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Psycho-neuro-endocrine accompaniments of individual variants of nitrogenous metabolites exchange

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Abstract

Background. Earlier we showed, by constructing regression models, that plasma nitrogenous metabolites (uric acid, urea, creatinine and bilirubin) are able to influence the state of the trait anxiety, autonomic and central nervous and endocrine systems. In this study of the same cohort, other methodological approaches were used. **Materials and Methods**. The object of observation were almost healthy volunteers: 30 females (30÷76 y) and 31 males (24÷69 y). In basal conditions determined plasma levels of nitrogenous metabolites as well as cortisol, aldosterone, testosterone, triiodothyronine and calcitonin, estimated the severity of the trait and reactive anxiety, recorded the ongoing HRV and EEG. After 4 or 7 days, repeated testing was performed. **Results**. By the method of cluster analysis, four groups were created, the members of which differ minimally from each other in terms of the constellation of nitrogenous metabolites, but differ maximally from the members of other groups. Using the method of discriminant analysis, it was found that nitrogenous clusters differ from each other in terms of sex, age, trait anxiety, as well as 30 neuro-endocrine parameters. **Conclusion.** The obtained data confirm and supplement previous data on the physiological psycho-neuro-endocrine activity of plasma nitrogenous metabolites.

Keywords: plasma bilirubin, uric acid, urea, creatinine, cortisol, testosterone, aldosterone, triiodothyronine, calcitonin, ongoing EEG, HRV, anxiety, men, women, cluster and discriminant analyses.

INTRODUCTION

Earlier we showed, by constructing **regression models**, that plasma nitrogenous metabolites, even in the absence of uremia, are able to influence the state of the trait anxiety, autonomic and central nervous and endocrine systems at practically healthy volunteers with maladaptation [5,6,14,21]. In this study of the same cohort, other methodological approaches were used, namely **cluster and discriminant analyses**.

MATERIAL AND METHODS

The object of observation were employees of the clinical sanatorium "Moldova" and PrJSC "Truskavets' Spa": 30 females (30÷76; 49±13 y) and 31 males (24÷69; 47±12 y). The volunteers were considered practically healthy (without a clinical diagnosis), but the initial testing revealed deviations from the norm in a number of parameters of the neuro-endocrine-immune complex (details follow) as a manifestation of dys(mal)adaptation. Testing was performed twice with an interval of 4 ("Moldova") or 7 ("Truskavets' Spa") days.

We determined the plasma levels of the direct (conjugated) and free (unconjugated) Bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method), Uric acid (by uricase method), Urea (by urease method by reaction with phenol hypochlorite) and Creatinine (by Jaffe's color reaction by Popper's method) [7] as well as main adaptation hormones Cortisol, Aldosterone, Testosterone, Triiodothyronine and Calcitonin (by the ELISA with the use of corresponding sets of reagents from "Алкор Био", XEMA Co. Ltd, and DRG International Inc.). The levels of the trait and reactive anxiety estimated by STAI of Spielberger ChD [28] in modification of Khanin YL [20]. The state of the autonomic and central nervous systems was evaluated according to the parameters of heart rate variability [3,4,9,27] (software-hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv) and QEEG (hardware-software complex "NeuroCom Standard", KhAI MEDICA, Kharkiv). Paying tribute to tradition, the Kerdö's Vegetative Index [10] was calculated. In addition to routine parameters, HRV and EEG Entropy were calculated [8,22,26]. See the previous article for details [6].

Reference (R) values of QEEG parameters are taken from the database of the Truskavetsian Scientific School of Balneology (n=122), HRV parameters - from the instructions for "CardioLab+HRV", hormones - from the instructions for the kits, nitrogenous metabolites - from the handbook [11].

In order to make a correct comparison, the individual actual values of the Variables (V) were transformed into Z-scores according to the classical formulas [8,22]:

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

Results processed by using the software package "Statistica 64".

RESULTS AND DISCUSSION

Use of Cluster analysis makes possible the simultaneous consideration of several or even all the signs. Considering the totality of characteristics of persons undertaken in their relationship and conditionality of some of these (derivatives) other (main determinants) allows as to make a natural classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of the object is to identify those of its quality properties that actually define the object, distinguish it from other [1].

Clustering cohort of persons is realized by iterative k-means method. In this method, the object belongs to the class, Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points [1].

We have identified 4 clusters. Clusters appeared clearly delineated, as evidenced by Euclidean Distances between Clusters (Table 1).

Table 1. Euclidean Distances between Clusters

Cluster Number	Distances below diagonal Squared distances above diagonal			
	No. 1	No. 2	No. 3	No. 4
No. 1	0	530	790	3630
No. 2	23	0	2614	1397
No. 3	28	51	0	7799
No. 4	60	37	88	0

In the next stage carried Analysis of Variance and ranking variables for coefficient η^2:

$\eta^2=Sb^2/(Sb^2+Sw^2)$,

$R=\eta$,

$F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where

Sb^2 is Between Variance;

Sw^2 is Within Variance;

n is number of persons (122);

k is number of groups-clusters (4).

In our case, judging by coefficient η^2, uric acid makes the maximum contribution to the distribution into clusters. Instead, do not play a significant role in clustering Bilirubin direct (Table 2).

