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Original Article

Prevention of adverse effects of balneofactors at Truskavets' Spa on gastroenterologic patients through phytoadaptogens and therapeutic physical education: mechanisms of rehabilitation

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Abstract
Background. The impact of the standard balneotherapeutic complex at Truskavets' Spa on physical performance in both rats and resort patients yielded mixed results. Previous research has indicated that the phytoadaptogen "Balm Truskavets" can mitigate the negative effects of Naftussya bioactive water on dynamic muscle performance in healthy rats and patients with post-radiation encephalopathy. This study aims to evaluate the potential of this phytocomposition to prevent unfavorable actotropic effects of Truskavets' Spa balneofactors on gastroenterologic patients. **Material and methods.** The study observed 40 former female athletes (aged 30÷76 years, body weight 55÷98 kg) with chronic cholecystitis in remission phase, undergoing rehabilitation at Truskavets' Spa. Parameters including PWC150, adaptation hormone levels, HRV, EEG, immunity, metabolism and gas discharge visualization (GDV) were recorded. Members of the control group received for two weeks standard balneotherapy: drinking of Naftussya bioactive water, application of Ozokerite, baths with mineral water, therapeutic physical education. Members of the main group additionally received a phytoadaptogen. **Results.** The analysis of individual changes revealed that normal levels of PWC in control group fell to the lower zone of the norm. Phytoadaptogen prevents PWC decrease. This is accompanied by the prevention of both a decrease in power spectral density (PSD) T4-θ EEG and VLF HRV, leukocytes level as well as area and symmetry of GDV, as well as an increase in vagal tone and entropy of HRV as well as a rightward shift in the symmetry of the virtual first Chakra of GDV. In addition, phytoadaptogen reverses balneotherapy-induced moderate decrease in the frequency of α-rhythm, PSD O1-β, sympathetic tone, serum levels of catecholamines, testosterone and IgG, activity of Na,K-ATPase of erythrocyte shadows as well as Energy of the first, third and fourth virtual Chakras. Phytoadaptogen potentiates the reduction of PSD P4-β, IgM and cholesterol as well as initiates the reduction of δ-rhythm variability, PSD of α-rhythm in C3, C4, P4 and Fp2 loci, entropy in F4 locus as well as serum potassium while increasing in serum cortisol and calcitonin, blood B-lymphocytes levels as well as PSD Fp2-δ. **Conclusion.** The phytoadaptogen "Balm Truskavets'" prevents the adverse effect of the standard balneotherapeutic complex of the Truskavets' Spa on PWC by, apparently, its neuro-endocrine effects.
Keywords: physical working capacity, adaptation hormones, HRV, EEG, GDV, immunity, metabolism, phytoadaptogen, Truskavets' Spa, women, chronic cholecystitis.

Introduction

The researchers of the Truskavetsian Scientific School of Balneology have demonstrated the adaptogenic properties of the main curative factors of the Truskavets' Spa such as Naftussya bioactive water, Ozokerite and mineral baths, which together make up a standard balneotherapeutic complex (Flyunt IS et al., 2002; Popovych IL et al., 2003; Kostyuk PG et al., 2006; Flyunt IS et al., 2008; Popovych IL, 2011; Popovych IL et al., 2022).

However, in contrast to the beneficial effect of the latter on stress resistance and the neuro-endocrine-immune complex, the effect on the physical performance of both rats and resort patients is ambiguous (Popovych IL et al., 2005; Zukow W et al., 2020; Zukow W et al., 2021; Zukow W et al., 2022), which prompted the additional use of aerobic training (Tserkovnyuk AV & Ruzhylo SV, 2001) and/or phytoadaptogens, both well-known (ginseng, Bittner's balsam), and the Ukrainian phytocomposition "Balm Kryms'kyi" (Hrinchenko BV, 1998; Hrinchenko BV et al., 1999; Flyunt IS et al., 2002; Kostyuk PG et al., 2006), the adaptogenic properties of

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which first discovered by representatives of the Truskavetsian Scientific School of Balneology (Panasyuk YM et al., 1994; Alyeksyeyev OI et al., 1996).

A detailed analysis of the causes of the adverse actotropic effect of balneotherapy revealed the following.

In an experiment on female rats, it was found that after 3 weeks of Naftussya water use adverse changes in swimming time to fatigue were observed only in 4 rats with initial very high performance, ie a reduction in swimming time from 61±7 min to 39±3 min. Instead, in 6 animals the performance increased from 13.0±1.4 min to 52.3±5.9 min, and in 8 animals did not change significantly (from 24.5±3.9 min to 37.3±5.9 min; $p>0.05$). In 73 children and adolescents of both sexes aged 10-17 with maladaptation, who received Naftussya water together with ozokerite applications and mineral baths, three variants of actotropic effects were also revealed. In particular, PWC170, assessed by the step test, decreased only in 31.5% of children with a PWC170 level significantly higher than the sex-age norm: from 125.8±3.5% to 119.6±3.0% (direct difference: -6.2±1.1%). On the other hand, the normal level of PWC170 in 21.9% of children did not change, and in 46.6% it increased from 113.7±2.7% to 125.6±2.8% (direct difference: 11.9±1.8%). A similar pattern also occurred in 42 adult gastroenterology patients of both sexes. In particular, PWC150, assessed by two-stage bicycle ergometry, decreased from 2.82±0.32 W/kg to 2.42±0.26 W/kg (by 11.0±5.0%) in 26.2% of patients, whereas in 47.6% of patients with lower work capacity, it increased from 2.32±0.18 W /kg to 2.45±0.14 W/kg (by 11.0±5.2%), and in another 26.2% it did not change significantly (Popovych IL et al., 2005). The analysis of individual changes in another sample (19 men and 3 women with urate urolithiasis and chronic pyelonephritis) revealed that in 54.5% patients the normal level of fitness fell to the lower zone of the norm, however, in 45.5% patients reduced fitness was completely normalized (Zukow W et al., 2022).

It is important to note that, firstly, various responses of fitness to balneofactors are accompanied by characteristic changes in metabolic, HRV, EEG, immune, and other parameters; secondly, based on the constellation of such initial parameters, first of all, the level of fitness, as well as lipids and electrolytes, it is possible to predict not only the direction, but also the severity of the fitness reaction (Popovych IL et al., 2005; Zukow W et al., 2021; Zukow W et al., 2022).

We have previously explored effects of phytocomposition “Balm Truskavets’”, which is analogous to the “Balm Kryms’kyi”, on parameters of neuro-endocrine-immune complex and biophotonics in humans with maladaptation (Ruzhylo SV et al., 2015; Fihura OA et al., 2021; Fihura OA et al., 2022; Fihura OA et al., 2023). It was shown, that this phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance both in healthy rats (Fihura OA et al., 2023) and in patients with post-radiation encephalopathy (Fihura OA, 2023).

The purpose of this study is to test the ability of this phytocomposition prevention of unfavourable actotropic effect of balneofactors of Truskavets’ Spa on gastroenterologic patients.

Material and methods

Participants. At the preliminary stage, for 8 days, we examined 63 former athletes women with chronic cholecystitis in remission phase (age 29÷76 years, body weight 48÷105 kg), who came for rehabilitation at Truskavets' Spa. For estimation of physical working capacity (PWC) a bicycle ergometer “Tunturi” (Finland) was used. The power of the first load was 0.5 W/kg, the second load (after 3 min) 1.5 W/kg at a pedaling frequency of 60-75 rpm. This corresponded to the recommendations for ergometer testing in occupational medicine (Trappe H-J & Löllgen H, 2000; Farazdaghi GR & Wohlfart B, 2001; Finger JD et al., 2013; Chatterjee M & Schmeißer G, 2017). We calculated submaximal PWC_{150} with the mechanical power in Watt per kilogram body weight (W/kg) as indicator of cardiorespiratory fitness (Finger JD et al., 2013).

The range of cardiorespiratory fitness was 1.30÷4.42 W/kg. The next study purposefully included women with fitness in the upper normal range (2.04÷4.42 W/kg), of which 40 former athletes women were found (age 30÷76 years, body weight 55÷98 kg).

Procedure / Test protocol / Skill test trial / Measure / Instruments.

The next day after bicycle ergometry, we recorded an electrocardiogram in II lead to assess the parameters of heart rate variability (HRV) (software and hardware complex "CardioLab+HRV" produced by "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters HRV were selected. Temporal parameters (Time Domain Methods): HR, the mode (Mo), the standart deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater than 50 msec (pNN_{50}); triangular index (TNN). Spectral parameters (Frequency Domain Methods): absolute ($msec^2$) and relative (%) power spectral density (PSD) bands of HRV: high-frequency (HF, range 0.4÷0.15 Hz), low-frequency (LF, range 0.15÷0.04 Hz), very low-frequency (VLF, range 0.04÷0.015 Hz) and ultralow-frequency (ULF, range 0.015÷0.003 Hz). Calculated classical indexes: LF/HF; (VLF+LF)/HF; LFnu=100%•LF/(LF+HF) (HRV, 1996; Berntson et al., 1997; Baevskiy R & Ivanov G, 2001).

