Original Article

Neuro-endocrine, hemodynamic and metabolic accompaniments of effects of balneotherapy at Truskavets' Spa on PWC in men with maladaptation

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Abstract
Background. The researchers of the Truskavetsian Scientific School of Balneology have demonstrated that effect of balneotherapy at the Truskavets' Spa on the physical working capacity of both healthy rats and different categories of patients is ambiguous. It is shown that various variants of actotropic effects are accompanied by characteristic changes in a number of body parameters. The purpose of this study was to identify neuro-endocrine, hemodynamic and metabolic accompaniments of actotropic effects of balneotherapy in men with maladaptation. Material and methods. The object of observation were 34 men (aged 23÷70 years) with maladaptation against the background of chronic pyelonephtis in remission phase, who came for rehabilitation at the Truskavets' Spa. The object of study: PWC, hemodynamics, HRV, EEG, adaptation hormones, blood and urine metabolites. The survey was conducted twice: on admission and after 7-10 dais of rehabilitation. Results. The analysis of individual changes revealed that balneotherapy in 18 patients did not significantly affect the level of fitness. In 9 patients balneotherapy caused an increase in PWC_{150} (direct difference: 0.57±0.12 W/kg) while in the other 9 patients the level of fitness decreased (direct difference: -0.42±0.03 W/kg). Discriminant analysis revealed 24 variables as characteristic accompaniment of the three variants of actotropic effects of balneotherapy. Among them, 2 are hemodynamic, 13 are neural, which reflect the entropy of EEG and HRV as well as delta-, theta- and beta-rhythms, 2 are endocrine, and 7 are metabolic. Classification accuracy is 100%.
Conclusion. The multivariate actotropic effects of balneofactors are due to their multivariate effects on neuro-endocrine regulation and metabolism, which, apparently, is determined by the peculiarities of the individual reactivity of the body.
Keywords: physical working capacity, hemodynamics, HRV, EEG, metabolism, Truskavets' Spa.

Introduction

The researchers of the Truskavetsian Scientific School of Balneology have demonstrated that effect of balneotherapy at the Truskavets' Spa on the physical working capacity is ambiguous. Multidirectional actotropic effects were found in experiments on healthy female rats using the swim-to-exhaustion test (Ruzhylo et al., 2003; Fihura et al., 2022), in clinical-physiological observations of children and adolescents of both sexes aged 10-17 with maladaptation by PWC_{170}, assessed by the step test (Ruzhylo et al., 2003; Popovych et al., 2005; Zukow et al., 2020), adult gastroenterology patients of both sexes (Popovych et al., 2005) and women (Zukow et al., 2024), patients with urate urolithiasis and chronic pyelonephritis (Hrinchenko, 1998; Hrinchenko et al., 1999; Zukow et al., 2022) by PWC_{150}, assessed by two-stage bicycle ergometry.

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 et al., 2005; Zukow et al., 2021; Zukow et al., 2022).

In a comparative study, our group discovered sexual dimorphism in the neuro-endocrine regulation of bicycle ergometric test parameters in untrained individuals with dysfunction of the neuro-endocrine-immune complex (Ruzhylo et al., 2022).

The purpose of this study was to identify neuro-endocrine, hemodynamic and metabolic accompaniments of actotropic effects of balneotherapy in **men** with maladaptation.

Material and methods

Participants. The object of clinical-physiological observation were 34 men (aged 23÷70 years, weight 65÷107 kg, height 160÷183 cm, body mass index 19,8÷32,0 kg/m^2) with maladaptation against the background of chronic pyelonephtis in remission phase, who came for rehabilitation at the Truskavets' Spa.

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

Systolic (Ps) and diastolic (Pd) blood pressure as well as heart rate (HR) was measured (by tonometer "Omron M4-I", Netherlands) in a sitting position three times in a row. On the basis of the received data, the Ps2/Ps1, Ps3/Ps1, Pd2/Pd1, and Pd3/Pd1 indices recently proposed by our group (Popovych et al., 2022a) were calculated. After that, the parameters of hemodynamics were determined (by echocamera "Toshiba-140", Japan): ejection time (ET), end-diastolic (EDV) and end-systolic (ESV) volumes of left ventricle with the following ejection fraction (EF), cardiac output (CO), and general peripheral vessels resistance (GPVR), calculation by classic formulas (Bobrov et al., 1997):

$$EF = 100•(EDV - ESV)/EDV; CO = (EDV - ESV)•HR; GPVR = 80•(0,67•Pd + 0,33•Ps)/HR•(EDV - ESV).$$

In addition, we calculated the Ruzhylo's&Popovych's contractile activity index (CAI) of left ventricle (Popovych et al., 2005):

$$RPCAI = 0,1332•(0,67•Pd + 0,33•Ps)•(EDV - ESV)/EDV•ET.$$

We recorded an electrocardiogram in II lead for 7 minutes in the supine position and 2 minutes after transition from the supine to upright position (lying-to-standing test) to assess the parameters of heart rate variability (HRV) (software and hardware complex "CardioLab+HRV" produced by "KhAI-MEDICA", Kharkiv). For further analyses the following parameters HRV were selected. Temporal parameters (Time Domain Methods): HR, the mode (Mo), the standard 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) as well as Baevsky's Activity of Regulatory Systems Index (BARSI) and Autonomous Reactivity Index (ARI) as the difference between BARSI in standing up and supine positions (HRV, 1996; Berntson et al., 1997; Baevsky & Ivanov, 2001; Baevsky & Chernikova. 2017)

Than we quantitative EEG 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, we 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 et al., 2001).

We calculated also for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych's (Popadynets et al., 2020; Gozhenko et al., 2021) equations based on classic Shannon's (1948) equation:

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

$$hHRV =- [PSDHF•log_2PSDHF + PSDLF•log_2PSDLF + PSDVLF•log_2PSDVLF + PSDULF•log_2PSDULF]/log_2 4.$$

In portion of the venous blood the serum levels of major hormones of adaptation: Cortisol, Testosterone, Aldosterone, Triiodothyronine, PTH and Calcitonin as well as Interleukins 1β and 6 assayed with ELISA kits according to the SOP provided by the manufacturer ("Алкор Био", XEMA Co Ltd, DRG International Inc, "Vector-Best") with the use of analyzers "RT-2100C" and "Stat Fax 303".

In addition, we estimated a number of metabolic parameters.

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, 1987); 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); calculated the Dobiásová's&Frohlich's atherogenic index (AGI) as TG/HDLP Ch ratio (Dobiásová et al., 2011).

State of lipids peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract) (Gavrilov & Mishkorudnaya, 1983) and malondialdehyde (test with thiobarbituric acid) (Andreyeva et al, 1988), as well as the activity of antioxidant enzymes: catalase serum (by the speed of decomposition hydrogen peroxide) (Korolyuk et al, 1988) and superoxide dismutase erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH) (Dubinina et al, 1988).

Electrolytes: calcium (by reaction with arsenase III); magnesium (by reaction with colgamite); phosphates (phosphate-molybdate method); chloride (mercury-rhodanidine method); sodium and potassium (flamming

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photometry). Nitrogenous metabolites: creatinine (by Jaffe's color reaction by Popper's method); urea (urease method by reaction with phenolhypochlorite); uric acid (uricase method).

