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Metabolic and immune accompaniments of electrocardiographic and morphologic gastric mucosa parameters in naïve and stressed rats

Oksana A. Fihura[1,2,5], Mykhaylo M. Korda[2], Ivan M. Klishch[2], Sofiya V. Ruzhylo[1], Oksana I. Melnyk[3], Walery Zukow[4], Roman I. Yanchij[5], Alyona A. Vorobienko[5,6], Oleksandr I. Plyska[6], Igor L. Popovych[5]

[1]Ivan Franko State Pedagogical University, Drohobych, Ukraine
oksanafigura08@gmail.com; doctor-0701@ukr.net
[2]IY Horbachevskyi National Medical University, Ternopil, Ukraine
cordamm@yahoo.com; klishch@tdmu.edu.ua
[3]Danylo Halytskyi National Medical University, Lviv, Ukraine
omelnyk7@gmail.com
[4]Nicolaus Copernicus University, Torun, Poland
w.zukow@wp.pl
[5]Bohomolets Institute of Physiology of National Academy of Sciences, Kyïv, Ukraine
i.popovych@biph.kiev.ua; tas@biph.kiev.ua
[6]Mykhajlo Dragomanov Ukrainian State University, Kyïv, Ukraine
a.a.vorobiinko@npu.edu.ua; plys2005@ukr.net

ORCID
OF: https://orcid.org/0000-0002-5711-0484
MK: https://orcid.org/0000-0003-0676-336X
IK: https://orcid.org/0000-0001-6226-4296
SR: https://orcid.org/0000-0003-2944-8821
OM: https://orcid.org/0000-0001-7928-4760
WZ: https://orcid.org/0000-0002-7675-6117
RJ: https://orcid.org/0000-0001-7129-7698
AV: https://orcid.org/0009-0008-4982-1551
OP: https://orcid.org/0000-0001-7002-7637
IP: https://orcid.org/0000-0002-5664-5591

Abstract

Introduction and aim. Earlier, by applying the water-immersion and restraint stress (WIRS) model, we reproduced primary attributes of stress and found that the severity of the damage to the gastric mucosa correlates with changes in ECG parameters, which indicate myocardial dystrophy. Further, it was found that such a connection is caused by the damaging effect on both targets of increasing the level of parathyroid hormone, as well as the production of aldosterone and catecholamines by enlarged adrenal glands. In addition, an increase in the level of corticosterone and sympathetic tone with a simultaneous decrease in vagal tone as well as serum calcitonin and testosterone cause damage to the gastric mucosa, but not to the myocardium. Such a constellation of neuro-endocrine reactions to stressors determines the severity of damage to the gastric mucosa and myocardium by 73%. The purpose of this study is to find out metabolic and immune accompaniments of electrocardiogram and gastric mucosa parameters in naïve and stressed rats.

Material and methods. The experiment is at 18 male and 20 female Wistar rats. Over the 10 days, one animal remained intact and 3 other rats were exposed to WIRS. The next day after stressing, immune and metabolic parameters as well as ECG and gastric mucosa injuries was recorded.

Results. Serum levels of Phosphates, Catalase and α-LP Cholesterol as well as erythrocyte level of Potassium and Na,K-ATPase activity of the erythrocyte are positively correlated with ECG markers of myocardial damage, and negatively correlated with visual markers of damage to the gastric mucosa. Erythrocyte level of Sodium and serum levels of Potassium and Alkaline Phosphatase reflect the intactness of the gastric mucosa only. While serum level of Calcium reflects damage to the gastric mucosa. Taken together, the listed metabolic factors determine the morpho-functional state of the gastric mucosa and myocardium by 72% (R=0.851). Damage to the gastric mucosa and myocardium is more severe, the lower the bactericidal activity of blood neutrophils, and the greater the mass of the thymus. The spleen mass and the content of fibroblasts in the thymus are negatively correlated only with the severity of damage to the gastric mucosa, while the percentages of reticulocytes and lymphoblasts in the spleen are positively correlated with it. Finally, the higher the percentage of macrophages in the thymus, the deeper the damage to the myocardium. The canonical correlation between the listed immune parameters and markers of the two targets of stressors is very strong (R=0.809).

Conclusion. Water-immersion and restraint stress causes changes in the neuro-endocrine-immune complex, which lead to changes in the metabolome and damage to the gastric mucosa and myocardium.

Keywords: acute water-immersion and restraint stress, damage to the ECG and gastric mucosa, immunity, metabolome, relationships, rats.

Introduction

Earlier, by applying the water-immersion and restraint stress (WIRS) model, we [18] reproduced Selye's [15,26,68,71] primary attributes of stress: an increase in adrenal mass, an increase in corticosterone levels, damage to the gastric mucosa and myocardium, on the one hand, and Cannon's attributes [2,7,43-45,83,84]: an increase in the level of circulating catecholamines and sympathetic tone and a reciprocal decrease in vagal tone - on the other hand side. We found that the severity of the gastric mucosa damage significantly correlates with changes in ECG parameters, in particular, depression of the T wave and S-T joint, which indicate myocardial dystrophy. Further, it was found that such a connection is caused by the damaging effect on both targets of increasing the level of parathyroid hormone, as well as the production of aldosterone and catecholamines by enlarged adrenal glands. In addition, an increase in the level of corticosterone and sympathetic tone with a simultaneous decrease in

vagal tone as well as serum calcitonin and testosterone cause damage only to the gastric mucosa, but not to the myocardium. Such a constellation of neuro-endocrine reactions to stressors (cold, immobilization, hunger, etc.) determines the severity of damage to the gastric mucosa and myocardium by 73% [18].

As Dhabhar FS [11-13] aptly pointed out, stress is known to suppress immune function and increase susceptibility to infections and cancer. Paradoxically, stress is also known to exacerbate asthma, and allergic, autoimmune and inflammatory diseases, although such diseases should be ameliorated by immunosuppression. Moreover, the short-term fight-or-flight stress response is one of nature's fundamental defense mechanisms that enables the cardiovascular and musculoskeletal systems to promote survival, and it is unlikely that this response would suppress immune function at a time when it is most required for survival (e.g. in response to wounding and infection by a predator or aggressor). These observations suggest that stress may suppress immune function under some conditions while enhancing it under others. Author propose that it is important to study and, if possible, to clinically harness the immunoenhancing effects of the acute stress response, that evolution has finely sculpted as a survival mechanism, just as authors study its maladaptive ramifications (chronic stress) that evolution has yet to resolve. In view of the ubiquitous nature of stress and its significant effects on immunoprotection as well as immunopathology, it is important to further elucidate the mechanisms mediating stress-immune interactions and to meaningfully translate findings from bench to bedside.

It is well known that the immune system closely interacts with the nervous and endocrine systems through common mediators and receptors [33, 35,48,52,57,59,60,63,64,73,74,82] creating a triune neuro-endocrine-immune complex [3,4,30,34,65,66,67]. It is also known that stress effectors affect metabolism, and metabolites, in particular nitrogenous and electrolytes, in turn affect neurons, endocrinocytes, and immunocytes [22,67].

Based on the above, we set ourselves a goal: to find out metabolic and immune accompaniments of electrocardiogram and gastric mucosa parameters at naïve and post stressed rats.

Material and methods

Ethics approval

All animals were kept in room having temperature 22±2ºC, and relative humidity of 44-55% under 12/12 hours light and dark cycle with standard laboratory diet and water given ad libitum. Studies have been conducted in accordance with the rules and requirements of the “General Principles for the Work on Animals” approved by the I National Congress on Bioethics (Kyïv, Ukraine, 2001) and agreed with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine “On the Protection of Animals from Cruelty” of 26.02.2006. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Participants

The experiment is at 38 Wistar rats: 18 males (Weight Mean=227 g; SD=25 g) and 20 females (Mean=214 g; SD=27 g).

Study design and procedure

Due to the purposeful formation of groups, the potential predictors of post-stress reactions of the neuro-endocrine-immune complex and the metabolome [46] were almost identical both in mean values and, to a lesser extent, in variance (SD). In particular, the hypoxic test (sec) was: 136±59 and 133±81; swimming test (min): 19±11 and 19±17; HRV Stress index (units) as $(AMo/2•Mo•MxDMn)^{1/3}$: 0,14±0,08 and 0,14±0,05 in intact animals and those exposed to acute stress.

Over the 10 days, one animal remained intact and 3 other rats were exposed to water-immersion and restraint stress (WIRS) according to the method of Nakamura J et al. [51] in the modification of Popovych IL [62], which is to reduce the duration of stay of the rat in a fixed standing position in cold water (t^0 20-21^0 C) to the level of the xiphoid process from 8 to 4 hours. Prior to the experiments, rats were fasted for 24 h, but allowed access to tap water *ad libitum*.

The next day after stressing, a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of Leukocytogram (LCG), ie the percentage of lymphocytes (L), monocytes (M), eosinophils (E), basophils (B), rod-shaped (RN) and polymorphonucleary (PMNN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the equation derived by Popovych IL [21,22,61,66] on the basis of the classical Shannon's [69] equation:

$$hLCG = - (L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + B \bullet \log_2 B + RN \bullet \log_2 RN + PMNN \bullet \log_2 PMNN)/\log_2 6.$$

Than the ECG under light ether anesthesia was re-recorded in order to assess the state of the myocardium and HRV [2], and right away the animals removed from the experiment by decapitation in order to remove the stomach, adrenal glands, thymus, spleen, and collect the maximum possible amount of blood in which was determined some endocrine, metabolic, and immune parameters. The endocrine set was the subject of the previous article [18].

