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Original Article

The role of innate muscular endurance and resistance to hypoxia in reactions to acute stress of neuroendocrine, metabolic and ECGs parameters and gastric mucosa in rats

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Abstract
Background. It is known about aerobic fitness variability between individuals explained by genetics factors. On the other hand, it is also known about a wide variety of individual reactions to stress. From the above it follows the hypothesis that interindividual differences in normal conditions determine the characteristics of the body's response to acute stress. The purpose of this study is to test the hypothesis. Material and methods. The experiment is at 58 rats (28 males) Wistar line. Animals were first tested for resistance to hypoxic hypoxia. One week later, aerobic muscular performance was determined by swimming test. On the basis of the received data two qualitatively equivalent groups in a ratio 10/48 were formed. After a week of recovery over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress. The next day after stress, the ECG recorded and some endocrine and metabolic parameters determined as well as erosive-ulcerative lesions of the gastric mucosa evaluated. Results. Wide variability of hypoxic and swimming tests was revealed. 4 clusters were created retrospectively. Each cluster is characterized by specific (correctness of classification 100%) post-stress changes of 7 neuro-endocrine, 15 metabolic and 4 ECGs parameters, as well as the length of ulcers and the index of damage to the gastric mucosa. The swimming test determines the post-stress state of the registered parameters of the body by 64,4%, the hypoxic test - by 58,6%, and taken together - by 73,5%. Conclusion. Innate muscular endurance and resistance to hypoxia significantly determines the post-stress neuro-endocrine and metabolic parameters as well as injuries of myocardium and gastric mucosa in rats.
Key words: swimming and hypoxic tests, acute stress, hormones, HRV, metabolism, gastric mucosa, rats.

Introduction

Both aerobic and strength exercise training lower the incidence of many chronic diseases via a number of mechanisms, including increased skeletal muscle mitochondrial function, modulation of the sympathetic nervous and immune systems, and optimization of the neuroendocrine system. These mechanisms act as buffers against chronic diseases, minimizing inflammatory state, and enhancing neuroplasticity and growth factor expression. However, large inter-individual differences exist in the physiological responses to any given exercise training (also called "trainability"). Large trainability has been observed in many physical fitness parameters, including maximal oxygen uptake, resting heart rate, exercise heart rate, aerobic threshold, anaerobic threshold, resting muscle glycogen content, muscle enzyme activity, as well as muscle mass and strength. The heritable component of trainability is large, with genetics explaining 47% of the variance in VO_2 peak trainability, and around 52% in resistance variability. The contribution of familial factors (genetics and environment) to trainability was demonstrated in the seminal HERITAGE family study. This study indicated that VO_2max was more variable between families than within families at baseline, and in response to exercise training, thus suggesting that DNA sequence variations could modulate exercise responses. Pinpointing the responsible gene variants could illuminate the fundamental mechanisms driving this heterogeneity in response to exercise training. Twin and family studies indicate that ~22–57% of aerobic fitness variability between individuals can be explained by genetics and therefore plays an important role in the range of aerobic phenotypes observed in a population (review: Alvarez-Romero et al., 2021; Guimarães et al., 2018; Rovniy et al., 2017a; Naisidou et al., 2017; Rovniy et al., 2017b).

One of the important manifestations of the body's overall resistance is its susceptibility to stress-induced damage of gastric mucosa and the myocardium. The variability of stress responses in different animal strains of the same species is well established. For example, selective breeding-based cholinergic hypersensitivity and hyposensitivity Flinders rat lines (Overstreet et Wegener, 2013); hyperanxious (HAB-M) and hypoanxious

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(LAB-M) mouse lines (Krömer et al., 2005); high-resistant and low-resistant to hypoxic hypoxia Wistar rats (Markova et al, 1997; Ordynskyi et al., 2017; 2019). The importance of individual vulnerability and resilience factors is increasingly acknowledged in mechanistic research and may exhibit a genetic (Savignac et al., 2011) and an epigenetic (Zannas et West, 2014) basis, and this is possibly based on "synaptic rewiring" of stress-sensitive neurons (Singh-Taylor et al., 2015). In all cases, however, it is likely that the "three-hit concept" of vulnerability and resilience persists: a genetic predisposition and early life adverse events are necessary so that a later-in-life stressor can exhibit negative health outcomes, and one or more missing may result in higher resilience (Daskalakis et al., 2013; Elsenbruch et Enck, 2017).