The same metabolic parameters, with the exception of lipids, were determined in daily urine collected the day before. The analysis carried out according to instructions (Goryachkovskiy, 1998) with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) as well as flame photometer "СФ-47" and corresponding sets of reagents. 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. We calculated submaximal PWC_{150} with the mechanical power in Watt per kilogram body weight (W/kg) as indicator of cardiorespiratory fitness (Finger et al., 2013; Steinhilber et al., 2022). In addition, for the assessment of cardiorespiratory fitness, the good old tests for the duration of breath retention after deep inhalation (Stange's test) and exhalation (Henchy's test) were used.

After the initial testing, the patients received for 7-10 days 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).

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

Data collection and analysis / Statistical analysis.

Data were collected through various physiological measurements, including: Physical Working Capacity (PWC150) assessed by bicycle ergometry. Hemodynamic parameters measured by echocardiography. Heart Rate Variability (HRV) parameters recorded using ECG. Quantitative EEG recorded at rest. Serum levels of adaptation hormones and interleukins measured by ELISA. Metabolic parameters in blood and urine.

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

The following statistical methods were applied:

1. Descriptive Statistics: Means and standard errors (SE) were calculated for all variables.

2. Data Normalization: Raw parameters were normalized using Z-score transformation: $Z = 4*(V - N)/(Max - Min) = (V - N)/SD = (V/N - 1)/Cv$, where V is the actual value, N is the normal (reference) value, SD is the standard deviation, and Cv is the coefficient of variation. This allowed comparison of variables with different units and variabilities.

3. Correlation Analysis: Pearson's correlation coefficients were calculated to examine relationships between variables.

4. Cluster Analysis: Hierarchical clustering was used to group similar variables into clusters.

5. Discriminant Analysis: 5.1. Forward stepwise discriminant analysis was performed to identify variables characteristic of the three variants of actotropic effects. 5.2. Discriminant functions were calculated and used to visualize patients in the discriminant root space. 5.3. Mahalanobis distances between clusters were calculated. 5.4. Classification functions were derived for retrospective identification of actotropic effects. 5.5. Classification accuracy was assessed.

6. Canonical Correlation Analysis: Used to examine relationships between sets of variables (e.g., EEG and HRV parameters). Canonical roots were calculated and their statistical significance was assessed.

7. Multiple Regression Analysis: Stepwise multiple regression was used to model relationships between predictor variables and outcome variables. Regression coefficients, R2 values, and statistical significance of models were reported.

8. Factor Analysis: Factor structure of canonical roots was examined. Factor loadings for variables were reported.

9. Calculation of Mahalanobis Distances: Squares of Mahalanobis distances between clusters were calculated. F-tests were used to assess the significance of differences between clusters.

The level of statistical significance was set at $p < 0.05$. Detailed statistical results, including correlation coefficients, regression coefficients, discriminant function coefficients, factor loadings, etc., were provided in tables and figures throughout the paper. This comprehensive statistical approach allowed for a thorough examination of the complex relationships between the studied variables and identification of characteristic patterns accompanying various actotropic effects of balneotherapy.

Results

It was established that balneotherapy in most patients (18) did not significantly affect the level of fitness: PWC_{150} (Mean±SE) was 2.01±0.15 W/kg and 1.98±0.15 W/kg before and after balneotherapy, respectively (reference level: 2.67±0.09 W/kg). At the same time, in 9 patients balneotherapy caused an increase in working capacity from 1.40±0.26 W/kg to 1.97±0.22 W/kg (direct difference: 0.57±0.12 W/kg) while in the other 9 patients the level of fitness decreased from 2.44±0.15 W/kg to 2.01±0.15 W/kg (direct difference: -0.42±0.03 W/kg).

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Adhering to the Truskavetsian Scientific School's analytical algorithm, in order to correctly compare variables expressed in different units and with different variability, the actual/raw parameters were normalized by recalculation by the equations:

$$Z = 4 \cdot (V - N)/(Max - Min) = (V - N)/SD = (V/N - 1)/Cv, \text{ where}$$

V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively (Polovynko et al, 2016; Popovych et al., 2022).

Screening revealed a number of EEG, HRV, hemodynamic and metabolic variables, the changes of which are unidirectional or inverse with respect to changes in PWC.

Further, profiles (Fig. 1) of changes in normalized values after balneotherapy were created.
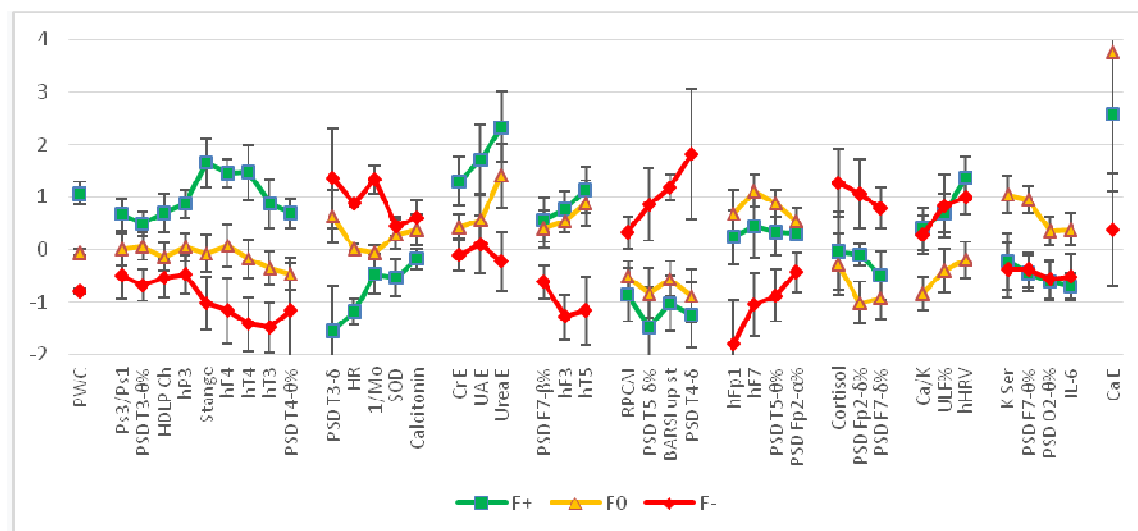
Fig. 1. Profiles of changes (Z±SE) in PWC_{150} (Fitness) and accompanied variables after balneotherapy. *See please also Table 5 for details*