On lipid metabolism judged by the level of triglycerides (metaperiodate-acetylacetone colorimetric method), total cholesterol (direct method by reaction Zlatkis-Zach) and its distribution as part of α-lipoprotein (applied enzymatic method Hiller G [25]) after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as described in the manual [20]. State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract [19]) and malondyaldehide (test with thiobarbituric acid [1]), as well as the activity of antioxidant enzymes: catalase of serum and erythrocytes (by the speed of decomposition hydrogen peroxide [31]) and superoxide dismutase of erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH [14]). On electrolytes exchange judged by the level of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method) and chloride (mercury rodanide method) in the serum, sodium and potassium both in the serum and erythrocytes (flame photometry method) as described in the manual [20]. In addition, the activity of Na,K-ATPase of the shadows of erythrocytes was determined (by the increase of Pi in the supernatant of the incubation medium [42]).

Alanine and aspartate aminotranspherase, alkaline and acid phosphatase as well as creatine phosphokinase determined by uniform methods as described in the manual [20].

Use analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" ("Boehringer Mannheim", BRD) and flame spectrophotometer "СФ-47".

The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the amount of ulcers and their length was measured, evaluated erosive and ulcerative damage on scale by Popovych IL [62] (0÷1 points). This scale is based on the qualitative-quantitative Harrington EC [24] scale.

The parameters of immunity were determined, as described in the manual [58]. The percentage of theophylline-resistant (TR) and theophylline-susceptible (TS) T-lymphocytes, B-lymphocytes, plasma cells (Pla), and natural killers (NK) were identified. For these components the Entropy of the Immunocytogram (hICG) was calculated by Popovych IL [62] equation:

$$hICG = - (TR \bullet \log_2 TR + TS \bullet \log_2 TS + B \bullet \log_2 B + Pla \bullet \log_2 Pla + NK \bullet \log_2 NK + 0L \bullet \log_2 0L)/\log_2 6.$$

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes), the microbial count (number of microbes absorbed by one phagocyte) and the killing

index (percentage of dead microbes) for *Staphylococcus aureus* (ATCC N25423 F49). Based on these parameters, taking into account the absolute content of neutrophils and monocytes, their bactericidal capacity was calculated (BCC N&M) [5].

The Spleen and Thymus were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram [6,26]. The components of the Thymocytogram (TCG) are lymphocytes (Lc), lymphoblastes (Lb), reticulocytes (Ret), macrophages (Mac), basophiles (B), endotheliocytes (En), epitheliocytes (Ep), and Hassal's corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblastes (Lb), plasma cells (Pla), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi), and eosinophils (Eo).

For them Shannon's entropy was calculated too [21]:

$hTCG = - (Lc \cdot \log_2 Lc + Lb \cdot \log_2 Lb + Ret \cdot \log_2 Ret + Mac \cdot \log_2 Mac + B \cdot \log_2 B + En \cdot \log_2 En + Ep \cdot \log_2 Ep + H \cdot \log_2 H)/\log_2 8;$

$hSCG = - (Lc \cdot \log_2 Lc + Lb \cdot \log_2 Lb + Pla \cdot \log_2 Pla + R \cdot \log_2 R + Ma \cdot \log_2 Ma + F \cdot \log_2 F + Mi \cdot \log_2 Mi + Eo \cdot \log_2 Eo)/\log_2 8.$

Statistical analysis

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

Results and discussion

As a result of the analysis of the metabolic accompaniment of post-stress injuries, the following was found (Table 1).

Table 1. Correlation Matrix for Neuro-endocrine and Gastric mucosa&ECG variables

Variables	GU amount	GU length	GM damage	T wave	ST joint
Phosphates of Serum	**-0.766**	**-0.759**	**-0.728**	**0.595**	**0.439**
Catalase of Serum	-0.245	*ns*	-0.313	0.285	*ns*
K of Erythrocytes	-0.324	-0.224	-0.260	**0.415**	0.280
α-LP Cholesterol	*ns*	-0.274	-0.283	*ns*	0.287
Na of Erythrocytes	**-0.342**	**-0.440**	**-0.450**	*ns*	*ns*
Na,K-ATPase of Erythr	*ns*	*ns*	*ns*	0.310	0.241
Alkaline Phosphatase	-0.306	**-0.412**	**-0.405**	*ns*	*ns*
K of Serum	*ns*	-0.276	-0.310	*ns*	*ns*
Ca of Serum	*ns*	0.300	0.274	*ns*	*ns*

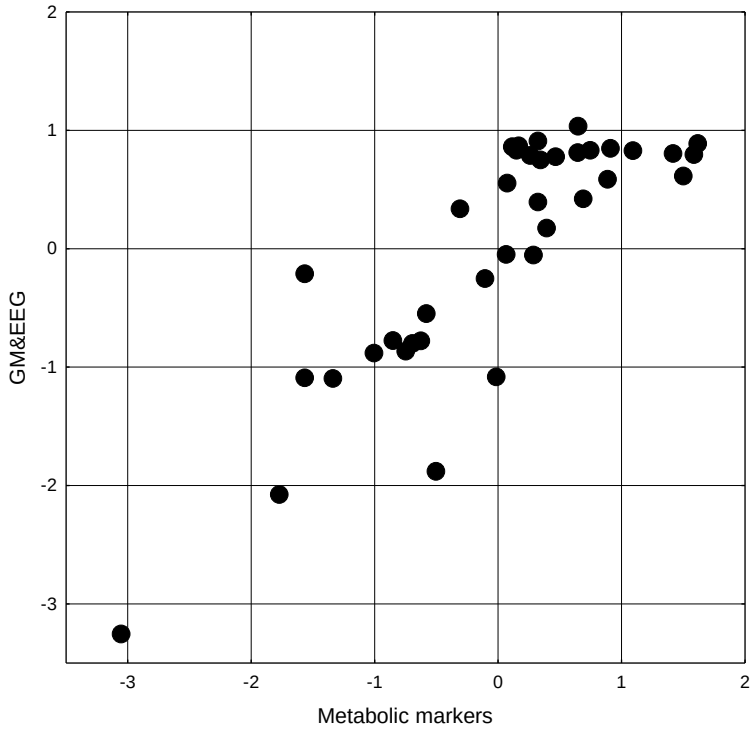
Note. According to the equation: $|r|=\{\exp[2t/(n-1.5)^{0.5}]-1\}/\{\exp[2t/(n-1.5)^{0.5}]+1\}$
for a sample of n=38 critical value |r| at $p<0.05$ ($t>2.02$) is **0.323,** at $p<0.01$ ($t>2.70$) is **0.420,** at $p<0.001$ ($t>3.55$) is **0.528**.

Serum levels of Phosphates, Catalase and α-LP Cholesterol as well as erythrocyte level of Potassium (as marker of Kalihistia) and Na,K-ATPase activity of the shadows of erythrocytes (as marker of membranes of myocardiocytes) are positively correlated with ECG markers of myocardial damage such as T wave amplitude and ST junction, and negatively correlated with visual markers of damage to the gastric mucosa, i.e. reflect intactness (normality) of both targets of stressors - myocardium and gastric mucosa. Erythrocyte level of Sodium (as marker of Natrihistia) and serum levels of Potassium and Alkaline Phosphatase reflect the intactness of the gastric mucosa only. While serum level of Calcium reflects damage to the gastric mucosa.

The canonical correlation between the listed metabolic parameters and state markers of the two targets of stressors is very strong (Table 2 and Fig. 1).

Table 2. Factor structure of Metabolic and Gastric mucosa&ECG Roots

Left set	**R**
Phosphates of Serum	**0.918**
Alkaline Phosphatase	**0.411**
Sodium of Erythrocytes	**0.407**
Potassium of Erythrocytes	**0.395**
Catalase of Serum	**0.362**
α-LP Cholesterol	**0.319**
Potassium of Serum	**0.311**
Calcium of Serum	**-0.270**
Na,K-ATPase of Erythrocytes	**-0.037**
Right set	**R**
Gastric Mucosa damage	**-0.961**
Gastric Ulcers amount	**-0.961**
Gastric Ulcers length	**-0.959**
ECG T wave	**0.752**
ECG ST joint	**0.585**



R=0.851; R^2=0.723; $\chi^2_{(45)}$=71; p=0,008; Λ Prime=0,090

Fig. 1. Scatterplot of canonical correlation between metabolic markers (X-line) and markers of state of gastric mucosa and myocardium (Y-line) at intact and stressed rats

In order to compare the results obtained by us with data from the literature, we consider it necessary to focus attention on the activity of alkaline phosphatase in view of its closest correlation with indicators of post-stressor damage to the gastric mucosa. Osteoblasts are considered the main source of this enzyme entering the blood; however, leukocytes and, especially, the epithelium of the gastrointestinal tract should not be neglected. The work of Stiel D et al. [70] is of greatest interest. A day after the subcutaneous administration of cysteamine to rats, the

authors noted the development of acute duodenal ulcers, which was accompanied by a significant decrease in the activity of enzymes involved in the secretion of bicarbonates by enterocytes - carbonic anhydrase and HCO_3^--activated ATPase, as well as - alkaline phosphatase, which, according to the authors, reflects the activity of the latter in the apical membranes of enterocytes. It is significant that the activity of other marker enzymes of neither the apical membrane nor intracellular organelles did not change, i.e., in this experimental ulcer model, there is no organelle pathology, but there is damage to the resistance of the duodenal mucosa, one of the markers of which is alkaline phosphatase, along with hypersecretion of acid by the stomach. Kuehl P et al. [36] believe that the loss of the activity of this enzyme in human duodenal enterocytes may be an early marker of the development of metaplasia of the gastric mucosa or, at least, a morphological manifestation of epithelial cell damage. Vetvik K et al. [78] found in patients with active duodenal ulcer an increase in the activity of alkaline phosphatase of the duodenal mucosa as a result of 4-week use of misoprostol - an analog of prostaglandin E1, known as a cytoprotector, compared to normal controls. The same group of authors [79] in another study showed that the activity of alkaline phosphatase increases in the duodenal mucosa of the same patients due to the use of omeprazole, an inhibitor of gastric H^+,K^+-ATPase. Mizunashi K et al. [49] in an in vitro study demonstrated that omeprazole also inhibits osteoresorption, which is known to be mediated by H^+-ATPase of osteoclasts, different from H^+,K^+-ATPase of parietal cells. In the same work, it was shown that omeprazole treatment of patients with gastric ulcer causes an increase in serum alkaline phosphatase activity associated with suppression of osteoresorption.