From the above it follows the hypothesis that inter-individual differences in normal conditions determine the characteristics of the body's response to acute stress. The purpose of this study is to test the hypothesis.

Material and methods

Participants. The experiment is at 58 rats Wistar line weighing 170-280 g: 28 males (Mean=216 g; SD=22 g) and 30 females (Mean=196 g; SD=19 g).

Procedure / Test protocol / Skill test trial / Measure / Instruments. At the preparatory stage, all animals were first tested for resistance to hypoxic hypoxia by the classical method of Berezovskyi (1975). To do this, each rat was placed in a pressure chamber with a transparent lid, in which the pump created a vacuum of air equivalent to a rise to a height of 12 km (20 kPa) and recorded the time of the second agonal breath or seizure. One week later, aerobic muscular performance was determined by the duration of swimming (t^0 water 26^0 C) with a load (5% of body weight) to exhaustion (falling to the bottom of the bath) (Brekhman, 1968).
After a week of recovery under light ether anesthesia for 15-20 sec recorded electrocardiogram (ECG) in standard lead II (introducing needle electrodes subcutaneously).

On the basis of the received data two qualitatively equivalent groups (equally females and males, practically identical average sizes and variances of swimming and hypoxic tests) in a ratio 10/48 were formed. Over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress according to the method of Nakamura et al. (1977) in the modification of Popovych (2007), 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. The next day after stressing, the ECG was re-recorded. The experiment was completed by decapitation of the animals in order to remove the adrenal glands and stomach and collect the maximum possible amount of blood in which was determined some endocrine and metabolic parameters. Among endocrine parameters determined plasma concentration of corticosterone, testosterone and triiodothyronine (by ELISA, reagents from JSC "Alkor Bio", RF) (Instructions, 2000).

On lipid metabolism judged by the level of plasma 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 (1987) after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as well as nonα-lipoprotein (turbidometric method Burstein-Samay) as described in the handbook (Goryachkovskiy, 1998).

State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract) (Gavrilov et Mishkorudnaya, 1983) and malondyaldehid (test with thiobarbituric acid) (Andreyeva et al, 1988), as well as the activity of antioxidant enzymes: catalase serum and red blood cells (by the speed of decomposition hydrogen peroxide) (Korolyuk et al, 1988) and superoxide dismutase erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH) (Dubinina et al., 1988; Makarenko, 1988).

On electrolytes metabolism judged by the level of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method) and chloride (mercury rodanide method) in the plasma, sodium and potassium in the plasma and erythrocytes (flame photometry method) as described in the handbook (Goryachkovskiy, 1998). Based on obtained data evaluated hormonal activities: mineralocorticoid MCA=$(Nap/Kp)^{0,5}$, parathyroid PTA=$(Cap/Pp)^{0,5}$ and calcitonin CTA=$(1/Cap•Pp)^{0,5}$, based on their classic effects and guidelines Popovych (2011; 2020), as well as Cap/Kp ratio, which is considered a marker of sympathic-vagal balance (Fajda et al, 2016). Alanine and asparagine aminotranspherase, alkaline and acid phosphatase as well as creatine phosphokinase determined by uniform methods as described in the handbook (Goryachkovskiy, 1998).

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

After a blood sample was removed adrenal glands and stomach. The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the number of ulcers and their length was measured, evaluated erosive and ulcerative damage on scale by Popovych (2007; 2011). This scale is based on the qualitative-quantitative Harrington (1965) scale.

Data collection and analysis / Statistical analysis. Statistical processing was performed using a softwarepackage "Microsoft Excell" and "Statistica 64 StatSoft Inc".

Results

Visualization of the sample on one plane shows significant variability of both the swimming test (range 6 ÷ 66 min, Cv = 0,672) and the hypoxic test (range 65 ÷ 317 sec, Cv = 0,515). The sex differences for the hypoxic test are completely absent (M±SD: 131±76 and 132±55 sec in males and females, respectively), while according to the swimming test males are dominated by females (M±SD: 22,6±14,1 vs 15,2±6,5 min, $p<0,05$). Obviously, this is partly due to body weight (r=0,26) and levels testosterone (r=0,31; 40,3±4,6 vs 3,53±0,53 nM/L) and corticosterone (r=-0,58; 290±114 vs 406±82 nM/L).

