

Balneotherapy and coenzyme Q₁₀ in clinical and experimental medicine

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1. ABSTRACT

Balneotherapy or Spa therapy is used in neurological, cardiovascular, musculoskeletal, dermatological and gynecological diseases, in infertility as well as in metabolic disturbances. Beneficial effects of balneotherapy at the metabolic level is not fully understood. Authors have documented enhancement of antioxidants concentrations (coenzyme Q₁₀ - CoQ_{10-OX} and alpha-tocopherol) of women with gynecological diseases by treatment with natural mineral water (Spa Lucky balneotherapy, Slovakia). In an experiment with rats, drinking of Spa Lucky mineral water decreased oxidative stress and enhanced concentrations of antioxidants CoQ_{9-OX}, CoQ_{10-OX} in the myocardium, and alpha-tocopherol in uterus, ovaries and myocardium. Drinking of Spa Lucky water by rats stimulated myocardial mitochondrial respiration and energy production, and diminished skeletal muscle mitochondrial function. Simultaneous ingestion of

coenzyme Q₁₀ with drinking spa water returned mitochondrial parameters to the values of the control group. This pilot study helps explain the role of antioxidants, oxidative stress and mitochondrial energy production in beneficial effects of Spa Lucky balneotherapy.

2. INTRODUCTION

2.1. Balneotherapy

Balneotherapy or Spa therapy is a part of „Health resort medicine“, which uses natural mineral waters, medical peloids, muds, natural gases (bathing, drinking, inhalation), massages and physical therapy for health promotion, prevention, therapy and rehabilitation. The use of tap water for therapy is called *hydrotherapy* and the use of climatic factors for therapy is called *climatotherapy* (1). Natural mineral waters (Spa waters) act primarily on the skin. The beneficial effects of balneotherapy is used in

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several diseases - dermatological (atopic dermatitis, psoriasis), chronic musculoskeletal diseases (rheumatoid arthritis, ankylosing spondylitis, fibromyalgia, osteoarthritis, low back pain, chronic pain), male/female infertility, metabolic disturbances (blood viscosity, platelet aggregability, plasma lipids, stress hormones) and psychological conditions such as depression (2). Mineral water based on amount of minerals is classified as low mineralized (0.6-2g/l), mildly mineralized (>2-10g/l) and highly mineralized (>10g/l). Water temperature is described as being cold (<20°C), hypothermal (20-30°C), thermal (>30-40°C) or hyperthermal (>40°C), (2).

2.2. Balneotherapy Around the World

The use of balneotherapy in various diseases has expanded during last few decades in several countries. In a review, authors from *Japan* used Medline and Cochrane Library database from 1966 to 2003 and summarized the major success of balneotherapy treatment in dermatologic and musculoskeletal diseases, as atopic dermatitis, psoriasis, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and low back pain (3). Authors from *Cuba* proved therapeutic effect of San Diego de los Baños peloid (natural product with biological activities of organic compounds) in the treatment of inflammatory and dermatological diseases, as analgesic, and male and female infertility (4). Mud pack therapy was reported by authors from Spain as an effective alternative treatment for patients with knee osteoarthritis, to achieve significant improvement in function and quality of life (5). In *Italian* studies, the effect of drinking sulphate-bicarbonate-calcium thermal water (33°C) on risk factors for atherosclerosis and cholesterol gallstone disease was studied in postmenopausal women with functional dyspepsia and/or constipation. Consumption of 500 ml sulphate-bicarbonate-calcium thermal water every day in the morning, over a 30 minutes period, during 12 days had a positive effect on the lithogenic risk and intestinal transit (6). Beneficial effect of CO₂-enriched spa water was shown in 25 patients with peripheral occlusive arterial disease, with the immersion of the legs and feet in CO₂-enriched water, 10 treatments during two weeks. Significant reduction of plasma reactive oxygen species (ROS) levels and rising antioxidant were documented. These authors assumed an improvement in the microcirculation (7). Bath in thermal mineral water of patients with chronic lumbar complaints had a beneficial effect on the activity of antioxidant enzymes as catalase, superoxide dismutase and glutathione peroxidase in plasma (8). In a review the authors (9) confirmed that *Hungarian* thermal mineral waters significantly reduced pain in degenerative joint and spinal disease, as well as in osteoarthritis of the hand and knee, and also alleviated chronic low back pain. Safety of balneotherapy with thermal water 38°C, in 15 sessions of 30 minutes in hypertensive and obese diabetics was documented by way of a lack of deleterious effects on the antioxidant, inflammatory or metabolic parameters. Balneotherapy was not contraindicated for hypertensive or obese diabetics (10). Drinking spa sulphurous mineral water significantly reduced plasma ROS in type 2 diabetes mellitus patients, especially with antidiabetic drugs treatment. Drinking this water may be useful for the improvement of redox state of

