NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ

Pedagogy and Psychology of Sport. eISSN 2450-6605.
Journal Home Page
https://apcz.umk.pl/PPS/index

ZUKOW, Walery, SAVYTSKYI, Ivan, KOVALCHUK, Halyna and MELNYK, Oksana. The effect of drinking mineral waters on the physical performance of female rats and its metabolic support. Pedagogy and Psychology of Sport. 2026;31:70045. eISSN 2450-6605. https://doi.org/10.12775/PPS.2026.31.70045

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 07.02.2026. Revised: 21.03.2026. Accepted: 21.03.2026. Published: 21.03.2026.

The effect of drinking mineral waters on the physical performance of female rats and its metabolic support

WALERY ZUKOW[1], IVAN V. SAVYTSKYI[2], HALYNA Y. KOVALCHUK[3], OKSANA I. MELNYK4

Walery Zukow1*, https://orcid.org/0000-0002-7675-6117; w.zukow@wp.pl
Ivan V. Savytskyi2, https://orcid.org/0000-0002-5841-9993; prof_s.i.v@ukr.net
Halyna Y. Kovalchuk3, https://orcid.org/0000-0002-5261-8422; galynakovalchuk5@gmail.com
Oksana I. Melnyk4, https://orcid.org/0000-0001-7928-4760; omelnyk7@gmail.com

[1]Nicolaus Copernicus University, Torun, POLAND
[2]International Academy of Ecology and Medicine, Kyïv, UKRAINE
[3]Ivan Franko State Pedagogical University, Drohobych, UKRAINE
[4]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologiev, Lviv, UKRAINE
*Member Editorial Board.

Abstract
Background and aim. Mineral waters are widely used in spa therapy and sports medicine, yet their effects on physical performance and underlying metabolic mechanisms remain incompletely understood. This study aimed to investigate the impact of three different mineral waters on swimming performance in female rats and identify key metabolic predictors of physical capacity.
Material and methods. Sixty female Wistar rats (mean weight 262±22 g) were randomly divided into four groups: intact controls (n=10) receiving tap water, and three experimental groups (n=50) loaded with mineral waters (MW N1, N2, N3) at 3 mL/200 g body mass for 12 days. Swimming test until exhaustion was performed, along with

comprehensive metabolic, hormonal, and electrolyte analyses in serum and urine. Discriminant analysis and multiple regression were used to identify predictors of physical performance.
Results. Swimming duration ranged from 90 to 330 minutes and correlated most strongly with mineralocorticoid activity estimated as $(K^+_{urine}/Na^+_{urine})^{0.5}$ ($r=-0.564$; $p<10^{-6}$). Multiple regression revealed that mineralocorticoid activity, calcitonin activity, glycemia, body mass, and uricemia together explained 65% of swimming test variance ($R^2=0.650$; $F_{(5,54)}=20.1$; $p<10^{-6}$). MW N2 (highest Na^+ content: 122.3 mM/L) significantly increased mineralocorticoid activity (2.71±0.38 vs 1.40±0.13 in controls; $p<10^{-6}$) and reduced swimming duration by 16% (156±8 vs 187±2 min; $p<0.001$). MW N3 enhanced performance by 13% (210±8 min; $p<0.05$). Discriminant analysis correctly classified 96% of animals based on 12 metabolic variables.
Conclusions. Mineralocorticoid activity is the primary determinant of physical performance in rats consuming mineral waters. High-sodium mineral water impairs performance through aldosterone-mediated potassium loss and electrolyte imbalance, while balanced mineral composition maintains or enhances physical capacity. These findings have important implications for mineral water selection in sports medicine and spa therapy.

Key words: drinking mineral waters, trace elements, organic substances, **physical performance,** metabolism, female rats.

Introduction

Mineral waters have been used therapeutically for centuries and remain an integral component of balneotherapy and spa treatment worldwide (Popovych et al., 2022). Beyond their traditional applications in treating metabolic and gastrointestinal disorders, mineral waters are increasingly recognized for their potential effects on physical performance and exercise capacity (Zukow et al., 2024). However, the mechanisms underlying these effects remain poorly understood, particularly regarding the role of electrolyte composition and hormonal regulation.

Physical performance is a complex physiological phenomenon dependent on multiple interacting systems, including cardiovascular function, muscle metabolism, neuro-endocrine regulation, and electrolyte balance (Gozhenko, 2016). The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in regulating sodium and potassium homeostasis, blood pressure, and fluid balance—all critical factors for optimal physical performance (Baffour-Awuah et al., 2024). Recent evidence suggests that aldosterone, the primary mineralocorticoid hormone, significantly influences muscle function, exercise capacity, and metabolic efficiency (Kluwe et al., 2023; Supiano, 2013).