Therefore, the gastroprotective effect of omeprazole on osteoresorption is similar to that of calcitonin, which also has a gastroprotective ability, revealed in a number of experiments [8,10,16,17,23,39,54,77], including ours [18]. However, according to Ward TL et al. [81], the increase (under the influence of zeolite-A) of the activity of alkaline phosphatase in the serum of young pigs, associated with a decrease in the concentration of calcium and inorganic phosphorus in it (a marker of an increase in the level of calcitonin), does not protect animals from ulceration of the gastric mucosa.

Catalase showed both gastroprotective and cardioprotective effects. In the course of our project, the data on the antioxidant activity of adaptogens [55,56] are of particular interest. In particular, the efficacy of Kangfuxin liquid in WIRS-induced gastric ulcer at rats in the form of reduce the area of ulcers accompanied by improvement the pathological changes of ulcerated tissue: catalase (39%) as well as superoxide dismutase (58%) and malondialdehyde (54%) [38].

The metabolome, in turn, is determined by the constellation of neuro-endocrine effectors of stress (Table 3). In particular, enzymes as markers of cytolysis, α-LP Cholesterol, Potassium of serum and Sodium of erythrocytes are upregulated by Testosterone and Calcitonin while are downregulated by Corticosterone, Aldosterone, PTH and, probably, judging by the negative correlation with the Sex index, female sex hormones such as Progesterone and Estradiol.

Superoxide dismutase, Diene conjugates, Calcium and Sodium of serum as well as Potassium of erythrocytes and Na,K-ATPase of the shadows of erythrocytes are downregulated by Testosterone, Calcitonin, Catecholamines and Sympathetic tone while are upregulated by Corticosterone, Aldosterone, PTH, Vagal tone and, probably, female sex hormones. Catalase and Malondialdehyde are downregulated by Corticosterone and PTH while upregulated by Calcitonin and Vagal tone. Phosphates are downregulated by PTH and Calcitonin.

Table 3. Correlation Matrix for Neuro-Endocrine and Metabolic variables

Variables	Testosterone	Sex Index	Corticoster	Aldosterone	Adrenal mas	PTH	Calcitonin	1/ Mode	AMo	MxD Mn
Alkaline Phosphatase	**0.788**	**-0.834**	**-0.512**	**-0.498**	**-0.647**	ns	**0.484**	ns	ns	ns

Acid Phosphatase	**0.547**	**-0.548**	**-0.421**	**-0.342**	ns	**-0.393**	**0.466**	ns	ns	ns
Asparagine Transam	ns	-0.321	ns	**-0.342**	ns	ns	0.286	ns	ns	ns
K of Serum	**0.544**	**-0.606**	**-0.377**	**-0.790**	**-0.606**	**-0.471**	**0.330**	0.296	0.233	*ns*
Na of Erythrocytes	ns	ns	-0.239	-0.269	-0.311	ns	ns	ns	ns	ns
α-LP Cholesterol	**0.473**	**-0.432**	**-0.328**	**-0.377**	ns	**-0.477**	0.292	ns	ns	0.234
SOD of Erythrocytes	**-0.516**	**0.626**	0.291	**0.484**	**0.351**	ns	**-0.487**	ns	ns	ns
Diene conjugates	-0.312	**0.384**	ns	ns	0.314	ns	ns	-0.304	**-0.348**	0.321
Ca of Serum	**-0.676**	**0.744**	**0.399**	**0.525**	**0.538**	**0.771**	**-0.774**	*ns*	-0.243	*ns*
Na of Serum	**-0.385**	**0.504**	*ns*	**0.644**	**0.359**	**0.418**	**-0.434**	**-0.343**	**-0.348**	*ns*
K of Erythrocytes	ns	ns	ns	ns	0.292	ns	ns	-0.244	**-0.322**	**0.375**
Na,K-ATPase of Ery	ns	ns	ns	ns	ns	ns	ns	-0.276	-0.227	**0.424**
Catalase of Erythroc	0.223	ns	**-0.380**	ns	ns	-0.264	0.257	ns	ns	ns
Catalase of Serum	ns	ns	-0.267	ns	ns	ns	-0.256	ns	ns	ns
Malondialdehyde	ns	-0.260	-0.280	-0.285	ns	-0.283	0.315	ns	ns	**0.490**
Phosphates of Serum	*ns*	*ns*	*ns*	*ns*	*ns*	**-0.510**	**-0.357**	*ns*	*ns*	*ns*

As Elsenbruch S & Enck P [15] rightly note, the putative connection linking gender/sex and sex hormones to stress is undoubtedly highly complex yet intriguing and in need of more dedicated research in animal models, healthy humans, and patients with attention to effects across the life span. After all, many sex differences exist in the central and peripheral response to stress because of dimorphic brain development.

Therefore, quite expectedly, the sex index also appeared in the factor structure of the neuro-endocrine canonical root [18]. It is maximally negatively correlated with serum level of testosterone (r=-0.89), strongly correlated with calcitonin (r=-0.63) and bordering on significance with sympathetic tone (r=-0.31). Instead, the sex index is positively correlated with the mass of the adrenal glands (r=0.80) and serum levels of corticoids such as aldosterone (r=0.63) and corticosterone (r=0.47), as well as PTH (r=0.61). The sex index determines the levels of registered neuro-endocrine parameters by 88% [18].

This is explained by significantly higher levels of PTH (in our sample by 20%), corticosterone (by 37%) and, to a lesser extent, aldosterone (by 9%) in females than in males, as well as undoubtedly not recorded in this study estradiol and progesterone, instead drastically lower levels of testosterone and, to a lesser extent, calcitonin (by 40%) and sympathetic tone (by 20%).

At the same time, the role of other sex-linked neuro-endocrine factors (gonadotropic hormones and their releasing factors, etc.) and, in particular, the recently discovered sexual dimorphism of a number of EEG parameters in humans [32] is quite likely. From the above, it becomes clear why the sex index correlates with metabolic parameters similarly to PTH and aldosterone, on the contrary, with sympathetic tone.

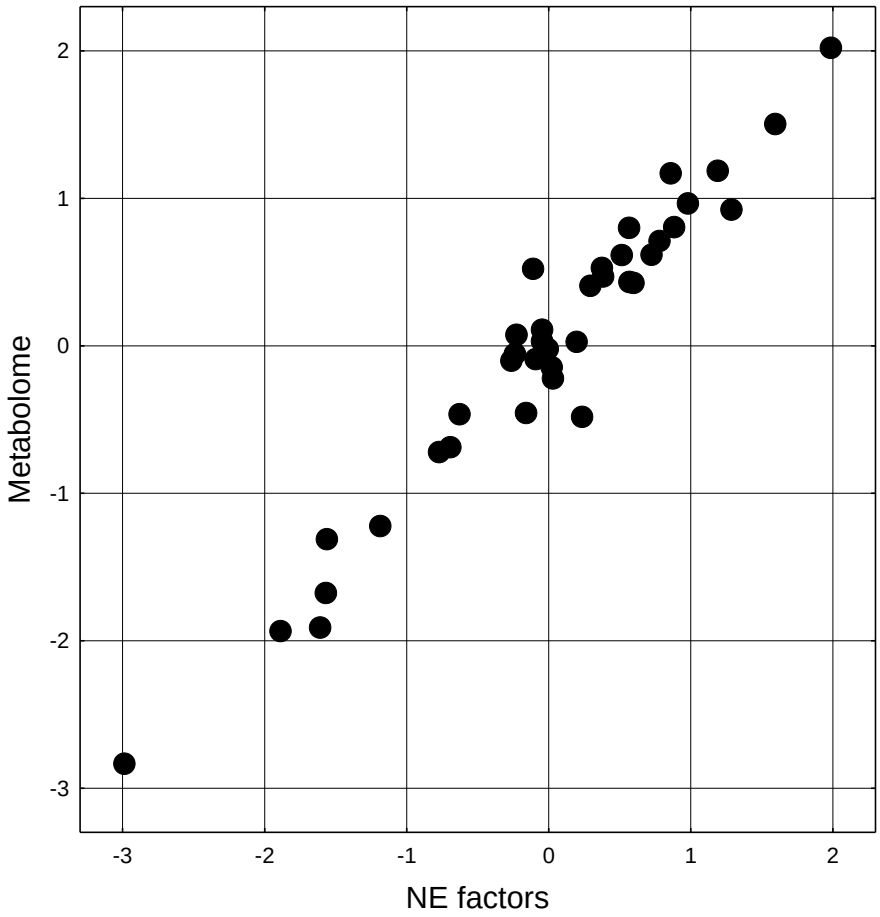
Keshavarzi Z et al. [28] shown that the Glutathione (GSH) concentration significantly decreased after induction of gastric ischemia-reperfusion (IR) in male rats. Estradiol and combined estradiol and progesterone significantly increased GSH levels. The myeloperoxidase (MPO) concentration significantly increased after induction of gastric IR. Different treatments significantly reduced MPO levels. The gastric acid concentration significantly increased after induction of gastric IR. Treatment with estradiol, progesterone and combined estradiol and

progesterone significantly reduced gastric acid levels. Superoxide dismutase (SOD) concentration decreased after induction of gastric IR. The SOD levels were not significant. The authors concluded that female sexual steroids have a therapeutic effect on gastrointestinal ischemic disorders (which, as is known, are manifested by damage to the mucosa [84]) by reduction of MPO and gastric acid, and increasing gastric GSH & SOD levels following gastric IR.