At the next stage of the analysis, more or less homogeneous variables were condensed into 11 clusters (Fig. 2).
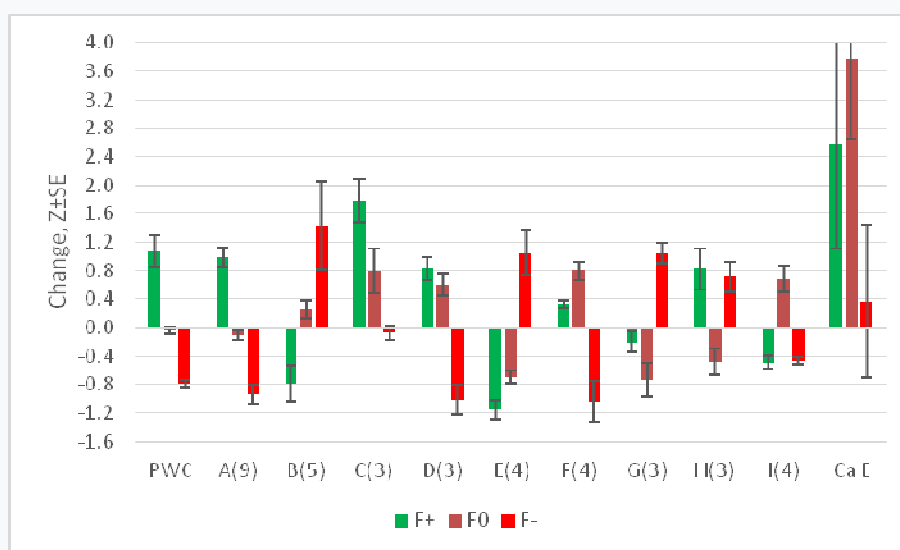
Fig. 2. Clusters of changes (number of variables below) in accompanied variables by various changes in PWC (Fitness) after balneotherapy. *See please also Table 5 for details*

In the first two clusters, variables whose changes under the influence of balneotherapy most directly (cluster A) or inversely (cluster B) approximate changes in PWC are collected. Excretion of creatinine, uric acid, and urea (cluster C) also increases with an increase in PWC, but to a lesser extent it also increases in the absence of changes in the latter, and does not change with a decrease in PWC (Figs. 2 and 3).
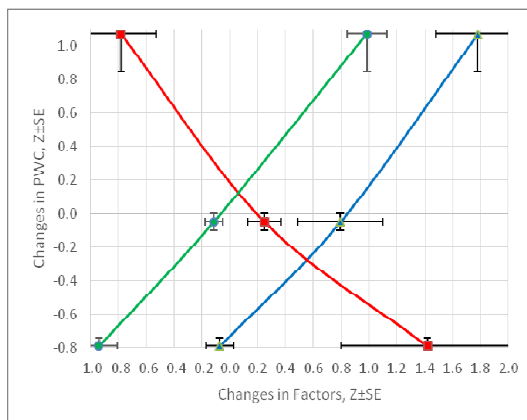
Fig. 3. Linear relationships between balneotherapy-induced changes in PWC and clusters A, B and C variables

The next pair of clusters (Figs. 2 and 4) contains variables whose relationships with changes in PWC are consistent but non-linear in nature. In particular, PSD of beta-rhythm in F7 locus as well as Entropy in F3 and T5 loci also decrease when PWC decreases, but increase both when it increases and when there is no change (cluster D). On the other hand, a decrease in PWC is accompanied by an increase in the PSD of delta-rhythm in T5 and T4 loci as well as Ruzhylo's&Popovych's and Baevsky's indices, however, the listed variables decrease both with positive and with quasi-zero actotropic effects of balneotherapy (cluster E).
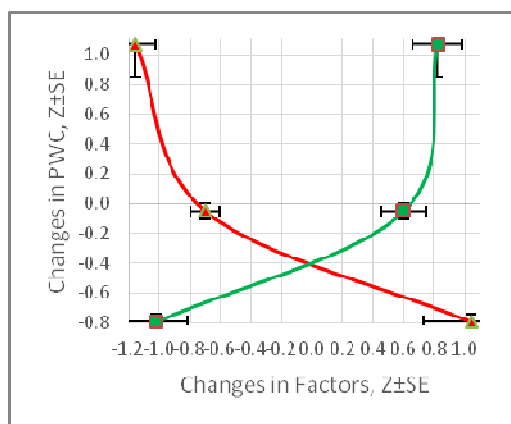
Fig. 4. Non-linear relationships between balneotherapy-induced changes in PWC and clusters D&E variables

The next pair of clusters illustrates the classic inverted U-shaped relationship between variables. In particular, the increase in PWC occurs in the absence of significant changes in the variables of clusters both F and G (Figs. 2 and 5). When cluster F variables increase or cluster G variables decrease, the PWC level does not change. Instead, fitness decreases both as cluster F variables decrease and as cluster G variables increase.
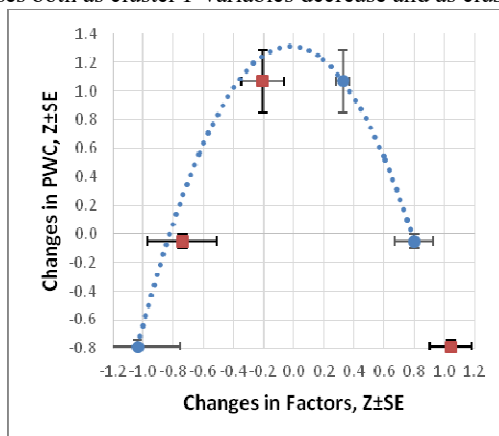

Fig. 5. Inverted U-shaped relationships between balneotherapy-induced changes in PWC and clusters F&G variables

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The last pair of clusters illustrates the absence of relationships between changes in PWC and clusters H&I variables (Figs. 2 and 6).
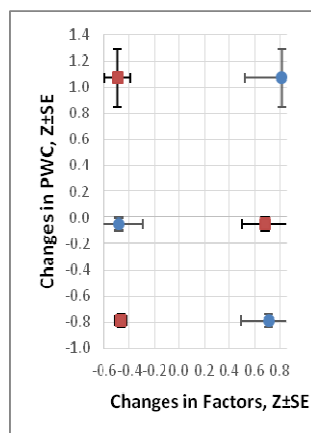
Fig. 6. Absence of relationships between changes in PWC and clusters H&I variables

Finally, the relationship between changes in calcium excretion and PWC has the form of a decaying sinusoid and is generally weak, but could not be ignored by us (spoiler: calcium excretion will be included in the discriminant model as a characteristic variable).

$$y = -1E\text{-}05x^6 + 0{,}0004x^5 - 0{,}005x^4 + 0{,}015x^3 + 0{,}088x^2 - 0{,}364x - 0{,}066$$
$$R^2 = 0{,}260$$
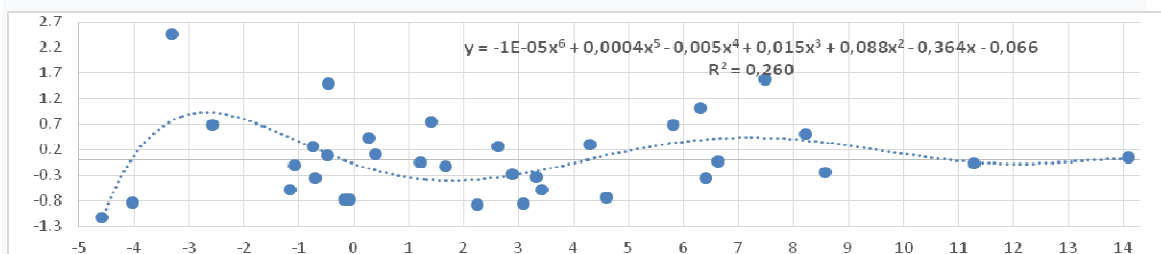
Fig. 7. Sinusoidal relationship between changes in Z-scores of calcium excretion (X axis) and PWC (Y-axis)

It is time to move on to the results of the discriminant analysis (Klecka, 1989). The first goal of such an analysis is to find out which changes of the mentioned variables are a characteristic accompaniment of the three variants of actotropic effects of balneotherapy. PWC itself was not the object of the analysis due to obviousness. The forward stepwise program included only 24 variables in the discriminant model. Among them, first of all, 2 are **hemodynamic**, 13 are **neural**, which reflect the entropy of EEG and HRV as well as delta-, theta- and beta-rhythms, 2 are **endocrine**, and 7 are metabolic, which reflect the exchange of **electrolytes** and **nitrogenous** metabolites (Table 1 and 2).

