The inner membrane is filled with proteins that are involved in electron transfer and ATP synthesis. This membrane surrounds the mitochondrial matrix, where the citric acid cycle produces electrons that move from one protein complex to the next protein complex in the inner membrane. At the end of this electron transfer chain, the final electron receptor is oxygen, which ultimately forms the water molecule.

Proton motive force (PMF): During electron transfer, protein complexes of the inner mitochondrial membrane transfer protons from the mitochondrial matrix to the intermembrane space. This process is repeated until the electrons released from the electron carriers (NADH and FADH2) lose all their energy as they move through the electron transfer chain and reduce oxygen during conversion to the water molecule. This transfer creates a concentration gradient, or proton motivation force (PMF), which uses the chemical and electrical potential and protein complex ATP synthase that it uses to synthesize ATP (Bcher1965 & Scholz).

Chemical potential:

$$\Delta G_{\Delta pH} = \Delta\mu_{\Delta pH} = RT \ln (C_{IN} / C_{OUT}) = 2.3\ RT\ \Delta pH$$

Where R is the general constant of gases and T is the temperature in Kelvin. ΔpH is the unequal distribution of protons that causes the pH in the space between the two membranes (outside) to be lower than the matrix (inside). The concentration gradient is the source of potential energy; Equilibrium occurs when balanced gradients and equal concentrations are formed on both sides of the membrane.

Electric potentioal:

$$\Delta G = -zF\ \Delta\Psi$$

Because the proton is a charged particle, the outside of the membrane (outside) has a more positive charge and the inside (inside) is more negative, and the difference is also called $\Delta\Psi$ and sometimes ΔE. z is the particle charge, which is +1 for proton. Based on this, the free energy of the proton ΔGPMF or the driving force of the proton is defined as the sum of the two mentioned potentials:

$$PMF = \Delta\Psi + 2.3\ (RT/F)\ \Delta pH$$

$$\Delta G_{PMF} = 2.3RT\Delta pH + F\ \Delta\Psi$$

$$\Delta G_{PMF} = F \times PMF$$

The PMF in mitochondria is about 0.205 volts and the Faraday constant is 96.5 $kJmol^{-1}\ V^{-1}$. About 70% of PMF in mitochondria is due to $\Delta\Psi$ and a smaller share belongs to ΔpH; The free energy of transfer of each proton to produce ATP in the synthase enzyme is calculated as follows:

$$\Delta G_{PMF} = F \times PMF \qquad \Delta G_{PMF} \sim -19.8\ kJmol^{-1}$$

On the other hand, in the standard condition free energy of ATP production is (Pänke & Rumberg 1997):

$$ADP + Pi \rightarrow ATP \qquad \Delta G^{0} = 31.1\ kJmol^{-1}$$

Table 1 shows the overall reaction of glucose oxidation and all major micro reactions involved in the ATP production process in biological systems and related thermodynamic parameters based on the experimental studies (Manchester 1980; Burton & Krebs 1953; Krebs et al., 1953).

Table 1. Reactions related to ATP production processes according to biological mechanism in living cells and related thermodynamic parameters

Reaction name		Chemical Reaction	$\Delta G1/\ kJmol^{-1}$	$\Delta H/\ kJmol^{-1}$	$\Delta S/\ JK^{-1}.mol^{-1}$
Glucose oxidation (Summary of total reactions)		C6H12O6(aq) + 6O2(g)→ 6CO2(g)+ 6H2O(l)	–28702	+2880	+19286
Glycolysis reactions[3]		Glc+ATP→Glc-6-P+ADP	-17	-	-
		Glc-6-P↔Fru-6-P	+2		
		Fru-6-P→ Fru-1,6-P	-14		
		Fru-1,6-P→GA3P+DHAP	+24		
		DHAP→ GA3P	+8		
		GA3P →1,3-BPG	+6		
		1,3-BPG→3PG	-19		
		3PG→2PG	+4		
		2PG→PEP	+2		
		PEP→pyruvate	-32		
Krebs cycle[3]		pyruvate→acetate	-50	-	-
		acetate + oxaloacetate→citrate	-2		
		Citrate ↔isocitrate	+6		
		Isocitrate → α-ketoglutarate	-8		
		α-ketoglutarate → succinyl CoA→ succinate	-64		
		Succinate → fumarate	+84		
		Fumarate → malate	-4		
		Malate → oxaloacetate	+68		
Electron transport chain	NADH oxidation-water formation	(NADH + H+ + ½ O2 → NAD+ + H2O)	-259	-257	+9
	FADH2 oxidation-water formation	FADH2 + ½ O2 → FAD + H2O	-278	-226	+172
ATP synthase	ATP production	ADP + Pi → ATP	+31.3	+28.1	-10.7