the diabetic subject (11). In an experiment with rats drinking chloride-sulphur-bicarbonate mineral water for two weeks, results suggest a possible antioxidant effect of mineral water treatment on diseases involving gastroenteric oxidative stress diseases with consequent improvement in the intestinal physiology (12). In the *Austrian* Spa balneotherapeutic applications study (carbon dioxide, mud bath, massages and physical therapy) 35 patients with high blood pressure were given the treatment between 2 and 5 times a week, in 20 minutes duration and 24-hours blood pressure variability was measured. Blood pressure after 3 weeks of balneotherapy significantly decreased in patients with medium or high initial blood pressure, whereas no change was observed in patients with low blood pressure. Authors demonstrated the benefits of balneotherapy for patients with medium and higher initial blood pressure (13). In the *Serbian* study, thirty one patients with osteoarthritis (OA) received mud pack and sulphur bath for 20 minutes a day, over 3 weeks. Significantly decreased plasma lipid peroxidation as well as pain intensity in patients with OA were found. Increased hemoglobin level suggested beneficial effect of spa therapy in OA patients (14).

Natural thermal mineral waters in Slovakia come from springs. In the biggest *Spa* in Slovakia – *Piešťany* is a unique thermal water which rises from a depth of over 2,000 metres, is trapped at a depth of 60 m which guarantees a constant chemical composition and temperature. One liter of water contains 1500 mg of mineral substances, containing sulphates, hydrogen carbonate, calcium, sodium, and sulphur compounds. High hydrogen sulphide (4,7 mg per litre of water) and high sulphur (from 6 to 10 mg per litre of water) contents are the most important features for the treatment of diseases. The water temperature of springs is 67 - 69°C. This water is the main factor in the formation of curative sulphur mud. A unique peculiar peloid is the result of long-term reactions of thermal water with local soft rocks and specific bacterial microflora. Several others spa in Slovakia are used to treat heart diseases, high blood pressure, atherosclerosis, post operative states of cardiovascular system, cancer, digestive diseases, disorders of metabolism and glands with internal secretion, gynecologic diseases, rheumatic and neurological diseases, kidney and urinary tract diseases, inflammations of the male reproductive organs and chronic inflammation of prostate gland.

Spa Lucky is among the oldest spas in Slovakia, dated 1777 with mild mineral thermal, sulphate-hydrogen-carbonic, calcium-magnesium, carbonic, and hypotonic water. In baths procedures carbon dioxide (CO₂) is absorbed through the skin. It can cause hyperemia of the skin, even if it is a hypothermic baths, the skin stimulates to the release of catabolites. The skin turns red easily, but there is not acceleration of the heart rate as the hot bath, in turn, slows the heart rate, and blood pressure drops. This facilitates peripheral circulation. Important is the chemical effect of sulphurous water. During bathing and after bath, sulphur deposits in the skin penetrate into deeper tissues, where they bind in the form of chondroitin and inhibit hyaluronidase activity resulting in increasing the

concentration of hyaluronic acid in serum. This effect of sulphurous water is used in the treatment of osteoarthritis. Sulphur also has a keratolytic, antiseptic and vasodilatory effects, which are used in the treatment of selected skin diseases. At present, in Spa Lucky patients with gynecological disorders, infertility of men and women, patients with musculoskeletal and oncological diseases, after a stroke, in the prevention and treatment of osteoporosis are treated. In the past, patients with cardiovascular diseases were treated in this spa. Mineral water Spa Lucky contains trace elements with antioxidant properties, which is a prerequisite for anti-stress and antioxidant effect. *Composition of natural mineral water Spa Lucky*: Ca (561,2 mg/L), Mg (109,44 mg/L), Fe (1,10 mg/L), Li (0,30 mg/L), SO_4^{2-} (1223,6 mg/L), HCO_3^- (823,5 mg/L), pH= 6,25). Total amount of minerals in thermal water is 2,719 mg/L (mildly mineralized). Temperature 31.5 -32°C.

2.3. Balneotherapy, Infertility and Gynecology Diseases

Imbalance between the production of ROS and antioxidant functional capacity of the organism leads to oxidative stress (OS); OS is implicated in the development of many diseases, including infertility and gynecologic diseases. OS is presumed to contribute to mitochondrial DNA damage in the spermatozoa, in necrozoospermia and asthenospermia (15, 16, 17). *Mitochondria*, subcellular organelles, play an important role in intracellular energy (ATP) production and also in the generation of free radicals. A key component of the respiratory chain in mitochondria is coenzyme Q10 (CoQ10), an essential factor in cellular bioenergetics. As an antioxidant it can scavenge free radicals and inhibit lipid peroxidation, and decreases oxidative stress. Dysfunctional mitochondria produce excessive amount of ROS, which can cause oxidative damage to the components of respiratory chain as well as to the mtDNA. Mitochondrial coenzyme Q functions include regulation of electron transport in the respiratory chain, transferring electrons from complex I and complex II to complex III and transfer of protons from fatty acids across the inner mitochondrial membrane in the Q-cycle, producing a protonmotive force necessary for ATP synthesis (18), (Figure 1).