Table 1. Summary of the analysis of Discriminant Functions in relation to the changes in PWC and accompanying neural, endocrine, hemodynamics and metabolic variables

Step 24, N of vars in model: 24; Grouping: 3 grps; Wilks' Λ: 0,0003; approx. $F_{(48,2)}$=18,5; p<10^{-6}

Variables currently in the model	Clusters of changes in Fitness (n)			Parameters of Wilks' Statistics				
	F+ (9)	F0 (16)	F- (9)	Wilks Λ	Parti-al Λ	F-re-move (2,8)	p-level	Tole-rancy
Heart Rate, beats/min	-9,7 2,1	0,2 0,6	7,2 0,6	0,009	0,034	114,9	10^{-6}	0,024
Ps3/Ps1 Ratio	0,06 0,03	0,00 0,03	-0,04 0,03	0,001	0,366	6,929	0,018	0,045
PSD Fp1 Entropy	0,02 0,05	0,07 0,05	-0,18 0,08	0,002	0,131	26,59	10^{-3}	0,018
PSD F3 Entropy	0,09 0,04	0,06 0,02	-0,14 0,05	0,002	0,150	22,62	0,001	0,006
PSD F4 Entropy	0,17 0,03	0,01 0,05	-0,14 0,08	0,000	0,725	1,517	0,276	0,094
PSD T5 Entropy	0,14 0,06	0,11 0,06	-0,15 0,08	0,002	0,172	19,23	0,001	0,005
PSD P3 Entropy	0,12 0,03	0,01 0,03	-0,06 0,05	0,003	0,095	38,02	10^{-4}	0,011
PSD HRV Entropy	0,13 0,04	-0,02 0,03	0,09 0,03	0,001	0,405	5,871	0,027	0,121
PSD F7-δ, %	-11 10	-21 9	18 9	0,001	0,516	3,745	0,071	0,003

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Variable								
PSD T5-δ, %	-27 14	-15 9	16 13	0,001	0,293	9,633	0,007	0,047
PSD T3-δ, μV²/Hz	-140 76	57 45	347 213	0,003	0,115	30,66	10^{-4}	0,017
PSD F7-0, %	-2,1 1,6	4,4 1,1	-1,8 1,6	0,002	0,179	18,32	0,001	0,014
PSD T5-0, %	1,5 1,9	4,0 1,1	-4,0 2,2	0,005	0,069	54,36	10^{-4}	0,007
PSD T4-0, %	3,2 1,3	-2,1 1,4	-5,4 4,1	0,001	0,259	11,45	0,004	0,092
PSD F7-β, %	9,8 7,4	6,7 6,1	-10,5 5,3	0,001	0,245	12,33	0,004	0,002
Mode HRV, msec	47 38	5 14	-136 28	0,000	0,837	0,777	0,492	0,297
Calcitonin, ng/L	-1,3 1,3	2,6 2,0	4,2 2,3	0,005	0,061	61,47	10^{-5}	0,021
Superoxide dismutase, U/mL	-9,4 6,1	5,2 5,1	8,0 3,2	0,001	0,240	12,65	0,003	0,042
Potassium Serum, mM/L	-0,11 0,25	0,50 0,17	-0,18 0,24	0,005	0,057	65,54	10^{-5}	0,003
(Ca/K)⁰·⁵ Serum Ratio	0,02 0,02	-0,05 0,02	0,02 0,02	0,002	0,149	22,76	10^{-3}	0,005
Calcium Excretion, mM/24h	2,41 1,38	3,54 1,06	0,35 1,01	0,003	0,124	28,23	10^{-3}	0,050
Creatinineuria, mM/24h	4,29 1,55	1,40 0,84	-0,34 1,01	0,006	0,050	76,09	10^{-5}	0,014
Uricosuria, mM/24 h	1,28 0,50	0,42 0,47	0,08 0,42	0,001	0,322	8,416	0,011	0,039
Urea Excretion, mM/24h	198 58	119 51	-20 49	0,001	0,360	7,116	0,017	0,077

Note: the mean direct difference and SE are given for the variables

Table 2. Summary of forward stepwise analysis. Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Heart Rate, beats/min	49,0	10^{-6}	0,240	49,0	10^{-6}
(Ca/K)⁰·⁵ Serum Ratio	6,36	0,005	0,169	21,5	10^{-6}
Calcitonin, ng/L	4,19	0,025	0,131	17,0	10^{-6}
PSD HRV Entropy	7,29	0,003	0,086	16,9	10^{-6}
PSD F7-0, %	3,02	0,065	0,070	14,9	10^{-6}
PSD T5-0, %	3,38	0,050	0,056	14,0	10^{-6}
PSD T3-δ, μV²/Hz	2,06	0,148	0,048	12,7	10^{-6}
Creatinineuria, mM/24h	1,29	0,294	0,043	11,4	10^{-6}
Ps3/Ps1 Ratio	1,78	0,191	0,037	10,6	10^{-6}
Uricosuria, mM/24 h	1,32	0,287	0,033	9,83	10^{-6}
Potassium Serum, mM/L	1,18	0,327	0,030	9,10	10^{-6}
PSD F7-β, %	2,54	0,104	0,024	9,09	10^{-6}
PSD T5-δ, %	2,72	0,091	0,019	9,24	10^{-6}
Urea Excretion, mM/24h	2,96	0,077	0,014	9,57	10^{-6}
PSD P3 Entropy	1,60	0,230	0,012	9,30	10^{-6}
PSD T4-0, %	2,16	0,148	0,009	9,37	10^{-6}
Mode HRV, msec	1,69	0,217	0,008	9,25	10^{-6}
PSD F3 Entropy	1,61	0,236	0,006	9,13	10^{-6}
PSD Fp1 Entropy	2,42	0,128	0,004	9,52	10^{-6}
Calcium Excretion, mM/24h	4,31	0,039	0,003	11,1	10^{-6}
PSD T5 Entropy	3,64	0,061	0,002	12,7	10^{-6}
Superoxide dismutase, U/mL	6,67	0,014	0,001	17,0	10^{-6}
PSD F7-δ, %	2,56	0,132	0,000	18,5	10^{-6}
PSD F4 Entropy	1,52	0,276	0,000	18,5	10^{-6}

The rest of the variables were outside the discriminant model, probably due to duplication or redundancy of identifying information (Table 3).

