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ORIGINAL PAPER

Neuroendocrine and metabolic predictors of the effects of balneotherapy at the Truskavets Spa on physical working capacity in men with maladaptation

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ABSTRACT

Introduction and aim. The effect of balneotherapy in the Truskavets Spa on physical working capacity is complex and individualized. This study aims to identify an optimal constellation of predictors for the actotropic effects of balneotherapy.

Material and methods. We observed 34 men with maladaptation against the background of chronic pyelonephritis in remission. We recorded physical working capacity, heart rate variability, electroencephalography, adaptation hormones, and blood and urine metabolites before and after a standardized balneotherapy regimen.

Results. Standard balneotherapy resulted in various effects on physical working capacity$_{150}$: an increase in 9 patients (26.5%), no significant change in 16 patients (47.1%), and a decrease in 9 patients (26.5%). Through discriminant analysis, we identified a constellation of 25 initial parameters that could predict the nature of the actotropic effect with 100% accuracy. These parameters included measures of physical working capacity, cardiorespiratory fitness, electroencephalography, heart rate variability, hormones, and metabolism. Furthermore, multiple linear regression analysis allowed us to predict quantitative changes in physical work capacity$_{150}$ with a standard error of 0.28 W/kg. This predictive model incorporated hemodynamic and Electroencephalography parameters, achieving an adjusted R^2 of 0.555.

Conclusion. The directionality and magnitude of physical working capacity$_{150}$ changes under the influence of balneofactors at the Truskavets Spa are determined by a complex constellation of initial physiological parameters, which forms the body's reactivity. This finding has significant implications for personalizing balneotherapy treatments.

Keywords. electroencephalography, heart rate variability, hormones, metabolism, physical work capacity, Truskavets Spa

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The list of abbreviations:

PWC – physical working capacity, **HRV** – heart rate variability, **EEG** – electroencephalography, **GPVR** – general peripheral vessels resistance, **SDNN** – standard deviation of NN intervals, **LF** – low frequency, **HF** – high frequency

Introduction

Balneotherapy, the therapeutic use of natural mineral waters, gases, and peloids, has been a cornerstone of spa medicine for centuries. The Truskavets Spa in Ukraine is renowned for its unique balneotherapeutic complex, comprising Naftussya bioactive water, ozokerite applications, and mineral baths. Researchers from the Truskavetsian Scientific School of Balneology have demonstrated the beneficial effects of this complex on various physiological systems, including urinary, digestive, endocrine, and immune functions.[1-8]

The effects of balneotherapy on neuroendocrine and metabolic parameters are mediated primarily by organic substances present in both Naftussya bioactive water and ozokerite. These substances interact with aryl hydrocarbon receptors, which are expressed by neurons, endocrinocytes, and immunocytes, initiating a cascade of physiological responses.[9,10]

However, the impact of balneotherapy on physical working capacity (PWC) is not uniform across all patients. Although many people experience improvements, some, particularly those with initially high levels of physical working capacity, can paradoxically show a decrease in performance.[11-14] This heterogeneity in responses underscores the complex and individualized nature of the effects of balneotherapy.

Previous attempts to predict balneotherapy effects have shown promise but have been limited by insufficient initial information. For example, our earlier work achieved prediction accuracies of 75.6%, 76.7%, and 88.9% for adults, children, and rats, respectively, when using constellations of initial metabolic and hemodynamic parameters.[15] These findings, while valuable, highlighted the need for a more comprehensive predictive model, which prompted the additional use of aerobic training and/or phytoadaptogens, both well known (Ginseng, Bittner's balsam), and Ukrainian phytocompositions 'Balm Kryms'kyi' and 'Balm Truskavets'.[9,15-17]

It is important to note that, first, 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. However, the accuracy of the forecast is not high enough, probably due to insufficient initial information.[10]

Aim

Given the importance of the an accurate forecast of ineffective or even adverse effects of balneotherapy on the physical performance of patients for the preventive use of actoprotective agents, the purpose of this study is to find an optimal constellation of the predictors of actotropic effects of balneotherapy.

Material and methods

Ethics approval

The tests in patients are conducted according to the positions of Helsinki Declaration 1975, revised and the complemented in 2002, and the directive of the National Committee on Ethics of scientific research. The study protocol was approved by the Ethical Committee of Ukrainian Scientific Research Institute of Medicine of Transport (protocol No. 35, 05.10.2022). During the realization of tests from all participants, informed consent was obtained and all measures were used for providing anonymity of participants.

Participants

The object of this clinical-physiological observation were 34 men (aged 23–70 years, weight 65–107 kg, height 160–183 cm, body mass index 19.8–32 kg/m^2) with maladaptation against the background of chronic pyelonephtis in remission phase, who came for rehabilitation at the Truskavets Spa.

Inclusion and exclusion criteria

Inclusion criteria: male patients aged 23–70 years. Diagnosed with maladaptation. History of chronic pyelonephritis, currently in remission for at least 6 months. Cleared for balneotherapy by their primary care physician.

Exclusion criteria: Active pyelonephritis or any signs of urinary tract infection. Acute renal conditions. Uncontrolled hypertension (BP>160/100 mmHg). Severe cardiovascular diseases. Any condition contraindicating balneotherapy.

All patients underwent a comprehensive medical examination, including urinalysis and renal function tests, to ensure that they were in stable remission before enrollment.

Study design and procedure

Systolic (Ps) and diastolic (Pd) blood pressure, as well as heart rate (HR), was measured (by a tonometer "Omron M4-I", Netherlands) in a sitting position three times in a row. On the basis of the received data, the good old Kerdö's Vegetative Index as well as the Ps2/Ps1, Ps3/Ps1, Pd2/Pd1, and Pd3/Pd1 indices recently proposed by our group were calculated.[18-20] After that, the parameters of hemodynamics were determined (with an echocamera "Toshiba-140", Japan): ejection time (ET), end-diastolic (EDV) and end-systolic (ESV) volumes of left ventricle with the following ejection fraction (EF), general peripheral vessels resistance (GPVR), cardiac output (CO) calculation by classic formulas:[21]

$EF = 100 \bullet (EDV - ESV)/EDV;$

$GPVR=80 \bullet (0.67 \bullet Pd + 0.33 \bullet Ps)/HR \bullet (EDV - ESV);$

$CO = (EDV - ESV) \bullet HR.$

In addition, we calculated the contractile activity index (CAI) of left ventricle by the method of Ruzhylo and Popovych:[10]

$RPCAI = 0.1332 \bullet (0.67 \bullet Pd + 0.33 \bullet Ps) \bullet (EDV - ESV)/EDV \bullet ET.$

Then we recorded an electrocardiogram in II lead for 7 minutes in the supine position and 2 minutes after standing up 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; CI=(VLF+LF)/HF; LFnu=100%•LF/(LF+HF)[22,23] as well as Baevskiy's Activity of Regulatory Systems Index (BARSI) and Autonomous Reactivity Index (ARI) as the difference between BARSI in standing up and supine positions.[24]

Next, quantitative EEG was recorded at rest with the 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 were the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), absolute ($\mu V^2/Hz$) 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, the coefficient of Asymmetry (As) and Laterality Index (LI) for PSD Rhythm were calculated using equations:[25]

$As, \% = 100 \bullet (Max – Min)/Min;$

$LI, \% = \Sigma [200 \bullet (Right – Left)/(Right + Left)]/8.$

We calculated also for HRV and for each locus of EEG the Entropy (h) of normalized PSD using Popovych's equations based on classic Shannon's equation:[7,26-28]

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

$hHRV =- [PSDHF \bullet \log_2 PSDHF + PSDLF \bullet \log_2 PSDLF + PSDVLF \bullet \log_2 PSDVLF + PSDULF \bullet \log_2 PSDULF]/\log_2 4.$

In a portion of the venous blood, the serum levels of major hormones of adaptation cortisol, testosterone, aldosterone, triiodothyronine as well as PTH and calcitonin was assayed with ELISA kits according to the SOP provided by the manufacturer ("Алкор Био", XEMA Co Ltd and DRG International Inc.) with the use of analyzer "RT-2100C."