Balneotherapy with natural mineral water Spa Lucky has a long tradition in the treatment of gynecological disorders, and male and female infertility. We hypothesized a beneficial effect of balneotherapy by mitigation of OS by antioxidants in women with gynecologic diseases. Molecular mechanisms of the therapeutic effects of balneotherapy are not clear. We therefore carried out studies to examine the effect of drinking Spa water in both humans and rats. Our aim was to investigate the metabolic basis of natural mineral water ingestion in clinical and experimental trials: a/ The effect of balneotherapy on plasma antioxidants and oxidative stress in women with gynecology diseases; b/ The effect of drinking natural mineral water from Spa Lucky and simultaneous CoQ10 treatment on selected concentrations of antioxidants in plasma, tissues and mitochondrial oxidative phosphorylation in rats.

3. MATERIALS AND METHODS

3.1. Biochemical Analysis

3.1.1. Plasma and tissues samples

Plasma samples for estimation of CoQ_{9-OX}, CoQ_{10-OX}, alpha-tocopherol and TBARS were prepared from blood, collected into tubes with heparin after centrifugation at 1.96 x g for 15 minutes. *Tissue samples* of rat myocardium, uterus and ovaries were used for CoQ_{9-OX}, CoQ_{10-OX}, and alpha-tocopherol determination.

3.1.2. Determination of CoQ_{9-OX}, CoQ_{10-OX}, and alpha-tocopherol

Concentration of CoQ_{9-OX}, CoQ_{10-OX}, and alpha-tocopherol were determined simultaneously by isocratic high-performance liquid chromatography (LKB Sweden) according to Lang *et al.* (19) with some modification by Kucharská *et al.* (20, 21). Plasma samples (500 µl) were extracted by mixture hexane/ethanol (5/2; v/v). Tissue samples from myocardial left ventricle, uterus and ovary (30-50 mg), isolated mitochondria (2-2.5 mg) were homogenized in water and extracted with the mixture of hexane/ethanol (5/2; v/v). Organic layer was evaporated under nitrogen, the residue dissolved in 99.9% ethanol and injected on the 7 µm column SGX C18 (Tessek, Czech Republic). Elution was performed with methanol/acetonitrile/ethanol (6/2/2; v/v/v); flow rate 0.85 ml/min. Concentrations of CoQ_{9-OX}, CoQ_{10-OX}, and alpha-tocopherol were detected spectrophotometrically at 275 nm using external standards (Sigma; Germany). Data were collected and processed using CSW32 chromatographic station (DataApex Ltd; Czech Republic).

3.1.3. Determination of TBARS in plasma

Thiobarbituric acid reactive substances (TBARS) in plasma were determined spectrophotometrically by the method Ohkawa *et al.* (22).

3.1.4. Mitochondrial respiration and oxidative phosphorylation

Mitochondria of the myocardium and skeletal muscle were isolated by differential centrifugation using method by Palmer *et al.* (23). Mitochondrial proteins were determined according to Lowry *et al.* (24) using bovine serum albumin as standard. Mitochondrial respiration was determined polarographically by means of Oxygraph Gilson 5/6H (USA) using Clark-oxygen electrode. All the measurements were performed at 30°C. Respiration medium contained 120 mM KCl, 5 mM KH₂PO₄, 0.5 mM EDTA, 10 mM HEPES and 2% dextran, pH 7.2. Mitochondria (3-5 mg prot.) were added to the medium in a water-jacked chamber of 1.8 ml volume. For measurements of Complex I. NAD-substrate glutamate was added (final concentration 5.0 mM), for Complex II. FAD-substrate succinate was added (final concentration 10 mM) in presence of inhibitor of Complex I rotenone. *State 3* respiration rates were determined from oxygen uptake after addition of 600 nmol of ADP. *State 4* respiration rates were determined from the oxygen uptake after ADP consumption counting that the solubility of oxygen in the medium was 222.5 nmol/cm³. The *oxidative phosphorylation rate (OPR)* was calculated from the

