Thyrotropic effects of balneotherapy in Truskavets' spa and their endocrine, vegetative and metabolic accompaniments

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Abstract

Background. Previous studies of the Truskavetsian Scientific School of Balneology have studied the effects of course use of bioactive water Naftussya - the main therapeutic factor of the spa - on plasma levels of thyroid hormones in males and females healthy rats and women with thyroid hyperplasia. In men with chronic cholecystitis, the immediate thyrotropic effects of a single use of Naftussya water have been studied. The aim of this study is thyrotropic effects of balneotherapy in Truskavets' spa and their endocrine, vegetative and metabolic accompaniments in men and women with chronic cholecystitis and pyelonephritis. **Materials and Methods**. The object of clinical-physiological observation were 34 men (23-70 years) and 10 women (39-76 years), who underwent rehabilitation treatment of chronic cholecystitis and pyelonephritis in remission in the Truskavets' spa. The examination was performed twice, before and after a 7-10-day course of balneotherapy. The main object of study was the plasma level of Triiodothyronine. We determined also content in plasma Cortisol, Aldosterone, Testosterone and Calcitonin as well as the parameters of the HRV and metabolism. **Results**. We find in 16 patients (12 men and 4 women) inhibitory thyrotropic effect, in 22 (16 men and 6 women) neutral, and in 6 men only – enhancing effect of balneotherapy. Each variant of the

thyrotropic effect is accompanied by characteristic changes in testosteronemia, calcitoninemia, parathyroid activity, Heart Rate, Baevskiy's Activity Regulatory Systems Index, HRV-markers of vagal tone, Calciemia as well as VLD and LD lipoproteins Cholesterol plasma. **Conclusion**. Our data confirmed and supplemented the provisions on the multivariate effects of balneotherapy in the spa of Truskavets' on the endocrine and autonomic nervous systems and metabolism of patients.

INTRODUCTION

Previous investigations of the Truskavetsian Scientific School of Balneology have studied the effects of course use of bioactive water Naftussya - the main therapeutic factor of the spa - on plasma levels of thyroid hormones in males [12-15,21] and females [17,21,31] healthy rats and women with thyroid hyperplasia [4,6,16,19,21,27,28,30]. In men with chronic cholecystitis, the immediate thyrotropic effects of a single use of Naftusya water have been studied [18,20,21]. The aim of this study is thyrotropic effects of balneotherapy in Truskavets' spa and their endocrine, vegetative and metabolic accompaniments in men and women with chronic cholecystitis and pyelonephritis.

MATERIALS AND METHODS

The object of clinical-physiological observation were 34 men (23-70 ys) and 10 women (39-76 ys), who underwent rehabilitation treatment in the Truskavets' spa of chronic cholecystitis and pyelonephritis in remission with of neuroendocrine-immune complex dysfunction. The examination was performed twice, before and after a 7-10-day course of balneotherapy.

The main object of study was the plasma levels of Triiodothyronine. We determined also content in plasma Cortisol, Aldosterone, Testosterone and Calcitonin (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co., Ltd and DRG International Inc.).

In basal conditions we estimated the state of the autonomous regulation by the method heart rate variability (HRV) [1,3,10,29], using a hardware-programmatic complex "CardioLab+HRV" (KhAI Medica, Kharkiv, Ukraine). The following parameters were object to analysis. Frequency Domain Methods: HF ($0,4 \div 0,15$ Hz), LF ($0,15 \div 0,04$ Hz), VLF ($0,04 \div 0,015$ Hz), ULF ($0,015 \div 0,003$ Hz) bands. Time Domain Methods: HR, Triangular Index, SDNN, RMSSD, pNN_{50}. Calculated also Baevskiy's Activity Regulatory Systems Index (BARSI) [1].

The day before, daily urine was collected, in which was determined the concentration of calcium (by reaction with arsenase III) and phosphates (phosphate-molybdate method).

The same metabolic parameters were determined in plasma as well as magnesium (by reaction with colgamite), chloride (mercury-rhodanidine method), sodium and potassium (flamming photometry), creatinine (by Jaffe's color reaction by Popper's method), urea

(urease method by reaction with phenolhypochlorite), uric acid (uricase method), triglycerides (by a certain meta-periodate method), total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of HD lipoproteins (by the enzyme method after precipitation of VLD and LD lipoproteins); VLD lipoproteins (expected by the level of triglycerides); LD lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition HD and VLD lipoproteins).