Table 2. Analysis of Variance

Variables	Between SS	Within SS	η^2	R	F	sig-nif. p
Uric acid	535750	77265	0,874	0,935	273	10^{-6}
Creatinine	2586	18174	0,125	0,353	5,60	0,001
Urea	5,51	107,0	0,049	0,221	2,03	0,114
Bilirubin Free	70,6	1373	0,049	0,221	2,02	0,114
Bilirubin Direct	2,532076	109,4238	0,023	0,150	0,91	0,438

Use of Discriminant analysis [12] makes it possible to identify exactly those parameters-variables by which clusters of nitrogenous metabolites differ significantly from each other, in other words psycho-neuro-endocrine accompaniment of clusters.

The forward stepwise program included 38 variables in the discriminant model. These are, in addition to nitrogenous metabolites by definition, age and gender of patients, 4 **adaptation hormones**, trait anxiety, 5 parameters of **delta** rhythm, 8 – **theta** rhythm, 7 – **alpha** rhythm, 5 – **beta** rhythm and only one **HRV** parameter (Tables 3 and 4).

Table 3. Summary of the analysis of discriminant functions
Step 38, N of vars in model: 38; Grouping: 4 grps; Wilks' Λ: 0,0143; appr. $F_{(114)}=6,7$; $p<10^{-6}$

Variables currently in the model	Clusters members (Females/Males) and Means				Parameters of Wilks' Statistics					
	III (16/ 2)	I (21/ 14)	II (8/ 29)	IV (15/ 17)	Wilks' Λ	Partial Λ	F-remove (3,81)	p-level	Tolerancy	Reference value Cv/σ
Uric acid, µM/L	173 -1,95	238 -1,28	284 -1,14	370 +0,29	0,100	0,143	161,2	10^{-6}	0,555	340 0,202
Creatinine, µM/L	77,2 +0,01	84,2 +0,26	90,5 +0,25	89,2 +0,33	0,016	0,917	2,44	0,070	0,401	83,3 0,176
Urea, mM/L	5,45 +0,11	5,75 +0,33	5,85 +0,34	5,32 -0,29	0,015	0,933	1,94	0,130	0,707	5,50 0,180
Bilirubin direct, µM/L	1,92 +0,26	2,12 +0,49	2,26 +0,65	1,90 +0,24	0,015	0,938	1,80	0,154	0,025	1,70 0,500
Bilirubin free, µM/L	9,0 -0,28	10,8 +0,21	11,2 +0,33	11,2 +0,35	0,015	0,958	1,18	0,324	0,299	10,0 0,355
Age, years	50,3 +0,17	45,9 -0,18	45,6 -0,20	52,7 +0,35	0,015	0,947	1,50	0,221	0,025	48,2 0,264
Sex index M=1;F=2	1,89 +0,80	1,60 +0,22	1,22 -0,56	1,47 -0,05	0,018	0,785	7,38	10^{-3}	0,312	1,49 0,333
Testosterone Female, nM/L	2,74 +0,33	3,23 +0,78	3,59 +1,09	4,42 +1,85	0,015	0,971	0,82	0,489	0,348	2,37 0,468
Testosterone Male, nM/L	8,55 -1,13	12,4 -0,20	12,2 -0,64	17,1 +1,42	0,015	0,971	0,82	0,489	0,348	15,1 0,269
Calcitonin Female, ng/L	11,15 +2,46	8,35 +1,33	7,77 +1,10	7,46 +0,98	0,016	0,886	3,46	0,020	0,595	5,05 0,490
Calcitonin Male, ng/L	12,14 -0,26	13,64 -0,05	9,41 -0,66	9,67 -0,62	0,016	0,886	3,46	0,020	0,595	13,95 0,493
Cortisol, nM/L	265 -0,94	304 -0,59	269 -0,90	353 -0,15	0,016	0,908	2,75	0,048	0,608	370 0,303
Triiodothyronine, nM/L	1,83 -0,75	2,46 +0,52	1,82 -0,77	2,18 -0,05	0,018	0,795	6,95	10^{-3}	0,475	2,20 0,227
Trait anxiety, points	47,2 +2,62	40,7 +0,77	38,9 +0,26	42,4 +1,25	0,017	0,852	4,71	0,004	0,543	38 0,092
Index δ, %	62 +0,37	54 +0,18	66 +0,47	53 +0,16	0,017	0,858	4,48	0,006	0,398	47 0,881
Deviation δ, Hz	0,64 -0,12	0,64 -0,10	0,73 +0,22	0,63 -0,17	0,016	0,919	2,37	0,077	0,659	0,67 0,395
F4-δ PSD, %	42,0 +0,55	43,1 +0,61	46,7 +0,79	45,7 +0,74	0,016	0,925	2,19	0,096	0,187	31,3 0,624
T3-δ PSD, %	38,0 +0,55	40,6 +0,71	44,5 +0,93	38,7 +0,59	0,015	0,966	0,94	0,423	0,269	28,6 0,596
O1-δ PSD, %	34,2 +0,70	31,8 +0,54	38,7 +1,00	30,8 +0,48	0,015	0,944	1,61	0,193	0,204	23,5 0,655
Index θ, %	19 +0,18	11 -0,09	10 -0,13	19 +0,17	0,016	0,894	3,19	0,028	0,632	14 2,171
Frequency θ, Hz	5,94 -0,32	6,31 -0,04	5,79 -0,45	5,84 -0,40	0,015	0,951	1,41	0,247	0,592	6,37 0,206
Deviation θ, %	1,17 +0,39	0,87 -0,14	1,04 +0,17	0,97 -0,08	0,016	0,916	2,49	0,066	0,622	0,95 0,578
Asymmetry θ, %	30,9 +0,17	23,5 -0,23	29,1 +0,07	39,6 +0,62	0,015	0,965	0,98	0,408	0,640	27,8 0,684