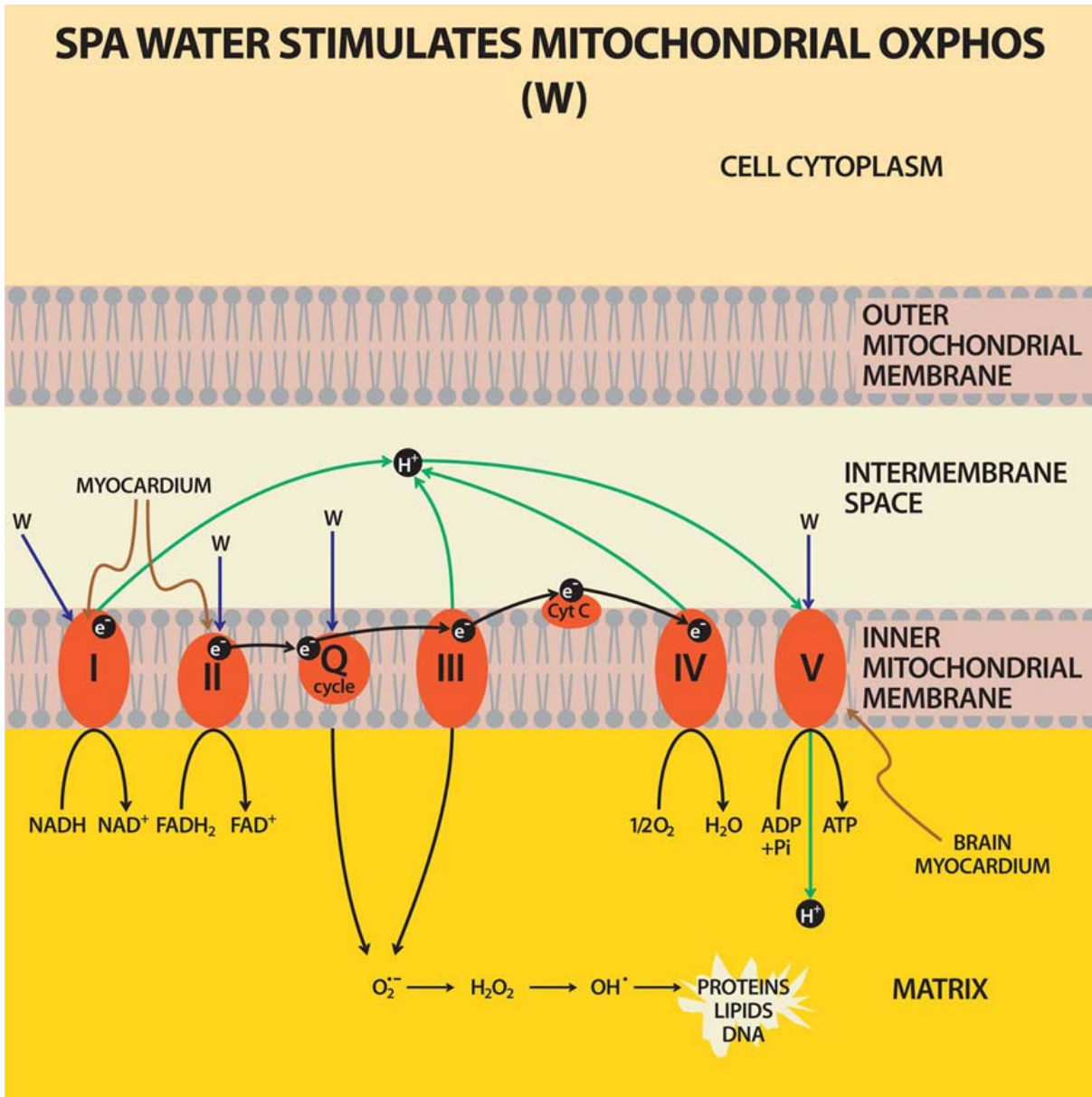


Figure 1. Spa Lucky water drinking stimulates myocardium mitochondrial oxidative phosphorylation in rats. W – Spa Lucky mineral water; OXPHOS – oxidative phosphorylation; CoQ – Coenzyme Q; ATP – adenosine triphosphate; ADP – adenosine diphosphate; Pi – inorganic phosphate; I, II, III, IV, V - respiratory chain complexes; H⁺ - proton; e⁻ - electron; Q-cycle – Coenzyme Q cycle; cyt c – cytochrome c; NADH - reduced nicotinadenindinucleotid; NAD⁺ - nicotinadenindinucleotid; FADH₂ – reduced flavinadenindinucleotid; FAD - flavinadenindinucleotid; O₂⁻ - superoxide radical; H₂O₂ – hydrogen peroxide; OH⁻ – hydroxyl radical; H₂O – water; O₂ – oxygen

nmoles of added ADP divided by time needed for its consumption and mg proteins in the chamber. *Respiratory control ratio (RCR)* was calculated as a ratio of State 3 to State 4. *Coefficient of oxidative phosphorylation (ADP/O)* was calculated from nmoles of added ADP divided by nmoles of oxygen utilized during State 3 respiration using method by Estabrook (25).

3.2. Clinical Study

3.2.1. Patients

In the study we included 51 women

a/ Healthy women (9 women), aged 25-50 years;

b/ Women with endometriosis sterility (13 women), aged 25-35 years. Of the 13 women, nine were diagnosed with primary sterility and four women with secondary sterility. Vaginal palpation findings (thickening, tenderness and shortening parametria) were available for four women at the onset of the treatment, for two women after diagnostic laparoscopy (LSK) for adhesions, for two women - after diagnostic laparoscopy (LSK) for endometriosis, one woman after laparotomy (fibroid

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removal), three women after diagnostic laparoscopy for tubal blockage both; four women – for long-lasting inflammation uterus pendants and uterus ligaments, and one woman – for recurrent miscarriages.

c/ Women after hysterectomy (29 women), age 45-60 years - the uterus after surgery and uterus pendants. Vaginal palpation finding was available for 12 women at the onset of the treatment.

