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CANONICAL ANALYSIS OF NEUROENDOCRINE-METABOLIC AND NEUROENDOCRINE-IMMUNE RELATIONSHIPS AT FEMALE RATS

Oksana I. Mel'nyk[1], Walery Zukow[2], Myroslava V. Hrytsak[3], Dariya V. Popovych[4], Yuriy V. Zavidnyuk[3,4], Volodymyra R. Bilas[5], Igor L. Popovych[3,5]

[1]Danylo Halyts'kyĭ National Medical University, L'viv, Ukraine omelnyk7@gmail.com
[2]Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl
[3]Ukrainian Scientific Research Institute of Medicine for Transport, Odesa, Ukraine hrytsak.myroslava@gmail.com
[4]IY Horbachevs'kyi State Medical University, Ternopil', Ukraine darakoz@yahoo.com
[5]OO Bohomolets' Institute of Physiology, Kyïv, Ukraine i.popovych@biph.kiev.ua

Abstract

Background. It has been shown before that drinking mineral waters of different composition has a significant effect on the neuroendocrine, metabolic and immune parameters of healthy rats. We analyzed the canonical correlations between neuroendocrine parameters, on the one hand, and metabolic and immune parameters, on the other hand. **Materials and Methods**. Experiment was performed on 58 healthy female Wistar rats 240-290 g. HRV parameters, major adaptation hormones, metabolism and immunity parameters were recorded. **Results**. The method of canonical correlation analysis revealed causal relationships between neuroendocrine and metabolic and neuroendocrine and immune parameters of the organism. The degree of neuroendocrine determination of individual sets of metabolic parameters ranges from 60% to 92%, and immune status from 87% to 96,5%. **Conclusion**. Effects of mineral waters on metabolism and immunity are realized through nervous and hormonal mechanisms.
Keywords. HRV, hormones, metabolites, immunity, relationships, female rats.

INTRODUCTION

It has been shown before that drinking mineral waters of different composition has a significant effect on the neuroendocrine, metabolic and immune parameters of healthy rats [24]. A priori, effects of mineral waters, by analogy with other natural and reshaped factors, on metabolism and immunity are realized through nervous and hormonal mechanisms [8,10,13-23]. To confirm this position, we analyzed the canonical correlations between neuroendocrine parameters, on the one hand, and metabolic and immune parameters, on the other hand.

MATERIALS AND METHODS

Experiment was performed on 58 healthy female Wistar rats 240-290 g. Ten animals remained intact, using tap water from drinking ad libitum, and others received mineral water and water-salt solution of various compositions [24].

The day after the completion of the drinking course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for leukocytogram analysis. Then they assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [2].

Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA); as well as electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (both in plasma and in erythrocytes) by flamming photometry; nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method), medium molecular polypeptides (by spectrophotometric method), bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method) [6]; lipid peroxidation products: diene conjugates (spectrophotometry of the heptane phase of the lipids extract) [5] and malonic dyaldehide (in the test with thiobarbituric acid) [1], antioxidant enzymes: superoxide dismutase erythrocytes (according to the degree of inhibition of reduction of nitroblue tetrazolium in the presence of N-methylphenazonium metasulphate and NADH) [11] and catalase plasma (at the rate of decomposition of hydrogen peroxide) [9], as well as amylase (Karavay's amyloclastic method with starch substrate) and glucose (glucose-oxidase method) [6].

Most of the listed parameters of metabolism were also determined in daily urine. By the size of the diuresis and the level of creatinine in plasma and urine, glomerular filtration and tubular reabsorption were calculated. In addition, the osmolarity of the urine was measured by the cryostatic method.

The analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a fiery spectrophotometer "СФ-47".

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients $(Cap/Pp)^{0,5}$ and $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficients $(1/Cap•Pp)^{0,5}$ and $(Cau•Pu/Cap•Pp)^{0,25}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Nap•Ku/Kp•Nau)^{0,25}$, based on their classical effects and recommendations by IL Popovych [10].

In the blood, the parameters of immunity were determined, as described in the manual [12]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep. Natural killers were identified as large granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocyte index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [3,4].

After decapitation, the spleen, thymus and adrenal glands were removed from the animals. Immune organs weighed and made smears-imprints for counting splenocytogram and thymocytogram [3]. For them, as well as leukocytogram, Shannon's entropy was calculated [7,15]. In the adrenal glands after weighing, the thickness of glomerular, fascicular and reticular zones was measured under a microscope [3].

Digital material is statistically processed on a computer using the software package "Statistica 5.5".

RESULTS AND DISCUSION

At the first stage, a matrix of neuroendocrine-metabolic correlations was created (Table 1).