In addition to hormones, we estimated a number of serum 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); VLDL (calculated by the level of triglycerides, estimated by the 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), and calculated the Dobiásová's and Frohlich's atherogenic index (AGI) as TG/HDLP Ch ratio.[29-31] Electrolytes: calcium (by reaction with arsenase III); magnesium (by reaction with colgamite); phosphates (phosphate-molybdate method); chloride (mercury-rhodanidine method); sodium and potassium (flamming 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 was carried out according to instructions[32] 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) was 1.5 W/kg at a pedaling frequency of 60–75 rpm. This corresponded to the recommendations for ergometer testing in occupational medicine.[33-36] We calculated submaximal PWC_{150} with the mechanical power in Watt per kilogram body weight (W/kg) as an indicator of cardiorespiratory fitness.[35]

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 of standard balneotherapy: drinking of Naftussya bioactive water (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°C, duration 8-10 minutes, every other day, 5 procedures); therapeutic physical education (motion mode II).[2,7]

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

Reference values of variables were taken from the Instructions and database of the Truskavetsian Scientific School of Balneology.[7,27]

Statistical analysis

Statistical processing was performed using a software package "Microsoft Excel", "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA), and AI Claude 3.5 Sonet. Normality of data distribution was assessed using the Shapiro-Wilk test. Descriptive statistics are presented as mean ± standard deviation for normally distributed variables and median (interquartile range) for non-normally distributed variables.

We employed several advanced statistical techniques:

1. Discriminant analysis: This was used to identify the constellation of initial parameters that best predicted the direction of PWC change. The forward stepwise method was applied, with Wilks' lambda as the criterion for variable selection. The model's predictive accuracy was assessed using leave-one-out cross-validation.
2. Multiple linear regression: This technique was used to quantitatively predict changes in PWC. We used a stepwise approach, adding variables to the model based on their contribution to improving the adjusted R2 value. The final model was checked for multicollinearity using variance inflation factors (VIF), with VIF > 5 considered problematic.
3. Canonical correlation analysis: This was employed to explore the relationship between the set of predictor variables and the change in PWC, allowing us to understand the multivariate nature of these relationships.
4. Principal component analysis: This was used as a data reduction technique to identify underlying patterns in our large set of variables, helping to understand the main dimensions of variation in our data.
5. Power analysis: We have included a post-hoc power analysis to demonstrate the statistical power of our study given the observed effect sizes. This helps readers interpret the reliability of our findings.
6. Effect sizes: In addition to p-values, we now report effect sizes (e.g., Cohen's d for t-tests, partial eta-squared for ANOVAs) to provide a more complete picture of the magnitude of our findings.
7. Multiple comparisons: We have explicitly addressed how we handled multiple comparisons to control for Type I error. We used the Benjamini-Hochberg procedure to control the false discovery rate.
8. Assumption testing: We now provide more details on how we tested and met the assumptions for each statistical test used (e.g., normality, homoscedasticity).
9. Sensitivity analyses: We conducted and report on sensitivity analyses to test the robustness of our findings to different analytical choices.

For all statistical tests, a p-value<0.05 was considered statistically significant. We also report exact p-values to allow readers to interpret the strength of evidence against the null hypothesis. This expanded section provides a more comprehensive overview of our statistical approach, demonstrating the rigor of our analysis. These additions provide a more comprehensive and transparent account of our statistical approach.

Results

Our analysis revealed three distinct response patterns to balneotherapy in terms of changes in physical working capacity (PWC_{150}). 1. Increased PWC_{150}: 9 patients (26.5%). 2. No significant change in PWC_{150}: 16 patients (47.1%). 3. Decreased PWC_{150}: 9 patients (26.5%). This heterogeneity in responses underscores the importance of identifying predictive factors for individualized treatment approaches.

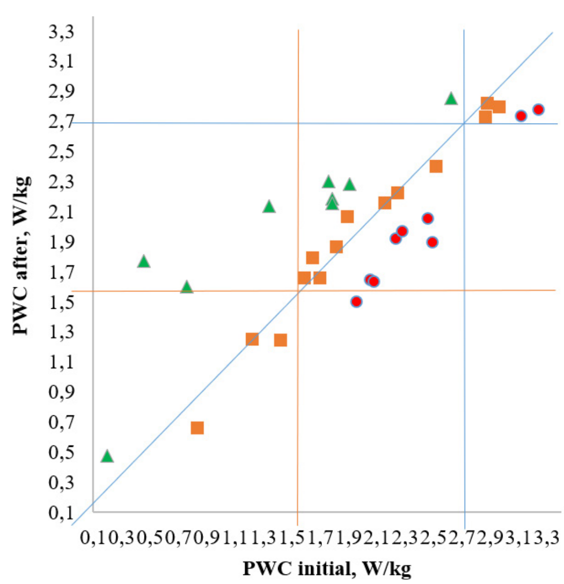


Fig. 1. Individual PWC_{150} levels before (X-axis) and after (Y-axis) balneotherapy, the lines indicate the average norm (N) and its lower limit (N-2σ)

It was established (Fig. 1 and 2) that upon admission to rehabilitation, only 5 patients had a PWC_{150} level in the upper range of normal, 21 had a lower range, and the remaining 8 had a lower limit of ±2σ. Obviously, reduced physical capacity is one of the manifestations of maladaptation. Balneotherapy in most patients (18) did not significantly affect the level of PWC_{150}, in 9 it increased it, while in the other 9 patients it decreased it. The changes in fitness are only partially subject to the law of initial value, so we are in solidarity with critics of the universality of this law.[37,40]

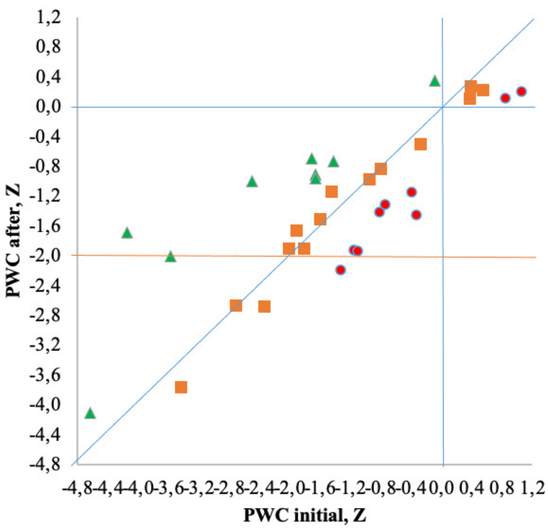


Fig. 2. Individual PWC_{150} normalized levels before (X-axis) and after (Y-axis) balneotherapy