Simultaneosly qEEG recorded at rest a hardware-software complex "NeuroCom Standard" (KhAI Medica, Kharkiv, Ukraine) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on the earlobes. Two minutes after the eyes had been closed, 25 sec of artifact free EEG data were collected by computer. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device. In addition, calculated coefficient of Asymmetry (As) and Laterality Index (LI) for PSD each Rhythm using equations:

As, % = 100•(Max – Min)/Min;

LI, % = Σ [200•(Right – Left)/(Right + Left)]/8 (Newberg AB et al., 2001).

We calculated also for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych's IL (Ruzhylo et al., 2015; Popadynets et al., 2020) equations based on classic Shannon's CE (1948) equation:

$hEEG = - [PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta]/\log_2 4$;

$hHRV = - [PSDHF \bullet \log_2 PSDHF + PSDLF \bullet \log_2 PSDLF + PSDVLF \bullet \log_2 PSDVLF + PSDULF \bullet \log_2 PSDULF]/\log_2 4$. Than we registered kirlianogram by the method of gas discharge visualization (GDV) by the device "GDV Chamber" ("Biotechprogress", SPb, RF). Method of GDV, essence of which consists in registration of photoelectronic emission of skin, induced by high-frequency electromagnetic impulses, allows to estimate integrated psycho-somatic state of organism. The first base parameter of GDV is Area of gas discharge image (GDI) in Right, Frontal and Left projections registered both with and without polyethylene filter. The second base parameter is a coefficient of shape (ratio of square of length of external contour of GDI toward his area), which characterizes the measure of serration/fractality of external contour. The third base parameter of GDI is Entropy, id est measure of chaos. It is considered that GDI, taken off without filter, characterizes the functional changes of organism, and with a filter characterizes organic changes. Program estimates also Energy and Asymmetry of *virtual* Chakras (Korotkov KG, 2014). The ability and informativeness of GDV method is confirmed by the research of Truskavetsian Scientific School (Babelyuk VYe et al., 2017; Babelyuk VE et al., 2017; Babelyuk VYe et al., 2023).

In portion of venous blood we determined serum levels of major hormones of adaptation: Cortisol, Testosterone, Aldosterone, Triiodothyronine and Calcitonin (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co Ltd and DRG International Inc.).

Immune status evaluated as described in the manual (Lapovets' LYe & Lutsyk BD, 2004). For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD56 (company "Granum", Kharkiv) with visualization under light microscope with immersion system.

Entropy of Immunocytogram (ICG) was calculated too:

$hICG = - [CD4 \bullet \log_2 CD4 + CD8 \bullet \log_2 CD8 + CD22 \bullet \log_2 CD22 + CD56 \bullet \log_2 CD56]/\log_2 4$.

The state of humoral immunity judged by the concentration in serum of Immunoglobulins of classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method). We estimated lipoprotein profile of plasma: total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of it in composition of HDL (by the enzyme method by Hiller); VLDL (calculated by the level of triglycerides, estimated by meta-periodate method, as ratio TG/2.1834); LDL (calculated by a difference between a total cholesterol and cholesterol in composition HD and VLD lipoproteins) according to instructions (Goryachkovskiy AM, 1998). On sodium and potassium exchange judged by their levels in the serum and erythrocytes (flame photometry method) as well as activity of Na,K-ATPase of erythrocyte shadows, determined by the increase of Pi in the supernatant of the incubation medium (Makarenko YeV, 1987). An analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents as well as flame photometer "СФ-47" was used for biochemical studies.

According to the data of daily bicycle ergometry of eight patients, two groups were formed from them with approximately the same levels of PWC, body weight and age, followed by blind allocation into the control or main group. Members of the control group received for two weeks standard balneotherapy: drinking of Naftussya bioactive water by 3 mL/kg for 1 hour before meals three times a day; application of Ozokerite on the lumbar region (temperature 45^0C, exposure 30 minutes, every other day, 5 procedures); baths with mineral water (Cl^--SO_4^{2-}-Na^+-Mg^{2+} containing salt concentration 25 g/L, temperature 36-37^0C, duration 8-10 minutes, every other day, 5 procedures); therapeutic physical education (motion mode II). Members of the main group additionally received a phytoadaptogen (5 mL pre-diluted in 200 mL of Naftussya water according to a similar scheme). Phytoadaptogen Balm "Truskavets'" produced by private research-production enterprise "Ukrainian Balms" (Mykolaïv, Ukraine). Here are the components of the phytocomposition: *Nepeta cataria, Mentha × piperita, Salvia officinalis, Echinacea purpurea, Cichorium inthybus, Achillea millefolium, Artemisia balchanorum, Acorus calamus, Althaea officinalis, Silybum marianum, Rubus idaeus, Rosa majalis* (ТУ У 15.8-24055046-005:2009).

The next morning after completing the treatment, retesting was performed.

Data collection and analysis / Statistical analysis.

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA).

The article employs discriminant analysis to classify patients into groups based on their examination results. Discriminant analysis is utilized when there is a categorical dependent variable (e.g., patient group) and multiple independent variables (e.g., laboratory test results). Discriminant analysis in this study.

This study analyzed the examination results of patients from three groups: Control group; Group treated with standard balneotherapy; Group treated with balneotherapy and an additive. The authors initially verified whether statistically significant differences existed in the baseline parameters between the groups. Subsequently, discriminant analysis was performed to incorporate all examination results and better differentiate between the groups. Discriminant analysis enables: Identification of a set of variables that best differentiate between the groups. Visualization of the examination results for individual patients. Assignment of new patients to groups based on their examination results.

Results of discriminant analysis. Discriminant analysis revealed that 31 examination parameters significantly differentiate between the three patient groups. These parameters included EEG, GDK, hormone, immunity, metabolism test results, and others. Based on these 31 parameters, two main discriminant factors were extracted, which together explain almost 100% of the variability between the groups.

Results

The starting positions of the members of the control and main groups turned out to be almost the same (Mean±SE): age 49.9±2.8 vs 47.3±2.6 years; body weight 71.6±3.1 vs 73.8±3.2 kg; PWC_{150} 2.45±0.13 vs 2.38±0.16 W/kg; cortisol 367±28 vs 322±20 nM/L; aldosterone 227±5 vs 221±6 pM/L; testosterone 3.17±0.50 vs 3.58±0.54 nM/L; triiodothyronine 2.05±0.22 vs 1.81±0.15 nM/L; calcitonin 5.33±0.69 vs 6.14±0.73 ng/L. A similar situation was established also with regard to the parameters of HRV, EEG, immunity and metabolism. This gave us the reason to combine members of both treatment groups into one.

As expected on the basis of previous studies, after standard balneotherapy, the PWC level dropped from the upper normal zone to its middle: from 2.45±0.10 to 2.02±0.08 W/kg. Additional use of phytoadaptogen minimized the adverse actotropic effect of standard balneotherapy to 2.24±0.10 W/kg.

Adhering to the Truskavetsian Scientific School's analytical algorithm, the actual/raw parameters were normalized by recalculation by the equations:

$Z = (V - N)/SD = (V/N - 1)/Cv$, where

V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively.

Reference values are taken from the database of the Truskavetsian Scientific School of Balneology (EEG, GDV, immunity) or instructions (HRV, ELISA, metabolism).

Further, profiles (Fig. 1) of normalized parameters were created, the levels of which differ significantly before and after standard or combined therapy, as well as several parameters which according to the following discriminant analysis were still recognizable, despite the insignificant value of Student's t criterion.