Table 1. Matrix of correlations between neuroendocrine and metabolic parameters of rats

Variables	Symp tone	Glom ZAC	Fasc ZAC	Retic ZAC	Adre Mass	T_3	Mo-de	Vag tone	Corti-coster	Testo-sterone
Na Urine	**-,39**	-,15	-,03	-,24	-,16	-,23	,24	,21	,03	,07
Cl Urine	-,22	-,18	,05	-,20	-,20	-,08	,06	,01	,02	,12
K Urine	**,29**	-,08	-,02	,06	-,09	,10	**-,28**	**-,30**	,02	,05
Mg Urine	,24	,03	**,46**	**,41**	,06	**,68**	-,20	-,18	,12	,10
Ca Urine	-,11	-,00	-,25	-,07	,17	**-,38**	,11	,04	**,35**	-,10
Pi Urine	-,02	,14	-,15	-,14	,17	-,23	,09	,05	,14	-,01
Urea Urine	-,18	,07	-,11	-,13	,03	-,11	,18	,23	,05	-,05
Creatinine Urine	**,30**	,13	,23	,11	-,03	**,28**	-,25	-,15	-,06	**,28**
Uric acid Urine	-,07	,07	**-,50**	,04	-,21	**-,54**	,14	,25	-,08	-,16
Amylase Urine	**,28**	**,30**	,18	,10	,16	-,03	**-,29**	-,19	-,06	,11
MMM Urine	-,07	-,12	-,20	-,16	,21	**-,26**	,09	-,03	,14	-,07
Katalase Urine	-,20	**-,33**	**-,28**	-,21	,15	**-,33**	,19	,05	,17	,08
MDA Urine	,01	,00	-,03	**,30**	-,19	,15	-,04	-,09	,00	,13
DC Urine	**-,33**	,04	,03	,17	-,25	,07	**,33**	,22	-,17	,07
Osmolality Urine	**-,29**	-,14	-,05	-,24	-,18	-,18	,14	,12	,05	,08
Na Excretion	**-,31**	**-,26**	-,08	**-,27**	-,01	-,24	,23	,23	,00	-,01
Cl Excretion	-,21	**-,28**	,04	**-,27**	-,10	-,11	,10	,07	,01	,00
K Excretion	,15	-,22	,00	,01	-,02	,02	-,14	-,24	,11	-,13
Mg Excretion	,15	-,03	**,45**	**,45**	,02	**,69**	-,13	-,16	,10	,01
Ca Excretion	-,07	-,18	-,23	-,12	,23	**-,29**	,07	,02	,21	-,07
Pi Excretion	-,07	-,17	-,12	-,09	,11	-,15	,10	,03	,08	-,13
Creatinine Excretion	,09	-,08	,18	,08	,02	,20	-,09	-,10	,04	,01
Urea Excretion	-,14	-,12	-,14	-,16	,11	-,14	,13	,12	,03	-,05
Uric acid Excretion	-,08	-,19	**-,44**	,06	-,12	**-,50**	,18	,20	-,00	-,20
Diurese	-,08	-,21	-,02	-,01	,07	-,03	,08	,02	,04	-,13
Canalicular Reabsorbtion	,25	,22	**,27**	,01	-,04	**,33**	-,14	-,02	**-,42**	,14
Glomerular Filtration	-,03	,11	**,27**	-,04	-,07	,20	,09	,12	-,23	-,10
Creatinine Plasma	-,02	-,19	-,15	,13	,05	-,12	-,09	-,17	**,49**	,03
Na Erythrocytes	**-,31**	-,19	-,09	-,14	-,06	**-,37**	,22	**,26**	,02	,04
K Erythrocytes	,15	,03	,16	,07	-,10	,14	-,20	-,13	,03	,10
Na Plasma	**,32**	,06	-,03	,02	,01	-,05	**-,28**	-,16	,08	,14
K Plasma	-,01	-,21	-,16	-,08	,03	**-,34**	,06	-,07	,13	-,09
Mg Plasma	-,24	,10	-,16	,14	-,10	-,14	,16	,22	,15	,13
Ca Plasma	-,01	,16	**,31**	,16	-,06	**,36**	,03	-,05	-,03	-,08
Pi Plasma	,20	,08	**,47**	**,29**	-,04	**,65**	-,16	-,19	,06	,12
Cl Plasma	,19	,06	-,04	,03	-,02	-,08	-,16	-,08	,09	,10

Glucose Plasma	-,17	,10	-,03	,18	-,08	-,01	,13	,11	-,04	-,01
Cholesterol Plasma	-,08	,06	,11	-,25	,02	-,16	,13	,11	-,01	-,11
Bilirubine Plasma	-,04	-,01	**-,28**	**-,30**	**,30**	**-,34**	,05	-,07	,03	-,23
Urea Plasma	,20	-,11	-,09	,13	,09	,02	-,25	**-,31**	**,38**	,11
Uric acid Plasma	-,23	,02	-,22	-,06	-,15	**-,27**	,24	**,37**	-,10	-,09
MMM	-,15	-,02	-,00	,01	,18	-,12	,11	-,02	,25	-,11
Amylase Plasma	,23	,21	,08	,20	,04	,23	**-,29**	-,23	-,12	,10
SOD Erythrocytes	,06	,24	,22	,23	-,14	,00	,00	-,10	,18	,01
Katalase Plasma	-,24	-,08	**-,29**	-,23	,11	**-,31**	,19	,07	,16	,06
MDA Plasma	**-,29**	-,02	**-,38**	,03	,04	**-,33**	,15	,12	,11	,06
DC Plasma	**-,65**	,19	-,13	,08	-,10	-,15	**,48**	**,52**	-,13	-,02

Note. For a sample of 58 animals, the critical level of the modulus of the correlation coefficient at p<0,05 (t>2,00) is **0,26**, at p<0,01 (t>2,66) is **0,34**, at p<0,001 (t>3,66) is **0,45**.