Large circles indicate animals that have not been exposed to stress.

The second phase was conducted cluster analysis of fitness variables (in stressed rats only). Clustering cohort of rats is realized by iterative k-means metod. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points (Aldenderfer et Blashfield, 1989).

We have identified 4 clusters (Table 1). The first cluster contains 9 females and 2 males, the second - only 3 males, the third - 13 males and 12 females and fourth cluster contains 5 males and 4 females. Note that the markers of intact rats superimposed on the plane are detected almost proportionally in each cluster. This is important given the subsequent assessment of stress-induced deviations from the norm of the registered parameters of the body.

Table 1. The average values of fitness variables intact rats and members of different clusters

Test	Cluster (n)	I (11)	II (3)	III (25)	IV (9)	Intact (10)
Hypoxic, sec	Mean SD	145 24	105 57	86 15	248 22	132 68
Swimming, min	Mean SD	14,7 7,4	53,0 1,0	16,8 6,4	17,3 9,0	18,8 12,6

For the purpose of comparative qualitative-quantitative assessment actual fitness variables (V) expressed as Z-scores (Table 2) calculated by formula: Z = (V/M – 1)/Cv, where M is Mean for the sample, Cv is Coefficient its variation.

Table 2. The average Z-scores of fitness variables intact rats and members of different clusters

Test	Cluster (n)	I (11)	II (3)	III (25)	IV (9)	Intact (10)
Hypoxic, Z	Mean SD	+0,20 0,36	-0,39 0,85	-0,67 0,22	+1,72 0,33	+0,04 0,39
Swimming, Z	Mean SD	-0,32 0,58	+2,71 0,08	-0,16 0,51	-0,12 0,71	+0,03 0,46

As can be seen, the characteristics of the members of the first cluster are normal, both resistance to hypoxia and muscular endurance. Rats of the second cluster are distinguished by a drastic duration of swimming to exhaustion. The third cluster is characterized by moderately reduced resistance to hypoxia, and the fourth - significantly increased.

In order to identify exactly those post-stress parameters (variables) whose constellation is characteristic for each cluster, the available informational field was subjected to discriminant analysis by the method of forward stepwise (Klecka, 1989). To include in the model (Tables 3), the program has selected 28 variables (7 neuro-endocrine, 7 metabolic for lipids&lipids peroxidation, 5 metabolic for electrolytes, 4 ECGs as well as 5 enzymatic markers of cytolysis and gastric mucosa injuries, while the other 15 were outside the discriminant model, but still worth noting.

Table 3. Discriminant Function Analysis Summary

Step 28, N of vars in model: 28; Grouping: 4 grps

Wilks' Lambda: 0,0035; approx. $F_{(85)}=3,5$; $p<10^{-6}$

	Clusters (n)				Parameters of Wilks' Statistics					
Variables currently in the model	I (11)	II (3)	III (25)	IV (9)	Wilks' Λ	Partial Λ	F-remove	p-value	Tolerancy	Norm (10)
Triiodothyronine, nM/L	3,05 0,19	2,58 0,80	3,70 0,08	3,22 0,08	0,006	0,605	3,71	0,032	0,188	3,43 0,31
Triglycerides, mM/L	0,99 0,03	1,17 0,07	1,08 0,02	1,07 0,01	0,008	0,412	8,08	0,001	0,211	1,07 0,02
Gastric Ulceris Length, mm	5,4 1,3	1,7 1,7	2,7 0,6	0,6 0,3	0,004	0,781	1,59	0,229	0,048	0
Chloride Plasma,	100,3	87,3	97,6	96,8	0,006	0,569	4,29	0,020	0,004	97,8