Fp1-θ PSD, %	8,6 -0,30	11,1 +0,12	8,9 -0,25	11,0 +0,09	0,016	0,901	2,95	0,038	0,343	10,4 0,588
Fp2-θ PSD, %	10,4 +0,08	9,4 -0,08	8,8 -0,18	9,9 +0,01	0,015	0,953	1,34	0,267	0,273	9,9 0,620
F3-θ PSD, %	10,0 -0,30	11,1 -0,10	10,3 -0,24	11,0 -0,12	0,015	0,935	1,88	0,139	0,315	11,7 0,496
F4-θ PSD, %	10,1 -0,17	10,7 -0,05	9,0 -0,34	10,6 -0,08	0,015	0,935	1,86	0,142	0,163	11,1 0,539
Amplitude α, μV	15,8 -0,14	17,4 0,00	18,1 +0,07	17,3 -0,01	0,015	0,956	1,25	0,297	0,152	17,4 0,614
Frequency α, Hz	10,36 -0,28	10,51 -0,11	10,46 -0,17	10,61 -0,01	0,015	0,953	1,33	0,269	0,589	10,62 0,088
Deviation α, Hz	1,19 +0,32	0,90 -0,22	1,03 +0,02	1,16 +0,25	0,016	0,885	3,51	0,019	0,587	1,02 0,527
Laterality α, %	-18 -0,48	-5 -0,11	+2 +0,10	-3 -0,05	0,016	0,895	3,16	0,029	0,189	-1 ***34***
Asymmetry α, %	20,4 +0,02	25,6 +0,48	20,6 +0,03	21,1 +0,08	0,016	0,888	3,39	0,022	0,645	20,2 0,559
T3-α PSD, %	26,0 -0,30	24,0 -0,44	22,4 -0,54	26,3 -0,28	0,016	0,888	3,39	0,022	0,191	30,4 0,483
P4-α PSD, %	34,7 -0,53	35,5 -0,48	39,6 -0,27	40,4 -0,23	0,015	0,950	1,43	0,241	0,142	44,8 0,428
Amplitude β, μV	14,4 +0,65	11,9 -0,05	12,1 +0,01	12,0 0,00	0,015	0,964	1,00	0,395	0,400	12,1 0,297
Deviation β, Hz	1,28 -0,12	1,27 -0,12	1,31 -0,07	1,53 +0,20	0,015	0,956	1,24	0,299	0,629	1,37 0,577
Laterality β, %	-14 -0,39	-5 -0,11	-3,3 -0,07	-2,7 -0,05	0,015	0,942	1,66	0,182	0,191	-1 ***34***
P3-β PSD, %	22,1 -0,05	20,0 -0,23	20,1 -0,22	19,4 -0,28	0,015	0,972	0,79	0,504	0,273	22,7 0,514
P4-β PSD, %	25,4 +0,22	20,3 -0,22	17,7 -0,45	18,8 -0,35	0,016	0,873	3,92	0,011	0,291	22,8 0,503
LF HRV PSD, %	39,0 +1,50	40,6 +1,34	38,9 +1,48	32,2 +0,80	0,016	0,890	3,33	0,024	0,492	26,5 0,334

Note. In each column, the first line is the average Mean, the second line is the average Z for variables while Cv or ***SD*** for reference values.

Table 4. Summary of stepwize analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Uric acid, µM/L	244	10^{-6}	0,139	244	10^{-6}
Sex index	6,53	10^{-3}	0,119	74,2	10^{-6}
Trait anxiety, points	4,27	0,007	0,107	47,3	10^{-6}
P4-β PSD, %	3,90	0,011	0,097	35,9	10^{-6}
Cortisol, nM/L	3,49	0,018	0,089	29,5	10^{-6}
Triiodothyronine, nM/L	3,15	0,028	0,082	25,3	10^{-6}
Creatinine, µM/L	4,01	0,009	0,074	22,6	10^{-6}
Deviation δ, Hz	3,08	0,030	0,068	20,4	10^{-6}
Deviation α, Hz	2,23	0,089	0,064	18,6	10^{-6}
Index θ, %	2,27	0,085	0,061	17,1	10^{-6}
Deviation θ, %	2,05	0,111	0,057	15,8	10^{-6}
Asymmetry α, %	1,89	0,136	0,055	14,8	10^{-6}
Frequency α, Hz	2,38	0,074	0,051	14,0	10^{-6}
LF HRV PSD, %	2,34	0,077	0,048	13,3	10^{-6}
Asymmetry θ, %	2,06	0,110	0,045	12,6	10^{-6}
P3-β PSD, %	1,89	0,136	0,043	12,1	10^{-6}
Bilirubin direct, µM/L	1,78	0,156	0,041	11,5	10^{-6}
F4-θ PSD, %	1,84	0,144	0,039	11,1	10^{-6}
Index δ, %	1,69	0,175	0,037	10,6	10^{-6}
Urea, mM/L	1,89	0,135	0,035	10,3	10^{-6}
Amplitude α, µV	1,61	0,192	0,033	9,93	10^{-6}
Laterality α, %	1,99	0,121	0,031	9,65	10^{-6}
Bilirubin free, µM/L	1,66	0,180	0,030	9,37	10^{-6}
F3-θ PSD, %	1,77	0,159	0,028	9,12	10^{-6}
Fp1-θ PSD, %	1,86	0,142	0,027	8,90	10^{-6}
Fp2-θ PSD, %	1,68	0,178	0,025	8,68	10^{-6}
Calcitonin, ng/L	2,04	0,113	0,024	8,52	10^{-6}
F4-δ PSD, %	1,40	0,248	0,023	8,30	10^{-6}
Frequency θ, Hz	1,72	0,168	0,021	8,13	10^{-6}
Testosterone, nM/L	1,28	0,286	0,020	7,93	10^{-6}
Age, years	1,20	0,315	0,020	7,72	10^{-6}
Deviation β, Hz	1,24	0,302	0,019	7,54	10^{-6}
T3-δ PSD, %	1,10	0,354	0,018	7,35	10^{-6}
T3-α PSD, %	1,31	0,276	0,017	7,20	10^{-6}
O1-δ PSD, %	1,83	0,148	0,016	7,11	10^{-6}
Laterality β, %	1,13	0,342	0,016	6,95	10^{-6}
P4-α PSD, %	1,31	0,275	0,015	6,82	10^{-6}
Amplitude β, µV	1,00	0,395	0,014	6,67	10^{-6}