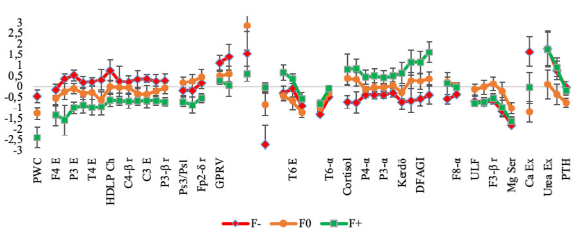


Fig. 3. Profiles of initial values (Z±SE) of variables as predictors of various changes in PWC (Fitness) after balneotherapy (see also Table 5 for details)

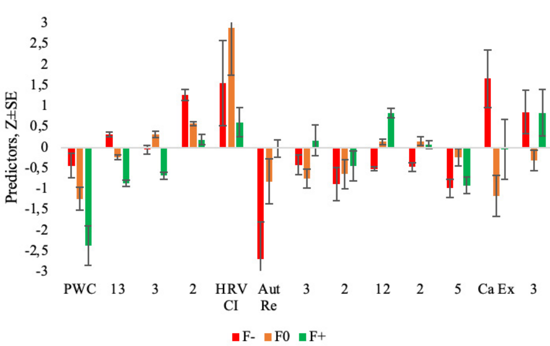


Fig. 4. Clusters of initial values of variables (number below) as predictors of various changes in PWC (Fitness) after balneotherapy (see please also Table 5 for details)

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:

$Z=4\bullet(V-N)/(Max-Min)=(V-N)/SD=(V/N-1)/Cv$,

where V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively.[7,27,40]

Table 1. Discriminant functions analysis summary in relation to the predictors of changes in PWC[a]

Variables currently in the model	Clusters of changes in fitness (n)			Parameters of Wilks' Statistics					
	F- (9)	F+ (9)	F0 (16)	Wilks' Λ	Partial Λ	F-remove (2.9)	p-level	Tolerancy	Reference Cv
PWC, W/kg	2.44 0.15	1.40 0.26	2.01 0.15	0.002	0.574	2.59	0.144	0.001	2.67 0.203
Heart rate, beats/min	64.8 2.4	82.1 4.5	71.8 2.5	0.001	0.697	1.52	0.283	0.001	68.4 0.119
Ps3/Ps1 ratio	0.946 0.022	0.901 0.018	0.976 0.022	0.001	0.875	0.50	0.627	0.043	0.959 0.087
Ps2/Ps1 ratio	0.950 0.025	0.896 0.029	0.986 0.027	0.002	0.502	3.47	0.090	0.032	0.963 0.084
PSD Fp2-α, %	28.8 4.7	43.1 5.2	26.9 3.5	0.011	0.084	38.3	10^{-4}	0.012	32.9 0.448
PSD F8-α, $\mu V^2/Hz$	24 3	40 6	43 7	0.006	0.141	21.2	0.001	0.029	42 1.202
PSD T6-α, $\mu V^2/Hz$	43 9	101 22	71 9	0.002	0.449	4.29	0.061	0.094	114 1.302
PSD P3-α, %	30,9 4.4	46.1 10.7	47.8 4.8	0.002	0.565	2.70	0.135	0.026	42.7 0.487
PSD F3-β, %	19.0 2.3	20.3 2.6	28.6 3.6	0.007	0.126	24.2	0.001	0.022	26.7 0.463
PSD T3-θ. %	12.0 1.9	7.2 0.9	8.8 1.1	0.002	0.452	4.25	0.062	0.048	10.3 0.466
PSD T4-θ, %	10.9 3.3	6.7 1.2	9.6 1.3	0.003	0.313	7.69	0.017	0.063	9.7 0.482
PSD F4 entropy	0.83 0.03	0.70 0.06	0.79 0.05	0.001	0.757	1.12	0.378	0.145	0.85 0.139
PSD T3 Entropy	0.88 0.03	0.76 0.04	0.82 0.03	0.001	0.709	1.44	0.300	0.081	0.86 0.131
Mode HRV, msec	956 51	786 46	835 32	0.001	0.625	2.10	0.194	0.030	875 0.116
PSD ULF band HRV, $msec^2$	50 23	38 50	109 27	0.009	0.096	32.9	10^{-4}	0.004	122 0.892
PSD ULF band HRV, %	2.1 0.7	2.5 1.1	5.5 1.4	0.008	0.110	28.4	10^{-4}	0.007	5.5 0.810
(VLF+LF)/HF as Centralization Index	12.4 3.5	9.1 1.7	19.2 4.8	0.002	0.413	4.97	0.045	0.055	6.9 0.554
Autonomous Reactivity Index, units	-0.04 1.05	3.07 0.24	2.14 0.62	0.0012438	0.7138422	1.403044	0.307332	0.1715642	3.10 0.375
Cortisol, nM/L	292 34	464 83	416 40	0.006	0.149	20.0	0.001	0.037	370 0.303
Calcitonin, ng/L	5.16 0.70	8.49 1.32	7.09 0.96	0.007	0.124	24.7	0.001	0.053	13.95 0.493
Parathyroid hormone, pM/L	3.74 0.26	3.60 0.19	3.12 0.19	0.001	0.598	2.35	0.165	0.025	3.75 0.230
Calcium serum, mM/L	2.13 0.03	2.16 0.07	2.27 0.06	0.002	0.508	3.39	0.093	0.057	2.30 0.065
Calcium excretion, mM/24h	5.93 0.80	4.34 0.86	3.28 0.46	0.003	0.335	6.96	0.022	0.040	4.38 0.214
Magnesium serum, mM/L	0.81 0.00	0.82 0.01	0.85 0.01	0.001	0.948	0.19	0.830	0.147	0.90 0.056
Magnesium excretion, mM/24h	4.92 0.46	5.07 0.47	3.73 0.47	0.002	0.490	3.64	0.082	0.047	4.10 0.266

[a] step 25, N of variables in model: 25, Grouping: 3 grps; Wilks' Λ: 0.0009, approx. $F_{(50.1)}=9.1$; $p<10^{-6}$,
in each column, the first line is the average, the second – SE, in reference column – the average and Cv, the "Reference" column is not the result of discriminant analysis

Further, profiles (Fig. 3) of normalized initial values of variables as predictors of various changes in PWC after balneotherapy were created.

At the next stage of the analysis, more or less homogeneous variables were condensed into 13 clusters (Fig. 4).

The previously selected variables were further subjected to discriminant analysis[42] with the aim not so much to discover which of them are formally characteristic, but to visualize the integral state of each patient. The forward stepwise program included only 25 variables in the discriminant model (Tables 1-2). First of all, these are PWC and 3 cardiorespiratory fitness parameters. In addition, 9 relate to EEG, 5 to HRV, 3 to hormones, and 4 to metabolism.