The analysis carried out according to instructions [7] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents, as well as the flaming spectrophotometer "СФ-47".

According to the parameters of calcium and phosphates exchange, parathyroid activity was evaluated by coefficient $(Ca_P \bullet P_U / Ca_U \bullet P_P)^{0,25}$, based on its classical effects and recommendations by Popovych IL [9,23-26].

Normal (reference) values of variables are taken from the database of the Truskavetsian Scientific School of Balneology.

For statistical analysis used the software package "Statistica 64".

RESULTS AND DISCUSSION

Preliminary analysis of direct differences between plasma levels of triiodothyronine after and before balneotherapy revealed both negative (in the majority) and positive (in the minority) changes. This is quite expected, based on previous studies of the thyrotropic effects of balneotherapy. Unlike previous authors, we have expanded the range of insignificant (neutral) changes from $\pm 0,5\sigma$ to $\pm \sigma$. Accepting this gradation, we find in 16 patients (12 men and 4 women) inhibitory thyrotropic effect, in 22 (16 men and 6 women) neutral, and in 6 men only - enhancing.

To identify these parameters, changes in which are characteristic of polyvariant thyrotropic effects, a discriminant analysis [11] of registered indicators was conducted. The program forward stepwise included in the discriminant model, except triiodothyronine by definition, 9 variables: 3 **autonomous (HRV)**, 3 **endocrine** and 3 **metabolic** (Tables 1 and 3). Given the drastic gender differences in plasma levels testosterone (average 14,8 and 2,30 nM/L) and calcitonin (average 13,95 and 5,50 ng/L), they were expressed in Z-units for a correct estimate: $Z=(V/N-1)/Cv$ [8,25,31].

Table 1. Summary of the analysis of discriminant functions in relation to the change in neuro-endocrine and metabolic parameters

Step 10, N of vars in model: 10; Grouping: 3 grps; Wilks' Λ: 0,0644; approx. $F_{(23)}=8,3$; $p<10^{-6}$

Variables currently in the model	Groups of changes (Men/Women)			Parameters of Wilks' Statistics					
	Thyr + (6/0)	Thyr ± (16/6)	Thyr - (12/4)	Wilks' Λ	Partial Λ	F-remove (2,31)	p-level	Tolerancy	Norm Cv (30)
Triiodothyronine Plasma, nM/L	1,83 2,78 +0,96	1,79 1,75 -0,04	2,64 1,74 -0,90	0,342	0,188	66,8	10^{-6}	0,661	2,20 0,227
Baevskiy's Activity Regulatory Systems Index, units	3,47 3,63 +0,15	2,64 2,52 -0,13	4,22 2,39 -1,83	0,070	0,915	1,43	0,254	0,752	1,5 0,500
ULF band HRV Spectral Power, msec²	59 126 +67	68 132 +67	101 63 -37	0,074	0,866	2,40	0,107	0,561	122 0,892
Testosterone standardized by sex and age, Z	+0,62 -0,74 -1,37	+1,23 +0,15 -1,08	+0,36 -0,28 -0,64	0,074	0,876	2,20	0,127	0,257	0 0,400
VLD Cholesterol Plasma, mM/L	0,66 0,79 +0,13	0,62 0,49 -0,13	0,41 0,60 +0,18	0,085	0,759	4,93	0,014	0,558	0,54 0,612
Heart Rate, beats/min	74,1 77,6 +3,5	70,2 69,9 -0,4	65,8 69,7 +4,0	0,077	0,835	3,06	0,061	0,712	68,4 0,120
Parathyroid activity, units	1,72 1,77 +0,05	1,83 1,82 -0,01	1,81 1,83 +0,02	0,070	0,921	1,34	0,277	0,741	1,82 0,230
LD Cholesterol Plasma, mM/L	3,13 2,44 -0,68	3,57 3,77 +0,20	3,71 3,09 -0,61	0,067	0,963	0,59	0,561	0,666	3,48 0,192
Calcitonin standardized by sex, Z	-0,85 -0,96 -0,11	-0,97 -0,37 +0,60	-0,63 -0,60 +0,03	0,079	0,817	3,48	0,043	0,698	0 0,493
Calcium Plasma, mM/L	2,19 2,15 -0,04	2,21 2,23 +0,02	2,19 2,17 -0,02	0,074	0,874	2,24	0,123	0,504	2,30 0,065