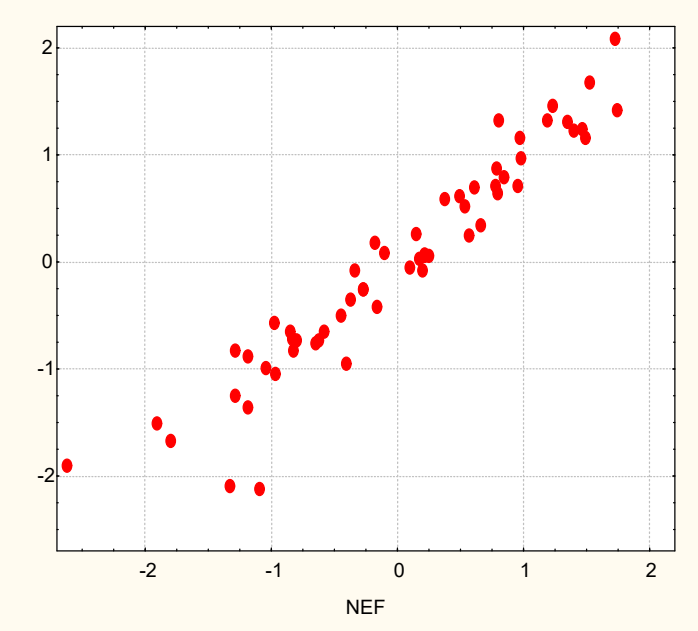
On the basis of the created matrix the canonical correlation analysis, ie the analysis of correlation between neuroendocrine and metabolic sets is carried out. The last set is divided into three subsets for convenience: urinary concentration, excretory and blood concentration. The program identified 6 pairs of canonical radicals. Neuroendocrine root was taken as causal, and metabolic - as consequential.

The factor structure of the first neuroendocrine radical is formed, in descending order of load, triiodothyronine, the thickness of the fascicular and reticular zones of the adrenal cortex as well as testosterone (Table 2).

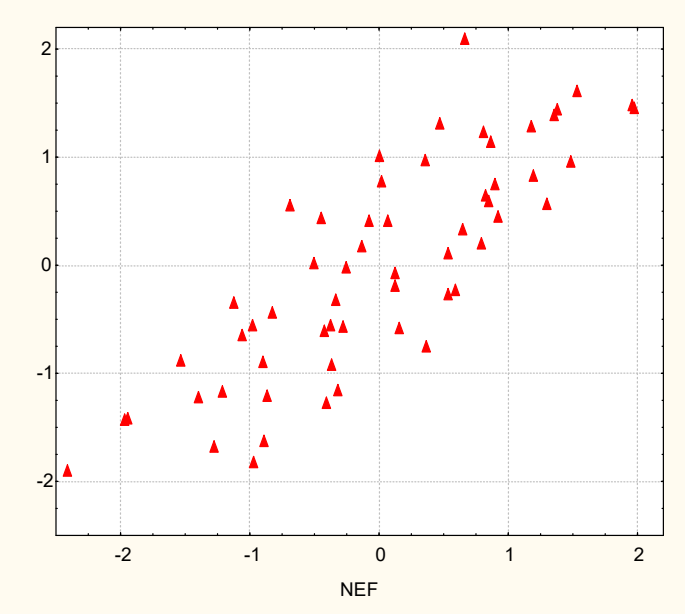
Table 2. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and concentration or activity in the urine of metabolites

Neuroendocrine factors	Root 1	Root 2
Triiodothyronine	**,973**	,015
Fascicular ZAC	**,712**	,172
Reticular ZAC	**,326**	-,245
Testosterone	**,268**	,169
Sympathetic tone	,286	**,639**
Catecholamines (1/Mode)	,285	**,585**
Adrenals Mass Index	,029	**,562**
Glomerular ZAC	-,035	**,274**
Corticosterone	-,045	**,166**
Vagal tone	-,283	**-,585**
Metabolic parameters	Root 1	Root 2
Uric acid Urine Concentr	**-,578**	-,220
Ca Urine Concentration	**-,422**	,225
Katalase activity Urine	**-,328**	-,049
Middle Mass Molecules U	**-,280**	,186
Pi Urine Concentration	**-,273**	,285
Mg Urine Concentration	**,717**	,057
Creatinine Urine Concent	**,317**	,155
Malonic dyaldehid Urine	**,137**	-,252
K Urine Concentration	**,112**	,082
Diene conjugates Urine	,066	**-,505**
Na Urine Concentration	-,209	**-,323**
Cl Urine Concentration	-,044	**-,269**
Osmolality Urine	-,165	**-,264**
Urea Urine Concentration	-,148	**-,048**
Amylase activity Urine	-,019	**,469**

The concentration or activity in the urine of metabolites is represented in the canonical radical by uric acid, calcium, catalase, medium mass molecules and phosphates inversely, therefore, their level is **negatively** affected by the listed hormonal constellation. In contrast, the **positive** effects of urine concentrations of magnesium, creatinine, malonic dialdehyde and potassium. As a result, we state the determination of endocrine factors levels in the urine of these metabolites by 92% (Fig. 1 above).