mM/L	1,4	3,0	0,9	2,3						0,8
Potassium Erythrocytes, mM/L	78 3	79 9	83 2	96 5	0,004	0,860	0,92	0,451	0,366	88 5
α-LP Cholesterol, mM/L	0,69 0,05	0,84 0,11	0,81 0,03	0,69 0,03	0,015	0,233	18,6	10^{-4}	0,018	0,84 0,05
Calcium Plasma, mM/L	3,68 0,18	2,20 0,00	3,32 0,16	2,98 0,34	0,005	0,638	3,22	0,049	0,006	3,18 0,27
Acid Phosphatase, IU/L	30,3 1,8	36,0 5,7	40,2 2,2	33,2 9,6	0,005	0,675	2,73	0,076	0,220	31,4 1,9
Malondialdehyde, μM/L	52,.4 1.7	79,3 15.4	53,8 1,9	61,3 3,3	0,006	0,590	3,94	0,027	0,236	63.5 5.6
Katalase Erythrocy-tes, μM/L•h	245 19	227 6	220 13	286 33	0,009	0,390	8,88	0,001	0,104	227 17
Nonα-LP/α-LP as Kli-mov's Atherogenity	1,43 0,17	0,97 0,12	1,01 0,08	1,18 0,18	0,011	0,305	12,9	10^{-4}	0,007	1,27 0,10
q-T/R-R Ratio ECG	0,53 0,03	0,63 0,05	0,57 0,02	0,54 0,03	0,006	0,570	4,27	0,020	0,282	0,61 0,01
Diene conjugates, E^{232}/mL	1,50 0,08	1,63 0,35	1,53 0,07	1,44 0,10	0,009	0,401	8,45	0,001	0,144	1,47 0,11
Corticosterone normalized by sex, Z	0,00 0,28	-0,58 0,21	+0,35 0,22	-0,40 0,31	0,007	0,491	5,88	0,006	0,258	0 0,30
Testosterone normalized by sex, Z	-0,26 0,35	-0,63 0,42	-0,88 0,25	-1,37 0,62	0,005	0,724	2,16	0,130	0,346	0 0,30
Alanine Aminotrans-pherase, μKat/L	0,59 0,04	1,00 0,26	0,62 0,04	0,68 0,13	0,006	0,604	3,71	0,032	0,161	0,53 0,05
Nonα-LP Cholesterol, mM/L	0,92 0,08	0,81 0,10	0,79 0,06	0,79 0,10	0,009	0,377	9,36	0,001	0,012	1,04 0,07
Injuries of Gastric Mucosa, points	0,41 0,07	0,20 0,15	0,29 0,05	0,11 0,05	0,006	0,544	4,75	0,014	0,032	0
$(Ca•P)^{-0,5}$ as Calcitonin Activity	0,50 0,02	0,61 0,03	0,51 0,02	0,53 0,03	0,007	0,592	3,90	0,027	0,009	0,51 0,03
P-q interval ECG, msec	52,1 0,5	49,3 5,8	47,7 1,4	51,0 3,7	0,008	0,441	7,19	0,003	0,204	55,6 0,8
Sodium Plasma, mM/L	134,2 0,8	124,7 2,5	132,5 0,6	131,8 1,7	0,005	0,633	3,28	0,047	0,004	132,8 0,5
qRS interval ECG, msec	30,5 0,5	29,7 1,4	29,7 0,7	29,9 1,2	0,006	0,631	3,31	0,045	0,300	29,5 0,1
$(Ca/P)^{0,5}$ as Parathyro-id Activity	1,81 0,08	1,35 0,06	1,62 0,07	1,50 0,08	0,005	0,774	1,65	0,214	0,014	1,53 0,07
R wave ECG, μV	433 52	442 186	406 30	275 71	0,005	0,661	2,90	0,065	0,346	330 18
Phosphate Plasma, mM/L	1,15 0,078	1,22 0,10	1,25 0,03	1,29 0,03	0,005	0,732	2,07	0,142	0,019	1,32 0,02
MxDMn HRV as Vagal tone, msec	38 9	23 14	32 5	16 3	0,006	0,597	3,83	0,029	0,111	84 10
Mode HRV as Humo-ral Channel, msec	182 9	167 34	159 5	155 9	0,005	0,682	2,64	0,083	0,133	184 6
Asparagine Amino-transpherase, μKat/L	0,26 0,02	0,39 0,09	0,26 0,02	0,26 0,04	0,004	0,795	1,46	0,261	0,203	0,21 0,02

Notes. In each column, the top row is the average, the bottom is the standard error. Testosterone and corticosterone levels normalized by sex.