Excess aldosterone causes sodium and water retention combined with potassium loss, leading to hypokalemia—a condition particularly detrimental to physical performance (Kwak et al., 2019). Potassium is essential for: (1) generation and propagation of action potentials in muscles and nerves; (2) optimal function of Na^+,K^+-ATPase, which maintains the electrochemical gradient necessary for muscle contraction; and (3) glycogen synthesis, a critical energy source during prolonged exercise. Conversely, blocking excessive aldosterone activity through mineralocorticoid receptor antagonists can improve muscle function, enhance vascular endothelial function, and increase exercise capacity in various clinical conditions (Supiano, 2013).

The chemical composition of mineral waters varies considerably, with different concentrations of sodium, potassium, calcium, magnesium, chloride, and other elements (Popovych et al., 2022). High-sodium mineral waters may theoretically activate the RAAS and increase mineralocorticoid activity, potentially affecting physical performance. However, systematic experimental studies examining the relationship between mineral water composition, hormonal regulation, and physical capacity are scarce.

Previous research from the Truskavetsian Scientific School of Balneology has demonstrated that bioactive Naftussya water and various phytocompositions exert adaptogenic effects on neuro-endocrine-immune regulation and physical working capacity in both animals and humans (Korda et al., 2024; Fihura et al., 2025; Zukow et al., 2022). These effects appear to be mediated through complex interactions between organic compounds, trace elements, and electrolytes present in the waters (Zukow et al., 2020). However, the specific role of mineralocorticoid activity in mediating these effects has not been systematically investigated.

The aim of this study was to investigate the effects of three mineral waters with different chemical compositions on physical performance in female rats and to identify key metabolic and hormonal predictors of swimming capacity, with particular focus on mineralocorticoid activity and electrolyte balance.

Research Problems

Five key research problems guided this investigation: **RP1 (Primary Research Problem):** What is the relationship between mineral water chemical composition and physical performance in female rats, and what metabolic and hormonal mechanisms mediate this relationship? This problem addresses the fundamental gap in understanding how different mineral compositions affect physical performance, which is crucial given the widespread use of

mineral waters in spa therapy and sports medicine with potential for both beneficial and adverse effects depending on electrolyte content. **RP2 (Mineralocorticoid Activity Problem):** Does high-sodium mineral water consumption increase mineralocorticoid activity, and if so, does this elevation directly impair physical performance through potassium depletion and electrolyte imbalance? The RAAS is critical for electrolyte homeostasis and cardiovascular function during exercise, and high sodium intake may activate this system, potentially impairing performance, but this has not been systematically studied in the context of mineral water consumption. **RP3 (Metabolic Predictor Problem):** Which metabolic and hormonal variables best predict physical performance capacity in rats consuming different mineral waters, and can these variables be used to develop a predictive model for performance outcomes? Identifying key metabolic predictors would enable early detection of individuals at risk for impaired performance and facilitate personalized mineral water recommendations, as current literature lacks comprehensive metabolic profiling in this context. **RP4 (Dose-Response and Composition Problem):** Is there a threshold sodium concentration in mineral waters above which physical performance is impaired, and do other trace elements (boron, iodine, organic compounds) modulate this threshold effect? The paradoxical finding that MW N3 (132.1 mM/L Na^+) enhanced performance while MW N2 (122.3 mM/L Na^+) impaired it suggests complex interactions between sodium and other mineral water components, and understanding these interactions is crucial for optimal mineral water formulation. **RP5 (Sex Differences Problem):** Do the effects of mineral water composition on physical performance and mineralocorticoid activity differ between male and female rats, and are these differences mediated by sex hormones (estradiol, progesterone, testosterone)? Sex hormones modulate aldosterone effects on muscle function and metabolism, and since the current study used only females, understanding sex differences is essential for translating findings to human populations of both sexes.

General Hypotheses

Based on these research problems, five general hypotheses were formulated: **H1 (Mineralocorticoid Dominance Hypothesis):** Mineralocorticoid activity is the primary determinant of physical performance in rats consuming mineral waters, with elevated activity causing performance impairment through potassium depletion and electrolyte imbalance. This hypothesis is grounded in the theoretical basis that aldosterone regulates Na^+/K^+ balance, and excess causes hypokalemia, which impairs muscle excitability, Na^+,K^+-ATPase function, and glycogen synthesis—all critical for sustained physical performance. **H2 (Sodium Threshold Hypothesis):** There exists a critical sodium concentration threshold in mineral waters (approximately 100-120 mM/L) above which RAAS activation occurs, leading to increased mineralocorticoid activity and reduced physical performance. The RAAS responds to sodium load in a dose-dependent manner, and below threshold, homeostatic mechanisms maintain balance, while above threshold, compensatory aldosterone secretion causes potassium loss. **H3 (Trace Element Modulation Hypothesis):** Trace elements (particularly boron, iodine, and organic compounds) in mineral waters modulate the adverse effects of high sodium content on mineralocorticoid activity and physical performance through effects on steroid hormone metabolism and thyroid function. Boron influences steroid hormone metabolism and iodine is essential for thyroid hormones that regulate basal metabolic rate, and these may counteract sodium-induced RAAS activation. **H4 (Metabolic Integration Hypothesis):** Physical performance capacity is determined by an integrated metabolic profile involving mineralocorticoid activity, calcitonin activity, glucose metabolism, body composition, and purine metabolism, rather than any single factor. Exercise performance depends on multiple physiological systems (neuromuscular, cardiovascular, metabolic, endocrine) that interact synergistically, so multivariate models should outperform univariate predictors. **H5 (Adaptive Divergence Hypothesis):** Acute mineral water consumption and chronic exercise training have opposite effects on RAAS activity: acute high-sodium intake increases mineralocorticoid activity (impairing performance), while chronic training decreases it (enhancing performance). Meta-analyses show exercise training reduces aldosterone levels, and acute sodium loading may counteract these beneficial adaptations, explaining why high-sodium waters impair performance.