R=0,957; R^2=0,916; $\chi^2_{(160)}$=286; p<10^{-6}; Λ Prime=0,001



R=0,831; R^2=0,690; $\chi^2_{(135)}$=178; p=0,007; Λ Prime=0,017

Fig. 1. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and urinary concentrations of metabolites (Y-axis)

The second neuroendocrine radical is represented directly by sympathetic tone, circulating catecholamines (marked by the inverse value of Mode HRV), adrenals mass, thickness of the glomerular zone of their cortex and corticosteronemia, while inverse by vagal tone. The metabolic canonical radical receives negative factor loads, primarily from the concentration of diene conjugates, as well as the osmolality of urine and its forming concentrations of sodium, chloride and urea. Instead, amylase activity gives a positive load.

As a result, the determination of neuroendocrine factors in the levels of urine of these metabolites is 69% (Fig. 1 below).

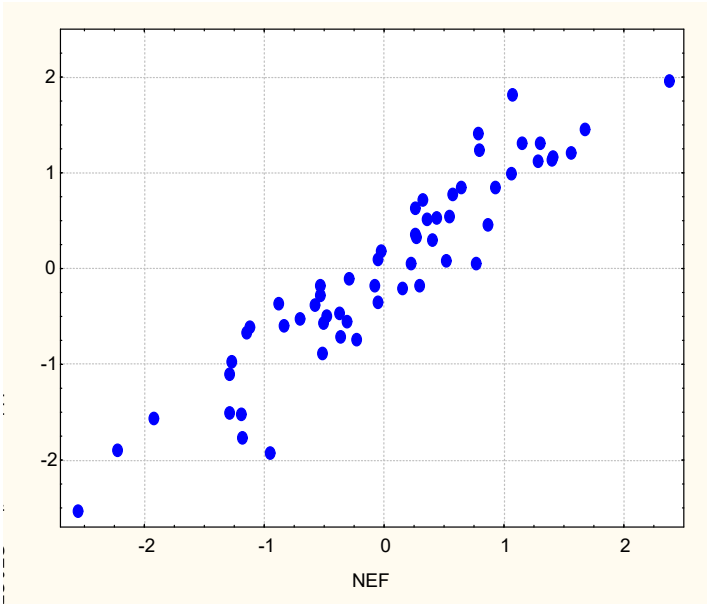
Canonical analysis of neuroendocrine-excretory connections revealed the following (Table 3).

Table 3. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and metabolites excretion parameters

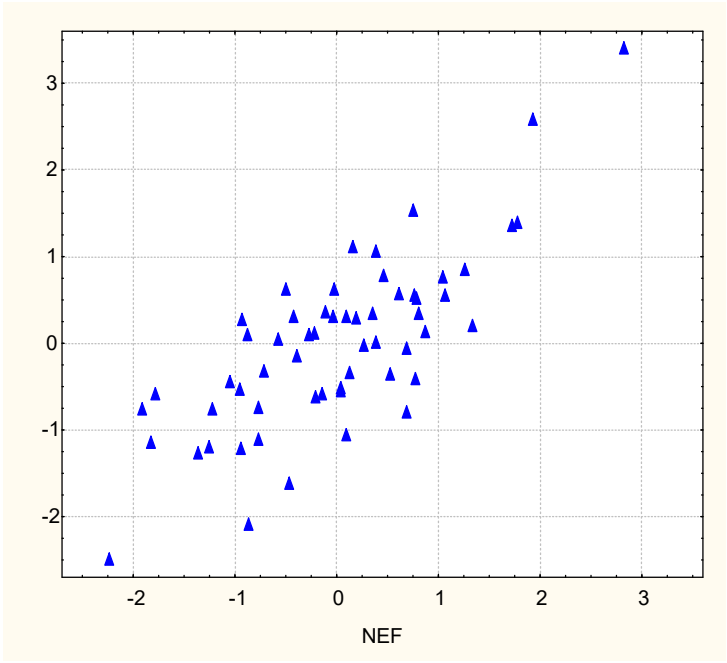
Neuroendocrine factors	Root 1	Root 2
Triiodothyronine	**,980**	,041
Fascicular ZAC	**,712**	-,157
Reticular ZAC	**,317**	,342
Testosterone	**,182**	,072
Catecholamines (1/Mode)	**,235**	,045
Vagal tone	**-,225**	,189
Sympathetic tone	,201	**,457**
Glomerular ZAC	,103	**,331**
Adrenals Mass Index	-,045	**,154**
Corticosterone	-,126	**-,362**
Metabolic parameters	Root 1	Root 2
Mg Excretion	**,713**	,070
Glomerular Filtration	**,268**	,025
Creatinine Excretion	**,214**	-,026
Uric acid Excretion	**-,532**	,196
Ca Excretion	**-,348**	-,106
Pi Excretion	**-,170**	-,119
Urea Excretion	**-,158**	-,116
Cl Excretion	-,074	**-,468**
Osmolality Urine	-,155	**-,440**
Na Excretion	-,226	**-,366**
Diurese	-,028	**-,156**
K Excretion	,020	**-,136**
Canalicular Reabsorbtion	,397	**,382**