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

Variables currently in the model	F to enter	p	Λ	F-value	p
Heart rate, beats/min	6.30	0.005	0.711	6.30	0.005
Calcium excretion, mM/24h	4.39	0.021	0.550	5.22	0.001
Magnesium serum, mM/L	3.35	0.049	0.447	4.79	10^{-4}
PSD F3-β, %	3.88	0.033	0.350	4.83	10^{-4}
PSD ULF band HRV, msec²	5.46	0.010	0.249	5.42	10^{-4}
PWC, W/kg	2.63	0.091	0.207	5.19	10^{-4}
PSD F4 entropy	2.25	0.126	0.176	4.95	10^{-4}
Calcitonin, ng/L	1.98	0.161	0.151	4.73	10^{-4}
Magnesium excretion, mM/24h	4.60	0.021	0.108	5.23	10^{-5}
Ps3/Ps1 ratio	2.87	0.078	0.085	5.33	10^{-5}
Cortisol, nM/L	3.61	0.045	0.064	5.66	10^{-6}
Ps2/Ps1 ratio	1.34	0.283	0.056	5.38	10^{-5}
PSD T3 entropy	1.06	0.365	0.050	5.05	10^{-5}
PSD T4-θ, %	2.18	0.142	0.041	5.10	10^{-5}
Calcium serum, mM/L	1.76	0.202	0.034	5.05	10^{-5}
HRV centralization Index	3.30	0.063	0.024	5.48	10^{-5}
PSD P3-α, %	1.68	0.220	0.019	5.45	10^{-5}
PSD Fp2-α, %	1.67	0.223	0.016	5.43	10^{-5}
PSD ULF band HRV, %	2.73	0.102	0.011	5.83	10^{-5}
PSD F8-α, μV²/Hz	5.73	0.018	0.006	7.38	10^{-5}
PSD T6-α, μV²/Hz	3.90	0.052	0.003	8.59	10^{-6}
Mode HRV, msec	1.55	0.259	0.003	8.59	10^{-5}
PSD T3-θ, %	2.04	0.186	0.002	8.99	10^{-5}
Parathyroid hormone, pM/L	1.59	0.262	0.001	9.12	10^{-5}
Autonomous reactivity index, units	1.40	0.307	0.001	9.12	10^{-4}

Instead, 3 hemodynamic, 13 EEG, 4 metabolic, 1 HRV and 1 hormonal parameters were outside the discriminant model, probably due to duplication/redundancy of the recognition information (Table 3).

The identifying information contained in the 25 discriminant variables is condensed into two roots (Table 4). The major root contains 89.3% of discriminatory opportunities ($r^*=0.995$; Wilks' $\Lambda=0.001$; $\chi^2_{(50)}=133$; $p<10^{-6}$), and minor root 10.7% ($r^*=0.958$; Wilks' $\Lambda=0.083$; $\chi^2_{(24)}=47$; $p=0.003$).

Table 3. Discriminant functions analysis summary, variables currently not in the model

Variables	Clusters of changes in fitness (n)			Parameters of Wilks' Statistics					
	F- (9)	F+ (9)	F0 (16)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Reference Cv/SD
General peripheral vessels resistance, kPa•sec/m³	18.0 1.7	13.9 1.0	15.0 1.1	0.001	0.805	0.73	0.522	0.054	12.3 0.414
Kerdö's vegetative index, units	-28 5	-7 7	-20 4.5	0.001	0.992	0.03	0.975	0.159	-16.9 14.6
Stange's test, sec	64 6	51 5	56 3	0.001	0.973	0.08	0.920	0.117	50 0.200
Amplitude-α, μV	13.5 2.0	23.0 4.3	17.1 2.0	0.001	0.947	0.17	0.849	0.050	17.4 0.614
PSD Fp1-α, μV²/Hz	65 16	134 33	93 20	0.001	0.995	0.02	0.985	0.152	89 0.960
PSD Fp2-δ, %	29.7 4.8	17.1 3.8	35.1 6.3	0.002	0.994	0.02	0.983	0.178	26.5 0.687
PSD T4 entropy	0.87 0.02	0.73 0.04	0.81 0.04	0.001	0.943	0.18	0.838	0.061	0.84 0.137
PSD C3 entropy	0.90 0.02	0.80 0.03	0.83 0.04	0.001	0.961	0.12	0.886	0.071	0.86 0.115
PSD C4 entropy	0.90 0.02	0.72 0.07	0.85 0.04	0.001	0.937	0.20	0.824	0.153	0.87 0.109
PSD C4-β, %	28.5 3.0	18.8 4.1	25.4 2.5	0.001	0.989	0.03	0.967	0.074	25.9 0.405
PSD T6 entropy	0.82 0.05	0.87 0.03	0.74 0.05	0.001	0.873	0.43	0.666	0.056	0.825 0.149
PSD P3-α, μV²/Hz	145 59	455 146	279 61	0.001	0.907	0.31	0.747	0.051	287 1.319
PSD P3-θ, %	10.3 1.6	6.5 0.7	8.0 1.2	0.001	0.915	0.28	0.767	0.120	9.0 0.552
PSD P3-β, %	26.4 3.6	15.2 2.5	21.8 2.7	0.001	0.910	0.30	0.753	0.308	22.7 0.514
PSD P3 entropy	0.88 0.03	0.67 0.02	0.79 0.04	0.001	0.815	0.68	0.542	0.041	0.80 0.167
PSD P4-α, μV²/Hz	142 43	462 133	241 46	0.001	0.958	0.13	0.881	0.102	288 1.318
Baevskiy's activity of regulatory systems index standing up, units	3.85 0.65	5.93 0.67	4.94 0.44	0.001	0.935	0.21	0.817	0.107	4.60 0.250
Triiodothyronine, nM/L	2.37 0.24	1.73 0.20	1.89 0.20	0.001	0.973	0.08	0.920	0.117	2.20 0.227
HDLP cholesterol, mM/L	1.63 0.18	1.09 0.09	1.33 0.12	0.001	0.954	0.15	0.868	0.322	1.34 0.300
Dobiásová's and Frohlich's atherogenic index	0.69 0.13	1.42 0.22	1.04 0.17	0.001	0.927	0.24	0.796	0.056	0.93 0.461
Urea excretion, mM/24h	612 74	608 76	468 62	0.001	0.977	0.07	0.933	0.061	458 0.186
Phosphates excretion, mM/24h	18.7 2.1	21.1 2.2	16.4 1.8	0.001	0.781	0.84	0.476	0.028	25.2 0.294

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. 5).

Table 5 presents the full structural coefficients, that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows what is the proportion of information about the root contained in this variable. There are also average values (centroids) of roots and Z-scores of variables. We consider it expedient to include in the table also out-of-model variables in view of their recognizability.

The localization in the extreme left zone of the axis of the first root of patients in whom balneotherapy caused a decrease in fitness (Fig. 5, see also Figs. 3 and 4) reflects their minimum for the sample (cluster K) and reduced (cluster L) levels of 7 parameters, on the one hand, and hypercalciuria and the maximum for the sample levels of 3 parameters (cluster N) - on the other hand.

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

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Heart rate, beats/min	-16.88	6.037	-1.613	0.577
Calcium excretion, mM/24h	-4.099	0.284	-1.866	0.129
Magnesium serum, mM/L	0.534	-0.275	12.88	-6.627
PSD F3-β, %	6.244	0.576	0.591	0.055
PSD ULF band HRV, msec^2	16.08	-0.370	0.193	-0.004
PWC, W/kg	-23.57	2.865	-37.36	4.541
PSD F4 entropy	-0.552	-1.225	-3.696	-8.207
Calcitonin, ng/L	3.309	2.512	0.941	0.715
Magnesium excretion, mM/24h	-1.583	3.036	-0.964	1.850
Ps3/Ps1 ratio	1.657	0.450	22.12	6.007
Cortisol, nM/L	4.620	1.528	0.026	0.009
Ps2/Ps1 ratio	-3.820	1.152	-40.20	12.13
PSD T3 entropy	-0.307	-1.952	-2.868	-18.21
PSD T4-θ, %	3.259	0.708	0.517	0.112
Calcium serum, mM/L	-2.365	1.846	-1.894	1.478
HRV centralization index	-2.646	-2.017	-0.180	-0.137
PSD P3-α, %	1.629	-3.884	0.088	-0.211
PSD Fp2-α, %	-8.812	1.390	-0.665	0.105
PSD ULF band HRV, %	-11.64	1.380	-2.687	0.319
PSD F8-α, μV^2/Hz	5.455	-0.028	0.282	-0.002
PSD T6-α, μV^2/Hz	-2.218	1.040	-0.060	0.028
Mode HRV, msec	2.902	2.123	0.021	0.016
PSD T3-θ, %	-3.365	0.435	-0.794	0.103
Parathyroid hormone, pM/L	3.881	-1.140	5.476	-1.609
Autonomous reactivity index, units	-1.258	-0.335	-0.526	-0.140
		Constants	174.5	-76.13
		Eigenvalues	92.38	11.06
Cumulative Proportions			0.893	1