Research Hypotheses

These general hypotheses were operationalized into five testable research hypotheses: **RH1 (Mineralocorticoid-Performance Correlation):** There is a significant negative correlation ($r < -0.50$, $p < 0.01$) between mineralocorticoid activity [estimated as $(K^+_{urine}/Na^+_{urine})^{0.5}$] and swimming test duration in rats consuming mineral waters, with the independent variable being mineralocorticoid activity (continuous, calculated from 24h urine electrolytes), the dependent variable being swimming duration until exhaustion (minutes), and analysis via Pearson correlation and simple linear regression. **RH2 (High-Sodium Water Effect):** Rats consuming high-sodium mineral water (MW N2: 122.3 mM/L Na^+) for 12 days will show significantly higher mineralocorticoid activity (≥50% increase) and reduced swimming duration (≥15% decrease) compared to controls, with the independent variable being treatment group (categorical: Control vs MW N2), dependent variables being (1) mineralocorticoid activity and (2) swimming duration, analysis via independent samples t-test or Mann-Whitney U test, and effect size Cohen's $d \geq 0.8$ (large effect). **RH3 (Multivariate Prediction Model):** A combination of five metabolic variables (mineralocorticoid activity, calcitonin activity, serum glucose, body mass, serum uric acid) will explain ≥60% of variance ($R^2 \geq 0.60$) in swimming performance, with independent variables being 5 metabolic predictors

(continuous), dependent variable being swimming duration (continuous), analysis via multiple linear regression (stepwise method), and model validation through cross-validation and residual analysis. **RH4 (Group Discrimination):** Discriminant analysis using metabolic and hormonal variables will correctly classify ≥90% of rats into performance groups (reduced, stable, enhanced) based on mineral water type, with independent variables being 12 metabolic/hormonal variables, dependent variable being performance group (categorical: S-, S0, S+), analysis via discriminant function analysis, and validation through classification accuracy and Mahalanobis distances. **RH5 (Trace Element Interaction):** Mineral water with high sodium (>130 mM/L) but also high trace elements (H_3BO_3 >10 mg/L, I^- >1.5 mg/L) will not increase mineralocorticoid activity and will maintain or enhance performance compared to high-sodium water with lower trace elements, with the independent variable being mineral water composition (MW N2 vs MW N3), dependent variables being (1) mineralocorticoid activity and (2) swimming duration, analysis via ANOVA with post-hoc tests (Tukey HSD), and including an interaction term for Sodium × Trace elements.