As a result of the canonical correlation analysis between Neuro-Endocrine and Metabolic variables, two pairs of canonical roots were obtained. It was found that the hormonal constellation and Vagal tone determine the state of metabolism by 95% (Table 4 and Fig. 2).

Table 4. Factor structure of first pair of Neuro-Endocrine and Metabolic Roots

Left set	**R1**
Parathyroid hormone	**-0,831**
Aldosterone	**-0,510**
Sex Index (M=1;F=2)	**-0,416**
Corticosterone	**-0,350**
Adrenals mass	**-0,294**
Calcitonin	**0,457**
Testosterone	**0,369**
MxDMn HRV as Vagal tone	**0,286**
Right set	**R1**
Potassium of Serum	**0,798**
Phosphates of Serum	**0,792**
α-LP Cholesterol	**0,502**
Alkaline Phosphatase	**0,488**
Malondialdehyde	**0,356**
Acid Phosphatase	**0,292**
Potassium of Erythrocytes	**0,287**
Sodium of Erythrocytes	**0,275**
Calcium of Serum	**-0,804**
Superoxide dismutase of Erythrocytes	**-0,543**
Sodium of Serum	**-0,334**
Diene conjugates	**-0,156**



R=0.974; R^2=0.949; $\chi^2_{(130)}$=237; p<10^{-6}; Λ Prime<10^{-4}

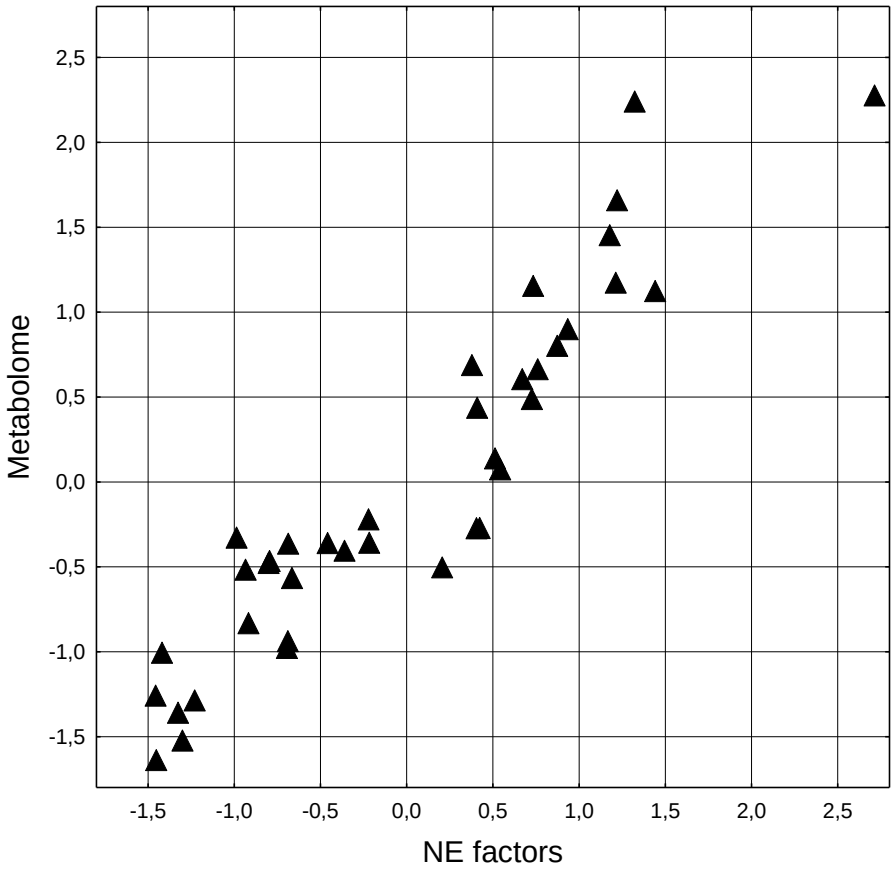
Fig. 2. Scatterplot of canonical correlation between neuro-endocrine parameters (X-line) and parameters of metabolome (Y-line) at intact and stressed rats. First pair of Roots

The metabolic constellation of the second pair is determined by hormones and Sympathetic tone by 87% (Table 5 and Fig. 3).

Table 5. Factor structure of second pair of Neuro-Endocrine and Metabolic Roots

Left set	**R2**
Sex Index (M=1;F=2)	**-0,866**
Adrenals mass	**-0,613**
Aldosterone	**-0,546**
1/Mode HRV as Catecholamines	**-0,416**
Corticosterone	**-0,365**
Parathyroid hormone	**-0,302**
Testosterone	**0,739**
Calcitonin	**0,565**
AMo HRV as Sympathetic tone	**0,460**
Right set	**R2**
Potassium of Serum	**0,800**
Alkaline Phosphatase	**0,696**
Acid Phosphatase	**0,533**
α-LP Cholesterol	**0,490**
Asparagine Transaminase	**0,283**
Malondialdehyde	**0,226**
Catalase of Erythrocytes	**0,133**
Sodium of Serum	**-0,526**

Superoxide dismutase of Erythrocytes	**-0,523**
Calcium of Serum	**-0,500**
Phosphates of Serum	**-0,458**
Potassium of Erythrocytes	**-0,415**
Diene conjugates	**-0,343**
Catalase of Serum	**-0,136**
Na,K-ATPase of Erythrocytes	**-0,110**



R=0.935; R^2=0.873; $\chi^2_{(108)}$=162; p=0.0006; Λ Prime=0.002

Fig. 3. Scatterplot of canonical correlation between neuro-endocrine parameters (X-line) and parameters of metabolome (Y-line) at intact and stressed rats. Second pair of Roots

Screening of correlations between immunity parameters and markers of morpho-functional state of the gastric mucosa and myocardium revealed the following (Table 6).

Table 6. Correlation Matrix for Immune and Gastric mucosa&ECG variables

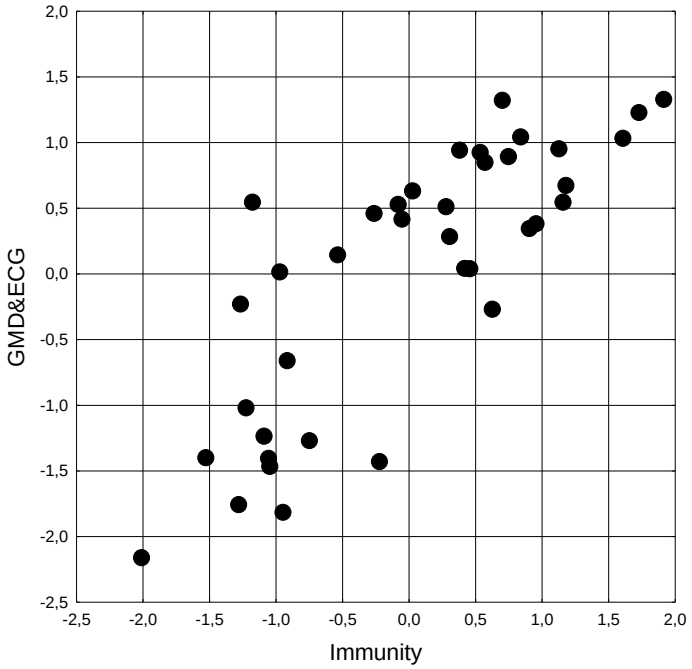
Variables	GU amount	GU length	GM damage	ST joint	T wave
Killing Index of Neutrophils	**-0.392**	**-0.504**	**-0.429**	0.236	0.213
Spleen Mass	*ns*	-0.226	**-0.331**	*ns*	*ns*
Reticulocytes of Spleen	**0.335**	**0.388**	**0.427**	*ns*	*ns*
Lymphoblastes of Spleen	*ns*	0.213	0.251	*ns*	*ns*
Thymus Mass	0.276	0.263	*ns*	-0.237	*ns*
Fibroblastes of Thymus	-0.245	*ns*	*ns*	*ns*	*ns*

Macrophages of Thymus	*ns*	*ns*	*ns*	-0.208	*ns*

Damage to the gastric mucosa and myocardium is more severe, the lower the bactericidal activity of blood neutrophils (the completion of phagocytosis of *Staph. aureus*), and the greater the mass of the thymus. The spleen mass and the content of fibroblasts in the thymus are negatively correlated only with the severity of damage to the gastric mucosa, while the percentages of reticulocytes and lymphoblasts in the spleen are positively correlated with it. Finally, the higher the percentage of macrophages in the thymus, the lower the ST junction, that is, the deeper the damage to the myocardium. Taken together, the listed immune factors determine the morpho-functional state of the gastric mucosa and myocardium by 65% (Table 7 and Fig. 4).