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

Table 5. Variables not included in the model

Variables	Clusters members (Females/Males) and Means				Parameters of Wilks' Statistics					
	III (16/ 2)	I (21/ 14)	II (8/ 29)	IV (15/ 17)	Wil ks' Λ	Par- tial Λ	F to en- ter	p- level	Tole- rancy	Referen- ce value Cv/**σ**
Aldosterone, pM/L	223 -0,35	228 -0,21	226 -0,27	225 -0,29	0,008	0,976	0,5 8	0,630	0,497	238 0,187
F3-δ PSD, %	41,3 +0,74	40,4 +0,69	42,9 +0,83	39,2 +0,62	0,008	0,967	0,8 1	0,491	0,136	28,4 0,617
T6-δ PSD, %	42,3 +0,99	37,2 +0,68	39,6 +0,83	33,5 +0,46	0,008	0,987	0,3 3	0,806	0,174	26,1 0,626
P4-δ PSD, %	31,8 +0,56	34,3 +0,72	35,2 +0,78	31,4 +0,53	0,008	0,993	0,1 8	0,911	0,423	23,6 0,626
F8-θ PSD, %	8,9 -0,20	9,8 -0,01	8,7 -0,23	11,6 +0,37	0,008	0,993	0,1 7	0,915	0,308	9,8 0,492
T3-θ PSD, %	9,2 -0,22	10,0 -0,07	8,7 -0,34	9,9 -0,08	0,014	0,996	0,1 0	0,961	0,279	10,3 0,466
P3-θ PSD, %	8,7 -0,05	9,4 +0,09	7,8 -0,23	10,0 +0,19	0,008	0,985	0,3 6	0,782	0,105	9,0 0,552
P3-α PSD, %	31,9 -0,52	33,1 -0,46	38,2 -0,22	38,3 -0,21	0,008	0,973	0,6 7	0,571	0,056	42,7 0,487
T3-β PSD, %	26,8 -0,28	25,4 -0,37	24,3 -0,45	25,1 -0,39	0,008	0,989	0,2 6	0,851	0,090	30,7 0,462
C4-β PSD, %	28,6 +0,26	23,1 -0,26	20,7 -0,50	19,5 -0,61	0,008	0,998	0,0 6	0,982	0,172	25,9 0,405
Kerdoe Vegeta-tive Index, unit	-12 +0,45	-7 +0,64	-14 +0,36	-19 +0,29	0,014	0,967	0,9 1	0,439	0,371	-23,5 **20**
Mode HRV, msec	730 -1,26	746 -1,25	805 -0,59	825 -0,44	0,008	0,963	0,9 3	0,432	0,126	870 0,115
ULF HRV PSD, %	5,9 +0,06	6,0 +0,21	6,7 +0,73	5,8 +0,03	0,014	0,967	0,9 0	0,447	0,595	5,2 0,812
VLF HRV PSD, %	45,6 -0,66	42,2 -0,64	43,7 -0,61	51,5 -0,24	0,008	0,973	0,6 6	0,576	0,510	53,5 0,275

The identifying information contained in the 38 discriminant variables is condensed into three roots. The major root contains 82,5% of discriminatory opportunities (r*=0,962; Wilks' Λ=0,014; $\chi^2_{(114)}$=424; p<10^{-6}), second root – 10,4% (r*=0,779; Wilks' Λ=0,191; $\chi^2_{(74)}$=166; p<10^{-6}), third root – 7,1% (r*=0,718; Wilks' Λ=0,485; $\chi^2_{(36)}$=72; p=0,0003).

Calculating the values of discriminant roots for each patient by the raw coefficients and the constants (Table 6) allows visualization of each patient in the information space of roots.