Summary of Stepwise Analysis for Variables ranked by criterion Λ

Variables currently not in the model Next, the 28-dimensional space of discriminant variables transforms into 3-dimensional space of a canonical discriminant functions (canonical roots), which are a linear combination of discriminant variables. The discriminating (differentiating) ability of the root characterizes the canonical correlation coefficient (r*) as a measure of connection, the degree of dependence between groups (clusters) and a discriminant function. It is for Root 1 0,965 (Wilks' Λ=0,0034; $\chi^2_{(84)}$=175; $p<10^{-6}$), for Root 2 0,919 (Wilks' Λ=0,0514; $\chi^2_{(54)}$=92; $p<10^{-3}$), for Root 3 0,817 (Wilks' Λ=0,3317; $\chi^2_{(25)}$=34; p=0,130). The first root contains 64,7% of discriminative opportunities, the second is 25,8% and the third only 4,5%.

It resents actual and standardized (normalized) coefficients for discriminant variables. The raw coefficient gives information on the absolute contribution of this variable to the value of the discriminative function, whereas standardized coefficients represent the relative contribution of a variable independent of the unit of measurement. They make it possible to identify those variables that make the largest contribution to the discriminatory function value.

Standardized and Raw Coefficients and Constants for Canonical Variables

The third discriminant parameter is the full structural coefficients, that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows how closely variable and discriminant functions are related, that is, what is the portion of information about the discriminant function (root) contained in this variable. The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots. According recommendation by Popovych (2011; 2020) variables obtained after acute stress (SV) expressed as Z-scores calculated by formula: Z = (SV/NV – 1)/Cv, where NV is Norm (obtained from intact rats) Variable, Cv is Coefficient its variation in intact rats. This approach allows us to compare the variables expressed in different units (μKat, %, nM/L, msec etc) in one scale. Shows, in addition to those included in the model, extramodel variables, which still carry differentiating information.

Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables The localization of the members of the **first** cluster in the extreme left zone of the axis of the first root reflects the most severe for sampling stress-induced damage to the gastric mucosa and myocardium, accompanied by elevated levels of nonα-LP Cholesterol, as much as possible for sampling by low levels of Phosphate plasma and Potassium erythrocytes. In contrast, the decrease in MxDMn as HRV-marker of Vagal tone (Baevskiy et al, 1984) is minimal for sampling, and the other two parameters of HRV and Testosterone are within normal limits. At the opposite pole of the axis are the members of the **fourth** cluster. This localization reflects minimal stress-induced injuries, which are accompanied by normal levels of nonα-LP Cholesterol and Phosphate plasma and Potassium erythrocytes, maximally for sampling reduced levels of Testosterone and Vagal tone, instead of maximum levels of Sympathetic tone and, apparently, circulating Catecholamines, judging by Mode HRV (Baevskiy et al, 1984). The intermediate location along the axis of the members of the other two clusters reflects the intermediate levels of these parameters. The delimitation of clusters is quite clear, without any mutual penetration. In addition, the third cluster is separated from the other three along the axis of the second root. The lowest localization of its members reflects the highest level of Acid Phosphatase and normal, but maximum for sampling levels of Triiodothyronine and Corticosterone in combination with the minimum for sampling levels of Katalase erythrocytes and P-q interval ECG.

Three males of the second cluster, in turn, occupy a top position along the axis of the third root. This reflects elevated levels of cytolysis, lipids peroxidation and Calcitonin activity markers, as well as normal but maximal for sampling level of α-LP Cholesterol. On the other hand, these animals are characterized by a stress-induced decrease in the activity of antioxidant enzymes and plasma levels of Na^+, Cl^- and Ca^{2+}, apparently caused by a decrease in Mineralocorticoid and Parathyroid activity. While the mass of the adrenal glands, level of Sodium erythrocytes (and myocardiocytes?) and the activity of creatine phosphokinase (as myocardiocytes damage marker) remain within normal limits, as well as qRS interval and q-T/R-R ratio ECG. The same discriminant parameters can be used to identify (classify) the belonging of one or another animal to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 8). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. The coefficients of the classifying functions are not standardized, therefore they are not interpreted. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant.