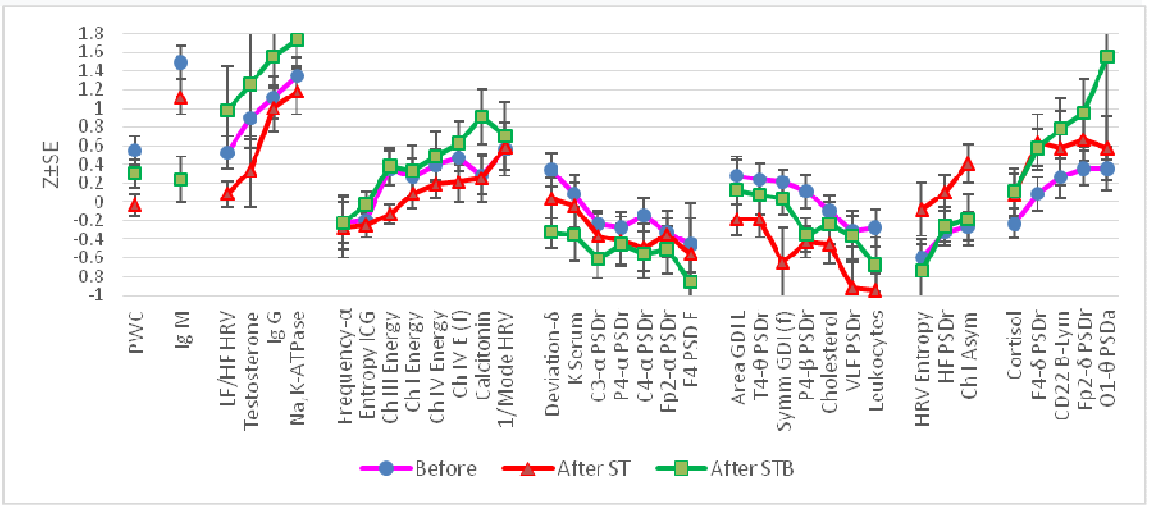

Fig. 1. Profiles of Z-scores of variables before and after standard therapy (ST) or supplemented with Balm

Next, the profiles were grouped into more or less homogeneous clusters (Fig. 2). It can be seen that, under the influence of standard balneotherapy, the increased levels of IgM, IgG and testosterone serum as well as Na,K-ATPase of erythrocyte shadows and LF/HF ratio as sympathetic tone decrease. Standard balneotherapy has practically no effect on the normal levels of the parameters of the two following clusters. On the other hand, the reduced entropy levels of HRV and PSD of HF band as vagal tone increase. That is, there is a **normalizing** (ambivalence-equilibratory) effect as one of the attributes of adaptogens (Balanovs'kyi VP et al., 1993; Kostyuk PG et al., 2006; Panossian AG et al., 2021) according to the good old "law of initial level".

At the same time, contrary to this law, the normal levels of cortisol, PSD of delta- and theta-rhythms in Fp2, F4 and O1 loci as well as B-lymphocytes also increase, and the normal levels of relative PSD of VLF band HRV, theta-rhythm in T4 locus and beta-rhythm in P4 locus, cholesterol, leukocytes as well as area and symmetry of GDI decrease. In addition, the left-sided asymmetry of the first Chakra is transformed into a right-sided one, which is evidenced by the transformation of a negative value into a positive one.

Supplementation of the balneotherapeutic complex with a phytoadaptogen potentiates both its inhibiting and enhancing effects on a number of parameters, instead it weakens or even reverses the effects on other constellations of parameters. A more detailed analysis will be conducted during the discussion.

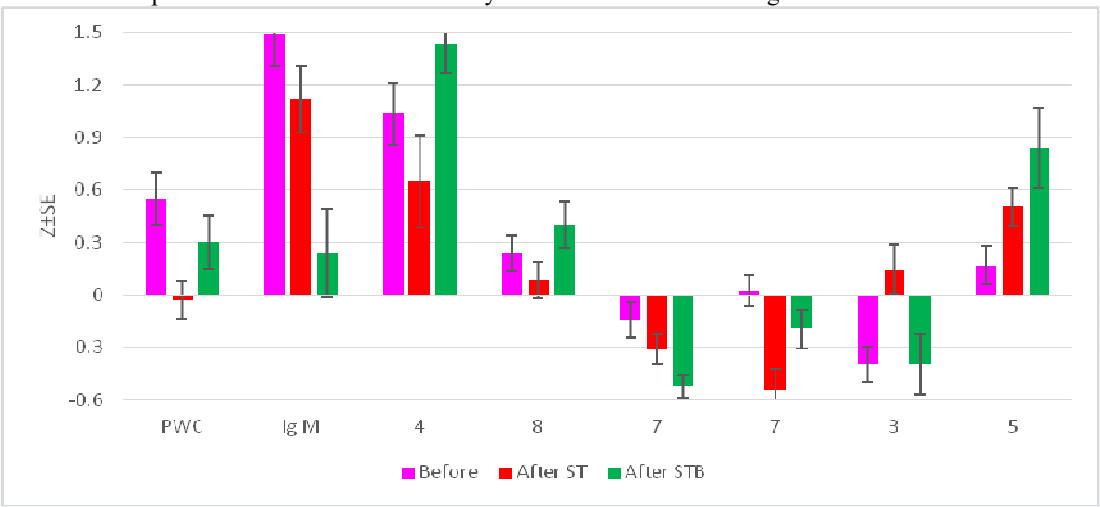

Fig. 2. Clusters of variables before and after standard therapy (ST) or combined with Balm (STB). ***The number of variables is indicated under the clusters***

In order not only to find out which of the listed parameters are characteristic (recognizable) for the three groups, but primarily to visualize each patient in the information field, the constellation of these parameters was subjected to a discriminant analysis (Klecka WR, 1989). The forward stepwise program included, except PWC by definition, 30 variables in the discriminant model (Tables 1-2). Among them, 12 relate to **EEG**, 5 – **GDV**, 3 – **Hormones**, 3 - **Immunity**, 3 – **Metabolism** and 4 – **GDV**.

Table 1. Discriminant Function Analysis Summary

Step 31, N of vars in model: 31; Grouping: 3 grps; Wilks' Λ: 0,1593; approx. $F_{(63)}$=2,3; $p<10^{-4}$

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	After Stan-dard Therapy (20)	After Standard Therapy + Balm (20)	Before Therapy (40)	Wilks' Λ	Par-tial Λ	F-re-move (2,47)	p-level	Tole-rancy	Refe-rence (Norm) (40) Cv; **SD**
Phys Working Capacity, W/kg	2,02 0,08	2,24 0,07	2,42 0,10	0,191	0,834	4,66	0,014	0,709	2,04 0,333
Frequency-α, Hz	10,36 0,30	10,42 0,27	10,41 0,21	0,178	0,894	2,78	0,072	0,450	10,62 0,088
Fp2-α PSD, %	27,8 3,6	25,3 3,7	28,3 2,2	0,194	0,822	5,09	0,010	0,058	32,9 0,448
C4-α PSD, %	27,4 3,8	26,4 3,7	32,6 2,5	0,179	0,891	2,88	0,066	0,046	34,8 0,432
C3-α PSD, %	29,3 4,2	25,2 3,6	31,5 2,3	0,174	0,916	2,16	0,127	0,071	35,4 0,468
P4-α PSD, %	36,8 4,9	35,9 4,5	39,5 3,2	0,167	0,957	1,06	0,353	0,047	44,8 0,428
Deviation-δ, Hz	0,68 0,06	0,58 0,04	0,76 0,05	0,179	0,890	2,91	0,064	0,408	0,67 0,395
Fp2-δ PSD, %	38,7 4,4	43,8 6,6	33,1 3,5	0,240	0,663	11,9	10^{-4}	0,164	26,5 0,687

F4-δ PSD, %	43,8 5,7	46,9 5,3	33,0 3,4	0,178	0,894	2,80	0,071	0,072	31,25 0,624
T4-θ PSD, %	8,9 0,9	10,0 0,9	10,8 0,8	0,198	0,806	5,66	0,006	0,332	9,7 0,482
O1-θ PSD, $\mu V^2/Hz$	53 11	83 34	46 7	0,187	0,851	4,13	0,022	0,255	35 0,878
P4-β PSD, %	17,9 1,9	18,8 2,0	24,1 2,1	0,183	0,872	3,45	0,040	0,169	22,8 0,503
Entropy of PSD in F4 locus	0,799 0,050	0,772 0,041	0,809 0,027	0,179	0,889	2,94	0,062	0,114	0,851 0,139
VLF band PSD, %	41,7 3,4	48,7 3,0	49,3 2,4	0,178	0,896	2,71	0,077	0,144	53,2 0,288
HF band PSD, %	15,3 1,9	11,9 1,7	11,4 1,0	0,195	0,816	5,28	0,008	0,557	15,1 0,682
HF/LF HRV ratio	3,01 0,26	4,73 0,88	3,91 0,36	0,168	0,949	1,27	0,290	0,188	2,84 0,717
Entropy of PSD of HRV bands	0,797 0,022	0,740 0,020	0,757 0,016	0,179	0,889	2,93	0,063	0,116	0,807 0,109
Mode HRV, msec	815 26	797 36	815 28	0,169	0,940	1,50	0,234	0,607	871 0,115
Testosterone, nM/L	2,74 0,42	3,77 0,65	3,36 0,37	0,217	0,734	8,51	0,001	0,111	2,37 0,468
Cortisol, nM/L	378 27	383 28	345 17	0,170	0,939	1,52	0,229	0,232	370 0,303
Calcitonin, ng/L	5,66 0,64	7,31 0,75	5,74 0,49	0,179	0,891	2,88	0,066	0,529	5,05 0,490
Leukocytes, $10^9/L$	5,05 0,18	5,33 0,29	5,72 0,20	0,167	0,955	1,10	0,342	0,372	6,00 0,167
CD22 B-Lymphocytes, %	22,0 1,4	22,8 1,1	20,9 0,8	0,175	0,913	2,25	0,116	0,136	20,0 0,175
Immunoglobulins M, g/L	1,46 0,05	1,22 0,07	1,56 0,05	0,168	0,946	1,34	0,273	0,404	1,15 0,239
Na,K-ATPase Erythr, M/L•h	1,02 0,05	1,14 0,06	1,06 0,04	0,169	0,944	1,38	0,260	0,802	0,76 0,288
Potassium Serum, mM/L	4,53 0,11	4,39 0,14	4,59 0,10	0,168	0,949	1,26	0,293	0,728	4,55 0,104
Cholesterol, mM/L	5,11 0,25	5,25 0,25	5,45 0,19	0,183	0,873	3,42	0,041	0,446	5,54 0,193
Symmetry of GDI (filter), %	92,29 0,53	93,25 0,24	93,50 0,19	0,185	0,861	3,80	0,029	0,542	93,20 0,015
Chakra I Asymmetry	0,01 0,05	-0,14 0,07	-0,16 0,04	0,177	0,902	2,55	0,089	0,485	-0,09 **0,25**
Chakra I Energy	0,13 0,06	0,22 0,10	0,20 0,07	0,167	0,954	1,14	0,329	0,132	0,10 **0,37**
Chakra III Energy	-0,13 0,04	0,08 0,09	0,07 0,08	0,178	0,896	2,71	0,077	0,161	-0,08 **0,42**