According to a study (Klingenberg & Pfaff 1966), the amount of oxidation energy per NADH is sufficient to produce 10 protons with a total energy of $(^{198}/_{259})$ kJ per mole, which leads to an oxidation efficiency of about 76% $(^{198}/_{259})$. Also, the oxidation energy of each FADH2 is sufficient to produce 6 protons with a total energy of about 119 kJ/mol, with an oxidation efficiency of 43% $(^{119}/_{278})$. According to experimental studies, the transfer of three protons leads to the formation of an ATP molecule. This means that the yield efficiency of ATP synthase protein is about 53% $(^{31.3}/_{59.4})$.

Calculations related to changes in heat content and pH in this study

The formula for calculating the amount of heat released in the environment and the resulting temperature changes was based on the following formula:

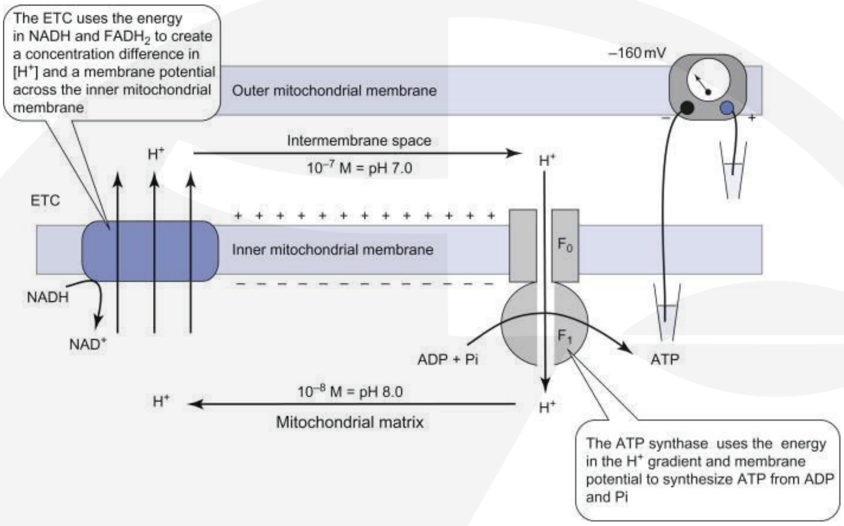


Figure 6. In the inner mitochondrial membrane, a high-energy electron is passed along an electron transfer chain. The released energy pumps hydrogen out of the matrix space. Using the generated hydrogen gradient energy, ATP synthesizes ATP synthase from a combination of ADP and Pi.

$$Q = m.C.\Delta T$$

Q in this study, the difference between the amount of energy released per transfer of each of the 3 H^+ ions from the mitochondrial intermembrane space into the matrix and the amount of energy stored per ATP molecule was calculated. The reason for choosing a 3:1 stoichiometry for H^+::ATP was that this ratio was obtained for the thermodynamic values reported under the standard conditions of ATP production and used in this study.

m and C are the specific mass and heat capacity of the energy delivery environment, respectively, which are taken into account in water biological systems, and based on this, temperature changes are calculated for the energy released in the mitochondria. It should be noted that since the study is performed at constant pressure, the heat exchanged is equal to the enthalpy of the reaction changes:

$$Qp = \Delta H$$