The factor structure of the first neuroendocrine radical receives positive loads from triiodothyronineemia, thickness of the fascicular and reticular zones of the adrenal cortex, testosteroneemia and catecholaminemia, while negative from the vagal tone. Glomerular filtration and excretion of magnesium and creatinine are directly represented in the effective canonical radical. Instead, negative loads give the levels of excretion of uric acid, calcium, phosphates and urea. As a result, the determination of neuroendocrine factors of these parameters of excretory function of the kidneys is 88% (Fig. 2 above).

The second neuroendocrine radical is represented directly by sympathetic tone, thickness of the glomerular zone of the adrenal cortex and their mass, while inverse by corticosteronemia. The metabolic canonical radical receives significant negative factor loads from urinary excretion of chloride and sodium, as well as related urine osmolality, and minor from diuresis and potassium excretion, which reflects the **negative** impact on these parameters of sympathetic tone and mineralocorticoids. In contrast, tubular water reabsorption is **negatively** related to plasma corticosterone levels. As a result, we state the determination by neuroendocrine factors of this set of parameters of excretory function of the kidneys by 60% (Fig. 2 below).



R=0,940; R^2=0,883; $\chi^2_{(130)}$=237; p<10^{-6}; Λ Prime=0,005



R=0,772; R^2=0,596; $\chi^2_{(108)}$=141; p=0,018; Λ Prime=0,044

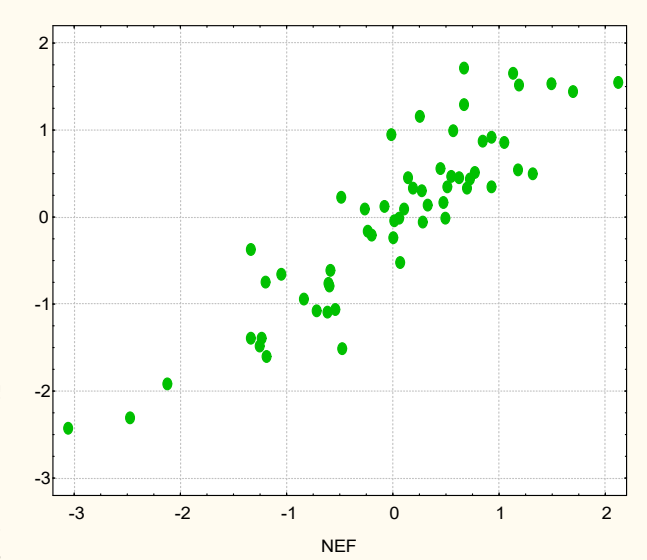
Fig. 2. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and indicators of renal excretion (Y-axis)

The analysis of the canonical correlation of regulatory factors with metabolic parameters of blood revealed that the factor structure of the first radical is exclusively endocrine and usually receives significant positive loads, in descending order, from triiodothyronemia, fascicular, reticular and glomerular zones thickness as well as testosteroneemia (Table 4).

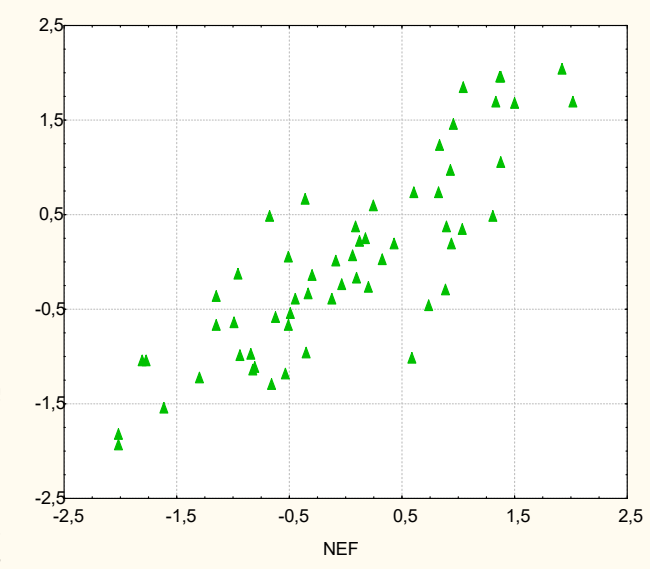
Table 4. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and blood concentration of metabolites