Table 3. Summary of Discriminant Functions analysis. Variables currently not in the model

Variables	Clusters of changes in Fitness (n)			Parameters of Wilks' Statistics				
	F+ (9)	F0 (16)	F- (9)	Wilks' Λ	Parti-al Λ	F to enter	p-level	Tole-rancy
Ruzhylo's&Popovych's Contr Activity Ind, kPa/sec	-3,7 2,2	-2,2 1,3	1,4 1,3	0,0003	0,928	0,271	0,770	0,235
PSD F7 Entropy	0,07 0,09	0,17 0,05	-0,16 0,09	0,0003	0,967	0,118	0,890	0,051
PSD T3 Entropy	0,10 0,05	-0,04 0,04	-0,17 0,05	0,0003	0,847	0,635	0,558	0,129
PSD T4 Entropy	0,17 0,06	-0,02 0,04	-0,16 0,06	0,0003	0,937	0,236	0,796	0,036

PSD Fp2-δ, %	-1,9 3,9	-18,5 7,4	19,5 12,3	0,0003	0,998	0,006	0,994	0,092
PSD T4-δ, μV²/Hz	-126 62	-88 52	183 124	0,0003	0,978	0,080	0,924	0,187
PSD T3-θ, %	2,4 1,1	0,3 1,3	-3,3 1,4	0,0003	0,981	0,068	0,935	0,352
PSD O2-0, %	-2,4 1,4	1,4 1,1	-2,2 1,4	0,0003	0,832	0,704	0,526	0,237
PSD Fp2-α, %	4,5 1,6	7,8 3,7	-6,3 5,7	0,0003	0,911	0,343	0,721	0,162
Baevsky's Activity Regula-tory Syst Ind up stand, unit	-1,18 0,61	-0,64 0,38	1,37 0,28	0,0003	0,981	0,070	0,933	0,280
ULF band HRV, %	2,3 1,4	-1,3 1,8	3,3 2,4	0,0003	0,891	0,428	0,668	0,062
Cortisol, nM/L	-4 84	-31 66	142 73	0,0003	0,958	0,154	0,860	0,305
HDLP Cholesterol, mM/L	0,28 0,14	-0,05 0,11	-0,20 0,15	0,0003	0,841	0,663	0,545	0,364
Interleukin-6, ng/L	-0,95 0,33	0,53 0,43	-0,71 0,61	0,0003	0,924	0,288	0,758	0,080
Stange's test, sec	16,5 4,6	-0,7 3,6	-10,2 5,1	0,0003	0,907	0,360	0,710	0,259

The identifying information contained in the 24 discriminant variables is condensed into two roots. The major root contains 99,0% of discriminatory opportunities (r*=0,999; Wilks' Λ=0,0003; $\chi^2_{(48)}$=157; p<10^{-6}), while minor root 1,0% only (r*=0,916; Wilks' Λ=0,1615; $\chi^2_{(23)}$=36; p=0,046).

The next goal of discriminant analysis is visualization of each patient in the information space of roots. It is achieved by calculating the values of discriminant roots for each patient by the raw coefficients and the constant (Table 4).

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

| Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Heart Rate, beats/min	6,294	-0,106	1,724	-0,029
(Ca/K)^{0,5} Serum Ratio	-13,07	0,160	-174,5	2,131
Calcitonin, ng/L	6,560	0,997	0,937	0,142
PSD HRV Entropy	0,033	2,419	0,282	20,85
PSD F7-θ, %	7,355	-2,514	1,760	-0,602
PSD T5-θ, %	-11,55	1,012	-2,453	0,215
PSD T3-δ, μV²/Hz	6,980	-2,078	0,022	-0,006
Creatinineuria, mM/24h	-8,114	0,777	-2,211	0,212
Ps3/Ps1 Ratio	3,388	-1,790	34,98	-18,48
Uricosuria, mM/24 h	4,111	-0,723	2,861	-0,503
Potassium Serum, mM/L	-17,72	0,761	-26,97	1,158
PSD F7-β, %	20,03	1,495	1,053	0,079
PSD T5-δ, %	3,864	-0,505	0,123	-0,016
Urea Excretion, mM/24h	-2,723	1,050	-0,015	0,006
PSD P3 Entropy	-9,173	-1,040	-76,71	-8,696
PSD T4-0, %	2,753	0,785	0,396	0,113
Mode HRV, msec	0,183	-0,784	0,002	-0,010
PSD F3 Entropy	-11,43	-1,765	-110,7	-17,10
PSD Fp1 Entropy	6,766	1,666	39,08	9,625
Calcium Excretion, mM/24h	-4,063	-1,092	-1,030	-0,277
PSD T5 Entropy	12,37	2,292	62,48	11,57
Superoxide dismutase, U/mL	-4,170	-0,886	-0,237	-0,050
PSD F7-δ, %	12,66	2,232	0,444	0,078
PSD F4 Entropy	1,610	-0,625	9,448	-3,666
	Constants		6,532	0,173
	Eigenvalues		516,9	5,191
Cumulative Proportion			0,990	1

Localization in the left zone of the axis of the first discriminant root (Fig. 8) of patients in whom balneotherapy caused an increase in PWC, reflects a concomitant increase in their response of systolic blood pressure to brachial artery cuff occlusion, duration of breath hold after inhalation (but not exhalation), HDLP Cholesterol level, excretion of nitrogenous metabolites, as well as a number of EEG parameters, primarily entropy in 6 loci and PSD of theta-rhythm in loci T3 and T4 and beta-rhythm in locus F7 (Table 5). Instead, heart rate and contractile activity index of left ventricle at rest, serum levels of circulating catecholamines (marker: 1/Mode HRV) and calcitonin, erythrocytes superoxide dismutase activity, PSD of delta rhythm in loci T3, T4 and T5 as well as reaction of HRV parameters on standing decreased in these patients. At the opposite pole are localized patients subject to the negative actotropic effect of balneotherapy. A decrease in PWC is accompanied by a decrease/increase in the listed parameters, respectively. An intermediate position along the axis of the first root is occupied by patients with a stable state of PWS. At the same time, changes in associated parameters fluctuate around zero.

Additional separation of this cluster from the other two occurs along the axis of the second root. The lowest localization of the neutral cluster reflects an increase in EEG entropy in loci Fp1 and F7, PSD of theta-rhythm in loci F7, O2 and T5 and alpha-rhythm in locus Fp2, serum levels of potassium and interleukin-6 as well as calcium excretion. Instead, PSD of delta-rhythm in loci Fp2 and F7, PSD of ULF band HRV and its entropy as well as serum cortisol and Ca/K ratio are reduced in these patients.