Table 7. Factor structure of Immune and Gastric mucosa&ECG Roots

Left set	**R**
Killing Index of Neutrophils	**0.602**
Spleen Mass	**0.234**
Fibroblastes of Thymus	**0.217**
Macrophages of Thymus	**0.155**
Reticulocytes of Spleen	**-0.517**
Thymus mass	**-0.433**
Lymphoblastes of Spleen	**-0.323**
Right set	**R**
GU length	**-0.932**
GM damage	**-0.881**
GU amount	**-0.864**
ST joint ECG	**0.470**
T wave ECG	**0.398**



R=0.809; R^2=0.654; $\chi^2_{(40)}$=74; p=0.0008; Λ Prime=0.084

Fig. 4. Scatterplot of canonical correlation between immune parameters (X-line) and parameters of gastric mucosa&ECG (Y-line) at intact and stressed rats

From a formal/mathematical point of view, it appears that four immune factors are **protective (stress-limiting)**, while the other three are responsible for **enhance** of post-stress injuries. According to an alternative interpretation, both immune parameters and morpho-functional state of the gastric mucosa&myocardium parameters together are subject to the regulatory influence of neuro-endocrine factors of acute stress. In other words, the detected immune parameters are only *companions*, but not *causal factors* of the morpho-functional state of the gastric mucosa&myocardium in naïve and stressed rats.

The solution to this problem is possible only by analyzing functional relationships supported by correlations (Tabl. 8).

Table 8. Correlation Matrix for Neuroendocrine, gastric mucosa&ECG and Immune variables

Variables	Testo-stero-ne	Calci-tonin	Vagal tone	Sym-patho-tone	Cate-chola-mines	Corti-coste-rone	Aldo-stero-ne	PTH	Sex Index	Adre-nals mass
Killing Ind Neutrophils	**0.430**	0.286	*0.204*	*ns*	*ns*	-0.291	-0.280	**-0.403**	**-0.509**	*ns*
Spleen Mass	**0.329**	*ns*	**0.459**	-0.274	*ns*	**-0.556**	-0.288	*ns*	**-0.339**	*ns*
Macrophages Thymus	**0.332**	0.244	*ns*	**0.415**	0.254	*ns*	*ns*	*-0.234*	-0.253	*ns*
Fibroblastes Thymus	*-0.201*	*ns*	**-0.411**	*ns*	*ns*	0.282	*ns*	*ns*	*0.220*	*ns*
Microbial Count Neutr	*ns*	0.315	*-0.219*	*0.221*	0.282	**-0.683**	*ns*	*-0.203*	-0.289	*ns*
Phagocytose Ind Neutr	**0.427**	**0.636**	*ns*	*ns*	*ns*	*ns*	-0.251	*ns*	*ns*	-0.283
B-Lymphocytes Blood	**0.321**	0.237	*ns*	0.233	**0.515**	*ns*	-0.216	*-0.212*	-0.276	*ns*
Eosinophiles Spleen	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	**-0.440**	*ns*	*ns*	*ns*
Reticulocytes Spleen	**-0.413**	*-0.222*	*-0.224*	*ns*	*ns*	0.281	0.311	0.319	**0.325**	*ns*
Thymus Mass	-0.241	**-0.435**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.295
Lymphoblastes Spleen	*ns*	*-0.210*	*-0.214*	*ns*	0.262	*ns*	*ns*	*0.226*	*ns*	*ns*
Monocytes Blood	-0.246	**-0.380**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Bactericidity Monocyte	-0.306	-0.310	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	**0.356**
NK-Lymphocyte Blood	*ns*	0.236	*ns*	*ns*	*0.226*	*ns*	*ns*	**-0.331**	*ns*	*ns*
Th T-Lymphoc Blood	*ns*	*ns*	*ns*	*ns*	*ns*	*0.234*	*ns*	*ns*	*ns*	*ns*
Tc T-Lymphoc Blood	*ns*	*ns*	*ns*	*ns*	0.249	*-0.215*	*ns*	*ns*	*ns*	*ns*
Gastric Ulcers Amount	*ns*	-0.229	*ns*	0.253	*0.188*	*ns*	0.226	**0.584**	*ns*	0.236
Gastric Ulcers Length	*-0.213*	-0.283	-0.230	*0.214*	*ns*	*0.206*	0.275	**0.621**	0.280	**0.384**
Gastric mucosa Damage	*-0.204*	-0.251	-0.283	0.273	*ns*	0.225	0.302	**0.516**	0.272	**0.391**
T wave ECG	*ns*	*ns*	*ns*	*ns*	**-0.356**	*ns*	*ns*	**-0.351**	*ns*	*ns*
S-T joint ECG	*ns*	*ns*	*ns*	*ns*	-0.284	*ns*	*ns*	**-0.342**	*ns*	*-0.192*

Note. Color highlighted Immune variables that **limited** or **enhanced** of post-stress injuries (see Table 6).

Let's limit ourselves to the most illustrative example, such as the bactericidal activity of blood neutrophils. It is known that phagocytosis is not an isolated cell response. It usually occurs together with other cell responses, including formation of reactive oxygen species

(ROS), secretion of pro-inflammatory mediators and production of cytokines [76]. The effect of ROS depends on the production site. Intracellular ROS suppressed IL-1β expression in these neutrophils, while extracellular ROS amplified IL-1β secretion. Production of extracellular H_2O_2 may thus affect cells of the surrounding tissue. Excessive neutrophil activity may cause tissue damage [50].

Back in 1992 Taché Y & Saperas E [72] showed that IL-1β is one of the most potent centrally acting inhibitors of gastric acid secretion in rats. Sites of action have been located in the anterior/preoptic area and paraventricular nucleus of the hypothalamus where other biological activities of IL-1 have also been described. IL-1β action is, so far, quite unique to this cytokine and its action is not reproduced by IL-2 or TNF-α. The IL-1 effect involves prostaglandin pathways and is unrelated to CRF. Similarly, systemic *injection* of IL-1 induces a long lasting inhibition of acid secretion through prostaglandin-dependent mechanisms. Several findings support the possibility that the effect of systemic IL-1 can be CNS-mediated and/or exerted at the periphery through local release of prostaglandin in the stomach. Exogenous IL-1 given into either the circulation or the cerebrospinal fluid also inhibits gastric injury induced by a variety of experimental models (stress, aspirin, ethanol). Such a protective effect is mediated through the inhibition of acid secretion and prostaglandin release, although other mechanisms may also contribute. Authors concluded: whether *endogenously* released IL-1β exerts a protective role in the gastric mucosa is still to be investigated.

In the same year, Uehara A et al. [75] found that in rats the *central* administration of IL-1 dose-dependently suppressed the development of gastric mucosal lesions induced by WIRS. These results clearly demonstrated that IL-1 has potent antiulcer (and antisecretory) effects that are mediated by the *central nervous* system. Moreover, these findings suggest that there may exist an "immune-brain-gut" axis, which is involved in the regulation of gastric secretion and mucosal homeostasis, especially under certain pathophysiological conditions that activate the immune system to release various cytokines including IL-1.

Hence, we assume that the negative correlation of neutrophil bactericidality with markers of gastric mucosa injury and the positive correlation with T wave and ST joint ECG reflects not the direct effect of factors released by them, at least IL-1, on the gastric mucosa and myocardium, but the upregulating influence of IL-1 on the anterior/preoptic area and paraventricular nucleus of the hypothalamus with subsequent indirect ***upregulation*** of serum levels of Testosterone and Calcitonin and Vagal tone, while ***downregulation*** of Corticosterone, Aldosterone, PTH and, probably, judging by the negative correlation with the Sex index, female sex hormones such as Estradiol and Progesterone, which, in turn, have a ***limiting*** or ***enhancing*** effect on post-stress injuries of the gastric mucosa and myocardium.

At the same time, an alternative hypothesis can be put forward that the neuroendocrine response caused by acute stress factors (immobilization, cooling, starvation, etc.) modulates the production of IL-1 by blood neutrophils (as well as thymic macrophages and blood B lymphocytes), which acts on the gastric mucosa and/or myocardium together with stress hormones and neurotransmitters. In particular, Testosterone, Calcitonin and Vagal tone have gastroprotective effect, while Sympathetic tone, Catecholamines, Corticosterone, Aldosterone, PTH and, probably, female sex hormones have gastro-and cardioaltering effects.

It should be noted that there is evidence of an adverse effect of both testosterone and interleukins on the gastric mucosa of rats.

The already mentioned Lu S et al. [38] showed that the efficacy of Kangfuxin liquid in WIRS-induced gastric ulcer in rats in the form of reducing the area of ulcers accompanied by inhibition of inflammatory reactions: decrease in TNF-α (9%) and IL-6 (11%) levels.

Konturek PC et al. [29] shown that caused by LPS injection the rise in plasma IL-1β and TNF-α levels resulted in a *delay* in ulcer healing associated with a significant decrease in gastric blood flow.

Machowska A et al. [40] compared the effects of major male hormone, testosterone, and female hormone, progesterone, on the healing of gastric ulcers induced by acetic acid technique in male rats with intact or removed testicles (testectomy) and female rats with intact or removed ovaries (ovariectomy). The authors shown that the area of gastric ulcers in placebo-control rats decreased significantly at day 7 upon ulcer induction and this effect was significantly *accelerated* by testectomy or ovariectomy. In contrast, testosterone significantly *delayed* ulcer healing while producing a significant fall in the gastric blood flow determined at the margin of ulcer. Treatment with progesterone significantly accelerated ulcer healing and increased the gastric blood flow. Testosterone applied alone or supplemented in testectomized animals produced the significant increment in plasma IL-1β levels as compared to the respective levels of this cytokine in placebo-control animals. The authors concluded that: 1) major male (testosterone) and female (progesterone) sex hormones exhibit opposite effect on healing of preexisting ulcers in the stomach because testosterone markedly delayed while progesterone significantly accelerated this healing; 2) testosterone-induced delay in ulcer healing involves the fall in the gastric microcirculation at the margin of gastric ulcers and the excessive production and release of proinflammatory cytokine IL-1β; and 3) testectomy improves the gastric ulcer healing due to inhibition of gastric acid secretion and the rise in plasma gastrin, which exerts gastroprotective, trophic and ulcer healing action on the gastric mucosa.