Table 2. Summary of stepwise analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-level
Immunoglobulins M, g/L	5,27	0,007	0,880	5,27	0,007
Physical Working Capacity, W/kg	3,83	0,026	0,799	4,51	0,002
Symmetry of GDI (filter), %	3,29	0,043	0,735	4,17	0,001
VLF band PSD, %	3,29	0,043	0,675	4,02	0,001
P4-β PSD, %	3,26	0,044	0,619	3,95	10^{-4}
Deviation-δ, Hz	2,20	0,119	0,584	3,71	10^{-4}
Na,K-ATPase Erythrocyte, M/L•h	1,79	0,174	0,556	3,46	10^{-4}
Fp2-δ PSD, %	1,70	0,190	0,530	3,27	10^{-4}
Testosterone, nM/L	2,40	0,098	0,495	3,23	10^{-4}
HF/LF HRV ratio	2,03	0,139	0,467	3,15	10^{-4}
Chakra I Asymmetry	1,91	0,157	0,442	3,07	10^{-4}
Cortisol, nM/L	1,73	0,185	0,420	2,98	10^{-4}
CD22 B-Lymphocytes, %	1,60	0,210	0,401	2,90	10^{-4}
Chakra III Energy	1,77	0,179	0,380	2,85	10^{-4}
O1-θ PSD, $\mu V^2/Hz$	1,60	0,210	0,361	2,79	10^{-4}
T4-θ PSD, %	2,10	0,131	0,338	2,79	10^{-4}
HF band PSD, %	1,95	0,151	0,318	2,77	10^{-4}
Frequency-α, Hz	1,35	0,266	0,304	2,71	10^{-4}
Fp2-α PSD, %	1,31	0,279	0,291	2,65	10^{-4}
Chakra I Energy	1,17	0,317	0,280	2,58	10^{-4}
F4-δ PSD, %	1,02	0,368	0,270	2,51	10^{-4}
Entropy of PSD in F4 locus	1,61	0,209	0,256	2,49	10^{-4}

Entropy of PSD of HRV bands	2,35	0,105	0,236	2,54	10^{-4}
Cholesterol, mM/L	1,19	0,311	0,226	2,49	10^{-4}
Mode HRV, msec	1,14	0,327	0,216	2,44	10^{-4}
C4-α PSD, %	1,69	0,195	0,203	2,44	10^{-4}
Calcitonin, ng/L	1,14	0,327	0,194	2,40	10^{-4}
C3-α PSD, %	1,57	0,219	0,183	2,39	10^{-4}
Potassium Serum, mM/L	1,22	0,303	0,174	2,36	10^{-4}
Leukocytes, 10^9/L	1,11	0,338	0,167	2,32	0,001
P4-α PSD, %	1,06	0,353	0,159	2,28	0,001

Other 5 variables, despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 3).

Table 3. Variables currently not in the model

Variables	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	After Stan-dard Therapy (20)	After Standard Therapy + Balm (20)	Before Therapy (40)	Wilks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy	Refe-rence (Norm) (40) Cv; **SD**
Entropy of Im-munocytogram	0,946 0,007	0,959 0,008	0,948 0,006	0,156	0,980	0,47	0,629	0,559	0,960 0,059
Immunoglobulins G, g/L	15,39 0,65	16,82 0,86	15,68 0,58	0,158	0,993	0,16	0,853	0,345	12,75 0,206
Area GDI Left, kPixels	23,97 0,54	25,01 0,50	25,55 0,65	0,157	0,984	0,38	0,683	0,105	24,47 0,150
Ch IV Energy	0,35 0,06	0,47 0,10	0,43 0,07	0,158	0,993	0,17	0,848	0,179	0,28 **0,38**
Ch IV Energy (filtered)	0,43 0,06	0,57 0,07	0,52 0,04	0,155	0,975	0,59	0,557	0,171	0,37 **0,31**

The identifying information contained in the 31 discriminant variables is condensed into two roots (Table 4). The first root contains 54,4% of discriminatory opportunities (r*=0,788; Wilks' Λ=0,159; $\chi^2_{(62)}$=114; $p<10^{-4}$), and second root 45,6% (r*=0,761; Wilks' Λ=0,421; $\chi^2_{(30)}$=54; p=0,005).

Table 4. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Immunoglobulins M, g/L	-0,269	0,390	-1,174	1,698
Physical Working Capacity, W/kg	0,562	0,254	1,053	0,476
Symmetry of GDI (filtered), %	0,590	0,264	0,505	0,226
VLF band PSD, %	0,409	-1,030	0,035	-0,087
P4-β PSD, %	1,103	-0,015	0,131	-0,002
Deviation-δ, Hz	0,419	0,527	2,090	2,630
Na,K-ATPase Erythrocyte, M/L•h	0,080	-0,336	0,365	-1,534
Fp2-δ PSD, %	0,447	-1,827	0,001	-0,004
Testosterone, nM/L	1,124	-1,672	42,81	-63,67
HF/LF HRV ratio	0,601	0,288	0,001	0,001
Chakra I Asymmetry	-0,402	0,418	-2,083	2,164
Cortisol, nM/L	0,370	-0,552	0,005	-0,008
CD22 B-Lymphocytes, %	-0,226	1,029	-0,056	0,253
Chakra III Energy	0,962	-0,347	2,976	-1,073
O1-θ PSD, μV^2/Hz	-0,804	0,562	-0,012	0,009
T4-θ PSD, %	0,969	0,031	0,280	0,009
HF band PSD, %	0,703	-0,199	0,057	-0,016
Frequency-α, Hz	0,400	-0,484	0,413	-0,499
Fp2-α PSD, %	1,247	-1,914	0,109	-0,168
Chakra I Energy	-0,687	0,311	-2,188	0,989
F4-δ PSD, %	0,556	-1,485	0,030	-0,080
Entropy of PSD in F4 locus	-0,497	-1,191	-3,535	-8,465
Entropy of PSD of HRV bands	-0,465	-1,191	-6,377	-16,35
Cholesterol, mM/L	0,659	0,159	0,566	0,137
Mode HRV, msec	0,339	0,218	0,002	0,001
C4-α PSD, %	1,926	0,303	0,158	0,025
Calcitonin, ng/L	0,479	-0,331	0,133	-0,092
C3-α PSD, %	-0,978	1,008	-0,081	0,083
Potassium Serum, mM/L	-0,226	0,257	-0,490	0,557
Leukocytes, 10^9/L	-0,281	0,350	-0,310	0,386
P4-α PSD, %	-1,064	-0,607	-0,069	-0,039
	Constants		-111,9	68,10
	Eigenvalues		1,641	1,377
Cumulative Proportion			0,544	1

Calculating the values of discriminant roots for each patient by raw coefficients and constants given in Table 4 allows visualization of each patient in the information space of roots (Fig. 3).

The shift along the axis of the first root of patients after standard balneotherapy to the left relative to their initial state reflects both a decrease in PWC and a constellation of other parameters positively related to the root (Table 5), as well as an increase in the inversely related entropy of HRV and vagal tone as well as a transformation of the left-sided asymmetry of the virtual first Chakra to the right.

Additional use of phytoadaptogen, firstly, prevents or minimizes changes in PWC and another 15 related parameters, as evidenced by the shuffling of the projections of patients on the axis of the first root; secondly, it causes an increase in 12 parameters correlated with the second root inversely, and a decrease in 8 parameters correlated with it directly, as evidenced by the lower position of patients of the main group along the axis of the second root.