Neuroendocrine factors	Root 1	Root 2
Triiodothyronine	**,875**	-,037
Fascicular ZAC	**,677**	-,122
Reticular ZAC	**,436**	,298
Glomerular ZAC	**,295**	,241
Testosterone	**,231**	,184
Vagal tone	-,210	**,630**
Sympathetic tone	,399	**-,603**
Catecholamines (1/Mode)	,287	**-,395**
Adrenals Mass Index	-,195	**-,250**
Corticosterone	-,054	**-,063**
Metabolic parameters	Root 1	Root 2
Bilirubine	**-,516**	**-,346**
Malonic dyaldehid	**-,447**	,352
Katalase activity	**-,439**	,036
Na Erythrocytes	**-,367**	,254
K	**-,346**	-,246
Middle Mass Molecules	**-,218**	-,090
Creatinine	**-,187**	,054
Cholesterol	**-,116**	-,162
Pi	**,704**	-,067
Amylase Activity	**,234**	,016
K Erythrocytes	**,210**	,026
Superoxide dismutase	**,185**	-,072
Na	**,090**	-,054
Cl	**,030**	,008
Diene conjugates	-,246	**,730**
Mg	-,102	**,490**
Uric acid	-,206	**,358**
Glucose	-,010	**,220**
Urea	-,009	**-,108**

This endocrine network has a **negative** effect on plasma levels of bilirubin, malonic dialdehyde, potassium, medium weight molecules, creatinine and cholesterol, catalase activity, as well as sodium levels in erythrocytes. In contrast, these endocrine factors have a **positive** effect on plasma phosphate, sodium and chloride levels, plasma amylase activity and erythrocyte superoxide dismutase, as well as their potassium content. The degree of endocrine-metabolic determination is 81% (Fig. 3 above).



R=0,901; R^2=0,813; $\chi^2_{(190)}$=282; p<10^{-4}; Λ Prime=0,001



R=0,851; R^2=0,723; $\chi^2_{(152)}$=212; p=0,006; Λ Prime=0,006

Fig. 3. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and metabolic parameters of blood (Y-axis)

The second neuroendocrine radical directly represents the vagal tone, while the inverse - sympathetic tone, catecholamines, adrenal mass and corticosteronemia. Positive factor loads on the corresponding metabolic radical from plasma levels of diene conjugates, magnesium, uric acid and glucose reflect their direct dependence on vagal tone and inverse - on sympathetic tone and catecholamines. Significant negative load on the radical from bilirubinemia reflects its **positive** relationship with the mass of the adrenal glands. In contrast, plasma urea levels are directly dependent on corticosteronemia, catecholaminemia, and sympathetic tone (see Table 3.4). As a result, the determination of neuroendocrine factors of this set of blood plasma metabolites is 72% (Fig. 3 below).

Following the accepted algorithm, a matrix of **noteworthy** correlations between neuroendocrine indicators, on the one hand, and immunity indicators, on the other, was first created (Table 5).

Table 5. Matrix of correlations between neuroendocrine and immune parameters

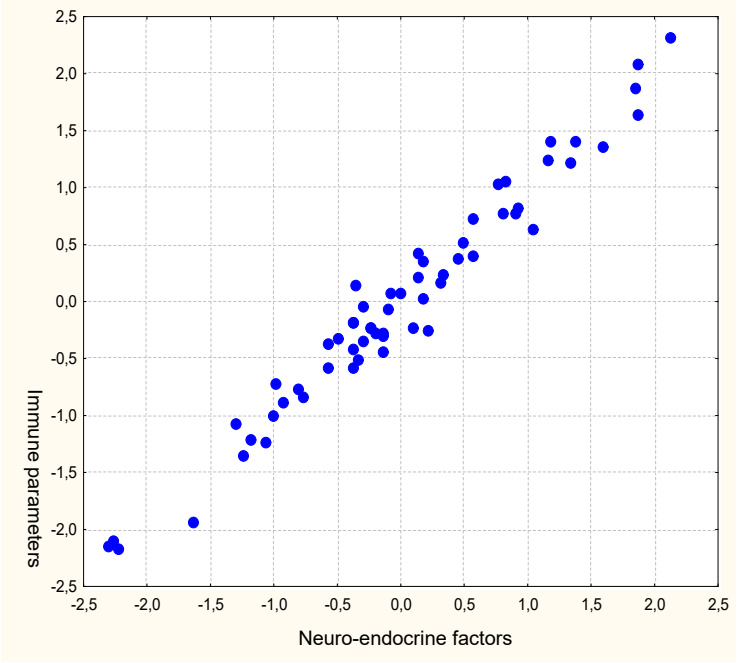
r	HL CG	HI CG	HT CG	FN N	FI M	FN M	FI N	Lb S	Pla S	Ret S	Fib S	Mac S	Thy mus	Lc T	Ret T	Epi T	Mo-noc	Ly-mph	NK	Th	Tc	B
T_3				**-,89**	,22	**,47**	**-,60**	**-,27**	**-,40**					-,24		**,36**	**,87**	-,23	**,90**			-,21
AMo			**,28**	**-,28**				**-,31**	**-,31**			**,69**		**-,30**		**,29**	**,29**		**,30**			
CA	**-,28**		,23	-,24				**-,34**	-,22			**,65**		**-,26**		**,31**	**,30**		**,27**			-,24
DX	,25	**,28**		**,27**		-,24	,24	**,30**				**-,43**		0,24		-,22	**-,33**	,24	**-,29**			,22
Med				**,28**				**,53**	**,52**			**-,29**	**-,38**	,23		**-,37**	**-,35**		**-,28**			
CTA	-,25			,24			**,28**	**,41**	**,35**			**-,29**				**-,36**	-,21		-,23			
PTA												-,25					**,29**		**,29**	-,22		
MC		,23		**-,34**					**-,30**	-,22			,24				**,28**		**,28**		,20	,20
Glo					-,21					-,25	**-,32**		**,46**								,24	
Cort	**-,27**	**-,28**																			-,22	**-,27**
Fasc				**-,61**		**,36**	**-,37**		-,25						,19	,23	**,63**		**,56**			-,23
Test			,23	**-,28**								**,32**										-,22
Ret				**-,26**									**,39**				**,31**		**,29**			