Table 5. Correlations between variables and roots, centroids of clusters and Z-scores of changes in variables

Variables	Correlations Variables-Roots		F+ (9)	F0 (16)	F- (9)	Cluster of profile
Root 1 (99,0%)	Root 1	Root 2	-26,0	-3,9	32,9	
Physical Working Capacity			1,07±0,22	-0,05±0,03	-0,79±0,05	Basic
Ps3/Ps1 Ratio	-0,016	0,045	0,67±0,31	0,00±0,31	-0,50±0,42	A
PSD T3-θ relative			0,50±0,23	0,06±0,26	-0,68±0,30	A
PSD P3 Entropy	-0,014	0,018	0,87±0,24	0,05±0,26	-0,47±0,36	A
HDLP Cholesterol			0,70±0,36	-0,14±0,27	-0,54±0,36	A
PSD F4 Entropy	-0,018	-0,003	1,45±0,28	0,07±0,40	-1,17±0,64	A
Stange's test			1,65±0,46	-0,07±0,36	-1,02±0,51	A
PSD T4 Entropy			1,47±0,51	-0,18±0,37	-1,42±0,51	A
PSD T3 Entropy			0,87±0,47	-0,36±0,32	-1,49±0,48	A
PSD T4-θ relative	-0,012	0,016	0,69±0,29	-0,46±0,31	-1,16±0,89	A
PSD T3-δ absolute	0,016	0,032	-1,55±0,84	0,63±0,50	1,36±0,95	B
Heart Rate	0,075	-0,220	-1,19±0,26	0,02±0,07	0,88±0,07	B
1/Mode HRV	0,040	0,068	-0,46±0,37	-0,05±0,14	1,33±0,27	B
Superoxide dismutase	0,015	-0,104	-0,53±0,35	0,29±0,29	0,45±0,18	B
Calcitonin	0,012	-0,060	-0,18±0,20	0,38±0,30	0,61±0,33	B
Creatinineuria	-0,020	0,071	1,30±0,47	0,42±0,26	-0,10±0,31	C
Uricosuria	-0,013	0,064	1,71±0,67	0,56±0,50	0,11±0,55	C
Urea Excretion	-0,020	-0,003	2,33±0,68	1,40±0,60	-0,23±0,57	C
PSD F7-β relative	-0,013	-0,065	0,57±0,43	0,39±0,35	-0,61±0,31	D
PSD F3 Entropy	-0,028	-0,149	0,77±0,33	0,53±0,21	-1,28±0,43	D
PSD T5 Entropy	-0,019	-0,102	1,13±0,43	0,88±0,44	-1,17±0,66	D
Ruzhylo's&Popovych's CAI			-0,86±0,51	-0,52±0,29	0,32±0,30	E
PSD T5-δ relative	0,016	0,055	-1,49±0,76	-0,83±0,47	0,85±0,69	E
Baevsky's ARSI up standing			-1,02±0,53	-0,56±0,33	1,19±0,25	E
PSD T4-δ absolute			-1,25±0,62	-0,88±0,51	1,82±1,23	E
Root 2 (1,0%)	Root 1	Root 2	2,52	-2,27	1,52	
PSD Fp1 Entropy	-0,017	-0,138	0,23±0,51	0,67±0,47	-1,80±0,84	F
PSD F7 Entropy			0,44±0,59	1,12±0,30	-1,04±0,60	F
PSD T5-θ relative	-0,018	-0,185	0,33±0,43	0,88±0,25	-0,88±0,49	F
PSD Fp2-α relative			0,31±0,11	0,53±0,25	-0,43±0,38	F
Cortisol			-0,03±0,75	-0,28±0,59	1,27±0,65	G
PSD Fp2-δ relative			-0,10±0,21	-1,01±0,41	1,07±0,67	G
PSD F7-δ relative	0,015	0,140	-0,49±0,46	-0,93±0,40	0,79±0,40	G
(Ca/K)^{0,5} Serum Ratio	0,002	0,217	0,39±0,41	-0,84±0,33	0,29±0,36	H
PSD ULF band HRV relative			0,69±0,37	-0,41±0,41	0,84±0,61	H
PSD HRV Entropy	-0,001	0,258	1,36±0,41	-0,20±0,34	1,00±0,35	H
Potassium Serum	-0,005	-0,182	-0,23±0,53	1,05±0,35	-0,39±0,51	I
PSD F7-θ relative	-0,008	-0,238	-0,45±0,35	0,95±0,25	-0,39±0,34	I
PSD O2-θ relative			-0,60±0,36	0,35±0,28	-0,57±0,37	I
Interleukin-6			-0,69±0,24	0,38±0,31	-0,51±0,44	I
Calcium Excretion	-0,011	-0,109	2,58±1,48	3,72±1,13	0,37±1,08	J
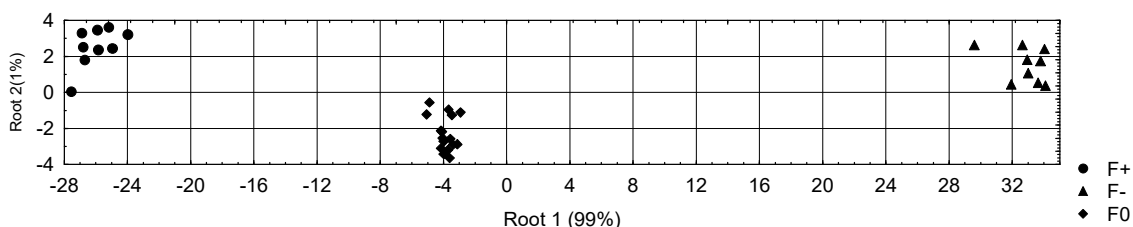
Fig. 8. Scattering of individual values of the first and second discriminant roots, which contain condensed information about changes in neuro-endocrine, hemodynamic and metabolic accompaniment of various effects of balneotherapy on fitness

The visual impression of a clear demarcation of the three clusters in the information field of the two roots is documented by calculating the distances of Mahalanobis (Table 6).

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Table 6. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=24,8) and p-levels (below the diagonal)

Clusters	F+ (9)	F- (9)	F0 (16)
F+ (9)	0	3462	512
F- (9)	168 10^{-6}	0	1363
F0 (16)	32 10^{-4}	84 10^{-6}	0

Another consequence of discriminant analysis is the possibility to retrospectively identify each variant of the actotropic effect of balneotherapy in each patient. This goal of discriminant analysis is realized with the help of coefficients and constants of classification functions (Table 7).

Table 7. Coefficients and constants of classification functions

Clusters	F+ (9)	F- (9)	F0 (16)
Variables	p=,265	p=,265	p=,470
Heart Rate, beats/min	-55,89	45,54	-17,64
$(Ca/K)^{0.5}$ Serum Ratio	5732	-4534	1865
Calcitonin, ng/L	-30,12	24,84	-10,09
PSD HRV Entropy	56,24	51,90	-37,57
PSD F7-0, %	-58,63	45,49	-16,84
PSD T5-0, %	80,87	-63,67	25,60
PSD T3-δ, μV^2/Hz	-0,715	0,559	-0,208
Creatinineuria, mM/24h	72,79	-57,52	22,88
Ps3/Ps1 Ratio	-1195	881,7	-332,5
Uricosuria, mM/24 h	-93,57	75,27	-27,89
Potassium Serum, mM/L	885,1	-702,8	283,2
PSD F7-β, %	-34,18	27,70	-11,27
PSD T5-δ, %	-4,078	3,163	-1,286
Urea Excretion, mM/24h	0,506	-0,377	0,149
PSD P3 Entropy	2483	-2021	829,0
PSD T4-0, %	-12,75	10,46	-4,529
Mode HRV, msec	-0,108	0,035	-0,011
PSD F3 Entropy	3560	-2933	1195
PSD Fp1 Entropy	-1252	1038	-433,7
Calcium Excretion, mM/24h	33,09	-27,25	11,63
PSD T5 Entropy	-2017	1647	-690,7
Superoxide dismutase, U/mL	7,727	-6,144	2,736
PSD F7-δ, %	-14,30	11,74	-4,861
PSD F4 Entropy	-301,4	258,1	-74,91
Constants	-534,8	-350,9	-60,15

Classification accuracy is 100%.