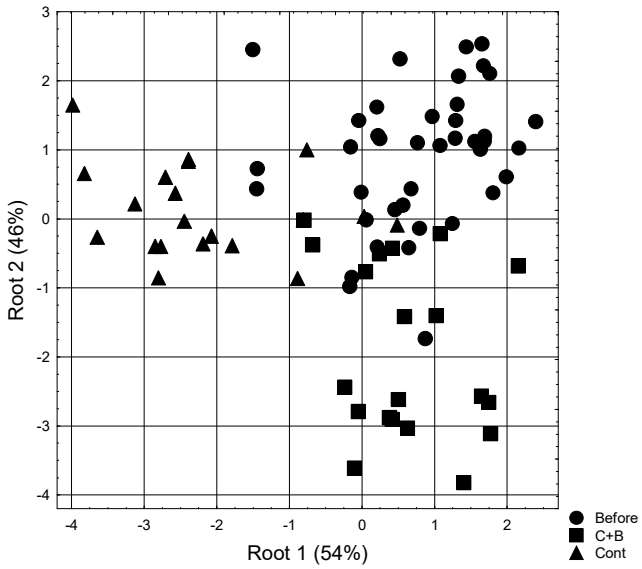

Fig. 3. Scattering of individual values of the first and second discriminant roots of patients before (circles) and after the course of standard balneotherapy (control, triangles) and in combination with Balm "Truskavets'" (squares)

The demarcation of three clusters in the information field of two discriminant roots is visualized more clearly by their centroids (Fig. 4) and is documented by calculating Mahalanobis distances (Table 6).

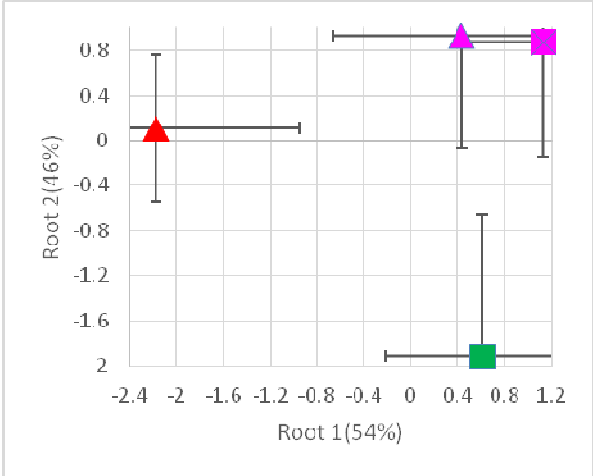

Fig. 4. Scattering of average values (M±SD) of the first and second discriminant roots of patients before and after the course of standard balneotherapy (triangles) and in combination with Balm "Truskavets'" (squares)

Table 5. Correlations between variables and roots; centroids of clusters and Z-scores of variables

Variables	Correlations Variables-Roots		After Standard therapy (20)	After Standard Thera-py + Balm (20)	Before Therapy (40)
Root 1 (54,4 %)	**Root 1**	**Root 2**	**-2,17**	0,61	0,78
PWC_{150}	**0,224**	0,101	**-0,03**	0,30	0,55
VLF PSDr	**0,206**	-0,027	**-0,92**	-0,36	-0,31
Symmetry GDI (filter)	**0,187**	0,032	**-0,65**	0,04	0,22
Ch III Energy	**0,123**	-0,015	**-0,13**	0,38	0,36
Leukocytes	**0,119**	0,080	**-0,95**	-0,67	-0,28
P4-β PSDr	**0,106**	0,122	**-0,43**	-0,35	0,11
T4-θ PSDr	**0,095**	0,042	**-0,18**	0,07	0,24
Cholesterol total	**0,083**	0,057	**-0,46**	-0,23	-0,09
Ch I Energy	**0,044**	-0,020	**0,09**	0,33	0,26
Frequency-α	**0,010**	-0,002	**-0,27**	-0,22	-0,22
Area GDI Left			**-0,19**	0,12	0,28
Ch IV Energy			**0,19**	0,49	0,39
Ch IV Energy (filter)			**0,21**	0,63	0,47
HRV Entropy	**-0,191**	0,085	**-0,08**	-0,74	-0,60
Ch I Asymmetry	**-0,163**	-0,008	**0,41**	-0,19	-0,26
HF PSDr	**-0,092**	0,073	**0,10**	-0,26	-0,33
Root 2 (45,6 %)	**Root 1**	**Root 2**	0,11	**-1,91**	0,90
Fp2-δ PSDr	0,044	**-0,256**	0,67	**0,95**	0,36
F4-δ PSDr	-0,047	**-0,178**	0,64	**0,58**	0,09
Na,K-ATPase Erythroc	0,106	**-0,128**	1,18	**1,74**	1,35
O1-θ PSDa	0,012	**-0,119**	0,57	**1,56**	0,36
Cortisol	-0,112	**-0,112**	0,07	**0,11**	-0,23
Calcitonin	0,011	**-0,100**	0,25	**0,91**	0,28
CD22 B-Lymphocytes	-0,026	**-0,092**	0,57	**0,78**	0,27
1/Mode HRV	-0,017	**-0,086**	0,59	**0,71**	0,56
Testosterone	0,039	**-0,084**	0,33	**1,26**	0,90
LF/HF HRV	0,011	**-0,057**	0,08	**0,98**	0,53
Entropy of ICG			-0,25	**-0,03**	-0,20
Immunoglobulines G			1,00	**1,55**	1,11
Immunoglobulines M	0,003	**0,315**	1,12	**0,24**	1,49
Deviation-δ	0,027	**0,184**	0,04	**-0,33**	0,34
Potassium Serum	-0,026	**0,110**	-0,04	**-0,35**	0,09
C3-α PSDr	0,006	**0,109**	-0,37	**-0,61**	-0,24
C4-α PSDr	0,055	**0,104**	-0,49	**-0,56**	-0,14
Fp2-α PSDr	-0,006	**0,054**	-0,35	**-0,51**	-0,32
F4 PSD Entropy	-0,001	**0,054**	-0,56	**-0,85**	-0,46
P4-α PSDr	0,021	**0,047**	-0,42	**-0,46**	-0,28

Table 6. Squares of Mahalanobis distances between groups (above the diagonal) and F-criteria (df=31,5) with p-levels (below the diagonal)

Groups	Before T (40)	After ST + Balm (20)	After ST (20)
Before Therapy	***	5,56	7,80
After Combined therapy	2,09; p=0,011	***	9,18
After Standard therapy	2,46; p=0,003	2,33; p=0,004	***

Until now, the object of discriminant analysis was actual parameters of patients before and after therapy. Our previous experience suggests that a more sensitive approach to estimating effects is to calculate individual direct differences between postprandial and basal parameter levels. In addition, the calculation of direct differences between the effects on individual parameters of standard balneotherapy and supplemented with phytoadaptogen allows to simulate the essential (per se) effects of the latter. The results of this approach are visualized in Figs. 5 and 6.

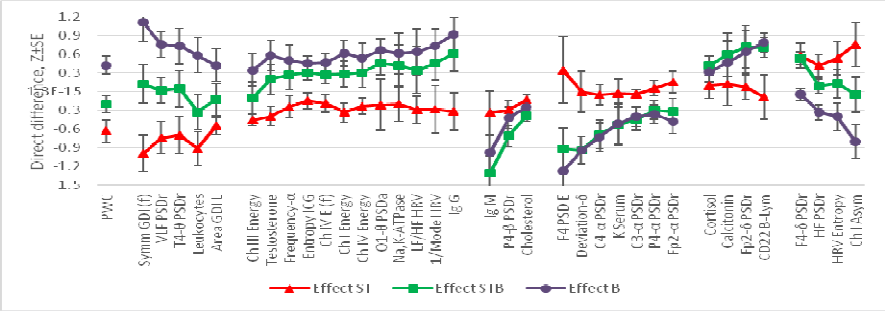

Fig. 5. Profiles of changes (direct differences) in PWC and accompanying parameters after standard (ST) and combined (STB) therapy as well as simulated essential effects of Balm (B)

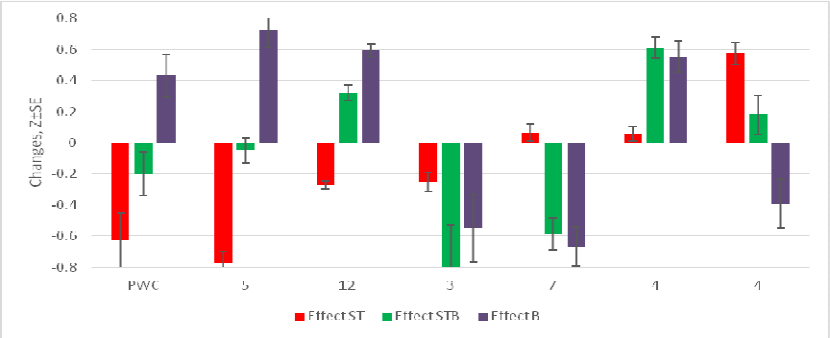

Fig. 6. Clusters of changes (direct differences) in PWC and accompanying parameters (number in parentheses) after standard (ST) and combined (STB) therapy as well as simulated essential effects of Balm (B)

Discussion

The decrease in the PWC_{150} level caused by standard balneotherapy reflects, in fact, an increase of 6.2±2.6 bpm in the HR response to a load of 1.5 W/kg, i.e., an increase in the autonomic reactivity of the heart. This is accompanied by a vagotonic shift in the sympatho-vagal balance (marker: LF/HF), an increase in vagal tone (marker: HF relative) and a decrease in the level of circulating catecholamines (marker: 1/Mode). The reduction of the relative PSD VLF band needs special attention, because its physiological interpretation is still a matter of debate.