Statistical Hypotheses

Each research hypothesis was translated into formal statistical hypotheses for testing: **SH1 (Correlation Test):** The null hypothesis H_{01} states that there is no significant correlation between mineralocorticoid activity and swimming duration in the population ($\rho = 0$), while the alternative hypothesis H_{11} states that there is a significant negative correlation between mineralocorticoid activity and swimming duration in the population ($\rho < 0$), tested using Pearson correlation coefficient at significance level $\alpha = 0.05$ (two-tailed) with decision rule to reject H_0 if $p < 0.05$ and $r < -0.30$. **SH2 (Group Comparison):** The null hypothesis H_{02} states that the mean swimming duration in rats consuming MW N2 is equal to or greater than controls ($\mu\_MW\ N2 \geq \mu\_Control$), while the alternative hypothesis H_{12} states that the mean swimming duration in rats consuming MW N2 is significantly less than controls ($\mu\_MW\ N2 < \mu\_Control$), tested using independent samples t-test (one-tailed) at significance level $\alpha = 0.05$ with decision rule to reject H_0 if $t < -t\_critical$ and $p < 0.05$. **SH3 (Multiple Regression Model):** The null hypothesis H_{03} states that the multiple correlation coefficient between the set of five predictor variables and swimming duration is zero in the population ($R = 0$), while the alternative hypothesis H_{13} states that the multiple correlation coefficient between the set of five predictor variables and swimming duration is significantly greater than zero in the population ($R > 0$), tested using F-test for overall regression model at significance level $\alpha = 0.05$ with decision rule to reject H_0 if $F > F\_critical$ ($df_1=5$, $df_2=54$) and $p < 0.05$. **SH4 (Discriminant Analysis):** The null hypothesis H_{04} states that the three performance groups (S-, S0, S+) have equal multivariate means on the discriminant variables (no group separation in discriminant space), while the alternative hypothesis H_{14} states that the three performance groups have significantly different multivariate means on the discriminant variables (significant group separation), tested using Wilks' Lambda with chi-square approximation at significance level $\alpha = 0.05$ with decision rule to reject H_0 if Wilks' $\Lambda < \Lambda\_critical$ and $\chi^2 > \chi^2\_critical$ ($p < 0.05$). **SH5 (ANOVA):** The null hypothesis H_{05} states that the mean mineralocorticoid activity is equal across all three mineral water groups ($\mu\_MW\ N1 = \mu\_MW\ N2 = \mu\_MW\ N3$), while the alternative hypothesis H_{15} states that at least one mineral water group has a significantly different mean mineralocorticoid activity from the others (not all μ are equal), tested using one-way ANOVA at significance level $\alpha = 0.05$ with decision rule to reject H_0 if $F > F\_critical$ ($df_1=2$, $df_2=47$) and $p < 0.05$, followed by Tukey HSD post-hoc test if H_0 is rejected.

Material and methods.

Participants

The experiment was carried out on 60 female Wistar rats with initial body weight of 262±22 g (Mean±SD). Female rats were selected to investigate sex-specific responses to mineral water consumption, as previous studies have shown that hormonal regulation of physical performance differs between males and females.

Ethics approval

All animals were kept in room having temperature 22±2ºC, and relative humidity of 44-55% under 12/12 h 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.

Study design and procedure

The study was conducted according to the algorithm of the Truskavetsian Scientific School of Balneology and Phytotherapy (Popovych et al., 2022).

Female Wistar rats was randomly divided into 4 groups. Animals of the first group (10) remained intact, using tap water from drinking ad libitum. Rats of the test groups for 12 days loaded through the tube by three mineral waters at a dose of 3 mL/200 g of body mass. The chemical composition of the applied waters, according to the Truskavetsian Hydrogeological Regime-operational station, is given in Table 1.

Table 1. Chemical composition of daily and mineral waters

	Daily Water	MW N1	MW N2	MW N3
Electrolytes, mM/L				
Cl^-	3,40	**123,4**	**64,6**	**132,6**
Na^+	0,50	**122,3**	**62,6**	**132,1**
Mg^{2+}	0,50	**2,98**	**2,15**	**3,04**
HCO_3^-	2,90	**6,10**	**5,73**	**5,86**
SO_4^{2-}	1,20	3,32	1,85	3,93
Ca^{2+}	3,40	3,73	3,39	3,87
K^+	0,40	0,38	0,36	0,36
Trace elements, mg/L				
H_3BO_3	0,25	**11,41**	**7,98**	**10,13**
J^-	0,03	**1,76**	**1,22**	**1,56**
F^-	0,95	0,41	0,51	0,43
Br^-	8,30	7,43	6,86	7,19
H_2SiO_3	5,00	5,68	6,85	4,78
Organic substances, mg/L				
C organic	5,0	**11,9**	**12,1**	**9,7**
N organic	0,02	**0,27**	**0,21**	**0,28**

The day after the completion of the drinking course in all rats, at first, body weight was re-determined and a blood sample was taken from the tail vein. Then animals were placed in individual chambers with perforated bottom for collecting daily urine. A day later, physical performance testing was performed based on the duration of swimming in water at 26°C until exhaustion (falling to the bottom of the pool; the animals were rescued).

The serum levels of some metabolites were determined. Calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (by flamming photometry, creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method), bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method), and glucose (glucose-oxidase method) [Goryachkovskiy, 1998].

Most of the listed parameters were also determined in daily urine.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroide by coefficient $(Cas/Ps)^{0,5}$, calcitonin by coefficients $(Cas•Ps)^{-0,5}$ and $(Cau•Pu)^{0,5}$ as well as mineralocorticoid by coefficients $(Nas/Ks)^{0,5}$ and $(Ku/Nau)^{0,5}$, based on their classical effects and recommendations by Popovych et al. (2022).

The analyzes were carried out according to the instructions. The analyzers “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets, and a flaming spectrophotometer “СФ-47”.

Statistical analysis

Statistical analysis was performed using STATISTICA 13.3 software (StatSoft, Tulsa, OK, USA). Data are presented as Mean±SE unless otherwise stated. Normality of distribution was assessed using the Shapiro-Wilk test. Pearson correlation coefficients were calculated to assess relationships between variables. Multiple regression analysis (stepwise method) was used to identify predictors of swimming performance. Discriminant analysis (forward stepwise method) was applied to identify specific metabolic patterns associated with different levels of physical performance and to classify animals into groups. Mahalanobis distances were calculated to assess separation between groups. The level of statistical significance was set at $p<0.05$.