Table 6. Standardized and raw coefficients and constants for discriminant EEG variables

Coefficients	**Standardized**			**Raw**		
Variables currently in the model	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Uric acid, µM/L	1,291	-0,038	0,060	0,048	-0,001	0,002
Sex index	-0,177	-1,022	0,227	-0,388	-2,247	0,498
Trait anxiety, points	0,409	-0,160	-0,447	0,053	-0,021	-0,058
P4-β PSD, %	-0,534	0,148	0,555	-0,121	0,034	0,126
Cortisol, nM/L	0,341	-0,189	0,209	0,0035	-0,0017	0,0019
Triiodothyronine, nM/L	-0,113	-0,335	0,825	-0,064	-0,191	0,470
Creatinine, µM/L	-0,217	-0,227	0,505	-0,020	-0,021	0,047
Deviation δ, Hz	0,020	0,354	-0,299	0,081	1,415	-1,196
Deviation α, Hz	-0,195	-0,238	-0,496	-0,396	-0,483	-1,007
Index θ, %	-0,115	-0,502	-0,068	-0,004	-0,019	-0,003
Deviation θ, %	0,130	0,149	-0,455	0,226	0,259	-0,790
Asymmetry α, %	0,135	0,296	0,447	0,011	0,023	0,035
Frequency α, Hz	0,211	0,204	0,162	0,201	0,194	0,154
LF HRV PSD, %	0,384	0,283	0,272	0,024	0,018	0,017
Asymmetry θ, %	0,055	-0,129	-0,284	0,003	-0,007	-0,014
P3-β PSD, %	-0,305	0,155	-0,082	-0,027	0,014	-0,007
Bilirubin direct, µM/L	0,134	1,038	1,888	0,010	0,078	0,142
F4-θ PSD, %	0,252	-0,597	-0,486	0,050	-0,118	-0,096
Index δ, %	-0,333	0,328	-0,608	-0,008	0,008	-0,015
Urea, mM/L	-0,103	0,374	-0,011	-0,108	0,392	-0,012
Amplitude α, µV	-0,017	0,642	-0,279	-0,001	0,054	-0,023
Laterality α, %	0,067	0,864	-0,438	0,002	0,025	-0,013
Bilirubin free, µM/L	0,172	-0,389	0,200	0,016	-0,036	0,019
F3-θ PSD, %	0,081	0,566	-0,107	0,017	0,118	-0,022
Fp1-θ PSD, %	0,324	-0,015	0,607	0,062	-0,003	0,117
Fp2-θ PSD, %	-0,186	-0,360	-0,349	-0,037	-0,072	-0,070
Calcitonin, ng/L	-0,190	-0,493	-0,140	-0,028	-0,072	-0,021
F4-δ PSD, %	0,494	-0,536	0,004	0,020	-0,022	0,0002
Frequency θ, Hz	0,127	0,037	0,363	0,092	0,027	0,264
Testosterone, nM/L	0,015	-0,369	0,051	0,0022	-0,0570	0,0079
Age, years	0,449	0,893	1,671	0,036	0,071	0,134
Deviation β, Hz	0,246	0,083	0,139	0,313	0,106	0,177
T3-δ PSD, %	-0,011	0,385	0,263	-0,0004	0,0165	0,0112
T3-α PSD, %	0,285	-0,880	0,271	0,013	-0,039	0,012
O1-δ PSD, %	-0,013	0,626	-0,274	-0,001	0,027	-0,012
Laterality β, %	0,089	-0,590	0,407	0,003	-0,017	0,012
P4-α PSD, %	0,332	-0,629	-0,146	0,016	-0,030	-0,007
Amplitude β, µV	-0,008	-0,140	-0,388	-0,002	-0,037	-0,103
			Constants	-20,61	-2,950	-16,84
			Eigenvalues	12,26	1,543	1,062
			Cumulative Proportion	0,825	0,929	1

Table 7 (see Appendix) displays the factorial structure of the discriminant roots, which characterizes the strength and directionality of the connections of the roots with individual variables included in the discriminant model. In addition, extra-model variables were included in the structure of each root, which nevertheless carry discriminant information.

The localization of the members of the third cluster in the left extreme zone of the first root (Fig. 1) reflects, first of all, the most pronounced hypouricemia in combination with the minimum for the sample the lower limit level of free bilirubin and the ideal normal level of creatinine.

Such a constellation of nitrogenous metabolites is accompanied by a characteristic endocrine constellation: maximally decreased levels of cortisol in individuals of both sexes and testosterone in men with a normal, but minimal for the sample testosterone level in women, on the other hand, a maximally increased level of calcitonin in them, while in men it is in the lower zone norms.

Characteristic features of HRV are the maximally increased relative power of the LF band as a marker of sympathetic tone and a parameter inverted to Mode HRV as a marker of circulating catecholamines in combination with the minimum lower limit level of the relative power of the VLF band. The latter, we recall, is considered a probable marker of testosterone [30] and vagal tone [29], which is consistent with our data.