Discussion

So, in this study, the statement about the multivariate effect of balneotherapy at the Truskavets' SPA on physical performance was confirmed. We consider it necessary to note that the method of determining physical capacity based on the reaction of heart rate to dosed exercise is not direct, but is based on its close correlation with VO_2max. Essentially, the PWC_{150} reflects the state and reactivity of the autonomic nervous system rated for HRV. This is confirmed by a close correlation between changes in PWC_{150} and HRV parameters (Table 8).

Table 8. Regression Summary for changes in PWC_{150}
**R=0.726; R^2=0.527; Adjusted R^2=0.480; $F_{(3.3)}$=11.2; p<10^{-4}**

N=34		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(30)}$	p-level
Variables	r		Intercpt	0,013	0,057	0,23	0,823
Heart Rate, beats/min	-0,68	-0,537	0,158	-0,022	0,007	-3,40	0,002
Baevsky's ARSI up standing	-0,53	-0,207	0,157	-0,049	0,037	-1,31	0,199
PSD HRV Entropy	0,26	0,205	0,126	0,655	0,405	1,62	0,116

At the same time, we hasten to argue the reality of the actotropic effect of Naftussya bioactive water (as the main component of the resort's balneofactors) based on the results of an experiment on rats, in which physical performance was assessed by a test of the duration of swimming until exhaustion (Ruzhylo et al., 2003; Zukow et al., 2022).

Returning to balneotherapy-induced changes in HRV parameters, we point out their close relationship with changes in EEG parameters (Table 9 and Fig. 9).

Table 9. Factor structure of canonical roots of changes in EEG and HRV parameters

Left set	R
PSD T4 Entropy	0,726
PSD T3 Entropy	0,719
PSD T5 Entropy	0,646
PSD F3 Entropy	0,583
PSD F4 Entropy	0,573
PSD F7 Entropy	0,567
PSD Fp1 Entropy	0,563
PSD P3 Entropy	0,426
PSD Fp2-α, %	0,521
PSD T3-θ, %	0,457
PSD T5-θ, %	0,442
PSD F7-θ, %	0,415
PSD T4-θ, %	0,331
PSD T3-δ, μV^2/Hz	-0,667
PSD Fp2-δ, %	-0,491
PSD T5-δ, %	-0,341
PSD F7-δ, %	-0,335
Right set	**R**
Heart Rate, beats/min	-0,907
1/Mode HRV, 1/msec	-0,872
Baevsky's ARSI up standing	-0,472
PSD ULF band HRV, %	0,166
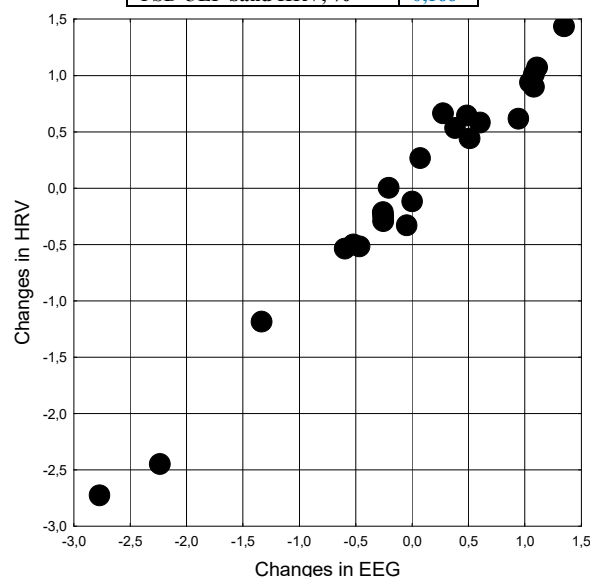
$R=0.987$; $R^2=0.974$; $\chi^2_{(68)}=92$; $p=0.028$; Λ Prime$<10^{-3}$

Fig. 9. Scatterplot of canonical correlation between changes in EEG (X-line) and HRV (Y-line) parameters

A number of publications are known, starting from Van Buren & Ajmone-Marsan (1960) and ending with Borghesi et al (2024), including our group (Popovych et al., 2014; Babelyuk et al., 2017; Zukow et al., 2024), about relationships between EEG and HRV parameters as well as between HRV and cortical thickness or brain activity in selected CAN regions (Sakaki et al., 2016; Winkelmann et al., 2017; Yoo et al., 2018; Carnevali et al., 2020; Matusik et al., 2023).

Therefore, the close connections between balneotherapy-induced changes in EEG and HRV parameters revealed in this study are quite expected. Although EEG changes are plotted along the X-axis as a factor feature and HRV changes along the Y-axis as an outcome feature, the actual cause-and-effect relationships are *bidirectional* and are realized within the framework of the *central autonomic network* (Benarroch, 1993; Thayer & Lane, 2009; Palma & Benarroch, 2014).

Cortisol and Interleukin-6, which appeared in the constellation of the characteristic accompanying actotropic effects of balneotherapy, have long been known as one of the key elements of the *neuro-endocrine-immune network* (Besedovsky & Sorkin, 1977; Besedovsky & del Rey, 1996). We found significant connections between changes in serum Cortisol and EEG parameters as well as autonomic reactivity (Table 10 and Fig. 10).

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Table 10. Regression Summary for changes in serum Cortisol
R=0.788; R^2=0.621; Adjusted R^2=0.456; F$_{(10.2)}$=3.8; p=0.004

| N=34		Beta	St. Err. of Beta	B	St. Err. of B	t$_{(23)}$	p-level
Variables	r		Intercpt	-27,5	44,9	-0,61	0,547
PSD Fp1 Entropy	**-0,48**	-1,334	0,326	-1806	441	-4,09	10^{-3}
PSD F7 Entropy	**-0,41**	1,144	0,360	1303	409	3,18	0,004
PSD F3 Entropy	**-0,32**	0,520	0,248	1071	511	2,10	0,047
PSD F7-θ, %	**-0,43**	-0,460	0,198	-24,92	10,72	-2,32	0,029
PSD Fp2-α, %	**-0,38**	0,353	0,291	6,818	5,608	1,22	0,236
PSD F7-δ, %	**0,42**	0,610	0,277	5,054	2,294	2,20	0,038
PSD Fp2-δ, %	**0,37**	-0,425	0,311	-3,767	2,757	-1,37	0,185
PSD T3-δ, µV^2/Hz	**0,37**	0,388	0,180	0,293	0,136	2,15	0,042
PSD T5-δ, %	**0,37**	0,638	0,265	4,955	2,062	2,40	0,025
Baevsky's ARSI up standing	**0,29**	0,226	0,141	32,34	20,27	1,60	0,124
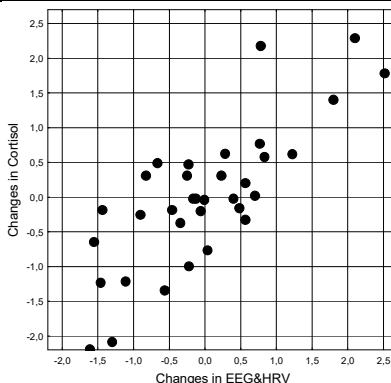
R=0.788; R^2=0.621; χ$^2_{(10)}$=26; p=0.004; Λ Prime=0.379

Fig. 10. Scatterplot of canonical correlation between changes in EEG&HRV parameters (X-line) and serum Cortisol (Y-line)

It is important that all EEG parameters, without exception, related to cortisol, are included in the factor structure of the EEG-root, which is related to the HRV-root (Table 9, **highlighted in bold**). This suggests that neural structures projecting to these loci regulate both HRV and Cortisol. The changes in serum Interleukin-6 are also associated with changes in EEG and HRV parameters as well as Calcitonin (Table 10 and Fig. 10).