3.2.2. Balneotherapy treatment

Treatment was complex, consisting of a drinking cure "mineral water Valentine's Day - BJ - 101" -1000 500 ml per day for 28 days. Drinking water was combined with therapeutic treatments. Procedures in the pool (swimming, therapeutic exercise, resting standing) in sterile women included - yoga, individual physical exercise, hydrotherapy (whirlpool bath, tub bath, aromatherapy), and classic or underwater massages. Thermal treatments (peat, paraffin) and electrotherapy were excluded. The patients received 3-4 treatments daily for a total of 50 to 60 treatments. Spa treatment for individual patients differed minimally.

3.2.3. Clinical examination

Vaginal gynecological examination (2 - 3 times per month), blood pressure measurement (once a - week), weight measurement (once a - week), collection of blood (hematocrit and leukocytes twice a month).

3.2.4. Antioxidants and TBARS

Concentration of antioxidants in blood and plasma (CoQ₁₀, alpha-tocopherol) and TBARS were determined before and after 4-weeks spa therapy.

3.3. Experimental Study

3.3.1. Experimental animals

Wistar rats, females, 3 months of age (220 - 245 g body weight), were randomly assigned to three groups. Each group consisted of eight rats. *C group* (control) - Rats given tap water from Bratislava (Slovakia); *W group* - Rats given natural mineral water from *Spa Lucky* (Slovakia); *W+Q10 group* - Rats given natural mineral water from *Spa Lucky* and received 20 mg of coenzyme Q10/kg body weight/day, administered by oral gavage. Duration of experiment was 3 months. The study was performed in accordance with institutional guidelines with approval by the State Veterinary and Food Administration of the Slovak Republic and by the Ethical Committee. All rats were housed under a 12 hour light and 12 hour darkness cycle, at constant temperature and humidity. Rats were fed with free access to standard laboratory chow and drinking water.

3.3.2. Biochemical analysis

Effects of mineral water *Spa Lucky* (W) were statistically evaluated in comparison with control group (C); effects of simultaneous W and coenzyme Q₁₀ administration (W+Q₁₀) were compared with W group: on plasma lipid peroxidation (TBARS) and the concentration of endogenous antioxidants (CoQ_{9-OX}, CoQ_{10-OX} and alpha-tocopherol) in plasma, tissues and mitochondria of

myocardium and skeletal muscle. Parameters of mitochondrial respiratory chain were evaluated between C group and after 3 months drinking of *Spa Lucky* natural mineral water (W) or in simultaneous treatment with coenzyme Q₁₀ (W+Q₁₀).

3.4. Statistics

Statistical significance of differences between experimental groups (C vs W and W vs W+Q₁₀) was evaluated using Student's t-test. Differences between groups of p<0.05 were considered statistically significant.

4. RESULTS

4.1. Sterility of Women

After graduating spa treatment, palpation findings were negative in all 13 women monitored and clinical parameters were improved in all women. TBARS values in all women were significantly higher compared with control values (p<0.01). After four-weeks of balneotherapy concentration of TBARS in plasma was not changed. Patients had deficiency of CoQ₁₀ in the blood and plasma, values were significantly reduced relative to control values (p<0.001, and p<0.05). CoQ₁₀ concentration in plasma (p<0.05) was significantly increased after four-weeks of balneotherapy. Alpha-tocopherol in whole blood and plasma showed extremely low values in comparison with the control group (p<0.001 and p<0.01). Balneotherapy increased concentrations of alpha-tocopherol in the blood (p<0.05) and plasma (p<0.001) of the sterile women, but they remained below control values (Table 1).

4.2. Women after Hysterectomy

Following spa treatment palpation findings were negative in 11 women, and in one woman continued to stay positive. TBARS values were increased in all women compared with the control group. Four weeks balneotherapy significantly reduced TBARS concentration (p<0.01) and slightly stimulated blood and plasma concentrations of antioxidants (CoQ₁₀ and alpha-tocopherol), (Table 1).

4.3. Rats

4.3.1. Plasma

a/ *Spa water* drinking during 3 months significantly decreased TBARS in plasma vs controls (p<0.015) and slightly stimulated concentrations of CoQ_{9-OX} and alpha-tocopherol.

b/ Simultaneous spa water + CoQ₁₀ treatment increased plasma concentrations of CoQ_{10-OX} (from not detectable to 0.449±0.103 μmol/L) and alpha-tocopherol (marginally significant) vs spa water treatment (Table 2).

4.3.2. Tissues

a/ *Spa water* treatment *in utero* did not change CoQ_{9-OX}, CoQ_{10-OX} concentrations, and diminished alpha-tocopherol (p<0.01) vs controls. *In ovaries* significantly increased alpha-tocopherol concentration (p<0.05) was observed. *In myocardium* significantly increased levels of CoQ_{9-OX} (p<0.01), CoQ_{10-OX} (p<0.01) and alpha-tocopherol (p<0.01) vs controls were found.

Table 1. Effect of Spa Lucky balneotherapy on plasma antioxidants and TBARS in women with gynecological diseases.