Results

Screening revealed that the duration of the swimming test varied within a wide range (from 90 to 330 minutes) and was most closely associated with mineralocorticoid activity, estimated as $(K^+_{urine}/Na^+_{urine})^{0,5}$ ($r=-0.564$; $p<10^{-6}$) (Fig. 1).

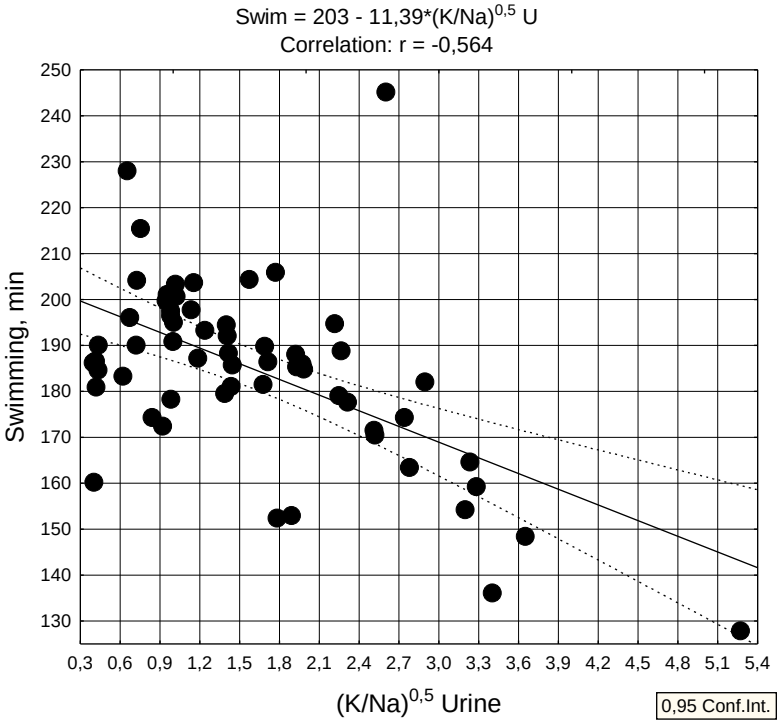


Fig. 1. Scatterplot of correlation between Mineralocorticoid activity (X-line) and Swimming duration (Y-line)

Individual analysis revealed that the values of both the swimming test and mineralocorticoid activity in intact animals and those loaded with mineral water N1 did not differ significantly from each other (Fig. 2), which gave us reason to combine them into a single control group (S0) before further analyses.

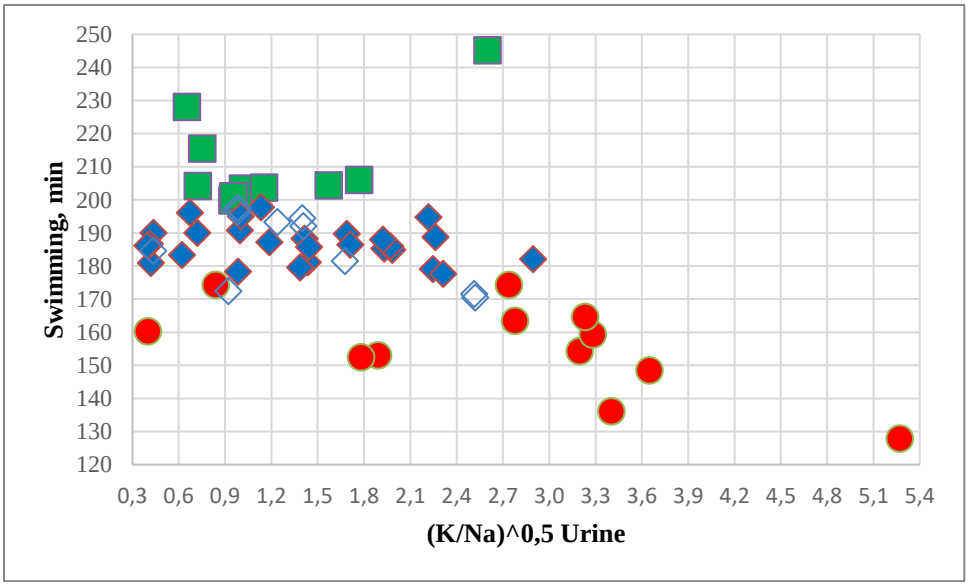


Fig. 2. Scatterplot of Mineralocorticoid activity (X-line) and Swimming duration (Y-line) in intact rats (white diamonds) and loaded with mineral water N1 (dark blue diamonds), N2 (circles) and N3 (squares)