Akselrod S et al. (1981) in pioneering experiment illustrated that after parasympathetic blockade the amplitude of the VLF peak is reduced; β-sympathetic blockade tends to reduce the VLF peak's amplitude, but this effect is not consistent because of the low tonic level of sympathetic activity in the resting dog. Increasing the activity of either the sympathetic or parasympathetic nervous system augments the area under the VLF peak. Therefore, both SNS and PSNS may mediate the VLF fluctuations. Selective blockade of renin-angiotensin system (by converting enzyme inhibitor) lead to 2-4.5-fold increase in the area under the VLF peak. Taylor JA et al. (1998) in young healthy subjects observed that β-adrenergic blockade had no significant effect on VLF power; ACE blockade modestly (approximately 21%) increased VLF power in the supine (but not upright tilt) position; atropine, given alone or with atenolol, decreased VLF band by 92%. Authors concluded that although VLF band are influenced by the renin-angiotensin-aldosterone system, they depend primarily on the presence of parasympathetic outflow. However, Del Valle-Mandragon L et al. (2022) showed that during hemodialysis angiotensin II had a **positive** correlation with VLF band (r=0.390) and with LF/HF (r=0.359) while a negative correlation with LF (r=-0.262) and HF (r=-0.383) bands. Therefore, the contradictions regarding the nature of VLF connections with vagal and sympathetic tone as well as the renin-angiotensin-aldosterone system remain unresolved. Besides it was shown that low VLF power has been correlated with low levels of testosterone, while cortisol have not (Theorell T et al., 2007). In our group study (Korda MM et al., 2021), was also found a direct correlation of **absolute** PSD VLF band with the HF band (r=0.65) and plasma testosterone in men (r=0.32), no correlation with LFnu (r=-0.16) as well as also inversely, but insignificantly, with aldosterone (p=-0.19). However, the **relative** PSD of VLF band correlates negatively with markers of vagal tone (r=-0.44÷-0.54), but positively with the stress index (r=0.27) and AMo (r=0.31), as well as cortisol (r=0.44) in the complete absence of a connection with both aldosterone (r=-0.05) and testosterone (r=-0.03). So, in this specific situation, the **relative** PSD of VLF band acts as a marker of sympathetic tone and cortisol (Ruzhylo SV et al., 2022).

In our situation, a decrease in **relative** PSD of VLF band is accompanied by a decrease in LF/HF (r=0.34) and testosterone level (r=0.26) in combination with an increase in relative PSD of HF (r=-0.60), i.e. it is a marker of sympathetic tone and testosterone.

Based on the nonoverlapping, bimodal distribution of circulating testosterone concentration with 95% references ranges of 7.7 to 29.4 nM/L in healthy men and 0 to 1.7 nM/L in healthy premenopausal women - making an allowance for women with the mild hyperandrogenism of polycystic ovary syndrome, who are overrepresented in elite athletics - the eligibility criterion for female athletic events should be a circulating testosterone concentration of <5.0 nM/L (Handelsman DJ et al., 2018). Greater increases in serum testosterone, but not fat-free mass, resulted in larger effects on performance. Larger increases in testosterone were observed in young males, but performance only improved in diseased and older males (Varanoske AN et al., 2020).

In our women, the initial level of PWC in the upper normal range was also combined with a moderately increased level of testosterone, and balneotherapy reduced both parameters.

A vagotonic shift in the sympatho-vagal balance is accompanied by a decrease in the PSD of θ-rhythm in the T4 locus, which probably projects to the right amygdala (Romodanov AP, 1993). The changes in PSD T4-θ are positively correlated with changes in the level of circulating catecholamines (r=0.48) and LF/HF (r=0.34),

but negatively with changes in relative PSD HF (r=-0.32). This is consistent with the concept of the central autonomic network (Benarroch EE, 1993). The amygdala is under tonic inhibitory control via prefrontal vagal pathways to intercalated cells in the amygdala. The activation of the central amygdala nucleus (CeA) inhibits the nucleus of the solitary tract which in turn inhibits inhibitory caudal ventrolateral medullary inputs to the rostral ventrolateral medullary (RVLM) sympathoexcitatory neurons, and simultaneously inhibits vagal motor neurons in the nucleus ambiguus and the dorsal vagal motor nucleus. In addition, the CeA can directly activate the sympathoexcitatory neurons in the RVLM. The enhancing of prefrontal activity (projected on F3/F4 loci) leads to the vagotonic shift in sympatho-vagal balance (Palma JA & Benarroch EE, 2014; Sakaki M et al., 2016; Carnevali L et al., 2020).

It is interesting that the decrease in PSD T4-θ is positively correlated with the decrease in testosterone level (r=0.57), and changes in the latter are directly related to changes in LF/HF (r=0.23), the level of circulating catecholamines (r=0.26), and PSD O1-θ (r=0.40), but inversely with changes in PSD F4-δ (r=-0.30).

In our patients, after standard balneotherapy, a decrease in testosterone level, CeA activity and sympathetic tone and an increase in vagal tone and prefrontal activity were found.

In a previous study of our group (Kozyavkina NV et al., 2021) it was also shown that the level of testosterone in both women and men is inversely correlated with PSD F3-θ (r=-0.29) and relative HF (r=-0.22), instead directly - with LFnu (r=0.21). That is, testosterone acts as a sympathoexcitatory factor by inhibiting prefrontal activity, which leads to disinhibition of the CeA.

From the evolutionary perspective testosterone is a **precursor** of estradiol, dihydrotestoterone and other metabolites rather than a hormone *per se.* Testosterone does not only act per se, but also *via* the products of its metabolism (Dolomatov S et al., 2018; Rychert K. 2022).

Reduction to dihydrotestosterone by 5-alpha reductase increases the androgen activity, conversion to estradiol by aromatase converts the androgen to estrogen activity (review: Celec P et al., 2015). Estrogen receptors Eα and Eβ are widely expressed in the brain along with androgen receptors. Thus, circulating testosterone and estradiol levels are intrinsically related (review: Almeida M et al., 2017).

It is obvious that the revealed neurotropic effects of testosterone are realized through androgen receptors which are widely expressed in the brain (review: Gillies GE & McArthur, 2010).

The publications on the effect of testosterone on EEG are collected in excellent review (Riddle J et al., 2021). Top-down signals from prefrontal cortex are theorized to converge with signals generated by reproductive steroid hormones in the medial temporal lobe. The amygdala, hippocampus, and bed nucleus of the stria terminalis within the medial temporal lobe include a large population of neurons with estrogen and androgen receptors. The medial temporal lobe projects to and regulates the periventricular nucleus of the hypothalamus, which is responsible for initiating the stress response. Thus, reproductive steroid hormones are theorized to exercise their anxiolytic effects via the **medial temporal** lobe. Similarly, connectivity between the prefrontal cortex and the medial temporal lobe has been identified in emotion regulation and is decreased in anxiety and depression. Activation of the HPA-axis via a stressful task or administration of cortisol both increases frontal alpha asymmetry and inhibits the production of anxiolytic reproductive steroid hormones in the gonads. Riddle J et al. (2021) shown that low levels of testosterone or E2 related to greater pathological frontal activity, left greater than right. Multiple linear regression revealed that **low levels of testosterone** correlated with greater frontal alpha asymmetry in women. Source localization found that frontal asymmetry was driven by **decreased alpha power in right inferior frontal gyrus** that correlated with increased behavioral inhibition in women. From this we conclude that in this study the level of testosterone was positively associated with frontal activity. This is not consistent with our data or with the concept that androgens are associated with sympathetic hyperactivity in females. Even the postmenopausal ovary continues to produce androgens; serum testosterone or other androgen levels do not change significantly across menopause. In polycystic ovary syndrome patients, who often suffer from hyperandrogenism, sympathetic activity was enhanced whereas parasympathetic signaling was suppressed (review: So SY & Savidge TC, 2021).