		Control	Sterility	Sterility	Hysterectomy	Hysterectomy
		C	(BB)	(AB)	(BB)	(AB)
TBARS	Plasma	4.24±0.31	5.68±0.21**	5.70±0.15	6.44±0.13	5.92±0.12++
CoQ _{10-ox}	Blood	0.516±0.038	0.267±0.045***	0.330±0.034	0.413±0.027	0.464±0.021
	Plasma	0.493±0.034	0.367±0.033*	0.494±0.039+	0.530±0.035	0.594±0.036
alpha-tocopherol	Blood	21.70±2.00	6.17±0.71***	12.41±1.79++	12.50±1.59	16.59±1.18
	Plasma	33.42±2.93	21.10±2.30**	24.82±2.88	25.38±1.72	28.16±1.46

µmol/L – micromol/liter; TBARS – thiobarbituric acid reactive substances (parameter of lipid peroxidation); CoQ_{10-ox} – oxidized coenzyme Q₁₀; Data are expressed as mean ± standard error of the mean; p – statistical significance; NS – not significant; Groups of patients: Control (C); Before balneotherapy (BB); After balneotherapy (AB); BB vs C: *p<0.05; **p<0.01; *** p<0.001; AB vs BB: ++p<0.05; +++p<0.01

b/ Simultaneous spa water + CoQ₁₀ treatment *in utero* significantly increased concentrations of plasma CoQ_{10-ox} (p<0.001), and alpha-tocopherol (p<0.05) vs spa water treatment. *In ovaries* marginally significantly increased concentrations of CoQ_{9-ox}, CoQ_{10-ox} (p<0.001) and alpha-tocopherol (marginally significant) vs spa water treatment were found. *In myocardium* significantly decreased CoQ_{9-ox} (p<0.01) and alpha-tocopherol (p<0.01) vs spa water treatment were found, with no change in CoQ_{10-ox} concentrations (Table 3).

4.3.3. Mitochondria

Effect of spa mineral water on myocardial mitochondria showed stimulation of mitochondrial energy production.

a/ *In myocardium mitochondria* spa water treatment significantly stimulated concentrations of CoQ_{9-ox} (p<0.01), CoQ_{10-ox} (p<0.01), and alpha-tocopherol (p<0.01) vs controls. *In mitochondria of skeletal muscle* spa water treated did not change levels of CoQ_{9-ox}, CoQ_{10-ox} and significantly increased level of alpha-tocopherol (p<0.001) vs controls.

b/ Simultaneous spa water + CoQ₁₀ treatment *in myocardium mitochondria* resulted in marginally increased concentrations of CoQ_{9-ox}, and alpha-tocopherol vs spa water treatment. *In mitochondria of skeletal muscle* significantly increased CoQ_{10-ox} (p<0.01) and alpha-tocopherol concentrations vs spa water treatment were found (Table 4).

4.3.4. Oxidative phosphorylation

Myocardium: at Complex I. of mitochondrial respiratory chain spa water treatment significantly increased OPR (p<0.01), State 3 (p<0.001), and RCR (p<0.01). State 4 and ADP:O were not changed vs controls. Spa water treatment increased OPR (NS), State 3 (p<0.01), and RCR (p<0.001) at *Complex II.*, State 4 and ADP:O were not changed vs controls. Simultaneous Spa water + CoQ₁₀ treatment returned these parameters to the control values in both complexes (Table 5; Figure 1).

Skeletal muscle: at Complex I. of mitochondrial respiratory chain Spa water treatment marginally significantly decreased OPR, State 3 and State 4. ADP:O and RCR were not affected vs controls. Spa water treatment parameters at *Complex II.* did not change vs controls. Simultaneous Spa water + CoQ₁₀ treatment increased OPR and state 3 to control values at Complex I (marginally significant); at Complex II. OPR and State 3 were

significantly decreased (p<0.01) vs Spa water treatment (Table 6).

5. DISCUSSION

Beneficial effects of balneotherapy – antioxidant protection and decreased oxidative stress were documented in several diseases (see Chapters 2.2. and 2.3.).

Oxidative stress in infertile women has been shown by several authors (14, 26, 27, 28). Uncontrolled formation of reactive oxygen species/reactive nitrogen species can cause reproductive diseases, including endometriosis, polycystic ovary syndrome, unexplained infertility and may cause various complications during pregnancy, such as spontaneous abort, pre-eclampsia, intrauterine growth retardation of the fetus (17).

Spa treatment has become an effective adjunct to hormonal therapy. Relationship between inner glands metabolic processes and neurovegetative functions could explain the failure of hormonal therapy in some dysghormonosis, with positive effects only in association with balneotherapy (27, 29).

Results of our study are in agreements with other authors (7, 11, 14) four-weeks of spa balneotherapy stimulated plasma antioxidants (concentrations of CoQ₁₀ and alpha-tocopherol) in women with sterility and decreased oxidative stress (TBARS) in women after hysterectomy (Table 1). We assume that the imbalance between antioxidants and oxidative stress to normal values could be achieved by simultaneous balneotherapy and antioxidants treatment. The importance of antioxidant supplementation in preventing preeclampsia is emphasized. The authors (30) in their report point to support antioxidant therapy before pregnancy. Adjunctive treatment with coenzyme Q₁₀ during pregnancy reduces the risk of preeclampsia (31). Authors (32) have suggested avoiding premature loss of the fetus using antioxidants before pregnancy. Other authors (33) found a decreased oxidative stress during pregnancy by administration of exogenous antioxidants. The importance of antioxidant therapy in men and women with fertility disorders have been suggested by several authors (15, 17, 34). The positive effect of coenzyme Q₁₀ supplementation in infertile men with idiopathic oligoasthenozoospermia has been reported (35).