Table 11. Regression Summary for changes in serum Interleukin-6
R=0.768; R^2=0.590; Adjusted R^2=0.436; F$_{(9.2)}$=3.8; p=0.004

N=34		Beta	St. Err. of Beta	B	St. Err. of B	t$_{(24)}$	p-level
Variables	r		Intercpt	-0,334	0,204	-1,64	0,115
PSD T3-δ, µV^2/Hz	**-0,44**	-0,346	0,172	-0,0014	0,0007	-2,01	0,055
PSD Fp2-δ, %	**-0,33**	0,653	0,329	0,030	0,015	1,99	0,059
PSD T5-δ, %	**-0,32**	-0,290	0,218	-0,012	0,009	-1,33	0,195
Heart Rate, beats/min	**-0,42**	-1,060	0,329	-0,140	0,043	-3,22	0,004
1/Mode HRV, 1/msec	**-0,30**	-0,999	0,364	-0,012	0,005	-2,75	0,011
PSD O2-θ, %	**0,45**	0,300	0,158	0,102	0,054	1,90	0,069
PSD Fp1 Entropy	**0,38**	0,224	0,179	1,578	1,262	1,25	0,223
PSD Fp2-α, %	**0,33**	0,404	0,285	0,041	0,029	1,42	0,168
Calcitonin, ng/L	**0,28**	0,194	0,142	0,036	0,026	1,37	0,184
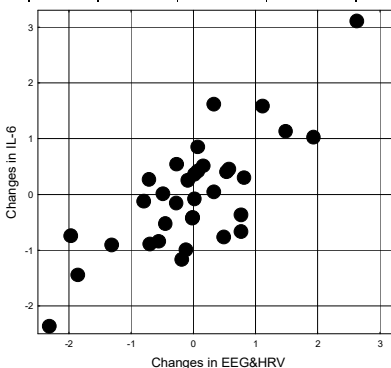
R=0.768; R^2=0.590; χ$^2_{(9)}$=25; p=0.004; Λ Prime=0.410

Fig. 11. Scatterplot of canonical correlation between changes in EEG&HRV parameters (X-line) and serum Interleukin-6 (Y-line)

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According to the concept of the Truskavetsian Scientific School of Balneology, the active factors of Naftussya water are polyphenols/aryl hydrocarbons, fatty acids and autochthonous bacteria, which through the corresponding receptors (Ah, GP40/120, TL/NL) affect neurons of the enteric nervous system and immunocytes of the gut-associated lymphoid tissue (GALT), as well as microbiota cells.

The neurotransmitters and cytokines released under this influence, in turn, exert autocrine and paracrine effects on the same or neighboring neurons and immunocytes, as well as on neurons of the autonomic and central nervous systems, reaching them through the blood and activating afferent terminals of n. vagus and n. splanchnicus. Next, the corresponding nerve nuclei of the cortex, brainstem, and medulla exert a modulating effect both on the immunocytes of the bone marrow, thymus, spleen, lymph nodes, and again GALT, and on the endocrinocytes of at least the adrenals, gonads, and thyroid and parathyroid glands, whose hormones, in turn, affect immunocytes and neurons. A direct effect of aryl hydrocarbons and fatty acids on endocrinocytes and blood/lymph immunocytes is also possible (Popovych, 2022; Popovych, 2022a; Popovych et al., 2022; Popovych et al., 2022a). It is important that aryl hydrocarbons are also present in the composition of Ozokerite and realize their effects through Ah receptors of immunocytes of the skin-associated lymphoid tissue. This is manifested in the similarity of the effects of both Naftussya and Ozokerite on neuro-endocrine-immune complex (Ruzhylo et al., 2021).

The results obtained in our study fit into the presented concept. The applied balneofactors, through the mediation of the central autonomic and neuro-endocrine-immune networks, affect the mechanisms responsible for physical performance, or rather its marker. Note that our study incorporates the recommendations of Armstrong et al. (2022) regarding research in this direction. The authors 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.

However, the question remains open, why the same factor(s) cause polyvariate actotropic effects? This situation has been known for a long time. Hildebrandt (1980) showed that a 4-week endurance training, performed at different times of day, yields different results: an early-morning training did not evoke a significant increase of the PWC_{130}, whereas the afternoon training gave maximum results. In relation to our study, it is important to note that different responses to therapeutical stimuli depended on the circadian phase were accompanied by different states of autonomic reactivity, also subject to circadian rhythms. Earlier we discovered a features of reactions to acute stress of neuro-endocrine-immune complex, metabolome, ECG and gastric mucosa in rats with various state of *innate* muscular endurance and resistance to hypoxia (Fil et al., 2021; Zukow et al., 2022; Melnyk et al., 2023).

In the next article, which has already been prepared for printing, we will show that using the method of discriminant analysis, 25 initial parameters identified, based on the totality of which the nature of the actotropic effect of balneotherapy can be predicted with 100% accuracy.

Conclusion
1. The multivariate actotropic effects of balneofactors are due to their multivariate effects on neuro-endocrine regulation and metabolism, which, apparently, is determined by the peculiarities of the individual reactivity of the body.
2. Our study confirms the heterogeneity of responses to balneotherapy at Truskavets' Spa, with some patients experiencing increased physical working capacity, others showing decreased capacity, and a third group maintaining stable levels.
3. The discriminant analysis revealed 24 variables as characteristic accompaniments of the three variants of actotropic effects, including hemodynamic, neural (EEG and HRV), endocrine, and metabolic parameters. This comprehensive approach allows for a more nuanced understanding of the physiological changes associated with balneotherapy.
4. The close connections between balneotherapy-induced changes in EEG and HRV parameters highlight the importance of the central autonomic network in mediating these effects.
5. The involvement of cortisol and interleukin-6 in the characteristic accompanying effects underscores the role of the neuro-endocrine-immune network in the body's response to balneotherapy.
6. The study demonstrates the potential of using initial physiological parameters to predict not only the direction but also the magnitude of fitness response to balneotherapy. This could lead to more personalized and effective treatment plans.
7. The observed sexual dimorphism in neuro-endocrine regulation of bicycle ergometric test parameters suggests that gender-specific approaches may be necessary when designing balneotherapy protocols.

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8. The findings support the concept of the Truskavetsian Scientific School of Balneology regarding the active factors in Naftussya water and their effects on the enteric nervous system, gut-associated lymphoid tissue, and microbiota.

9. This research contributes to the growing body of evidence supporting the complex systems approach in understanding overtraining syndrome and related physiological adaptations.

10. Future studies should focus on longitudinal effects of balneotherapy, potential molecular mechanisms underlying the observed changes, and the development of predictive models for individualized balneotherapy protocols.

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