Unfortunately, we did not have the opportunity to determine the level of estradiol in the observed women. However, there are reasons to assume that in this situation standard balneotherapy increased the level of estradiol.

So SY & Savidge TC (2021) in the recent excellent review note that sympathetic activity is often increased in the luteal phase of the menstrual cycle or during menopause when estrogen levels are reduced. Surgical-induced menopause reduced parasympathetic nervous system activity and shifted this towards sympathetic hyperactivity. Although some studies found no effect, others reported that estrogens in hormone replacement therapy facilitate parasympathetic activity and suppress sympathetic signaling in postmenopausal women. Estrogen may reduce sympathetic fiber density directly through affecting Eα receptor expressed in sympathetic neurons, or indirectly through affecting target tissue or specific molecules. However, another study reported that estrogen is positively correlated to sympathetic activity in men. Nevertheless, estrogens are generally reported to inhibit sympathetic activity while sex could possibly influence the effect. However, several studies reported that

androgens are positively correlated with parasympathetic activity in males. Also, a study found that males with low testosterone levels were unable to maintain cardiosympathetic and cardiovagal responses. These inconsistent findings suggest that autonomic control mediated by sex steroids could be sex-dependent, as well as modulated by health and hormonal status of the individual.

Hence, we conclude that the observed decrease in sympathetic tone combined with an increase in vagal tone may be caused not only by the documented decrease in testosterone level, but also by a hypothetical increase in estradiol level.

In our imagination, we get the impression that the decrease in the level of PWC caused by standard balneotherapy is accompanied, first of all, by a decrease in the activity of the right amygdala (PSD T4-θ as marker) and serum testosterone level as well as PSD O1-θ and frequency of alpha-rhythm while enhancing in prefrontal activity (PSD F4-δ as marker). We consider the vagotonic shift of the sympatho-vagal balance due to an increase in vagal tone (PSD HFr as marker) and a decrease in sympathetic tone (PSD VLFr as marker) as well as the level of circulating catecholamines (1/Mode HRV as marker) as second-order effects. The effects of the third order seem to us to be a decrease in the level of leukocytes and immunoglobulins G and M in the blood as well as serum cholesterol and activity of Na,K-ATPase of erythrocyte shadows.

Such ideas fit into classical concepts of the central autonomic network (Benarroch EE, 1993; Palma JA & Benarroch EE, 2014), neuro-endocrine-immune network (Besedovsky H & Sorkin E, 1977; Besedovsky H & del Rey A, 1996; Nance DM & Sanders VM, 2007; Tracey KJ, 2007; Thayer JF & Sternberg EM, 2010; Chavan SS et al., 2017; Pavlov VA et al., 2018; Korneva EA, 2020) and functional-metabolic continuum (Gozhenko AI, 2016). By the way, all these concepts are cornerstones of Truskavetsian Scientific School of Balneology (Popovych IL, 2009; Popovych IL, 2011; Popovych IL et al., 2020; Popovych IL et al., 2022; Popovych IL et al., 2023). The decrease in PWC and accompanying changes in other parameters provide grounds for comparing the condition of our patients with overtraining syndrome.

Armstrong LE et al. (2022) in review about overtraining syndrome noted that complex systems in nature are not aptly characterized or successfully analyzed using the classic scientific method (i.e., simplifying complex problems into single variables in a search for cause-and-effect) because they result from myriad (often non-linear) concomitant interactions of multiple determinants. Authors proposes evidence-based areas for future overtraining syndrome investigations, including concomitant multi-domain analyses incorporating brain neural networks, dysfunction of hypothalamic-pituitary-adrenal responses to training stress, the intestinal microbiota, immune factors, and low energy availability. As you can see, such recommendations are implemented in our study. Overtraining syndrome is characterised by diminished sport-specific physical performance, accelerated fatiguability and subjective symptoms of stress. In addition to the determination of substrates and enzymes, the possibilities for monitoring of training by measuring hormonal levels in blood are currently being investigated. Endogenous hormones are essential for physiological reactions and adaptations during physical work and influence the recovery phase after exercise by modulating anabolic and catabolic processes. Testosterone and cortisol are playing a significant role in metabolism of protein as well as carbohydrate metabolism. Both are competitive agonists at the receptor level of muscular cells. The **testosterone/cortisol** ratio is used as an indication of the anabolic/catabolic balance. This ratio **decreases** during periods of intense training or repetitive competition, and can be **reversed** by regenerative measures. It seems more likely that the testosterone/cortisol ratio indicates the actual physiological strain in training, rather than overtraining syndrome. The sympatho-adrenergic system might be involved in the pathogenesis of overtraining. Overtraining appears as a disturbed autonomic regulation, which in its **parasympathicotonic** form shows a diminished maximal secretion of catecholamines (Urhausen A et al., 1995; Galychyn et al., 2020; Tkachuk et al., 2020). We will remind that in our patients vagotonic shift of sympatho-vagal balance and decrease in testosterone level, but without increase in cortisol level. Uusitalo AL et al. (1998) examined different hormonal responses to heavy endurance training and overtraining in female athletes. Hormone responses to exercise load indicated decreased sympathoadrenal and/or adrenocortical activity (or exhaustion of the adrenal gland or the central nervous system). Marked individual differences were found in training- and overtraining-induced hormonal changes.

The so-called informational (non-material) parameters require special attention. We assume that the decrease in the Entropy of the immunocytogram and the increase in the HRV Entropy will be accepted by readers at least tolerantly, similar to our previous publications regarding the physiological correlates of Entropy (Popadynets' OO et al., 2020; Gozhenko AI et al., 2021; Popovych IL et al., 2022). We also hope for an understanding regarding the reduction of GDI symmetry and its Area in the left projection. Although there is still skepticism about the GDV/PEI method, it has a biophysical basis (Korotkov KG et al., 2010; Muehsam et al., 2015; Korotkov KG, 2018; Korotkov KG, 2018a) and physiological correlates (Babelyuk VYe et al., 2017; Babelyuk VE et al., 2017; Babelyuk VY et al., 2021; Babelyuk VY et al., 2022; Bista S et al., 2022; Babelyuk VY et al., 2023). Instead, the reduction of the Energy of the third, fourth and first *virtual* Chakras, as well as the rightward shift of the symmetry of the first Chakra, we predict, will be criticized by readers, but will be accepted by the adepts of the Eastern medicine paradigm.

According to Ayurvedic medicine, Chakras are power centers, related to the endocrine glands and neural plexus as well as to some organs. Chase CR (2018) provides a table according to which the **first** Chakra is associated with **adrenals**, pelvic nerve plexus, spine, kidneys, bladder, large intestine; **second** Chakra with testes/**ovaries**, inferior mesenteric ganglion, ileum, organs of reproduction; **third** Chakra with [endocrine] pancreas, celiac plexus ganglion, liver, gall bladder, stomach, duodenum, pancreas, **spleen**; **fourth** Chakra with **thymus**, celiac plexus, heart, circulation, **vagus nerve**; **fifth** Chakra with thyroid and parathyroid glands, inferior cervical ganglion, lungs, bronchus, larynx, pharynx, large intestine, **vagus nerve**; **sixth** Chakra with pituitary and pineal glands, thalamus, hypothalamus, superior cervical ganglion, left and lower **brain**, ears/nose, left eye; **seventh** Chakra with pineal gland, right and upper **brain**, right eye.

Korotkov KG (2007) believes that GDV method measures the distribution of electron densities in human systems and organs. These electron densities are the main basis of physiological energy, so there is reason to say that the GDV method allows us to measure the body's potential energy reserve. At the same time, the GDV method is a bridge between the logical science of the West and the intuitive science of the East. It allows us to represent the same phenomena in different languages, in different systems, to look at the same things from different points of view. Author put forward the concept that each Chakra is associated with a part of the finger. This approach is embodied in the "GDV Chakras" program, which allows us to quantify the state of ***virtual*** Chakras.

A decrease in the Energy of the third Chakra associated with the thymus and the fourth Chakra associated with the spleen and vagus nerve, respectively, is consistent with a decrease in blood leukocytes and Igg G&M, but not with an increase in vagal tone. In this study, no changes in the Energy of the fifth to seventh Chakras, which are associated with the vagus nerve, pituitary gland and brain, were found, but such correlations were found in other studies (Babelyuk VY et al., 2021; Babelyuk VY et al., 2023). A decrease in testosterone level is accompanied by a decrease in the Energy of the first Chakra, which is associated with the adrenals, but not the second Chakra, which is associated with the ovaries. However, it should be borne in mind that the reticular zone of the adrenal glands is a source of androgens. In women, 50% of testosterone is produced by the conversion of androgens in the periphery, while the ovaries and the adrenal glands contribute equally to the rest of the testosterone that circulates in the blood (25% each) (Kanakis GA et al., 2019; Witchel SF et al., 2020; Dumontet T & Martinez A, 2021).