Previous studies showed increased antioxidant and bioenergetic protection of men and women, as well as

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Table 2. Concentrations of antioxidants (CoQ_{9-ox}, CoQ_{10-ox}, alpha-tocopherol) and TBARS in rats plasma (in μmol/L)

	C	W	W+Q10
CoQ _{9-ox}	0.193±0.012	0.218±0.018	0.213±0.034
CoQ _{10-ox}	n.d.	n.d.	0.449±0.103
alpha-tocopherol	10.90±1.38	13.00±0.44	18.00±2.68+m.s.
TBARS	11.56±0.57	9.80±0.31*	9.19±0.42

C (Control rats); W (spa water treated); W+Q₁₀ (spa water + Coenzyme Q₁₀ treated); CoQ_{9-ox} (Coenzyme Q₉ - oxidized); CoQ_{10-ox} (Coenzyme Q₁₀ -oxidized); TBARS (thiobarbituric acid reactive substances); μmol/L (micromol/liter). Data are expressed as mean ± standard error of the mean; p (statistical significance); n.d. – not detectable. Number of rats in each group was 8. W vs C: *p<0.05; W+Q₁₀ vs W: +m.s. – marginally significant.

Table 3. Concentrations of antioxidants (CoQ_{9-ox}, CoQ_{10-ox}, alpha-tocopherol) in rat tissues (in nmol/g ww)

	C	W	W+Q10
Uterus	CoQ _{9-ox}	10.40±0.807	11.70±1.11
	CoQ _{10-ox}	2.15±0.334	3.16±0.326
	alpha-tocopherol	41.10±5.12	18.90±2.04**
Ovaries	CoQ _{9-ox}	40.50±3.93	34.60±5.29
	CoQ _{10-ox}	8.02±1.56	8.92±1.84
	alpha-tocopherol	475.10±39.20	593.00±52.00*
Myocardium	CoQ _{9-ox}	204.70±12.00	260.40±11.80**
	CoQ _{10-ox}	22.30±1.28	30.00±1.70**
	alpha-tocopherol	56.70±2.53	75.90±4.50**

C (Control rats) W (spa water treated); W+Q₁₀ (spa water + Coenzyme Q₁₀ treated); CoQ_{9-ox} (Coenzyme Q₉ - oxidized); CoQ_{10-ox} (Coenzyme Q₁₀ -oxidized); nmol/g ww (nanomol/gram wet weight). Data are expressed as mean ± standard error of the mean; p (statistical significance). Number of rats in each group was 8. W vs C: *p<0.05; **p<0.01; W+Q₁₀ vs W: +p<0.05; ++p<0.01; +++p<0.001, +m.s. (marginally significant).

Table 4. Concentrations of antioxidants (CoQ_{9-ox}, CoQ_{10-ox}, alpha-tocopherol) in rat mitochondria (in nmol/mg prot.)

	C	W	W+Q10
Myocardium	CoQ _{9-ox}	3.580±0.119	4.910±0.399**
	CoQ _{10-ox}	0.432±0.004	0.523±0.028**
	alpha-tocopherol	0.520±0.014	1.030±0.157**
Skeletal muscle	CoQ _{9-ox}	3.040±0.156	3.070±0.013
	CoQ _{10-ox}	0.228±0.015	0.189±0.007
	alpha-tocopherol	0.337±0.008	0.389±0.008***

C (Control rats); W (spa Water treated); W+Q₁₀ (spa water + Coenzyme Q₁₀ treated); CoQ_{9-ox} (Coenzyme Q₉ - oxidized); CoQ_{10-ox} (Coenzyme Q₁₀ -oxidized); nmol/mg prot. (nanomol/miligram proteins). Data are expressed as mean ± standard error of the mean; p (statistical significance). Number of rats in each group was 8; W vs C: **p<0.01; ***p<0.001; W+Q₁₀ vs W: +m.s. (marginally significant); ++p<0.01

Table 5. Oxidative phosphorylation of myocardium mitochondria in rats.