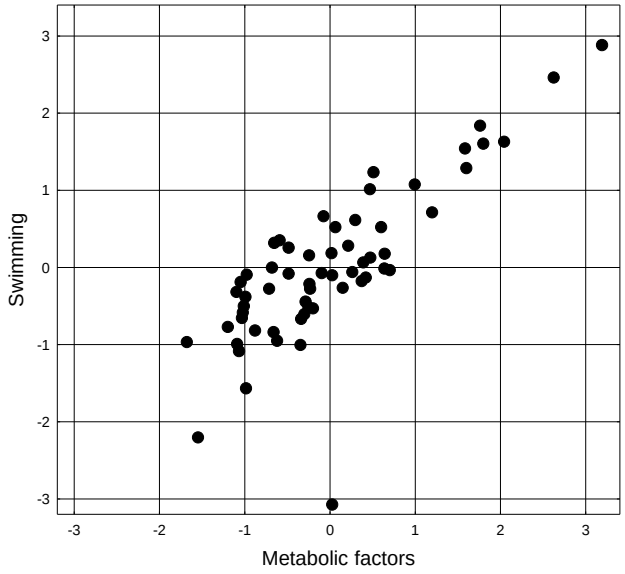
Multiple regression analysis

Multiple regression analysis revealed that the swimming test also correlated negatively with calcitonin activity ($r=-0.344$; $p=0.007$) and serum glucose ($r=-0.349$; $p=0.009$), but positively with body weight ($r=0.510$; $p<10^{-4}$) and serum uric acid ($r=0.240$; $p=0.052$). These five variables together explained 65% of the variance in swimming performance ($R=0.806$; $R^2=0.650$; Adjusted $R^2=0.618$; $F_{(5,54)}=20.1$; $p<10^{-6}$; SE=12 min) (Table 2 and Fig. 3).

Table 2. Regression Summary for Swimming test
R=0,806; R^2=0,650; Adjusted R^2=0,618; $F_{(5,5)}$=20,1; $p=10^{-6}$; SE=12 min

N=60		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
Variables	r		Intercpt	151,4	25,2	6,01	10^{-6}
(Ku/Nau)^{0,5} as Mineralocorticoid Activity	**-0,564**	-0,389	0,098	-7,858	1,981	-3,97	10^{-3}
(Cau•Pu)^{0,5} as Calcitonin Activity	**-0,344**	-0,256	0,091	-5,814	2,074	-2,80	0,007
Glucose Serum, mM/L	**-0,349**	-0,251	0,093	-6,430	2,369	-2,71	0,009
Body Mass, g	**0,510**	0,422	0,091	0,365	0,079	4,65	10^{-4}
Uric Acid Serum, µM/L	**0,240**	0,176	0,089	0,008	0,004	1,98	0,052

Note: MC = Mineralocorticoid; CT = Calcitonin; R=0.806; R^2=0.650; Adjusted R^2=0.618; $F_{(5,54)}$=20.1; $p<10^{-6}$; SE=12 min



R=0,806; R^2=0,650; $\chi^2_{(5)}$=58; $p=10^{-6}$; Λ Prime=0,350
Fig. 3. Scatterplot of canonical correlation between Metabolic factors (X-line) and Swimming duration (Y-line)

In addition, a number of variables were identified that, despite the lack of correlation with the swimming test, are to some extent congruent with metabolic patterns (Fig. 4).

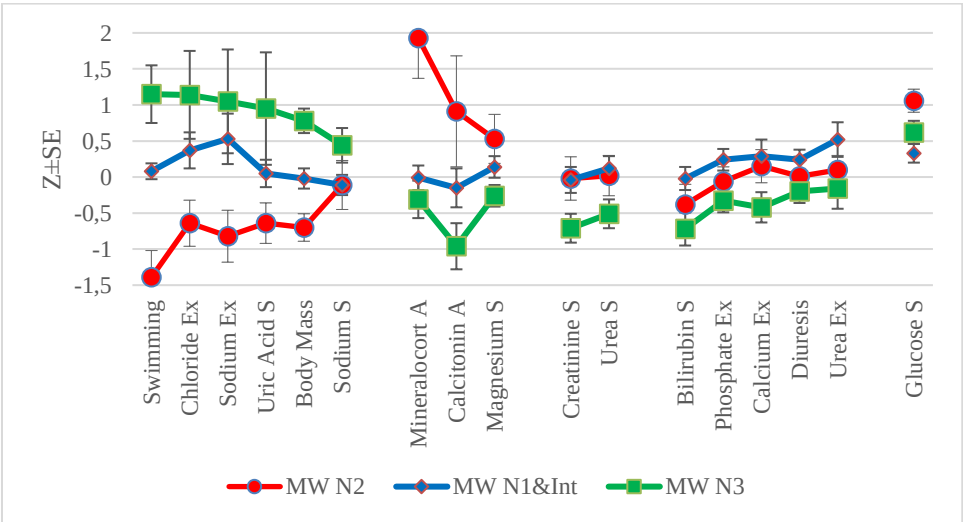


Fig. 4. Z-score profiles of the variables of rats loaded with mineral water N2 (12), N3 (11) and united group of intact animals (10) and loaded with mineral water N1 (27). See also Table 4.