Phytoadaptogen, mixed with Naftussya bioactive water, prevents the decrease of PWC. This is accompanied by the prevention of both a decrease in PSD T4-θ and VLFr, leukocytes level as well as area and symmetry of GDI, as well as an increase in vagus tone and entropy of HRV as well as a rightward shift of the symmetry of the first Chakra. In addition, phytoadaptogen reverses balneotherapy-induced moderate decrease in the frequency of α-rhythm, PSD O1-β, sympathetic tone, serum levels of catecholamines, testosterone and IgG, activity of Na,K-ATPase of erythrocyte shadows as well as Energy of the first, third and fourth virtual Chakras. Phytoadaptogen potentiates the reduction of PSD P4-β, IgM and cholesterol as well as initiates the reduction of δ-rhythm variability, PSD of α-rhythm in C3, C4, P4 and Fp2 loci, entropy in F4 locus as well as serum potassium while increasing in serum cortisol and calcitonin, blood B-lymphocytes levels as well as PSD Fp2-δ.

Screening for correlations between changes in PWC and the listed parameters revealed the following. PWC varied unidirectionally with changes in PSD T4-θ (r=0.46), Fp2-δ (r=0.30), VLFr (r=0.32), 1/Mode (r=0.41), testosterone (r=0.41), B-lymphocytes (r=0.53) as well as Energy of first (r=0.39) and fourth (r=0.34) Chakras, however varied opposite of changes in deviation of δ-rhythm (r=-0.36), PSD Fp2-α (r=-0.37), HFr (r=-0.35), HRV entropy (r=-0.31), IgM (r=-0.31) as well as Asymmetry of first Chakra (r=-0.23).

Meta-analysis carried out by Riiser A et al. (2023) testified that glucocorticoids had a small positive effect on maximal physical performance compared to placebo and improved aerobic performance but not anaerobic performance. According to the authors, these results are consistent and should be of interest to WADA and anyone concerned about fair play. And will these authors and/or WADA be interested in Ukrainian phytoadaptogen?

Nordsborg N et al. (2008) investigated the effect of dexamethasone (2x2 mg per day or placebo for 5 days) on Na^+,K^+ pump subunit expression and muscle exchange of K^+ during exercise in nine healthy male. It was found, that catalytic alpha1 and alpha2 subunit expression was approximately 17% higher and the structural beta1 and beta2 subunit expression was approximately 6-8% higher after dexamethasone compared with placebo. During one-legged knee-extension for 10 min femoral venous K^+ and thigh K^+ release was lower in dexamethasone compared with placebo. In our women, phytoadaptogen also increased serum cortisol and activity of Na,K-ATPase and decreased serum potassium.

There is a well-founded opinion that the adaptogenic properties of classical phytoadaptogens are caused by polyphenolic compounds (Panossian AG et al., 2021). The latter are also present both in the phytocomposition "Balm Kryms'kyi" (Alyeksyeyev OI et al., 1996) and Naftussya bioactive water (Dats'ko OR et al., 2008; Ivassivka SV et al., 1994; Ivassivka SV, 1997; Zukow W et al., 2022), which also have adaptogenic ability (Popovych IL, 2011; Popovych IL, 2022; Popovych IL et al., 2022). It is interesting that Ozokerite, an integral

component of the standard balneotherapeutic complex of the Truskavets' Spa, has a number of effects similar to those of Naftussya, both when taken orally and when applied to the skin (Popovych AI, 2018; Popovych AI, 2019; Ruzhylo SV et al., 2021), as well as in vitro (Ivassivka SV, 1997). According to the hypothesis of the Truskavetsian Scientific School of Balneology, polyphenolic compounds of adaptogens of various nature are ligands of aryl hydrocarbon receptors (AhR) (Popovych IL, 2022; Fihura OA et al., 2022; Fihura OA et al., 2023), which are known to be expressed by almost all types of cells of all organisms, starting from unicellular. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants, the activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the neural, endocrine and immune response (Esser C & Rannug, 2015; Murray IA & Perdew GH, 2020; Kou Z & Dai W, 2021; Rejano-Gordillo CM et al., 2022).

Taking into account literature data on the direct neurotropic effect of phytoadaptogens in vitro and in vivo (Panossian AG et al., 2021), previously published data on changes in EEG&HRV parameters as well as relationships between EEG and HRV, EEG&HRV and adaptogene hormones, EEG&HRV and immunity parameters (Korda MM et al., 2021; Popovych IL, 2022; Fihura OA et al., 2022; Fihura OA et al., 2023; Hrynzovskyi A et al., 2023; Zabielska J et al., 2018), the neurogenic mechanism of the revealed effects of balneotherapy factors and phytoadaptogens seems to be very real.

At the same time, there is a right to the hypothesis that the primary target of polyphenolic compounds are immunocytes, which through their cytokines affect neurons of the central and autonomic nervous and endocrine systems (reviews: Besedovsky H & del Rey A, 1996; Popovych IL, 2022; Popovych IL et al., 2022).

The presence of polyphenols in the composition of both the phytoadaptogen and the balneotherapy complex, it would seem, should enhance the effects of the latter when they are used simultaneously. However, contrary to expectations, the phytoadaptogen exerted effects opposite to those of the balneotherapeutic complex on PWC and 21 other parameters, and also affected 14 other parameters that did not respond to the balneotherapeutic complex (see please Figs 5 and 6). The reasons for this situation should be sought in differences in the composition of both factors. First, the term polyphenols covers hundreds of compounds with different properties, including both AhR agonists and blockers (Murray IA & Perdew GH, 2020). Secondly, the composition of Naftussya bioactive water contains autochthonous microflora, which can significantly affect the neuro-endocrine-immune complex (Bilas VR & Popovych IL, 2009; Popovych IL et al., 2022). This will be discussed in the next article.

Conclusions

1. The phytoadaptogen "Balm Truskavets'" prevents the adverse effect of the standard balneotherapeutic complex of the Truskavets' Spa on PWC by, apparently, its neuro-endocrine effects.
2. The initial positions of the members of the control and main groups were almost identical, which allowed for the merging of the members of both treatment groups into a single group.
3. As predicted based on previous studies, after standard balneotherapy, the PWC level decreased from the upper normal range to its middle range. The additional use of the phytoadaptogen minimized the negative actotropic effect of standard balneotherapy.
4. The application of the analytical algorithm of the Truskavets Scientific School allowed for the normalization of real/raw parameters.
5. Under the influence of standard balneotherapy, elevated serum levels of IgM, IgG, and testosterone, as well as erythrocyte shadow Na,K-ATPase and the LF/HF ratio as a sympathetic tone, decrease. Standard balneotherapy practically does not affect the normal parameter levels of the two following clusters.
6. Supplementation of the balneotherapeutic complex with a phytoadaptogen enhances both its inhibitory and strengthening effects on a number of parameters, while weakening or even reversing the effects on other parameter constellations.
7. To determine which of the listed parameters are characteristic (recognizable) for the three groups, and primarily to visualize each patient in the information field, the constellation of these parameters was subjected to discriminant analysis.
8. The forward stepwise program included 30 variables in the discriminant model, excluding PWC by definition. Among them, 12 were related to EEG, 5 to GDV, 3 to hormones, 3 to immunity, 3 to metabolism, and 4 to GDV.
9. As shown in Table 1, different variables had different effects on the results of standard therapy and standard therapy with the addition of Balm. Many of these variables showed significant differences between the groups after therapy.
10. As shown in Table 2 summarizes the stepwise analysis of discriminant variables by the Λ criterion. These variables are characteristic for the three groups and are key to distinguishing between them. The results suggest that standard therapy and therapy with the addition of Balm have different effects on different variables, which may have important implications for understanding and optimizing these therapies.

11. As shown in Table 3, different variables had different effects on the results of standard therapy and standard therapy with the addition of Balm. Many of these variables showed significant differences between the groups after therapy.
12. As shown in Table 4 summarizes the stepwise analysis of discriminant variables by the Λ criterion. These variables are characteristic for the three groups and are key to distinguishing between them. Calculating the values of discriminant roots for each patient using the raw coefficients and constants given in Table 4 allows for the visualization of each patient in the information space of the roots.
13. The shift along the axis of the first root of patients after standard balneotherapy to the left relative to their initial state reflects both a decrease in PWC and a constellation of other parameters positively correlated with the root, as well as an increase in HRV entropy and vagal tone inversely correlated with the root, and the transformation of the left-sided virtual asymmetry of the first Chakra into the right-sided one.
14. The additional use of the phytoadaptogen, firstly, prevents or minimizes changes in PWC and the next 15 related parameters, which confirms the rearrangement of the patients' projections on the axis of the first root; secondly, it causes an increase in 12 parameters correlated with the second root inversely, and a decrease in 8 parameters correlated with it directly, which confirms the lower position of the patients of the main group along the axis of the second root.

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Accordance to ethics standards

Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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