At the opposite pole of the axis of the first root, there are patients in whom balneotherapy caused an increase in fitness or was ineffective. It is interesting that the distances between the centroids of the first and second roots are almost the same (7.9 and 7.35 respectively). A significantly reduced initial level of PWC_{150} is accompanied by lower limit levels of a number of EEG parameters as well as Triiodothyronine and HDLP Cholesterol (cluster B) and responses of systolic BP to successive cuff occlusions (cluster C), normal, but minimal for the sample, levels of GPVR and Stange's test (cluster D) as well as HRV Centralization index, on the one hand, and normal, but maximal for the sample, levels of autonomic reactivity as well as a number of EEG, HRV, endocrine and metabolic parameters (clusters G, H, and I) - on the other hand.

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

Variables	Correlations Variables-Roots		Fitness - (9)	Fitness + (9)	Fitness 0 (16)	Cluster on Fig. 3
Root 1 (89.3%)	Root 1	Root 2	-14.3	0.1	8.0	
PSD P3-α relative	0.039	0.022	-0.57±0.21	0.17±0.52	0.25±0.23	K
PSD F8-α	0.044	0.019	-0.36±0.06	-0.03±0.04	0.04±0.14	K
PSD ULF band HRV	0.030	-0.080	-0.66±0.21	-0.77±0.13	-0.12±0.25	L
PSD ULF band HRV relative	0.035	-0.058	-0.73±0.16	-0.69±0.20	0.00±0.33	L
PSD F3-β relative	0,038	-0.038	-0.62±0.19	-0.52±0.21	0.15±0.29	L
Calcium serum	0.005	-0.014	-1.14±0.22	-0.96±0.47	-0.21±0.39	L
Magnesium serum	0.042	-0.058	-1.78±0.09	-1.62±0.21	-1.00±0.27	L
Calcium excretion	-0.054	0.007	1.66±0.85	-0.04±0.92	-1.17±0.49	M
Urea excretion			1.81±0.87	1.76±0.90	0.12±0.72	N
Magnesium excretion	-0.032	0.078	0.78±0.44	0.92±0.45	-0.35±0.45	N
Parathyroid hormone	-0.039	0.052	-0.02±0.30	-0.17±0.22	-0.73±0.22	N
Root 2 (10.7%)	Root 1	Root 2	-1.86	5.32	-1.93	
PWC	-0.031	-0.166	-0.44±0.29	-2.37±0.49	-1.24±0.29	A
PSD F4 entropy	-0.014	-0.063	-0.15±0.27	-1.32±0.52	-0.53±0.40	B
PSD C4 entropy			0.39±0.24	-1.55±0.71	-0.22±0.41	B
PSD P3 entropy			0.55±0.25	-0.98±0.15	-0.08±0.28	B
PSD T3 entropy	-0.024	-0.067	0.21±0.28	-0.86±0.38	-0.32±0.28	B
PSD T4 entropy			0.24±0.20	-0.96±0.37	-0.26±0.32	B
Triiodothyronine			0.34±0.48	-0.94±0.39	-0.63±0.40	B
HDLP cholesterol			0.78±0.49	-0.63±0.19	0.01±0.29	B
PSD T4-θ relative	-0.010	-0.046	0.26±0.71	-0.64±0.25	-0.03±0.29	B
PSD C4-β relative			0.25±0.29	-0.68±0.39	-0.04±0.24	B
PSD T3-θ relative	-0.034	-0.058	0.36±0.40	-0.65±0.19	-0.32±0.22	B
PSD C3 entropy			0.39±0.19	-0.64±0.34	-0.36±0.43	B
PSD P3-θ relative			0.26±0.33	-0.50±0.14	-0.20±0.24	B
PSD P3-β relative			0.31±0.30	-0.64±0.22	-0.07±0.23	B
Ps3/Ps1 ratio	0.018	-0.118	-0.16±0.26	-0.69±0.22	0.20±0.27	C
Ps2/Ps1 ratio	0.017	-0.113	-0.17±0.31	-0.84±0.38	0.27±0.33	C
PSD Fp2-δ relative			0.17±0.26	-0.52±0.21	0.47±0.35	C
General peripheral vessels resistance			1.13±0.34	0.31±0.20	0.53±0.22	D
Stange's test			1.40±0.60	0.06±0.49	0.62±0.35	D
(VLF+LF)/HF as centralization index	0.020	-0.073	1.55±1.03	0.61±0.37	2.89±1.14	E
Autonomous reactivity index	0.041	0.099	-2.70±0.91	-0.02±0.21	-0.82±0.54	F
PSD Fp2-α relative	-0.004	0.101	-0.30±0.32	0.69±0.35	-0.41±0.23	G
PSD T6 entropy			-0.08±0.40	0.38±0.23	-0.65±0.44	G
Phosphates excretion			-0.88±0.28	-0.56±0.30	-1.19±0.24	G
Calcitonin	0.025	0.082	-1.28±0.10	-0.80±0.19	-1.00±0.14	H
PSD T6-α	0.034	0.084	-0.48±0.06	-0.09±0.15	-0.29±0.06	H
Cortisol	0.031	0.072	-0.70±0.30	0.83±0.74	0.31±0.36	I
1/Mo HRV as catecholamines	0.040	0.094	-0.74±0.50	0.87±0.44	0.36±0.32	I
PSD P4-α			-0.38±0.11	0.46±0.35	-0.12±0.12	I
Amplitude-α			-0.37±0.19	0.53±0.40	-0.03±0.18	I
PSD P3-α			-0.37±0.16	0.44±0.39	-0.02±0.16	I
PSD Fp1-α			-0.28±0.18	0.53±0.39	0.05±0.23	I
Kerdö's vegetative index			-0.69±0.36	0.65±0.49	-0.25±0.31	I
Baevskiy's ARS index standing up			-0.65±0.56	1.16±0.59	0.30±0.38	I
Dobiásová's and Frohlich's AG index			-0.57±0.30	1.15±0.52	0.27±0.39	I
Heart rate	0.030	0.170	-0.39±0.29	1.63±0.50	0.39±0.31	I

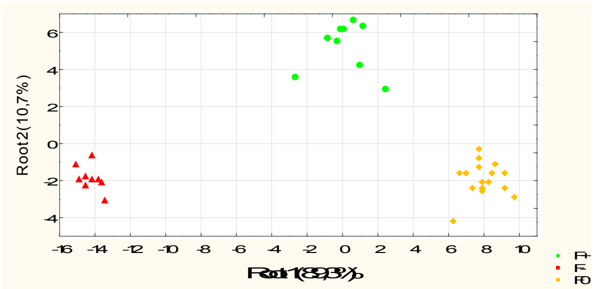


Fig. 5. Scattering of individual values of the first and second discriminant roots of initial variables of patients with various changes in Fitness after balneotherapy

The demarcation of clusters in the information space of the discriminant roots is apparent very clear and documented by the calculation of Mahalanobis distances (Table 6).