For further consideration, two pairs of significantly related pairs of canonical radicals were selected (Table 6).

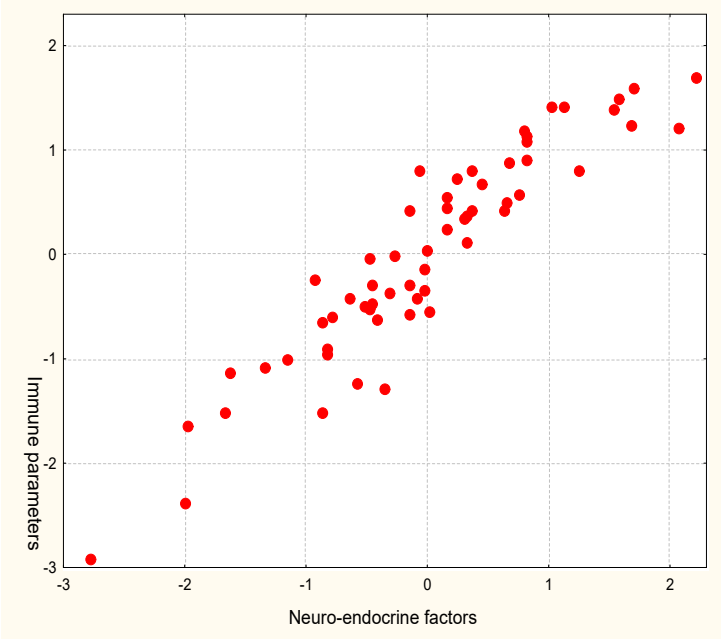
Table 6. Factor structure of two pairs of canonical roots, which represent neuroendocrine and immune parameters

Neuro-endocrine factors	**Root 1**	**Root 2**
Triiodothyronine	**0,935**	0,181
Fascicular Zone Adrenal Cortex	**0,596**	0,348
Mineralocorticoid activity	**0,408**	-0,357
Reticular Zone Adrenal Cortex	**0,335**	-0,082
Parathyroid activity	**0,290**	0,009
Catecholamines (1/Mode)	**0,289**	-0,287
Testosterone	**0,173**	-0,144
Medullar Zone Adrenal	**-0,435**	0,303
Calcitonin activity	**-0,319**	0,074
Vagal tone (MxDMn)	**-0,285**	0,011
Glomerular Zone Adrenal Cortex	0,138	**-0,488**
Sympathetic tone (AMo)	0,344	**-0,484**
Corticosterone	-0,056	**0,379**
Immunity	**Root 1**	**Root 2**
NK Lymphocytes Blood	**0,928**	0,134
Monocytes Blood	**0,909**	0,128
Microbial Count Monocytes	**0,496**	0,248
Epitheliocytes Thymus	**0,435**	-0,214
EntropyThymocytogram	**0,256**	-0,287
Reticulocytes Thymus	**0,213**	0,078
Phagocytic Index Monocytes	**0,185**	0,201
Reticulocytes Spleen	**0,139**	0,172
Microbial Count Neutrophils	**-0,908**	-0,144

Phagocytic Index Neutrophils	**-0,610**	-0,275
Plasmocytes Spleen	**-0,510**	0,223
Lymphoblastes Spleen	**-0,334**	0,188
Lymphocytes Thymus	**-0,310**	0,261
Pan-Lymphocytes Blood	**-0,257**	-0,043
T-helper Lymphocytes Blood	**-0,129**	-0,079
Macrophages Spleen	0,218	**-0,568**
Thymus Mass Index	**0,277**	**-0,425**
Entropy Immunocytogram	-0,028	**-0,423**
T-cytolytic Lymphocytes Blood	0,097	**-0,347**
Entropy Leukocytogram	0,181	**-0,344**
B-Lymphocytes Blood	-0,126	**-0,232**
Fibroblastes Spleen	0,094	**0,155**