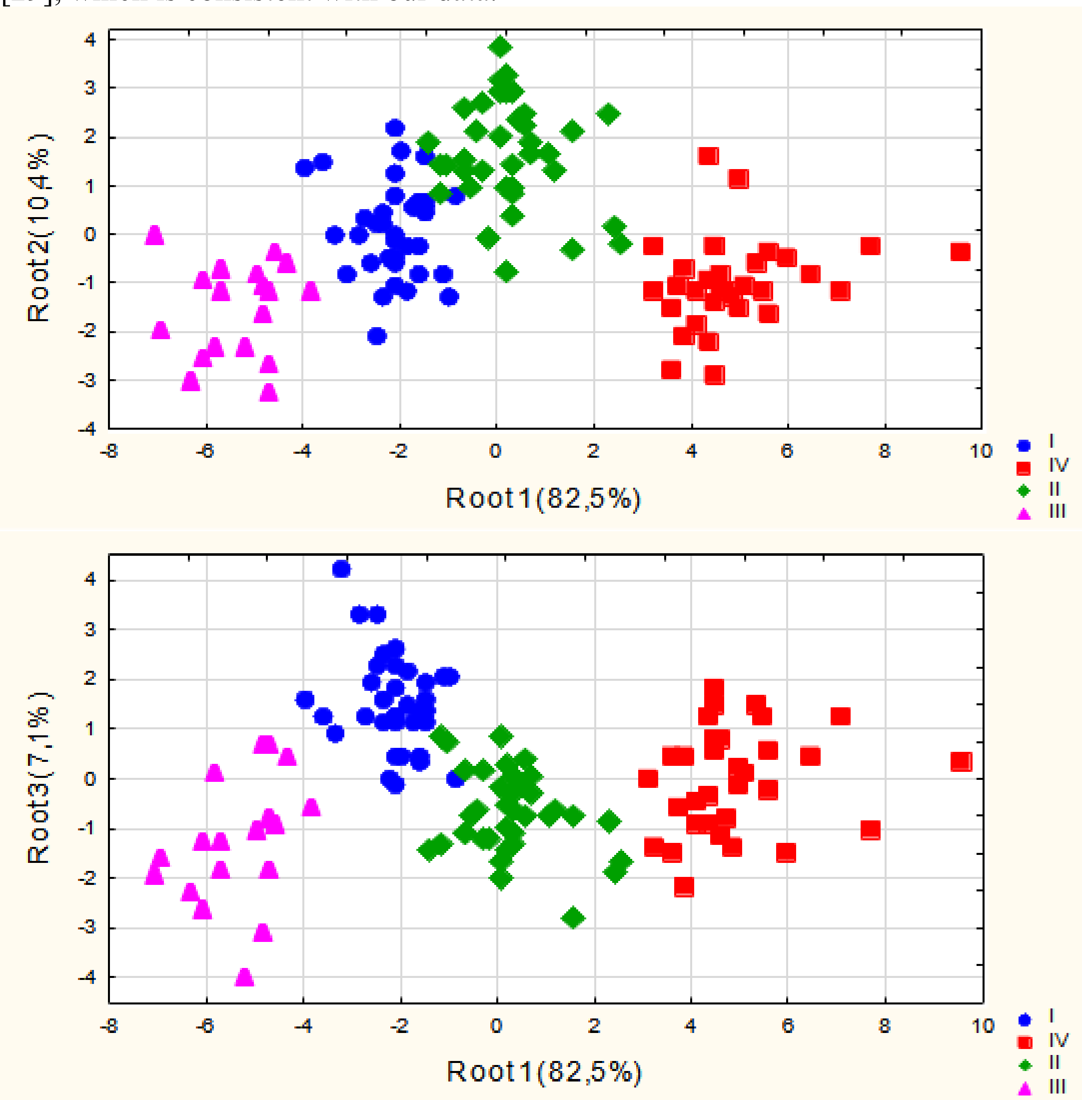

Fig. 1. Scattering of individual values of the first and second (top) and first and third (bottom) discriminant roots of members of clusters

The characteristic features of EEG are the following. The maximum for the sample upper limit amplitude of the beta rhythm and its left lateralization; the maximally increased PSD level of the delta rhythm in the T6 locus; normal, but maximum for the sample PSD levels of the beta rhythm in the C4 and P3 loci; minimum for the sample lower limit PSD levels of the alpha rhythm in the P3 and P4 loci. Judging by the schemes of Winkelmann T et al [31], the transverse temporal cortex of the right hemisphere (RH) projects to the T6 locus, the precentral gyrus of the RH to the C4 locus, and the supramarginal gyrus or isthmus cingulate cortex to the parietal loci. The same authors found an inverse correlation (r=-0,45) between the thickness of the isthmus cingulate cortex LH and HRV-marker of vagal influences (HF band). This is consistent with the maximum level of sympathetic influences in this cluster, however, it is not consistent with the data on the positive correlation (r=0,56; 0,44; 0,43 respectively) with the HF band thickness of those regions of the cortex that project to the other mentioned loci.

Localization of members of the fourth cluster at the opposite pole of the axis of the first root reflects their qualitatively opposite level of uricemia, as well as free bilirubin and creatinine - in the upper normal range. This is accompanied by pronounced hypertestosteroneemia in both sexes and a normal, but maximal for the sample, cortisol level, on the other hand, minimally pronounced hypercalcitoninemia in women and a lower limit level of plasma calcitonin in men. Levels of sympathetic tone and circulating catecholamines are also minimal for the sample. The parameters of the delta and beta rhythm are minimal for the sample, and the beta rhythm is practically symmetrical. Instead, the parameters of the alpha rhythm are maximal for the sample. That is, the neuro-endocrine accompaniment of nitrogen metabolism is also qualitatively opposite.