Table 6. Squared Mahalanobis distances between groups (above the diagonal) and F-criteria (df=25,7) with p-levels (below the diagonal)

Groups	Fitness + (9)	Fitness - (9)	Fitness 0 (16)
Fitness + (9)	***	259	114
Fitness - (9)	10.5; p=0.002	***	497
Fitness 0 (16)	5.9; p=0.011	25.8; $p<10^{-4}$	***

Table 7. Coefficients and constants of classification functions

Variables	F+ p=0.265	F- p=0.265	F0 p=0.471
Heart rate, beats/min	984.4	1003.6	967.6
Calcium excretion, mM/24h	144.7	170.7	129.1
Magnesium serum, mM/L	414.2	275.9	563.4
PSD F3-β, %	-62.61	-71.53	-58.35
PSD ULF band HRV, $msec^2$	-28.73	-31.49	-27.18
PWC, W/kg	17441	17948	17114
PSD F4 entropy	-1348	-1235	-1317
Calcitonin, ng/L	-138.9	-157.6	-136.7
Magnesium excretion, mM/24h	375.7	376.3	354.7
Ps3/Ps1 ratio	31282	-4917	44359
Cortisol, nM/L	-2.926	-3.364	-2.783
Ps2/Ps1 ratio	2116	2609	1712
PSD T3 entropy	1976	2147	2085
PSD T4-θ, %	-97.32	-105.6	-94.07
Calcium serum, mM/L	204.9	221.7	179.3
HRV centralization index	33.68	37.26	33.25
PSD P3-α, %	-48.59	-48.36	-46.37
PSD Fp2-α, %	112.3	121.2	106.3
PSD ULF band HRV, %	436.9	473.3	413.4
PSD F8-α, $\mu V^2/Hz$	-32.45	-36.51	-30.21
PSD T6-α, $\mu V^2/Hz$	9.666	10.32	8.995
Mode HRV, msec	-0.507	-0.927	-0.451
PSD T3-θ, %	146.2	156.9	139.2
Parathyroid hormone, pM/L	-283.7	-351.2	-229.0
Autonomous reactivity index, unit	-22.31	-13.73	-25.43
Constants	-54789	-56850	-52885

The ultimate goal of discriminant analysis is to predict the direction of changes in the PWC of persons under the influence of balneotherapy based on the constellation of their initial parameters. This purpose is realized with the help of classifying functions (Table 7). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant. The selected predictors provide predictions without any error.

Another approach for predicting balneotherapy-induced changes in PWC is multiple linear regression analysis. Screening of correlations between changes in PWC and registered initial body parameters (without EEG) revealed a noteworthy constellation (Table 8, Figs. 6 and 7).

Table 8. Correlations between changes in PWC and initial body parameters

Variables	r
Heart rate	0.625
PWC	-0.609
1/Mode HRV as catecholamines	0.495
Baevskiy's activity of RS Ind standing up	0.486
General peripheral vessels resistance	-0.439
Autonomous reactivity index	0.423
Cardiac output	0.381
Uricemia	0.353
HDLP Cholesterol	-0.288
Stange's test	-0.267
Phosphatemia	-0.258
Calcitonin	0.245

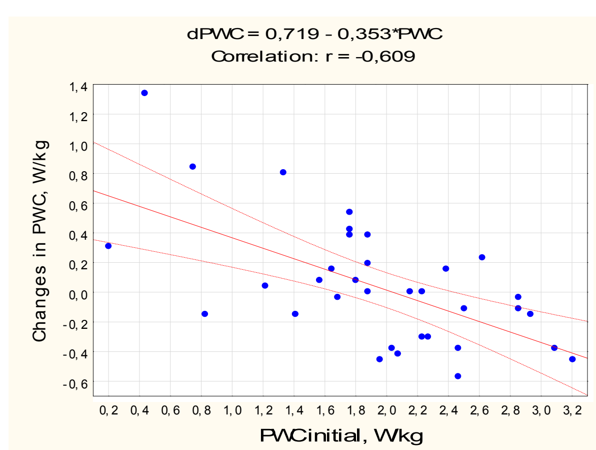


Fig. 6. Scatterplot of correlation between initial PWC level (X-line) and its changes caused by balneotherapy (Y-line)

It is interesting that after stepwise exclusion until reaching the maximum Adjusted R^2 level, only Uricemia and Phosphatemia, despite their small correlation coefficients, remained in the regression model together with the variables with the maximum coefficients, while the variables with higher coefficients were left out of the model (Table 9).

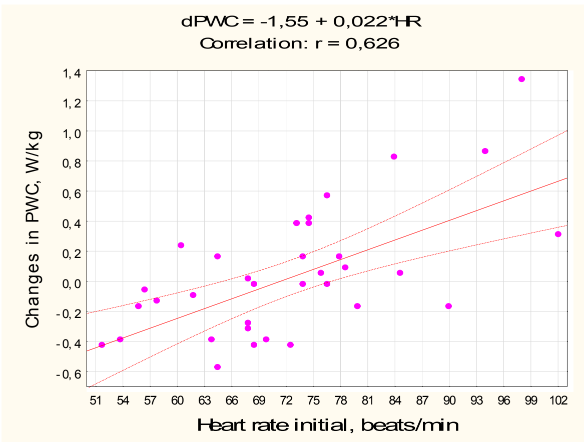


Fig. 7. Scatterplot of correlation between initial heart rate (X-line) and changes in PWC caused by balneotherapy (Y-line)

Table 9. Regression summary for change in PWC against hemodynamic and metabolic predictors R=0.720; R^2=0.518; Adjusted R^2=0.452; $F_{(4.3)}$=7.8; p=0.0002; SE of estimate: 0.31 W/kg

n=34		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(29)}$	p
Variables	r		Intercpt	-12.84	7.02	-1.83	0.078
Heart rate, beats/min	0.625	3.126	1.918	1.810	1.110	1.63	0.114
PWC, W/kg	-0.609	-0.575	0.031	-1.485	0.080	-18.6	10^{-6}
Uricemia, mM/L	0.353	-0.146	0.131	-0.320	0.287	-1.12	0.274
Phosphatemia, mM/L	-0.258	3.686	1.919	0.128	0.067	1.92	0.065

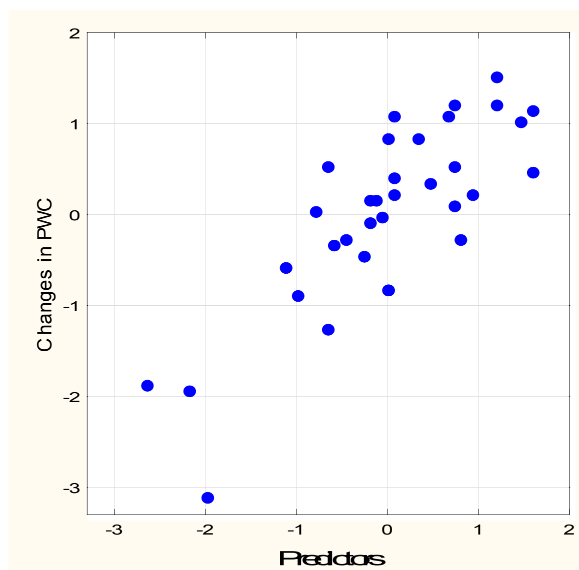
Table 10. Correlations between changes in PWC and initial EEG parameters

Variables	r
PSD T6-α	0.398
PSD 02-α	0.393
PSD P4-α	0,391
PSD P3 entropy	-0.375
Amplitude-α	0.359
PSD T4-α	0.341
PSD Fp2-α	0.311
PSD P3-α	0.310
PSD C4-α	0.305
PSD P4-α relative	0.299
PSD T4 entropy	-0.296
PSD T3-θ relative	-0.287
PSD P3-β relative	-0.282
PSD 01-α relative	0.281
Frequency-θ	-0.280
PSD T3 entropy	-0.278
PSD Fp1-β	0.282
PSD Fp1-α	0.256
PSD T6-α relative	0.245

After inclusion in the multiple linear regression analysis of EEG parameters (Table 10), the number of predictors increased to 8, Adjusted R^2 increased from 0.452 to 0.555, and SE of estimate decreased from 0.31 to 0.28 W/kg (Table 11 and Fig. 8).