Coefficients and Constants for Classification Functions The accuracy of classification (retrospective recognition) is **100%**. Another approach to clarifying the role of swimming and hypoxic tests in post-stress changes in the body is to build regressive models with step-by-step exclusion to reach the maximum level of Adjusted R^2. It is shown that the swimming test downregulates post-stress levels of Corticosterone (actual to a greater extent than normalized by sex), Chloride, both relative and actual Adrenals mass, as well as markers of Sympatho-Vagal balance and Parathyroid activity. Instead, to upregulation is subordinate the levels of both Aminotransferases, Triglycerides, Sodium erythrocytes and R wave ECG.

Regression Summary for Swimming test $R=0,802$; $R^2=0,644$; Adjusted $R^2=0,522$; $F_{(12)}=5,3$; $p<10^{-4}$
The constellation of the listed post-stress parameters is determined by the duration of swimming to exhaustion by 64,4%. Resistance to hypoxia determines by 58,6% a completely different constellation of post-stress parameters. **Regression Summary for Hypoxic test** $R=0,766$; $R^2=0,586$; Adjusted $R^2=0,488$; $F_{(9)}=6,0$; $p<10^{-4}$
Taken together, both innate factors of cardiorespiratory fitness determine the acute stress-induced changes in endocrine, metabolic and EEG parameters of rats by 73,5%.

Discussion

The different responses of animals and humans to an apparently equivalent stimulus are called inter-individual response variability. This phenomenon has gained more and more attention in research in recent years. Among others, this increased interest was driven by the intervention literature because the intervention-related individual differences in outcome measures have great practical relevance (e.g., in therapy, rehabilitation, health

care, prevention, and sports medicine) (Kozyavkina et al., 2015; Popovych et al., 2020; Shestopal et al., 2021; Nogas et al., 2019; Novopysmennyi et al., 2020; Kindzer et al., 2020; Dido, et al., 2021). Several factors constitute a potential source for inter-individual response variability. According to the literature, these factors can be categorized as follows: non-modifiable, modifiable, and other influencing factors. Non-modifiable factors comprise factors that are predetermined, such as genetics, sex, and age. There is considerable evidence highlighting the prominent influence of the *genotype* on the responsiveness of a single individual in physical performance parameters, brain structure and function etc. However, the exact influence of genetic factors on inter-individual response variability, at least for physical performance, is not yet exactly known and is currently under debate (Herold F et al., 2021).

Thus, low or unhealthy CRF is a strong, independent predictor of cardiovascular disease and all-cause mortality in adults. In youth, CRF is a predictor of a number of health indicators including cardiometabolic health and premature cardiovascular disease. Studies have investigated the relationship between CRF and various non-modifiable and modifiable factors including genetics, age, sex, race/ethnicity, physical activity and dietary patterns, obesity, sedentary time, built environment, and socioeconomics (Ross et al., 2016; Raghuveer et al., 2020). Cheng et al., (2000) have shown that active men had a significantly reduced risk for duodenal ulcers (relative hazard for the active group 0,38 vs 0,54 for the moderately active group). No association was found between physical activity and gastric ulcers for men or for either type of ulcer for women. Authors concluded that physical activity may provide a non-pharmacologic method of reducing the incidence of duodenal ulcers among men. Peel et al., (2009) have found that a low level of fitness is an independent predictor of digestive cancer mortality and morbidity.

It is especially interesting to compare our data with the data of Lu et al., (2019) who studied gastroprotective effects of the adaptogen Kangfuxin (KFX) against water-immersion and restraint stress (WIRS)-induced gastric ulcer in rats. They showed that pre-treatment with KFX could effectively reduce the area of gastric ulcers and improve the pathological changes of ulcerated tissue. Moreover, KFX increased the prostaglandin E2 (52%) and cyclooxygenase-1 (30%) levels, and improved malondialdehyde (54%), superoxide dismutase (58%), catalase (39%), and nitric oxide (11%) and TNF-α (9%), IL-6 (11%), MMP-9 (54%) and MMP-2 (53%) of ulcer tissue. Furthermore, pre-treatment with KFX dramatically increased IGF-1, PTEN, and Akt protein expression. Thus, results suggest that KFX has protective effects on WIRS-induced gastric ulcer via inflammatory reactions, oxidative stress inhibition, and pro-survival action.