Later Machowska A et al. [41] studied the effects of depletion of testosterone on the healing of acetic acid-induced ulcers at rats. The authors shown that testosterone (0.01-10 mg/kg/day i. m.) dose-dependently *delayed* gastric ulcer healing. When applied in an optimal dose of 1 mg/kg/day, this hormone significantly raised gastric acid secretion and plasma IL-1β and TNF-α levels. Attenuation of plasma testosterone levels via bilateral orchidectomy inhibited gastric acid secretion and accelerated the healing of gastric ulcers, while increasing plasma gastrin levels and these effects were reversed by testosterone. The authors propose that testosterone delays ulcer healing via a fall in blood flow at the ulcer margin, a rise in plasma levels of IL-1β and TNF-α and, an increase in gastric acid secretion.

Wang M et al. [80] have shown that male myocardium demonstrates greater loss in cardiac function in the presence of a given TNF level compared to female. In addition, estrogen has little influence on reducing TNF-caused myocardial dysfunction in female hearts, suggesting that male hormone - testosterone may be responsible for gender differences in TNF-mediated myocardial damage. Later the authors shown on isolated mouse hearts that TNF infusion significantly depressed left ventricular developed pressure, but not heart rate in males. Myocardial rate pressure product (RPP=LVDP•HR) was markedly decreased in male hearts compared to females in exposure to TNF, which was associated with higher levels of TNF-induced caspase-8&3. Importantly, depletion of endogenous testosterone by castration or blockade of androgen receptor by flutamide treatment abolished TNF-decreased RPP in male hearts. However, castration or flutamide treatment did not affect TNF production and myocardial expression of TNFR1 and TNFR2. The authors concluded that testosterone is critical to the gender difference in TNF-induced detrimental effects on myocardium.

However, a systematic review and meta-analysis shown that a lower total testosterone level was associated with a higher risk of cardiovascular disease mortality and all-cause mortality in males with chronic kidney disease CKD [53].

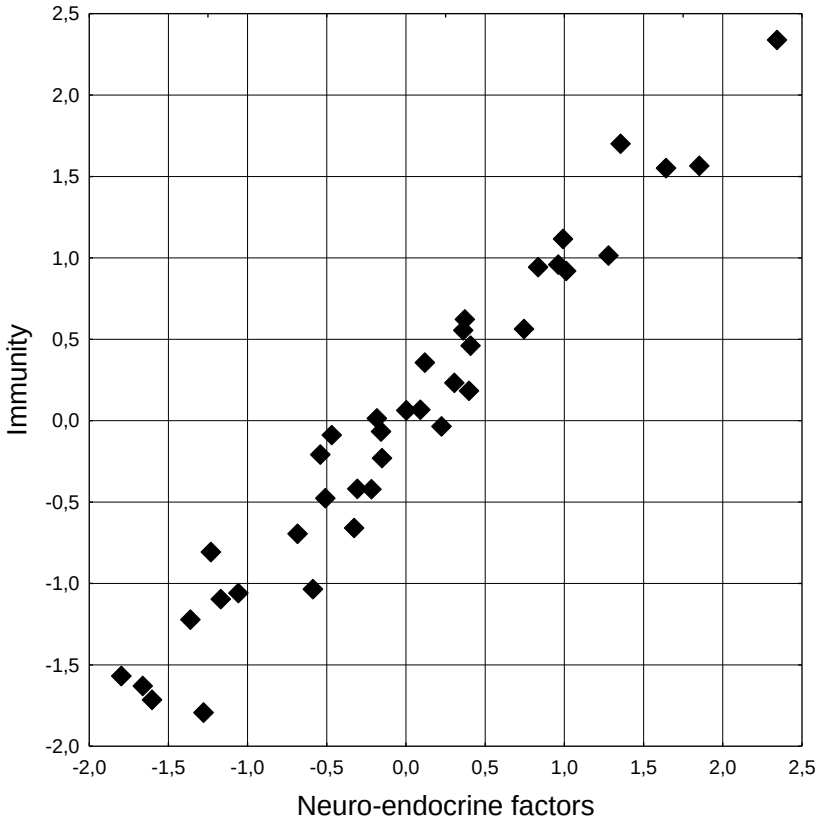
Regarding our research, the facts should be given that in a mouse model of ischemia-reperfusion injury, intraperitoneal treatment with CGRP significantly reduced gastric mucosal edema, hemorrhage, apoptosis, mucosal separation and inflammatory cell infiltration. It is known that after stimulation, capsaicin-sensitive sensory nerve fibers may release CGRP, and then CGRP increases the levels of prostacyclin and prostaglandin E2 in gastric mucosa, thereby inhibiting the activation of neutrophils and degranulation of mastocytes, reducing the secretion of inflammatory mediators (*e.g.*, histamine), and alleviating gastrointestinal inflammation [84].

Interestingly, a similar mechanism underlies the carbon monoxide-induced gastroprotection against stress ulcerogenesis. The exposure of rats to 3.5 h of WIRS resulted in numerous hemorrhagic gastric lesions and significantly decreased the gastric blood flow, raised MDA content and significantly decreased the mucosal SOD and GSH contents compared with intact gastric mucosa, and these changes were exacerbated in rats with capsaicin denervation. The sensory nerve endings releasing CGRP can contribute, at least in part, to the CO-induced gastric hyperemia, the attenuation of gastric mucosal lipid peroxidation and prevention of oxidative stress and stress ulcerogenesis [37].

In conclusion, in order to create a complete picture, we consider it necessary to present our own illustration of the neuroendocrine-immune complex [3,4] created on the basis of the correlation matrix for neuroendocrine and immune variables (Table 8). Despite moderate pairwise correlations, the canonical correlation between neuroendocrine and immune variables turns out to be very strong (Tables 9 and 10, Figs. 5 and 6)

Table 9. Factor structure of first pair of Neuroendocrine and Immune Roots

Left set	**Root 1**
Corticosterone	**-0.753**
Parathyroid hormone	**-0.483**
Sex Index (M=1; F=2)	**-0.430**
Adrenals mass	**-0.300**
1/Mode HRV as Catecholamines	**-0.226**
AMo HRV as Sympathetic tone	**-0.193**
Aldosterone	**-0.165**
Testosterone	**0.273**
MxDMn HRV as Vagal tone	**0.330**
Calcitonin	**0.529**
Right set	**Root 1**
Fibroblastes of Thymus	**-0.372**
Theophylline-resistant T-Lymphocytes	**-0.257**
Thymus mass	**-0.247**
Lymphoblastes of Spleen	**-0.205**
Reticulocytes of Spleen	**-0.188**
Eosinophiles of Spleen	**0.229**
Killing Index of Neutrophils	**0.312**
Phagocytosis Index of Neutrophils	**0.383**
Spleen Mass	**0.469**
Microbial Count of Neutrophils	**0.636**

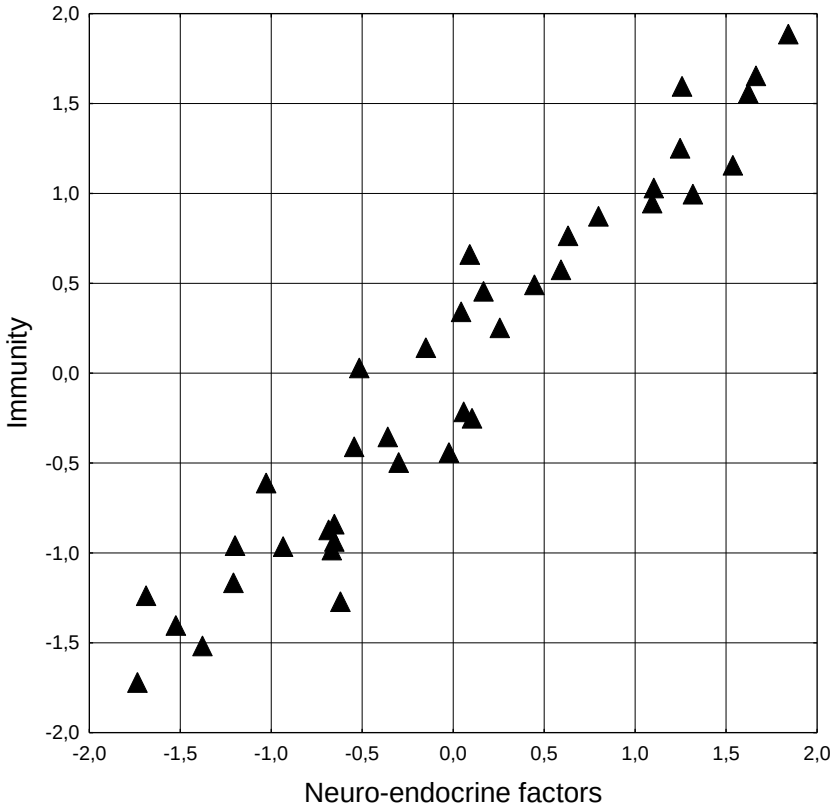


R=0.976; R^2=0.952; $\chi^2_{(160)}$=284; p<10^{-6}; Λ Prime<10^{-5}

Fig. 5. Scatterplot of canonical correlation between Neuroendocrine (X-line) and Immune (Y-line) parameters at intact and stressed rats. *First pair of Roots*