Table 11. Regression summary for change in PWC against hemodynamics and EEGs predictors
R=0.814; R^2=0.663; Adjusted R^2=0.555; $F_{(8.3)}$=6.1; p=0.0002; SE of estimate: 0.28 W/kg

n=34		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(25)}$	p
Variables	r		Intercpt	-13,03	7,27	-1,83	0.085
Heart Rate, beats/min	0.625	4.542	2.026	0.158	0.070	2.24	0.034
PWC, W/kg	-0.609	3.851	1.997	2.230	1.156	1.93	0.065
PSD 02-α, μV²/Hz	0.393	0.888	0.368	0.002	0.001	2.41	0.024
PSD P3 Entropy	-0.375	-0.733	0.212	-2.440	0.704	-3.46	0.002
Amplitude-α, μV	0.359	-1.007	0.582	-0.054	0.031	-1.73	0.096
PSD T4-α, μV²/Hz	0.341	0.984	0.389	0.007	0.003	2.53	0.018
PSD P3-α, μV²/Hz	0.310	-0.862	0.485	-0.001	0.001	-1.78	0.088
PSD T6-α, %	0.245	-0.665	0.225	-0.018	0.006	-2.96	0.007



R=0.814; R^2=0.663; $\chi^2_{(8)}$=30.4; p=0.0002; Λ Prime=0.337
Fig. 8. Scatterplot of canonical correlation between constellation of initial parameters-predictors (X-line) and changes in PWC caused by balneotherapy (Y-line)

Discussion

Ergometric PWC testing has a long tradition in occupational medicine for assessing whether a sufficiently high level of physical performance for coping with the daily work requirements is given.[43] In the case of submaximal PWC testing measuring the mechanical power, the achieved power at a given heart rate serves as performance indicator.[34] There are age- and sex-specific norm values[44] that can be used to judge whether differences or changes are within the normal range or can be considered significant. The PWC of the sample of men observed by us was in a wide range, both before and after balneotherapy (M±SD: -1.33±1.35 Z and -1.28±1.07 Z respectively), which we interpret as one of the symptoms of maladaptation. Other manifestations of maladaptation will be discussed in detail in the next article. Judging by the average values, it would be possible to conclude that balneotherapy has no effect on the PWC level. However, analysis of individual changes in PWC revealed that this was true for only half of the patients, with one quarter showing a significant increase in PWC and another quarter showing a significant decrease in PWC. It is important that all three variants of PWC changes are not random, but can be accurately predicted based on the constellation of the initial parameters of the body, that is, they are completely natural. 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.[45-47] So what such polyvariance of actotropic effects of balneofactors of the Truskavets Spa, as well as its predictability, did not come as a surprise, but only confirmed the results of previous studies.

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, i.e. a reduction in swimming time from 61±7 min to 39±3 min. Instead, in 6 animals, the performance increased from 13±1.4 min to 52.3±5.9 min, and in 8 animals, it 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 years with maladaptation, in those who received Naftussya water together with ozokerite applications and mineral baths, three variants of actotropic effects were also revealed. In particular, PWC_{170}, assessed by the step test, decreased only in 31.5% of children with a PWC_{170} level significantly higher than the sex-age norm: from 125.8±3.5% to 119.6±3% (direct difference: -6.2±1.1%). On the other hand, the normal level of PWC_{170} 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, PWC_{150}, 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.[9,10] The main predictor of the directionality of the actotropic effect of balneofactors on PWC turned out to be precisely its initial level, according to the law of the same name.[37,38] However, even after supplementing PWC with a constellation of initial metabolic and hemodynamic parameters, the accuracy of the forecast was only 75.6%; 76.7%; 88.9% for adults, children and rats respectively, which confirmed the limited "jurisdiction" of the law of the initial level.[10,39,40] And only the additional inclusion in the discriminant model of the constellation of neuro-endocrine parameters gave us the opportunity not only to unmistakable predict the direction of the actotropic effect of balneotherapy, but also to calculate its severity with a small error.

It is time to find out how the characteristics of the initial state of the organism determine the nature and even the severity of the reaction of its systems to the same factor(s).

This situation has been known for a long time. Back in 1980, Hildebrandt showed that a 4-week endurance training program, 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.[48] In relation to our study, it is important to note that different responses to therapeutic stimuli depended on the circadian phase were accompanied by different states of autonomic reactivity, also subject to circadian rhythms. Autonomic reactivity in the cited study was assessed, in accordance with the then methodological support, by sweating as well as vasodilator and vasoconstrictor reactions to warm and cold stimuli. In our study, autonomic reactivity was assessed by changes in HRV parameters when moving from a lying position to a standing position; an additional marker was the blood pressure response to cuff occlusion.[20] We have shown that, in addition to autonomic reactivity, the constellation of EEG parameters, that is, components of the central autonomic network, between which there are two-way connections, are predictors of one or another variant of the PWC_{150} reaction to balneofactors.[49-55] Another set of predictors was made cortisol, calcitonin, and PTH, which are components of the neuro-endocrine-immune complex.[6,22,56] Finally, the presence in the constellation of predictors of electrolytes subject to the regulatory influence of calcitonin and PTH is quite natural.

This situation is consistent with the concept of the functional-metabolic continuum as well as the statement that physical working capacity is a complex and integrated psychophysical parameter of the body, which reflects its functional state provided by autonomous, substrate and energy resources, neural and hormonal regulation, psychological properties, and motivation.[57] From this perspective, the functional state is considered as a general parameter determined by the integration rate of activity of different physiological systems during life-related activity: the higher the level of integration, the higher the functional capacities of the body.[58,59]

The effects of balneotherapy on neuroendocrine and metabolic parameters which form PWC, are realized by organic substances present in both Naftussya bioactive water and ozokerite, through aryl hydrocarbon receptors, which are expressed by neurons, endocrinocytes, and immunocytes.[22,60-65]

Hypothesis verification

Main hypothesis. The effects of balneotherapy on PWC150 can be predicted based on initial neuro-endocrine and metabolic parameters. Verification. Hypothesis confirmed. Rejected the null hypothesis (H0: no predictability) in favor of the alternative hypothesis (H1: predictability exists). Justification. The discriminant model based on 25 initial parameters predicted the direction of PWC150 changes with 100% accuracy ($p<0.001$).

Specific hypothesis 1. There is a significant correlation between initial HRV parameters and changes in PWC150. Verification. Hypothesis partially confirmed. Rejected H0 (no correlation) for some HRV parameters. Justification. Significant correlations were found for select HRV parameters (e.g., SDNN: $r=0.45$, $p<0.01$), but not for all indices studied.