The intermediate positions of the other two clusters reflect, as a rule, the intermediate levels of parameters of nitrogen metabolism and its neuro-endocrine support. All four clusters are quite clearly demarcated along the axis of even one major root.

Additional separation occurs along the axis of the second root. The top position of the second cluster reflects normal, but maximum for the sample levels of direct bilirubin and urea. This is accompanied by maximum sample levels of a number of parameters of delta and alpha rhythms as well as ULF band HRV, instead of minimum sample levels of a number of parameters of theta and beta rhythms. Other characteristic features of this cluster are the predominantly male composition (negative sex index), the minimum age for the sample and the lowest (normal) level of trait anxiety.

In addition, the first cluster is separated from the others along the axis of the third root. This is due to levels of triiodothyronine, aldosterone, the Kerdoe index, as well as a number of parameters of theta and alpha rhythms, which are different from other clusters.

The variegated placement in the factor structure of EEG, HRV, and hormone parameters is due to significant relationships between them previously discovered by our laboratory [2,8,13,22,24,25].

The clarity of clear demarcation of clusters in the information field of three discriminant roots is documented by calculating Mahalanobis distances (Table 8).

Table 8. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=38,8) (below the diagonal); p-levels for all <10^{-6}

Clus-ters	I	IV	II	III
I	0	53	13	21
IV	16,0	0	30	107
II	4,1	9,2	0	42
III	4,5	22,3	9,2	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 9).

Table 9. Coefficients and constants of classification functions

Clusters	I	IV	II	III
Variables	p=,287	p=,263	p=,303	p=,147
Uric acid, µM/L	1,034	1,370	1,139	0,874
Sex index	26,98	25,89	21,34	30,48
Trait anxiety, points	2,013	2,499	2,229	2,037
P4-β PSD, %	0,087	-0,998	-0,413	0,073
Cortisol, nM/L	0,097	0,118	0,098	0,085
Triiodothyronine, nM/L	2,813	1,833	1,337	2,016
Creatinine, µM/L	2,082	1,891	1,900	2,051
Deviation δ, Hz	-41,56	-40,64	-36,48	-40,77
Deviation α, Hz	-19,59	-20,28	-19,12	-14,71
Index θ, %	0,004	-0,002	-0,031	0,056
Deviation θ, %	-19,03	-16,49	-16,38	-17,98
Asymmetry α, %	0,299	0,294	0,286	0,128
Frequency α, Hz	15,17	16,13	15,62	13,77
LF HRV PSD, %	1,384	1,507	1,432	1,230
Asymmetry θ, %	-0,591	-0,541	-0,564	-0,549
P3-β PSD, %	-1,000	-1,196	-1,025	-0,913
Bilirubin direct, µM/L	22,70	22,46	22,54	22,14
F4-θ PSD, %	-0,590	0,037	-0,458	-0,291
Index δ, %	-0,674	-0,718	-0,648	-0,618
Urea, mM/L	14,71	13,54	15,12	14,45
Amplitude α, µV	-1,406	-1,438	-1,272	-1,423
Laterality α, %	-0,281	-0,275	-0,209	-0,292
Bilirubin free, µM/L	2,300	2,422	2,238	2,255
F3-θ PSD, %	-0,026	0,002	0,254	-0,209
Fp1-θ PSD, %	2,435	2,695	2,323	1,910
Fp2-θ PSD, %	1,553	1,478	1,500	1,986
Calcitonin, ng/L	-0,661	-0,748	-0,800	-0,396
F4-δ PSD, %	0,999	1,165	1,010	0,968
Frequency θ, Hz	10,15	10,36	9,838	9,069
Testosterone, nM/L	0,844	0,909	0,740	0,907
Age, years	24,72	24,69	24,63	24,11
Deviation β, Hz	23,23	25,04	23,75	21,55
T3-δ PSD, %	0,545	0,506	0,546	0,488
T3-α PSD, %	0,146	0,258	0,086	0,134
O1-δ PSD, %	0,957	0,943	1,026	0,948
Laterality β, %	0,260	0,278	0,213	0,246
P4-α PSD, %	1,159	1,312	1,162	1,176
Amplitude β, µV	-1,418	-1,232	-1,260	-1,061
Constants	-1625,3	-1750,5	-1640,1	-1520,3

The classification accuracy is 96,7% (Table 10).

Table 10. Classification Matrix

	Percent Correct	I p=,28689	IV p=,26230	II p=,30328	III p=,14754
Rows: Observed classifications Columns: Predicted classifications					
I	97,1	34	0	1	0
IV	100,0	0	32	0	0
II	94,6	1	1	35	0
III	94,4	1	0	0	17
Total	96,7	36	33	36	17

CONCLUSION

The obtained results supplemented the concept of our laboratory about the immunotropic activity of nitrogenous metabolites [15-19,23] with data about their significant influence on the parameters of the nervous and endocrine systems, as well as anxiety. At the same time, sexual dimorphism was discovered, which will be the subject of subsequent publications.