Table 10. Factor structure of second pair of Neuroendocrine and Immune Roots

Left set	**Root 2**
Parathyroid hormone	**0.549**
Sex Index (M=1; F=2)	**0.389**
1/Mode HRV as Catecholamines	**0.237**
Adrenals mass	**0.206**
Calcitonin	**-0.289**
Corticosterone	**-0.312**
Testosterone	**-0.370**
MxDMn HRV as Vagal tone	**-0.424**
Right set	**Root 2**
Thymus mass	**0.486**
Microbial Count of Neutrophils	**0.463**
Monocytes of Blood	**0.456**
Lymphoblastes of Spleen	**0.281**
Theophylline-susceptible T-Lymphocytes	**0.258**
Bactericidal Capacity of Monocytes	**0.221**
Macrophages of Thymus	**-0.179**
Theophylline-resistant T-Lymphocytes	**-0.242**
Phagocytosis Index of Neutrophils	**-0.250**
B-Lymphocytes of Blood	**-0.261**
Killing Index of Neutrophils	**-0.328**
NK-Lymphocytes of Blood	**-0.372**



$R=0.962$; $R^2=0.926$; $\chi^2_{(135)}=212$; $p<10^{-4}$; Λ Prime$<10^{-4}$

Fig. 6. Scatterplot of canonical correlation between Neuroendocrine (X-line) and Immune (Y-line) parameters at intact and stressed rats. *Second pair of Roots*

The morpho-functional basis of the relationships between the three systems of the complex are: receptors for hormones, neurotransmitters, and neuropeptides in immune cells; receptors for immune cytokines in endocrine glands; receptors for immune cytokines in the nervous system; neuro-endocrine agents in lymphoid organs; immune cytokines in endocrine and nervous systems; endocrine effects on the immune system; neural effects on the immune system; immune mechanisms that can be affected by neuro-endocrine agents; effects of immune-derived products on endocrine mechanisms; effects of immune-derived products on the nervous system; metabolic effects of cytokines [4].

Conclusion

Water-immersion and restraint stress causes changes in the neuro-endocrine-immune complex, which lead to changes in the metabolome and damage to the gastric mucosa and myocardium.

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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. Baevsky RM, Berseneva AP. *Use KARDIVAR system for determination of the stress level and estimation of the body adaptability*. Standards of measurements and physiological interpretation. Moscow-Prague;2008:41.
3. Besedovsky H & Sorkin E. Network of immune-neuroendocrine interaction. *Clin Exp Immunol*. 1977.

27(1):1-12. PMCID: PMC1540898
4. Besedovsky H & del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocrine reviews.* 1996. 17(1), 64–102. https://doi.org/10.1210/edrv-17-1-64
5. 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.
6. Bilas VR, Popadynets' OO, Flyunt ISS, et al. Entropies of thymocytogram, splenocytogram, immunocytogram and leukocytogram in rats are regulated by sex and the neuroendocrine parameters while regulates immune parameters. *JEHS*. 2020;10(7):266-288.
7. Berger EN. *Neurohumoral Mechanisms of Disturbed Tissue Trophism* [in Russian]. Kyïv: Zdorovya;1980:104.
8. Clementi G, Caruso A, Fiore CE, et al. Effect of parathyroid hormone, centrally or peripherally injected, on gastric activity in male rats. *Eur J Pharmacol*. 1989;166(3):549-552. doi: 10.1016/0014-2999(89)90374-9.
9. Castle C, Tietjens J. Perforated gastric ulcer as the initial manifestation of hyperparathyroidism. *BMJ Case Rep*. 2021;14(4):e240570. doi: 10.1136/bcr-2020-240570.
10. Clementi G, Prato A, Rapisarda E, et al. Inhibition of restraint stress by systemic (Asu1,7) eel-calcitonin. *J Endocrinol Invest*. 1985;8(6):543-546. doi: 10.1007/BF03348558.
11. Dhabhar FS, McEwen BS. Acute stress enhances while chronic stress suppresses immune function in vivo: a potential role for leukocyte trafficking. *Brain Behav Immun*. 1997;11:286–306.
12. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *NeuroImmunoModulation*. 2009;16:300–317.
13. Dhabhar FS. The short-term stress response – mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Front Neuroendocrinol*. 2018;49:175–192.
14. Dubinina YY, Yefimova LF, Sofronova LN, Geronimus AL. Comparative analysis of the activity of superoxide dismutase and catalase of erythrocytes and whole blood from newborn children with chronic hypoxia [in Russian]. *Laboratornoye Delo.* 1988;8:16-19.
15. Elsenbruch S, Enck P. The stress concept in gastroenterology: from Selye to today. F1000Res. 2017;6:2149. doi: 10.12688/f1000research.12435.1.
16. Evangelista S. Role of calcitonin gene-related Peptide in gastric mucosal defence and healing. *Curr Pharm Des*. 2009;15(30):3571-3576. doi: 10.2174/138161209789207024.
17. Evangelista S, Renzi D. A protective role for calcitonin gene-related peptide in water-immersion stress-induced gastric ulcers in rats. *Pharmacol Res*. 1997;35(4):347-350. doi: 10.1006/phrs.1997.0142.
18. Fihura OA, Melnyk OI, Ruzhylo SV, et al. Relationships between neuro-endocrine, electrocardiogram, and gastric mucosal damage parameters in naïve and stressed rats. Journal of Education, Health and Sport. 2023; 18(1): 162-190.
19. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of plasma levels of lipid hydroperoxides [in Russian]. *Laboratornoye Delo.* 1983;3:33-36.
20. Goryachkovskiy AM. Clinical biochemistry [in Russian]. Odesa: Astroprint,1998:608.
21. Gozhenko AI, Korda MM, Popadynets OO, Popovych IL. *Entropy, Harmony, Synchronization and Their Neuro-Endocrine-Immune Correlates* [in Ukrainian]. Odesa: Feniks;2021:232.
22. Gozhenko AI, Korda MM, Smagliy SS, et al. *Uric Acid, Metabolism, Neuro-Endocrine-Immune Complex* [in Ukrainian]. Odesa: Feniks;2023:266. doi.org/10.5281/zenodo.7575158
23. Guidobono F, Netti C, Pagani F, et al. Effect of unmodified eel calcitonin on gastric acid secretion and gastric ulcers in the rat. *Farmaco*. 1991;46(4):555-563.
24. Harrington EC. The Desirability Function. *Industrial Quality Control.* 1965;21:494-498.
25. Hiller G. Test for the quantitative determination of HDL cholesterol in EDTA plasma with Reflotron®. *Klin Chem.* 1987;33:895-898.
26. Horizontov PD, Belousova BI, Fedotova MI. *Stress and the Blood System* [in Russian]. Moskva: Meditsina;1983:240.
27. Hotta H, Onda A, Suzuki H, Milliken P, Sridhar A. Modulation of Calcitonin, Parathyroid Hormone, and Thyroid Hormone Secretion by Electrical Stimulation of Sympathetic and Parasympathetic Nerves in Anesthetized Rats. *Front Neurosci*. 2017;11:375. doi: 10.3389/fnins.2017.00375.
28. Keshavarzi Z, Mohebbati R, Mohammadzadeh N, Alikhani V. The protective role of estradiol & progesterone in male rats, following gastric ischemia-reperfusion. *Acta Endocrinol (Buchar)*. 2018;14(1):30-35. doi: 10.4183/aeb.2018.30.
29. Konturek PC, Brzozowski T, Konturek SJ, et al. Influence of bacterial lipopolysaccharide on healing of chronic experimental ulcer in rat. Scand J Gastroenterol. 2001 Dec;36(12):1239-47. doi: 10.1080/003655201317097065. PMID: 11761011.