	C	W	W+Q10
Complex I.	OPR	325.60±17.10	431.80±28.30**
	State 3	133.00±8.20	186.00±5.86***
	State 4	22.40±1.65	9.40±2.02
	ADP:O	2.47±0.047	2.40±0.064
Complex II.	RCR	6.45±0.536	10.80±1.32**
	OPR	283.10±12.10	350.40±33.10
	State 3	217.30±13.75	269.90±5.69**
	State 4	123.90±6.46	113.50±1.93
	ADP:O	1.320±0.036	1.330±0.056
	RCR	1.750±0.031	2.380±0.064***

C (Control rats); W (spa Water treated); W+Q₁₀ (spa water + Coenzyme Q₁₀ treated); *Complex I.* (respiratory parameters of myocardium mitochondria with glutamate as substrate); *Complex II* (respiratory parameters of myocardium mitochondria with succinate/rotenone as substrate); OPR (oxidative phosphorylation rate), in nmol ATP/mg prot./min; State 3 (ADP stimulated respiration) and State 4 (basal respiration), in nAtO/mg prot./min; ADP:O (coefficient of oxidative phosphorylation), in nmol ADP/nAtO; RCR (respiratory control ratio), in State 3/ State 4. Data are expressed as mean ± standard error of the mean; p (statistical significance). Number of rats in each group was 8. W vs C: **p<0.01; ***p<0.001; W+Q₁₀ vs W: ++ p<0.01; +++ p<0.001

Table 6. Oxidative phosphorylation of skeletal muscle mitochondria in rats

	C	W	W+Q10
Complex I.	OPR	453.40±25.80	391.00±17.10*m.s.
	State 3	184.10±8.46	165.60±5.41*m.s.
	State 4	45.10±6.01	30.00±1.93*
	ADP:O	2.49±0.038	2.38±0.033
Complex II.	RCR	4.48±0.460	5.59±0.359
	OPR	456.00±29.10	446.60±10.40
	State 3	309.60±13.80	333.00±10.30
	State 4	182.00±8.07	185.50±1.67
	ADP:O	1.46±0.028	1.35±0.033
	RCR	1.71±0.057	1.79±0.043

C (Control rats); W (spa Water treated); W+Q₁₀ (spa water + Coenzyme Q₁₀ treated); *Complex I.* (respiratory parameters of skeletal muscle mitochondria with glutamate as substrate); *Complex II* (respiratory parameters of skeletal muscle mitochondria with succinate/rotenone as substrate); OPR (oxidative phosphorylation rate), in nmol ATP/mg prot./min; State 3 (ADP stimulated respiration) and State 4 (basal respiration), in nAtO/mg prot./min; ADP:O (coefficient of oxidative phosphorylation), in nmol ADP/nAtO; RCR (respiratory control ratio), in State 3/ State 4. Data are expressed as mean ± standard error of the mean; p (statistical significance). Number of rats in each group was 8. W vs C: *m.s. (marginally significant); *p<0.05; W+Q₁₀ vs W: +m.s. (marginally significant); ++ p<0.01.

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the decreasing of oxidative stress using antioxidants CoQ₁₀ (Q-GEL FORTE® (coenzyme Q₁₀, vitamin E and C), which led to improving sperm parameters, fertility of men and achieving pregnancy in women with impaired fertility (36). Improvement of semen quality in infertile patients and subsequent pregnancy of women were found in supportive therapy with supplementation of CARNI-Q-GEL® (carnitine, ubiquinone, vitamin E and vitamin C) for 4-9 months (18). In another study, we focused on couples with fertility problems. Men and women have used the same supportive treatment with dietary supplement CARNI-Q-NOL® (carnitine, ubiquinol, vitamin E, vitamin C) for up to 6 months and pregnancy resulting in within 1-6 months in 47% of women (37, 38).

In our experimental study we demonstrated the beneficial effects of drinking Spa Lucky natural mineral water in rats on the stimulation of plasma antioxidants and reducing oxidative stress (Table 2), as well as stimulating effect on the concentrations of antioxidants in ovaries and myocardium tissues (Table 3). Due to the lack literature data we cannot compare our pilot results with those of other authors. We found also beneficial effect of drinking spa water on stimulation of *myocardium mitochondrial* respiratory chain function at Complex I, II. (State 3, OPR), coenzyme Q and Complex V, (ATP production), (Table 4, 5; Figure 1). These results may help to explain the mechanism of the beneficial effects of Spa Lucky in heart diseases. In isolated *skeletal muscle mitochondria* we did not found differences in CoQ_{9-OX} and CoQ_{10-OX} levels between controls and Spa water drinking. On the other hand, drinking this water in rats decreased skeletal muscle mitochondrial function, as respiration and energy production at Complex I. (Table 6.). These results may partly explain transient weakness of the skeletal muscle of patients treated with balneotherapy. Simultaneous effect of Spa Lucky water and coenzyme Q₁₀ returned these parameters to the values of control group.

Based on these results we recommend CoQ₁₀ supplementation to improve the quality of life of patients before, during or/and balneotherapy treatment. Our pilot data could contribute to the explanation of the role of antioxidants, oxidative stress and mitochondrial function in the beneficial effects of Spa Lucky mineral water, based on both clinical and animal studies.

6. SUMMARY AND PERSPECTIVES

Spa Lucky balneotherapy is beneficial to patients with gynecological diseases. Possible mechanisms of therapeutic action of natural mineral water in Spa Lucky are the reduction of oxidative stress, and increased antioxidant defense and energy production in the heart. We propose prevention of transient weakness and improvement in the health of patients by a targeted antioxidants supplementation before starting spa treatment or by simultaneous balneo-, antioxidant and energy therapy. The results help to clarify the positive metabolic action of natural mineral water in Spa Lucky.

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