Notes. In each column, the first line is the average initial, the second – final, the third – direct difference. In the "Norm" column, the first row is average, the second row is Cv. Only direct differences (effects) are the object of discriminant analysis.

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

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Triiodothyronine Plasma, nM/L	83,8	10^{-6}	0,196	83,9	10^{-6}
LD Cholesterol Plasma, mM/L	4,30	0,020	0,162	29,7	10^{-6}
VLD Cholesterol Plasma, mM/L	2,65	0,084	0,142	21,5	10^{-6}
Calcitonin standardized by sex, Z	2,82	0,072	0,124	17,5	10^{-6}
Heart Rate, beats/min	2,02	0,147	0,112	14,7	10^{-6}
Testosterone standardized by sex and age, Z	2,30	0,115	0,091	11,6	10^{-6}
Baevskiy's Activity RS Index, units	1,26	0,298	0,084	10,4	10^{-6}
Parathyroid activity, units	1,15	0,328	0,079	9,39	10^{-6}
ULF band HRV Spectral Power, msec^2	1,10	0,344	0,074	8,58	10^{-6}
Calcium Plasma, mM/L	2,24	0,123	0,064	8,28	10^{-6}

A number of variables, despite their recognizable properties, were outside the discriminant model (Tables 3 and 4).

Table 3. Neuroendocrine parameters currently not in the model

Variables	Groups of changes (Men/Women)			Parameters of Wilks' Statistics					
	Thyr + (6/0)	Thyr ± (16/6)	Thyr - (12/4)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv (30)
Triangular Index HRV, units	9,5 12,4 +2,9	10,1 11,2 +1,1	12,5 10,3 -2,2	0,064	0,989	0,17	0,841	0,532	11,2 0,217
SDNN HRV, msec	39 53 +14	45 50 +5	55 44 -10	0,064	0,990	0,14	0,866	0,465	56 0,516
RMSSD HRV, msec	17,8 28,3 +10,6	26,2 30,6 +4,3	34,5 27,7 -6,8	0,063	0,984	0,24	0,788	0,575	28,8 0,486
pNN$_{50}$ HRV, %	1,2 8,1 +6,9	8,8 9,6 +0,8	13,3 9,5 -3,9	0,063	0,983	0,27	0,768	0,563	9,0 0,820
Total Power HRV, msec^2	1570 3059 +1490	2324 2732 +408	3367 2286 -1081	0,064	0,991	0,14	0,874	0,448	2379 0,402
VLF HRV PS, msec^2	767 1297 +530	1067 1176 +110	1639 940 -700	0,064	0,992	0,12	0,891	0,696	1250 0,572
LF HRV PS, msec^2	633 1264 +631	771 856 +85	1235 837 -397	0,061	0,951	0,77	0,474	0,510	625 0,482
HF HRV PS, msec^2	111 373 +262	419 565 +146	560 446 -114	0,063	0,973	0,42	0,659	0,496	350 0,713
Aldosterone Plasma, pM/L	221 232 +11	227 233 +6	223 227 +4	0,062	0,963	0,58	0,567	0,814	238 0,187
Cortisol Plasma, nM/L	360 478 +117	371 425 +54	381 419 +38	0,064	0,992	0,11	0,892	0,759	370 0,303