30. Korneva EA. Pathways of neuro-immune communication: past and present time, clinical application [in Russian]. *Meditsinskaya Immunologiya.* 2020;22(3):405-418. doi:10.15789/1563-0625-PON-1974.
31. Korolyuk MA, Ivanova MI, Mayorova IG, Tokarev VYe. The method for determining the activity of catalase [in Russian]. *Laboratornoye Delo.* 1988;1:16-19.
32. Kozyavkina NV, Popovych IL, Popovych DV, et al. Sexual dimorphism in some psycho-neuro-endocrine parameters at human. *JEHS*. 2021;11(5):370-391.
33. Kozyavkina OV. The state of post-stress parameters of autonomic homeostasis and endocrine, metabolic and immune status and the relationship between them in rats with alternative types of pre-stress autonomic homeostasis induced by bioactive water Naftussya [in Ukrainian]. *Medical Hydrology and Rehabilitation*. 2009;7(2):40-56.
34. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, et al. *Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex* [in Ukrainian]. Kyïv: UNESCO-SOCIO;2015:349.
35. Kulchynskyi AB, Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017;7(3):53-71.
36. Kuehl P, Baczako K, Stanescu A, Malfertheimer P. Loss of alkaline phosphatase activity in duodenal mucosa: a marker for precursorsof gastric metaplasia? J Pathol. 1990. 162(4):317-322.
37. Kwiecien S, Magierowska K, Magierowski M, et al. Role of sensory afferent nerves, lipid peroxidation and antioxidative enzymes in the carbon monoxide-induced gastroprotection against stress ulcerogenesis. J Physiol Pharmacol. 2016 Oct;67(5):717-729. PMID: 28011952.
38. Lu S, Wu D, Sun G, et al. Gastroprotective effects of Kangfuxin against water-immersion and restraint stress-induced gastric ulcer in rats: roles of antioxidation, anti-inflammation, and pro-survival. *Pharmaceutical biology.* 2019;57(1):770–777. doi.org/10.1080/ 13880209. 2019.1682620
39. Luo XJ, Liu B, Dai Z, et al. Stimulation of calcitonin gene-related peptide release through targeting capsaicin receptor: a potential strategy for gastric mucosal protection. *Dig Dis Sci*. 2013;58(2):320-325. doi: 10.1007/s10620-012-2362-6.
40. Machowska A, Szlachcic A, Pawlik M, et al. The role of female and male sex hormones in the healing process of preexisting lingual and gastric ulcerations. *J Physiol Pharmacol*. 2004;55(Suppl 2):91-104. PMID: 15608364.
41. Machowska A, Brzozowski T, Sliwowski Z, et al. Gastric secretion, proinflammatory cytokines and epidermal growth factor (EGF) in the delayed healing of lingual and gastric ulcerations by testosterone. *Inflammopharmacology*. 2008;16(1):40-47. doi: 10.1007/s10787-007-1600-6.
42. Makarenko YeV. ATPase activity of erythrocytes in patients with chronic liver and stomach disease [in Russian]. *Laboratornoye Delo.* 1987;2:14-17.
43. Markova OO, Popovych IL, Tserkovnyuk AV, Barylyak LG. *Adrenaline Myocardiodystrophy and Reactivity of the Organism* [in Ukrainian]. Kyïv: Computerpress;1997:126.
44. Meerson FZ. *Adaptation, Stress and Prophylaxis* [in Russian]. Moskva:Nauka;1981:279.
45. Meerson FZ. Pathogenesis and prevention of stress and ischemic heart damage [in Russian]. Moskva: Meditsina;1984:272.
46. Melnyk OI, Chendey IV, Zukow W, et al. The 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. *JEHS*. 2023;38(1):96-128.
47. Melnyk OI, Struk ZD, Zukow W, Popovych IL. Vegetative, endocrine and metabolic accompaniments of individual immune responses to adaptogenic balneotherapy. Journal of Education, Health and Sport. 2019;9(12):207-229.
48. Melnyk OI, Zukow W, Hrytsak MV, et al. Canonical analysis of neuroendocrine-metabolic and neuroendocrine-immune relationships at female rats. *JEHS*. 2021;11(5):356-369.
49. Mizunashi K, Furukawa Y, Katano K, et al. Effect of omeprazole, an inhibitor of H+,K+-ATPase, on bone resorption in humans. Calcif Tissue Int. 1993; 53(61): 21-25.
50. Moghadam ZM, Henneke P, Kolter J. From flies to men: ROS and the NADPH oxidase in phagocytes. Front Cell Dev Biol. 2021; 9:628991. doi: 10.3389/fcell.2021.628991
51. Nakamura J, Takada S, Ohtsuka N, et al. An assessment of gastric ulcers in vivo: enhancement of urinary recovery after oral administration of phenolsulfonphthalein in rats. *J Pharmacobiodynamics*. 1984;7(7):485-491. doi.org/10.1248/bpb1978.7.485
52. Nance DM, Sanders VM. Autonomic innervation and regulation of immune system (1987-2007). *Brain Behav Immun*. 2007;21(6):736-745.
53. Oh ES, Steele CN, You Z, et al. Sex hormones and the risk of cardiovascular disease and mortality in male and female patients with chronic kidney disease: A systematic review and meta-analysis. Physiol Rep. 2022;10(22):e15490. doi: 10.14814/phy2.15490.

54. Ohno H, Noguchi M, Takayanagi N. Effect of elcatonin on experimental gastric and duodenal ulcers. *Jpn J Pharmacol*. 1985;37(1):67-75. doi: 10.1254/jjp.37.67.
55. Panossian AG, Efferth T, Shikov AN, et al. Evolution of the adaptogenic concept from traditional use to medical systems: Pharmacology of stress- and aging-related diseases. Med Res Rev. 2021;41:630–703. 10.1002/med.21743
56. Panossian A, Efferth T. Network Pharmacology of Adaptogens in the Assessment of Their Pleiotropic Therapeutic Activity. Pharmaceuticals (Basel). 2022;15(9):1051. doi: 10.3390/ph15091051.
57. Pavlov VA, Chavan SS, Tracey KJ. Molecular and functional neuroscience in immunity. *Annu Rev Immunol*. 2018;36:783-812.
58. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. *Immune Status, Principles of its Evaluation and Correction of Immune Disorders* [in Russian]. Kyïv: Zdorovya;1995:211.
59. 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.
60. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integral quantification of neuroendocrine and immune responses to chronic stress in male rats [in Ukrainian]. In: *Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016)*. Kharkiv: NPhU;2016:182-182.
61. Popadynets O, Gozhenko A, Badyuk N, et al. Interpersonal differences caused by adaptogen changes in entropies of EEG, HRV, immunocytogram, and leukocytogram. Journal of Physical Education and Sport. 2020; 20(Suppl. 2): 982-999.
62. Popovych IL. Factor and canonical analyzes of the parameters of the neuro-endocrine-immune complex, metabolism and erosive-ulcerative lesions of the gastric mucosa in rats under acute water-immersion stress [in Ukrainian]. *Medical Hydrology and Rehabilitation*. 2007;5(2):68-80.
63. Popovych IL, Kulchynskyi AB, Korolyshyn TA, Zukow W. Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. *JEHS*. 2017;7(10):11-23.
64. Popovych IL, Kulchynskyi AB, Gozhenko AI, et al. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. *JEHS*. 2018;8(2):135-156.
65. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. *Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment*. Riga:Scholars' Press;2020: 172. doi.org/10.5281/zenodo.3822074
66. Popovych IL, Gozhenko AI, Korda MM, Klishch IM, Popovych DV, Zukow W (editors). *Mineral Waters, Metabolism, Neuro-Endocrine-Immune Complex*. Odesa. Feniks;2022:252. doi.org/10.5281/zenodo.6604298
67. Popovych IL, Bombushkar IS, Żukow X, Kovalchuk HY. Uric acid, neuroendocrine-immune complex and metabolism: relationships. *JEHS*. 2023;36(1):135-159.
68. Selye H: A syndrome produced by diverse nocuous agents. 1936. *J Neuropsychiatry Clin Neurosci*. 1998;10(2):230–231. 10.1176/jnp.10.2.230a
69. Shannon CE. A mathematical theory of information. Bell Syst Tech J. 1948;27:379-423.
70. Stiel D, Murray DJ, Peters TJ. Mucosal enzyme activities, with special reference to enzymes implicated in bicarbonate secretion, in the duodenum of rats with cysteamine-induced ulcers. Clin Sci (Lond). 1983; 64(63):341-347.
71. Szabo S, Yoshida M, Filakovszky J, Juhasz G. "Stress" is 80 Years Old: From Hans Selye Original Paper in 1936 to Recent Advances in GI Ulceration. Curr Pharm Des. 2017;23(27):4029-4041. doi: 10.2174/1381612823666170622110046.
72. Taché Y, Saperas E. Potent inhibition of gastric acid secretion and ulcer formation by centrally and peripherally administered interleukin-1. Ann N Y Acad Sci. 1992;664:353-68. doi: 10.1111/j.1749-6632.1992.tb39774.x. PMID: 1456663.
73. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. *Brain Behav Immun*. 2010;24(8):1223-1228.
74. Tracey KJ. Understanding immunity requires more than immunology. *Nature Immunology*. 2010;11(7):561-564.
75. Uehara A, Okumura T, Kitamori S, et al. Gastric antisecretory and antiulcer actions of interleukin-1. Evidence for the presence of an "immune-brain-gut" axis. J Clin Gastroenterol. 1992;14 Suppl 1:S149-55. PMID: 1629572.
76. Uribe-Querol E & Rosales C. Phagocytosis: Our Current Understanding of a Universal Biological Process. *Frontiers in immunology.* 2020;11:1066. https://doi.org/10.3389/fimmu.2020.01066
77. Vasilenko VKh & Kochina EN (editors). *Neurohumoral Regulation of Digestion* [in Russian]. Moskva: Meditsina;1983:368.

78. Vetvik K, Schrumpf E, Andersen KJ. et al. Effect of misoprostol and antacids on gastric and duodenal mucosal enzyme activities in duodenal ulcer patients. Scand J Gastroenterol. 1991;26(64):385-391.
79. Vetvik K, Schrumpf E, Mowinckel P, et al. Effects of omeprazole and eradication of Helicobacter pylory on gastric and duodenal mucosal enzyme activities and DNA in duodenal ulcer patients. Scand J Gastroenterol. 1994;29(611): 995-1000.
80. Wang M, Gu H, Brewster BD, Huang C. Role of endogenous testosterone in TNF-induced myocardial injury in males. Int J Clin Exp Med. 2012;5(2):96-104. PMID: 22567171; PMCID: PMC3342716.
81. Ward TL, Watkins KL, Southern LL, et al. Interactive effects of sodium zeolite-A and copper in growing swine: growth, and bone and tissue mineral concentrations. J Anim Sci. 1991;69(62):726-733.
82. Zajats LM, Polovynko IS, Zukow W, et al. Neuroendocrine-immune relatioships in rat females. *JEHS*. 2017;7(10):59-78.
83. Zavodskaya IS & Moreva YeV. *Pharmacological Analysis of Mechanism of Stress and its Effects* [in Russian]. Moskva: Meditsina;1981:216.
84. Zhao DQ, Xue H, Sun HJ. Nervous mechanisms of restraint water-immersion stress-induced gastric mucosal lesion. *World J Gastroenterology.* 2020;26(20):2533–2549. doi.org/10.3748/wjg.v26.i20.2533