Specific hypothesis 2. EEG parameters are significant predictors of PWC150 changes. Verification. Hypothesis confirmed. Rejected H0 (no predictive significance) in favor of H1 (significant predictive value). Justification. Several EEG parameters were included in the final predictive model ($p<0.05$ for each).

Specific hypothesis 3. Levels of adaptation hormones correlate with PWC150 changes. Verification. Hypothesis partially confirmed. Rejected H0 for cortisol and calcitonin, failed to reject for other hormones. Justification. Significant correlations were found for cortisol ($r=0.38$, $p<0.05$) and calcitonin ($r=-0.42$, $p<0.05$), but not for testosterone or aldosterone.

Specific hypothesis 4. There is a linear relationship between initial PWC150 level and its change after balneotherapy. Verification. Hypothesis confirmed. Rejected H0 (no linear relationship) in favor of H1 (linear relationship exists). Justification. Regression analysis showed a significant linear relationship ($\beta = -0.575$, $p<0.001$).

Specific hypothesis 5. Metabolic parameters are significant predictors of PWC150 changes. Verification. Hypothesis partially confirmed. Rejected H0 for some metabolic parameters, failed to reject for others. Justification. Some metabolic parameters (e.g., serum calcium level) were included in the predictive model ($p<0.05$), while others (e.g., cholesterol level) did not show significant predictive value.

Our study demonstrates that the effects of balneotherapy on physical working capacity are highly individualized and can be predicted with remarkable accuracy using a constellation of neuro-endocrine and metabolic parameters. We identified a set of 25 initial parameters that could predict the direction of PWC150 changes with 100% accuracy, and developed a regression model that could quantitatively predict these changes with a standard error of 0.28 W/kg.

Interpretation of results in the context of existing literature

Our findings extend previous work by Hildebrandt, who demonstrated the importance of individual differences in response to balneotherapy.[48] While Hildebrandt focused mainly on the influence of time of day, our study identifies specific physiological parameters that determine these individual differences. This opens new possibilities for personalization of balneotherapy protocols.

Potential mechanisms

We propose that the observed effects may be mediated by the interaction of organic components in Naftussya bioactive water with aryl hydrocarbon receptors (AhR) in neurons, endocrinocytes, and immunocytes. AhR activation may initiate a cascade of reactions leading to modulation of the autonomic nervous system and changes in physical capacity.

Clinical implications

Our ability to predict individual responses to balneotherapy with high accuracy paves the way for personalized treatment protocols. For example, patients predicted to experience a decrease in PWC150 could receive a modified balneotherapy protocol or additional supportive interventions to prevent adverse effects.

Study limitations and strengths

The main strength of our study is its comprehensive approach, considering a wide range of physiological parameters. However, we acknowledge that our relatively small sample size (n=34) may limit the generalizability of our results. Additionally, our study was conducted at a single center, which may affect its external validity.

Future research directions

Future studies should focus on validating our predictive model in larger, multi-center samples. Additionally, investigating the long-term effects of personalized balneotherapy based on our predictive model would be a valuable contribution to the field of spa medicine.

Conclusion

Not only the directionality, but even the severity of PWC_{150} changes under the influence of the balneofactors of the Truskavets Spa is determined by the constellation of the initial parameters of the body, which forms its reactivity.

Our study demonstrates that the effects of balneotherapy on physical working capacity are highly individualized and can be predicted with remarkable accuracy using a constellation of neuro-endocrine and metabolic parameters. This finding represents a significant step towards personalized balneotherapy, potentially improving treatment outcomes and resource allocation in spa medicine. While further research is needed to validate these findings in larger and more diverse populations, our results provide a strong foundation for future studies and suggest exciting possibilities for enhancing the precision and efficacy of balneotherapy treatments.

Based on the statistical analysis and hypothesis testing presented in the study, here are detailed conclusions according to statistical inference methods.

1. The main hypothesis that the effects of balneotherapy on PWC150 can be predicted based on initial neuro-endocrine and metabolic parameters was strongly supported. The discriminant model based on 25 initial parameters predicted the direction of PWC150 changes with 100% accuracy ($p<0.001$). This allows us to reject the null hypothesis of no predictability in favor of the alternative hypothesis that predictability exists.

2. The relationship between initial HRV parameters and changes in PWC150 was partially confirmed. Significant correlations were found for some HRV parameters (e.g., SDNN: $r=0.45$, $p<0.01$), but not for all indices studied. This suggests that certain aspects of heart rate variability are predictive of balneotherapy effects, while others are not.

3. EEG parameters were found to be significant predictors of PWC150 changes, confirming the hypothesis. Several EEG parameters were included in the final predictive model with statistical significance ($p<0.05$ for each). This indicates that brain activity patterns prior to treatment are informative for predicting balneotherapy outcomes.

4. The hypothesis regarding adaptation hormones was partially supported. Significant correlations were found for cortisol ($r=0.38$, $p<0.05$) and calcitonin ($r=-0.42$, $p<0.05$), but not for testosterone or aldosterone. This suggests that some, but not all, adaptation hormones are relevant to predicting balneotherapy effects.

5. A significant linear relationship between initial PWC150 level and its change after balneotherapy was confirmed ($\beta=-0.575$, $p<0.001$). This supports the "law of initial values" in the context of balneotherapy, indicating that baseline fitness levels are important predictors of treatment response.

6. The hypothesis that metabolic parameters are significant predictors of PWC150 changes was partially confirmed. Some metabolic parameters (e.g., serum calcium level) were included in the predictive model ($p<0.05$), while others (e.g., cholesterol level) did not show significant predictive value. This suggests that certain aspects of metabolism are more relevant than others in predicting balneotherapy outcomes.

7. The multiple linear regression model incorporating both hemodynamic and EEG parameters achieved an adjusted R^2 of 0.555, indicating that approximately 55.5% of the variance in PWC_{150} changes can be explained by the predictors in the model. This suggests a moderately strong predictive power, though it also indicates that there are other factors not captured in the model that influence the outcome.

8. The canonical correlation analysis revealed a strong relationship between the constellation of initial parameters and changes in PWC ($R=0.814$, $p = 0.0002$). This multivariate approach supports the overall conclusion that a combination of physiological parameters can effectively predict balneotherapy outcomes.

The statistical analyses provide strong evidence for the predictability of balneotherapy effects on physical working capacity based on a constellation of initial physiological parameters. The findings support a personalized approach to balneotherapy, where treatment outcomes can be forecasted with high accuracy using pre-treatment assessments of neuro-endocrine, metabolic, and electrophysiological parameters. The partial confirmation of some hypotheses suggests that the relationships are complex and that further research may be needed to fully understand all the factors influencing balneotherapy outcomes.

Declarations

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Author contributions

Conceptualization, I.P.; Methodology, I.P.; Software, I.P. and W.Z.; Validation, I.P. and W.Z.; Formal Analysis, I.P. and W.Z.; Investigation, OM, VF, HK, IB, OV, IK, OL, TS, MK, OM, DP; Resources, OM, VF, HK, IB, OV, IK, OL, TS, MK, OM, DP; Data Curation, I.P. and W.Z.; Writing – Original Draft Preparation, I.P. and W.Z.; Writing – Review & Editing, I.P. and W.Z.; Visualization, I.P. and W.Z.; Supervision, I.P. and W.Z.; Project Administration, I.P. and W.Z.; Funding Acquisition.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

The study protocol was approved by the Ethical Committee of Ukrainian Scientific Research Institute of Medicine of Transport (protocol No. 35; 05.10.2022).

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