$R=0,982$; $R^2=0,965$; $\chi^2_{(345)}=533$; $p<10^{-6}$; Λ Prime$=10^{-6}$



R=0,933; R^2=0,871; $\chi^2_{(308)}$=400; p<10^{-3}; Λ Prime=0,00004

Fig. 4. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and immunity (Y-axis)

It was found that the neuro-endocrine root of the first pair receives the maximum positive factor load from triiodothyronine, less pronounced from markers of glucocorticoid, mineralocorticoid and androgenic functions of the adrenal cortex, circulating catecholamines, and parathyroid activity, instead negative from adrenaline-secreting adrenal medullary zone, vagal tone and calcitonin activity. And the immune root is represented by the parameters of the **blood**, **thymus**, as well as plasma cells and lymphoblastes of the **spleen**, which are subject to the types of **upregulation**/**downregulation**. The degree of neuroendocrine immunomodulation is very significant – 96,5%.

The neuro-endocrine root of the second pair is represented by sympathetic tone, glomerular zone of the adrenal cortex and, conversely, corticosterone. Sympathetic tone carries out **upregulation** of splenic macrophages. Corticosterone has a suppressive effect on T-killers and B-lymphocytes and reduces the entropy of the leukocytogram and thymocytogram. Mineralocorticoids are responsible for increasing the mass of the thymus and reducing the content of fibroblasts in the spleen. The degree of immunomodulation by this neuroendocrine constellation is less pronounced - 87%.

CONCLUSION

Effects of mineral waters on metabolism and immunity are realized through nervous and hormonal mechanisms.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil' National Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the

Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

REFERENCES

1. Andreyeva LI, Kozhemyakin LA, Kishkun AA. Modification of the method for determining the lipid peroxide in the test with thiobarbituric acid [in Russian]. Laboratornoye Delo. 1988; 11: 41-43.
2. Baevskiy RM, Kirillov OI, Kletskin SZ. Mathematical Analysis of Changes in Heart Rate by Stress [in Russian]. Moskva. Nauka; 1984: 221.
3. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(1): 68-102.
4. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil; 1981: 110.
5. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of plasma levels of lipid hydroperoxides [in Russian]. Laboratornoye Delo. 1983; 3: 33-36.
6. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa. Astroprint; 1998: 608.
7. Gozhenko AI, Korda MM, Popadynets' OO, Popovych IL. Entropy, Harmony, Synchronization, Harmony and Their Neuro-Endocrine-Immune Correlates [in Ukrainian]. Odesa. Feniks; 2021: 232.
8. Kolyada TI, Volyanskyi YL, Vasilyev NV, Maltsev VI. Adaptation Syndrome and Immunity [in Russian]. Kharkiv. Osnova; 1995: 168.
9. Korolyuk MA, Ivanova MI, Mayorova IG, Tokarev VYe. The method for determining the activity of catalase [in Russian]. Laboratornoye Delo. 1988; 1: 16-19.
10. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG., Popovych IL. Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex [in Ukrainian]. Kyiv. UNESCO-SOCIO; 2015: 349.
11. Makarenko YeV. A comprehensive definition of the activity of superoxide dismutase and glutathione reductase in red blood cells in patients with chronic liver disease [in Russian]. Laboratornoye Delo. 1988; 11: 48-50.
12. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. Immune status, principles of its evaluation and correction of immune disorders [in Russian]. Kyiv. Zdorovya; 1995: 211.
13. Polovynko IS, Zayats LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.
14. Polovynko IS, Zajats LM, Zukow W, Yanchij RI, Popovych IL. Quantitative evaluation of integrated neuroendocrine and immune responses to chronic stress in rats male. Journal of Education, Health and Sport. 2016; 6(8): 154-166.
15. Popovych IL. Functional interactions between neuroendocrine-immune complex in males rats [in Ukrainian]. Achievements of Clinical and Experimental Medicine. 2008; 2(9): 80-87.
16. Popovych IL. The concept of neuroendocrine-immune complex (Review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.
17. Popovych IL, Ivassivka SV. Role of organic substances of water Naftussya in its physiological activity [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(2): 6-26.
18. Popovych IL, Vis'tak HI, Gumega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Endocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv. UNESCO-SOCIO; 2014: 163.
19. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.
20. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
21. Tracey KJ. Reflex control of immunity. Nat Rev Immunol. 2009; 9(6): 418-428.
22. Uchakin PN, Uchakina ON, Tobin BV, Ershov FI. Neuroendocrine immunomodulation [in Russian]. Vestnik Ross AMN. 2007; 9: 26-32.

23. Zajats LM, Polovynko IS, Zukow W. Features neuro-endocrine support diversity of immune responses to chronic stress in male rats. Journal of Education, Health and Sport. 2017; 7(3): 97-105.
24. Zavidnyuk YV, Mysula IR, Klishch IM, Zukow W, Popovych IL, Korda MM. General non-specific metabolic, neuroendocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(3): 513-524.