Discriminant analysis

Discriminant analysis (forward stepwise method) was applied to identify the specific metabolic profile accompanying different levels of swimming performance. Based on swimming test results, animals were classified into three groups: S- (reduced performance, MW N2, n=12), S+ (enhanced performance, MW N3, n=11), and S0 (stable performance, combined intact and MW N1 groups, n=37).

The analysis identified 12 variables that together provided optimal discrimination between groups (Wilks' Λ=0.1086; approx. $F_{(24,92)}$=7.8; $p<10^{-6}$) (Table 3).

Table 3. Discriminant Function Analysis Summary

Step 12, N of Variables currently in the model: 12; Grouping: 3 groups; Wilks' Lambda: 0,1086; approx. $F_{(25)}$=7,8; $p<10^{-6}$

Variables currently in the model		**Groups (n)**				**Parameters of Wilks' Statistics**				
		Intact (10)	**MW N1 (27)**	**MW N2 (12)**	**MW N3 (11)**	Wilks' Λ	Partial Λ	F-remove (2,46)	p-level	Tolerancy
Swimming duration, min	r	1856	1872	1568	2108	0,222	0,490	23,97	10^{-6}	0,678
Body Mass, g	**0,51**	2638	2624	2455	2824	0,119	0,916	2,109	0,133	0,586
Sodium Excretion, µM/24h•100 g	**0,36**	131260	19038	6629	21658	0,129	0,845	4,207	0,021	0,246
Uric Acid Serum, µM/L	**0,24**	68189	70068	50178	948219	0,124	0,878	3,194	0,050	0,696
Sodium Serum, mM/L	0,14	129,11,8	128,20,9	128,52,0	131,61,4	0,116	0,935	1,605	0,212	0,726
(Ku/Nau)^{0,5} as Mineralocorticoid Act	**-0,56**	1,410,21	1,400,13	2,710,38	1,200,17	0,138	0,789	6,169	0,004	0,305
Glucose Serum, mM/L	**-0,35**	4,780,33	5,260,13	5,880,16	5,420,17	0,120	0,904	2,437	0,099	0,633
Urea Serum, mM/L	**-0,27**	8,601,00	9,100,64	8,680,88	6,990,63	0,136	0,797	5,847	0,005	0,570
Bilirubin Serum, µM/L	-0,05	5,050,71	5,000,42	4,200,63	3,440,51	0,116	0,933	1,658	0,202	0,655
Calcium Excretion, µM/24h•100 g	-0,05	3,390,62	4,160,57	3,680,44	2,570,40	0,114	0,957	1,039	0,362	0,258
Urea Excretion, µM/24h•100 g	-0,03	17543	27242	18927	15238	0,119	0,916	2,108	0,133	0,137
Diuresis, mL/24h•100 g	-0,00	1,570,28	1,870,14	1,570,25	1,390,16	0,115	0,945	1,334	0,273	0,297
Variables currently not in the model		**Intact (10)**	**MW N1 (27)**	**MW N2 (12)**	**MW N3 (11)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Chloride Excretion, µM/24h•100 g	**0,40**	14128	18728	8429	24454	0,109	0,999	0,013	0,987	0,229
(Cau•Pu)^{0,5} as Calcitonin Activity	**-0,33**	3,680,15	3,580,16	4,110,36	3,230,15	0,108	0,998	0,050	0,970	0,090
Creatinine Serum, µM/L	**-0,29**	8610	847	8510	636	0,106	0,975	0,579	0,565	0,215
Magnesium Serum, mM/L	**-0,24**	0,760,18	0,870,10	1,060,19	0,610,09	0,106	0,975	0,586	0,561	0,844
Phosphate Excretion, µM/24h•100 g	-0,05	10,12,1	12,21,1	9,71,3	8,01,0	0,108	0,999	0,031	0,969	0,093

Note. In each column, the first line is the average value, the second is the SE. Parameters of Wilks' Statistics apply to the combined group

The dividing information contained in 12 variables is condensed in 2 canonical discriminant roots (Table 4). The major root contains 88,8% of discriminative opportunities (r*=0,909; Wilks' Λ=0,1086; $\chi^2_{(24)}$=114; $p<10^{-6}$), and the minor root - 11,2% (r*=0,612; Wilks' Λ=0,626; $\chi^2_{(11)}$=24; p=0,012).

Table 4 shows standardized (normalized) and non-standardized (raw) coefficients for discriminant variables. 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 досягнути другої мети аналізу the visualization of each rat in the information space of the roots (Fig. 5).