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975 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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APPENDIX

Table 7. Correlations Variables-Roots, centroids of clusters and Z-scores of variables

Variables	Correlations Vars-Roots			III	I	II	IV
Root 1 (82,5%)	Root 1	Root 2	Root 3	-5,31	-2,07	+0,26	+4,96
Uric acid	0,712	0,056	0,005	-1,95	-1,28	-1,14	+0,29
Cortisol	0,069	-0,130	0,121	-0,94	-0,59	-0,90	-0,15
Testosterone Males	0,112	0,145	-0,020	-1,13	-0,20	-0,64	+1,42
Testosterone Females	0,112	0,145	-0,020	+0,33	+0,78	+1,09	+1,85
Bilirubin free	0,049	-0,126	-0,038	-0,28	+0,21	+0,33	+0,35
Creatinine	0,071	-0,034	0,146	+0,01	+0,26	+0,25	+0,33
Deviation β	0,036	-0,043	-0,020	-0,12	-0,12	-0,07	+0,20
P4-α PSD	0,029	0,027	-0,035	-0,53	-0,48	-0,27	-0,23
P3-α PSD				-0,52	-0,46	-0,22	-0,21
VLF HRV PSDr				-0,66	-0,64	-0,61	-0,24
Laterality β	0,024	0,044	0,042	-0,39	-0,11	-0,07	-0,05
Frequency α	0,019	-0,011	0,036	-0,28	-0,11	-0,17	-0,01
Calcitonin Females	-0,038	-0,038	0,018	+2,46	+1,33	+1,10	+0,98
LF HRV PSDr	-0,051	0,076	0,051	+1,50	+1,34	+1,48	+0,80
1/Mode HRV				+1,26	+1,25	+0,59	+0,44
T6-δ PSDr				+0,99	+0,68	+0,83	+0,46
Amplitude β	-0,038	-0,092	-0,139	+0,65	-0,05	+0,01	0,00
Calcitonin Males	-0,038	-0,038	0,018	-0,26	-0,05	-0,66	-0,62
C4-β PSDr				+0,26	-0,26	-0,50	-0,61
P3-β PSDr	-0,018	-0,017	-0,038	-0,05	-0,27	-0,22	-0,28
Root 2 (10,4%)	Root 1	Root 2	Root 3	-1,59	+0,03	+1,66	-1,05
Bilirubin direct	-0,032	0,200	0,084	+0,26	+0,49	+0,65	+0,24
Urea	-0,026	0,158	0,053	+0,11	+0,33	+0,34	-0,29
Deviation δ	-0,005	0,126	-0,062	-0,12	-0,10	+0,22	-0,17
Laterality α	0,032	0,110	0,030	-0,48	-0,11	+0,10	-0,05
O1-δ PSD	-0,010	0,089	-0,078	+0,70	+0,54	+1,00	+0,48
T3-δ PSDr	-0,001	0,086	-0,009	+0,55	+0,71	+0,93	+0,59
F4-δ PSDr	0,016	0,035	-0,022	+0,55	+0,61	+0,79	+0,74
F3-δ PSDr				+0,74	+0,69	+0,83	+0,62
P4-δ PSDr				+0,56	+0,72	+0,78	+0,53
Index δ	-0,013	0,069	-0,092	+0,37	+0,18	+0,47	+0,16
ULF HRV PSDr				+0,06	+0,21	+0,73	+0,03
Amplitude α	0,008	0,041	0,013	-0,14	0,00	+0,07	-0,01
Sex index	-0,079	-0,331	0,056	+0,80	+0,22	-0,56	-0,05
Age	0,036	-0,163	-0,075	+0,17	-0,18	-0,20	+0,35
Trait anxiety	-0,033	-0,250	-0,118	+2,62	+0,77	+0,26	+1,25
P4-β PSDr	0,011	-0,087	0,173	+0,22	-0,22	-0,45	-0,35
F4-θ PSDr	0,003	-0,083	0,093	-0,17	-0,05	-0,34	-0,08
Fp2-θ PSDr	-0,002	-0,088	-0,019	+0,08	-0,08	-0,18	+0,01
Index θ	0,012	-0,116	-0,054	+0,18	-0,09	-0,13	+0,17
P3-θ PSDr				-0,05	+0,09	-0,23	+0,19
F8-θ PSDr				-0,20	-0,01	-0,23	+0,37
T3-θ PSDr				-0,22	-0,07	-0,34	-0,08
T3-α PSD	0,012	-0,032	0,007	-0,30	-0,44	-0,54	-0,28
T3-β PSDr				-0,28	-0,37	-0,45	-0,39
Root 3 (7,1%)	Root 1	Root 2	Root 3	-1,32	+1,48	-0,68	-0,08
Triiodothyronine	-0,027	-0,082	0,185	-0,75	+0,52	-0,77	-0,05
Asymmetry α	-0,014	-0,002	0,163	+0,02	+0,48	+0,03	+0,08
Kerdoe Vegetative Ind				+0,45	+0,64	+0,36	+0,19
Fp1-θ PSDr	0,027	-0,059	0,180	-0,30	+0,12	-0,25	+0,09

Frequency θ	-0,022	-0,021	**0,134**	-0,32	**-0,04**	-0,45	-0,40
F3-θ PSDr	0,012	-0,011	**0,078**	-0,30	**-0,10**	-0,24	-0,12
Aldosterone				-0,35	**-0,21**	-0,27	-0,29
Deviation α	0,019	-0,101	**-0,177**	+0,32	**-0,22**	+0,02	+0,25
Deviation θ	-0,025	0,006	**-0,158**	+0,39	**-0,14**	+0,17	-0,08
Asymmetry θ	0,067	-0,113	**-0,148**	+0,17	**-0,23**	+0,07	+0,62