Table 4. Metabolic parameters currently not in the model

Variables	Groups of changes (Men/Women)			Parameters of Wilks' Statistics					
	Thyr + (6/0)	Thyr ± (16/6)	Thyr - (12/4)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv (30)
Calcium Urine, mM/L	2,65 2,53 -0,12	2,15 2,74 +0,59	2,45 2,72 +0,26	0,064	0,991	0,14	0,872	0,298	3,13 0,214
Phosphates Urine, mM/L	10,1 11,5 +1,4	9,9 13,5 +3,6	12,3 13,3 +1,0	0,063	0,979	0,33	0,724	0,314	18,0 0,294
Phosphate Plasma, mM/L	1,03 0,96 -0,07	1,03 1,01 -0,02	1,07 0,99 -0,08	0,062	0,970	0,46	0,633	0,433	1,20 0,167
Magnesium Plasma, µM/L	824 806 -18	849 829 -20	824 837 +13	0,064	0,998	0,03	0,974	0,652	900 0,056
Potassium Plasma, mM/L	4,01 4,11 +0,10	4,28 4,48 +0,20	4,43 4,39 -0,04	0,063	0,971	0,45	0,642	0,731	4,55 0,104
Chloride Plasma, mM/L	97,9 99,9 +2,0	102,9 103,8 +0,9	98,8 103,9 +5,0	0,064	0,991	0,14	0,874	0,720	101,5 0,032
Sodium Plasma, mM/L	138,0 140,5 +2,5	144,4 145,5 +1,1	139,2 145,5 +6,3	0,064	0,992	0,14	0,870	0,722	145,0 0,034
Creatinine Plasma, µM/L	93,0 86,3 -6,7	92,1 83,4 -8,7	83,7 80,5 -3,2	0,064	0,987	0,19	0,824	0,792	77,5 0,172
Uric acid Plasma, µM/L	289 295 +6	355 345 -9	283 300 +18	0,064	0,992	0,12	0,886	0,668	366 0,181
Urea Plasma, mM/L	6,78 6,22 -0,49	6,12 5,95 -0,17	6,00 5,50 -0,50	0,062	0,956	0,69	0,511	0,730	5,00 0,330
Total Cholesterol Plasma, mM/L	4,95 4,40 -0,55	5,60 5,67 +0,07	5,58 5,10 -0,48	0,064	0,987	0,19	0,827	0,105	5,15 0,192
HD Cholesterol Plasma, mM/L	1,17 1,17 0,00	1,41 1,41 0,00	1,47 1,42 -0,05	0,064	0,987	0,19	0,827	0,619	1,30 0,300

The identifying information contained in the 10 discriminant variables is condensed into two roots. The major root contains 84,3% of discriminatory opportunities (r*=0,928; Wilks' Λ=0,064; $\chi^2_{(22)}$=99; p<10⁻⁶), while minor root – 15,7% only (r*=0,732; Wilks' Λ=0,464; $\chi^2_{(10)}$=28; p=0,002).

Calculating the values of discriminant roots for each patient as the sum of the products of non-standardized (raw) coefficients for individual values of discriminant variables together with the constant (Table 5) allows visualization of each patient in the information space of roots (Fig. 1).

Table 5. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Triiodothyronine Plasma, nM/L	-1,185	0,181	-3,794	0,581
LD Cholesterol Plasma, mM/L	0,092	-0,298	0,096	-0,311
VLD Cholesterol Plasma, mM/L	0,183	0,868	0,560	2,662
Calcitonin standardized by sex, Z	-0,165	-0,668	-0,156	-0,636
Heart Rate, beats/min	-0,277	0,555	-0,029	0,058
Testosterone standardized by sex and age, Z	0,019	0,951	0,019	0,954
Baevskiy's Activity RS Index, units	-0,165	-0,407	-0,070	-0,172
Parathyroid activity, units	0,249	-0,317	0,742	-0,948
ULF band HRV Spectral Power, msec^2	-0,525	-0,063	-0,0029	-0,0003
Calcium Plasma, mM/L	0,536	0,081	2,900	0,440
Constants			-0,936	-0,111
Eigenvalues			6,206	1,154
Cumulative Proportion			0,843	1