In this regard, it is interesting to give the latest ideas about the mechanisms of WIRS-induced damage to the gastric mucosa (review: Zhao D. Q. et al., 2020). Some studies have found that RWIS leads to the elevation of blood corticosterone and adrenocorticotropic hormone levels in rats. This seems to indicate that the activity of the hypothalamic-pituitary-adrenal (HPA) axis is enhanced during RWIS. However, severing the subphrenic vagus nerves or consuming atropine can significantly alleviate and even cure RWIS-induced gastric mucosa lesion (GML), but removing the pituitary glands and adrenal glands or administering adrenergic α-receptor blocker has little impact on RWIS-induced GML, gastric hyperkinesia and RWIS-induced gastric acid secretion. This suggests that the HPA axis does not play a major role in RWIS-induced GML [on the contrary, Filaretova et al., (1998; 2008) consider corticosterone a gastroprotective factor] and the peripheral nervous mechanism of RWIS-induced GML is mainly through the enhanced parasympathetic activity. Therefore, the nervous mechanism of RWIS-induced gastrointestinal dysfunction in rats is mainly the "enhanced activity of parasympathetic nervous system", rather than the traditional ideas of the "enhanced activity of sympathetic-adrenal medulla system" and "HPA axis". The dorsal vagal complex (DVC) and vagal efferent play an outstanding role in the regulation of gastric mucosal resistance to injury. However, the role of the vagal nerve is likely to be dual, as it can mediate both mucosal damaging and protective effects. Biochemical and pharmacological studies have demonstrated that the mechanisms of vagal-mediated gastroprotective effects may be due to the activation of vagal cholinergic pathways, secretion of gastric prostaglandins and production of NO. Gastrointestinal excitatory motor neurons release excitatory transmitters, such as ACh and SP, thus promoting gastrointestinal smooth muscle contraction and glandular secretion. On the contrary, inhibitory motor neurons release inhibitory transmitters, such as NO and VIP, thus suppressing gastrointestinal smooth muscle contraction and glandular secretion. All these gastrointestinal excitatory and inhibitory motor neurons can interact with each other under a complex and delicate balance. If this balance is broken, gastrointestinal dysfunction may be induced.

Previous studies have demonstrated that NO can inhibit gastric acid secretion and neutrophil adhesion, improve gastric mucosal blood circulation and eliminate oxygen free radicals, thereby protecting the gastric mucosa from injury. It was reported that the expression level of iNOS increased significantly in the gastric mucosa of RWIS rats, while that of eNOS reduced significantly, indicating that the changes in iNOS and eNOS activities in the gastric mucosa are closely related to the incidence of GML. In stress-induced GML, NOS inhibitor can decrease the production of NO, thus exacerbating acute GML and inhibiting the healing process of chronic gastric ulcers, while NO precursor can obviously prevent the injury. Thus NO is involved in RWIS, and can promote the GML healing process.

The mechanisms of NO in protecting gastric mucosa are as follows. NO can reduce vascular permeability, inhibit platelet adhesion and aggregation in gastric mucosal vascular endothelium, and prevent thrombosis. Under physiological conditions, gastric mucosal vascular endothelium synthesizes NO, which in turn regulates vascular smooth muscle tension and maintains GMBF. In acute GML, NO increases GMBF by dilating the mucosal blood vessels, thus promoting gastric mucosal repair. In addition, the secretion of gastric acid can also be inhibited by NO as well as endogenous NO can inhibit the stimulation of histamine through parietal cells, thus reducing gastric acid secretion and protecting gastric mucosa. Gastric mucous cells promote NO synthesis by expressing high-level NOS, and enhance the mucous barrier through the NO effects of promoting mucin synthesis and secretion. RWIS-induced GMLs can weaken the synthesis and secretion of gastric mucus by reducing nNOS activity, while the NO donor can increase nNOS activity and mucus secretion.