Table 6 collects the Z-scores of discriminant variables together with those that are not included in the model, but still reflect the specificity of effects. This approach makes it possible to describe in more detail the neuro-endocrine and metabolic accompaniments of thyrotropic effects of balneotherapy.
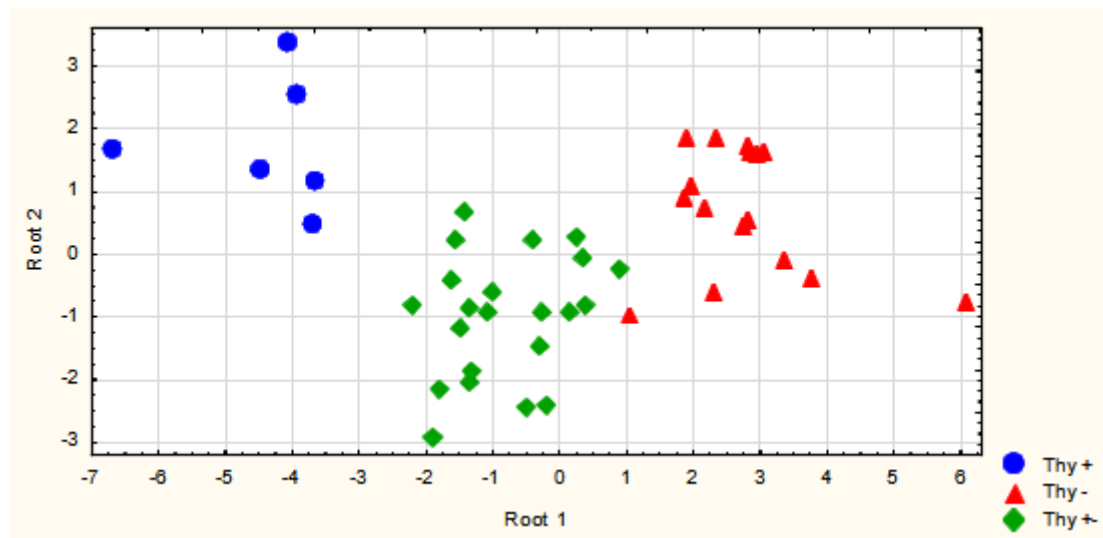
Table 6. Correlations between change in variables and roots, centroids of clusters and Z-scores of clusters

Variables	Correlations Variables-Roots		Thyr + (6)	Thyr ± (22)	Thyr - (16)
Root 1(84,3 %)	Root 1	Root 2	-4,42	-0,81	+2,77
Triiodothyronine Plasma	-0,811	0,086	+1,91	-0,07	-1,80
LF band HRV Spectral Power			+2,50	+0,39	-1,61
pNN$_{50}$ HRV			+1,14	+0,08	-0,59
HF band HRV Spectral Power			+1,57	+0,52	-0,54
RMSSD HRV			+0,96	+0,34	-0,56
Triangular Index HRV			+1,20	+0,47	-0,89
VLF band HRV Spectral Power			+0,84	+0,14	-0,98
SDNN HRV			+0,51	+0,18	-0,33
Baevskiy's Activity Regulatory Systems Ind	-0,137	-0,137	+0,21	-0,17	-2,44
ULF band HRV Spectral Power	-0,095	-0,126	+0,62	+0,62	-0,34
Testosterone standardized by sex and age	0,107	0,034	-1,37	-1,08	-0,64
Root 2(15,7 %)	Root 1	Root 2	+1,78	-0,98	+0,68
VLD Cholesterol Plasma	0,086	0,406	+0,37	-0,34	+0,53
Heart Rate	0,035	0,192	+0,42	-0,05	+0,49
Parathyroid activity	-0,005	0,037	+0,11	-0,01	+0,05
Creatinine Plasma			-0,50	-0,65	-0,24
Uric acid Plasma			+0,08	-0,13	+0,25
Chloride Plasma			+0,61	+0,27	+1,55
Sodium Plasma			+0,50	+0,22	+1,28
LD Cholesterol Plasma	-0,052	-0,403	-1,13	+0,31	-0,97
Calcitonin standardized by sex	-0,024	-0,274	-0,11	+0,60	+0,03
Calcium Urine			-0,18	+0,87	+0,39
Phosphates Urine			+0,26	+0,69	+0,18
Potassium Plasma			+0,22	+0,43	-0,08
Calcium Plasma	-0,004	-0,121	-0,24	+0,15	-0,11
Phosphates Plasma			-0,33	-0,08	-0,41
Urea Plasma			-0,30	-0,11	-0,30
Total Cholesterol Plasma			-0,58	+0,07	-0,47