Gozhenko et al (2000) believe that among a number of factors involved in the pathogenesis of acute gastric injury, the main ones are the activation by glucocorticoids of gluconeogenesis in the cells of the gastric mucosa, accompanied by the breakdown of proteins and increased release of ammonia, which activates acid secretion together with the vagus, also disturbance of microcirculation in the stomach wall due to vasoconstriction, upregulated by the catecholamines while downregulated by the vagus, the mediator of which is NO. The validity of the participation of these mechanisms is confirmed by the authors' data on the increase in NO synthesis in the stomach wall (in microvessels and secretory epithelium) in patients with acute ulcers.

Zhao et al (2020) found that catecholaminergic neurons in the nucleus of the medullary visceral center participate in the regulation of RWIS-induced GML, whereas catecholaminergic neurons in the nucleus of the anterior hypothalamus are rarely or not involved. Therefore, the neurons responsible for RWIS are not located in the anterior hypothalamus, but instead the neuronal activity in the nucleus may be regulated by medullary catecholaminergic neurons.

In a study close to ours, Ordynskyi et al., (2017; 2019) simulated chronic stress (4 times by an hour-long immobilization of rats the back down with an interval each 24 hours). In all groups of stressed animals, macroscopic damage to the gastric mucosa was noted, but the most vulnerable were low-resistance to hypoxic hypoxia (LRH) females. It was found that in control LRH male and female rats, compared with highly resistant to hypoxic hypoxia (HRH), is dominated by sympathetic tone. Under stress in males, the level of circulating catecholamines decreases, but sympathetic tone remains higher in LRH, and parasympathetic - in HRH. In LRH females under stress, an increase in circulating catecholamines and a decrease in vagus tone. In our study, post-stress levels of both circulating catecholamines and sympathetic tone were higher in HRH (IV cluster) than in LRH (III cluster) as well as vagal tone was lower.

Conclusion

Innate muscular endurance and resistance to hypoxia significantly determines the post-stress neuro-endocrine and metabolic parameters as well as injuries of myocardium and gastric mucosa in rats.

We found inter-individual variability, on the one hand, between innate two parameters of cardiorespiratory fitness (CRF), and responses of neuro-endocrine, metabolic and ECGs parameters as well as markers of gastric mucosa damage - on the other hand. In addition, we have shown that both aerobic muscular endurance (to a greater extent) and resistance to hypoxia (to a lesser extent) determine not only the severity but also the direction of stress-induced reactions of the autonomic nervous and endocrine systems, which in turn cause damage to the myocardium and gastric mucosa, the severity of which differs significantly in rats of different clusters.

Our data, in principle, are consistent with existing provision that cardiorespiratory fitness (CRF), an objective and more reproducible measure, reflects the functional consequences of physical activity habits of the individual, and therefore may provide a better exposure with which to evaluate associations with relevant health outcomes. Returning to our results, we note that minimal RWIS-induced injuries to both gastric mucosa and myocardium were found in rats with maximum resistance to hypoxia, which is to be expected, whereas the most severe damage occurred unexpectedly in animals with a completely normal state of cardiorespiratory fitness. Even more unexpected was the higher than in the previous cluster stress resistance of rats with minimal resistance to hypoxia at normal aerobic performance. And the combination of drastic “Ethiopian-Kenyan” duration of swimming to exhaustion with slightly lower than average resistance to hypoxia did not guarantee the “champion” stress resistance (only “silver” for the stomach and “bronze” for the myocardium).

However, this state of affairs is not accidental, because it is accompanied by specific post-stress changes in neuroendocrine and metabolic parameters. In particular, minimal RWIS-induced injuries are accompanied by the lowest levels of plasma testosterone (but not corticosterone) and vagal tone in combination with the highest sympathetic tone and circulating catecholamines while in the case of the most severe injuries, deviations of these tread parameters from the levels of intact animals are minimal or absent. On the other hand, the most severe injuries are accompanied by maximally increased mineralocorticoid and parathyroid activities, while with minimal damage their changes are insignificant. In rats of the second cluster, the protective factors are a decrease in corticosterone levels as well as mineralocorticoid and parathyroid activities in combination with increase in calcitonin activity. Our data are consistent with the provision that norepinephrine and dopamine are important endogenous inhibitory neurotransmitters that protect the integrity of gastric mucosa during stress.

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 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).

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Conflict of Interest The authors declare that they have no conflict of interest.

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