The first root represents changes in plasma triiodothyronine levels in the inverse way, so the cluster of patients subject to inhibitory thyrotropic effect is located in the extreme right zone of the root axis. This direction of the thyrotropic effect is accompanied by a significant decrease in the index of activity of regulatory systems, apparently due to parasympathetic, markers of which were not included in the discriminant model, apparently due to duplication and/or redundancy of information. At the opposite pole of the axis are the members of the cluster of enhancing thyrotropic effect, which is accompanied by an increase in vagal tone. And intermediate position is occupied by patients with insignificant changes in both triiodothyronine levels and vagal tone. Instead, changes in testosterone levels correlate with the root directly, so that the enhancing thyrotropic effect is accompanied by a maximum reduction in testosterone, the neutral effect - an intermediate reduction, and the inhibitory effect - the minimum for sampling reduction.

As we can see, all three clusters are quite clearly delineated along the axis of the major root. However, the cluster of neutral thyrotropic effect of balneotherapy is further different from the other two along the minor root axis. The lowest localization of the cluster reflects the decrease or absence of significant changes in the parameters that are directly related to the root, while both inhibitory and enhancing thyrotropic effects are accompanied by increasing levels of these parameters. Conversely, inversely related root parameters in patients with a neutral thyrotropic effect increase as much as possible for the sample or do not change, whereas in the other two clusters they are significantly lower.

Interestingly, changes in calcitonin levels are accompanied by corresponding changes in urinary calcium and phosphate concentrations, but not in plasma.

Fig. 1. Scattering of individual values of the roots of patients with enhancing, neutral and inhibititory thyrotropic effects of balneotherapy

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 7).

Table 7. Squares of Mahalanobis distances between clusters (above the diagonal) as well as F-criteria (df=11,3) and p-levels (below the diagonal)

Clusters	Thyr +	Thyr -	Thyr ±
Thyr + (6)	0	53	21
Thyr - (16)	15,9 10⁻⁶	0	16
Thyr ± (22)	6,7 10⁻⁵	9,9 10⁻⁶	0

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 8).

Table 8. Coefficients and constants of classification functions

Clusters	Thyr + (6)	Thyr - (16)	Thyr ± (22)
Variables	p=,136	p=,364	p=,500
Triiodothyronine Plasma, nM/L	13,77	-14,17	-1,560
LD Cholesterol Plasma, mM/L	-1,328	-0,295	-0,125
VLD Cholesterol Plasma, mM/L	3,560	4,648	-1,753
Calcitonin standardized by sex, Z	-0,164	-0,587	1,023
Heart Rate, beats/min	0,216	-0,055	-0,047
Testosterone standardized by sex and age, Z	0,599	-0,320	-1,964
Baevskiy's Activity RS Index, units	-0,190	-0,501	0,031
Parathyroid activity, units	-4,823	1,564	0,476
ULF band HRV Spectral Power, msec²	0,013	-0,008	0,003
Calcium Plasma, mM/L	-8,495	11,88	0,782
Constants	-10,78	-9,09	-1,99

Retrospective identification of all clusters is very accurate: with only one error (Table 9).

Table 9. Classification matrix
Rows: observed classifications; columns: projected classifications

	Percent	Thyr +	Thyr -	Thyr ±
Clusters	Correct	p=,136	p=,364	p=,500
Thyr +	100	**6**	0	0
Thyr -	93,8	0	**15**	1
Thyr ±	100	0	0	**22**
Total	97,7	6	15	23

CONCLUSION

Our data confirm and supplement the previous data on the polyvariance of thyrotropic effects of Naftusya bioactive water in healthy rats and patients of Truskavets' resort of both sexes. Such polyvariance is a separate manifestation of the polyvariance effect of balneotherapy on other body systems [2,5,8,21,28,30,31]. We confirmed and supplemented data on characteristic changes in other endocrine, HRV and metabolic parameters that interact closely within the neuroendocrine-immune complex [8,9,21,22,24,26].

ACKNOWLEDGMENT

ACCORDANCE TO ETHICS STANDARDS

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

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