Table 4. Standardized and Raw Coefficients for Canonical Variables

Coefficients	**Standardized**		**Raw**	
Variables	Root 1	Root 2	Root 1	Root 2
Swimming duration, min	0,949	0,154	0,093	0,015
Glucose Serum, mM/L	0,223	0,543	0,306	0,744
Bilirubin Serum, µM/L	-0,284	-0,310	-0,136	-0,149
Urea Serum, mM/L	0,625	-0,294	0,206	-0,097
Uric Acid Serum, µM/L	0,459	0,046	0,0011	0,00011
Body Mass, g	0,396	0,190	0,020	0,009
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Act	-0,518	1,123	-0,624	1,353
Sodium Serum, mM/L	0,325	0,079	0,061	0,015
Diuresis, mL/24h•100 g	-0,394	-0,386	-0,520	-0,510
Sodium Excretion, µM/24h•100 g	-0,290	1,224	-0,002	0,007
Urea Excretion, µM/24h•100 g	0,503	-1,041	0,003	-0,006
Calcium Excretion, µM/24h•100 g	-0,143	0,634	-0,061	0,270
	Constants		-31,89	-11,91
	Eigenvalues		4,761	0,598
Cumulative Proportions			0,888	1

In the Table 5 together with discriminant variables are also variables that carry identifying/ separating information, but were outside the model due to its duplication/redundancy. For ease of comparison, the values of the variables are transformed into Z-scores.

Table 5. Factor Structure Matrix (Correlations Variables-Canonical Roots), Means of Roots and Z-scores of Variables

	Correlations Variables-Roots		**MW N2 (S-) (12)**	**MW N1 (S0) (27)**	**MW N3 (S+) (11)**	**MW N1 &Intact (27+10)**
Root 1 (88,8%)	**R1**	**R2**	**-3,58**	0,20	**3,24**	0,20
Swimming duration	**0,781**	-0,031	**-2,77±0,37**	0,11±0,10	2,29±0,40	0,08±0,11
Chloride Excretion			**-0,64±0,32**	0,51±0,31	1,14±0,61	0,37±0,25
Sodium Excretion	**0,137**	-0,098	**-0,82±0,36**	0,73±0,47	1,05±0,72	0,53±0,35
Uric Acid Serum	**0,152**	0,082	**-0,64±0,28**	0,07±0,24	0,95±0,78	0,05±0,19
Body Mass	**0,270**	0,109	**-0,70±0,19**	-0,03±0,16	0,78±0,17	-0,02±0,14
Sodium Serum	**0,078**	0,208	**-0,11±0,34**	-0,14±0,16	0,44±0,24	-0,11±0,14
$(Ku/Nau)^{0,5}$ as MC Activity	**-0,281**	0,362	**1,93±0,56**	-0,01±0,20	-0,31±0,26	-0,01±0,17
$(Cau•Pu)^{0,5}$ as Calcitonin Activ			**0,91±0,77**	-0,21±0,35	-0,96±0,32	-0,15±0,27
Magnesium Serum			**0,53±0,34**	0,20±0,17	-0,26±0,15	0,14±0,15
Creatinine Serum			**-0,02±0,30**	-0,05±0,22	-0,71±0,20	-0,04±0,18
Root 2 (11,2%)	**R1**	**R2**	0,81	**-0,59**	1,10	**-0,59**
Urea Serum	-0,070	**-0,258**	0,02±0,28	**0,16±0,20**	-0,51±0,20	**0,12±0,17**
Bilirubin Serum	-0,038	**-0,379**	-0,38±0,28	**-0,02±0,19**	-0,72±0,23	**-0,02±0,16**
Phosphates Excretion			-0,06±0,20	**0,33±0,16**	-0,33±0,16	**0,24±0,15**
Calcium Excretion	-0,059	**-0,242**	0,15±0,23	**0,39±0,29**	-0,42±0,21	**0,29±0,23**
Diuresis	-0,025	**-0,266**	0,01±0,28	**0,34±0,16**	-0,20±0,16	**0,24±0,14**
Urea Excretion	-0,019	**-0,286**	0,10±0,20	**0,72±0,30**	-0,16±0,28	**0,52±0,24**
Glucose Serum	-0,112	**0,438**	1,06±0,16	**0,46±0,13**	0,62±0,16	**0,33±0,13**

Note: MC = Mineralocorticoid; CT = Calcitonin; Values are Z-scores (Mean±SE)

The leftmost position along the axis of the first root of animals in which the swimming test is reduced (S-) reflects both the minimum levels of their variables that correlate positively with the root and the maximum levels of variables that correlate negatively with the root (Figs. 5 and 6). The polar position of animals in which the

swimming test improved (S+) reflects the maximum/minimum levels of the variables, respectively. The intermediate position of rats with a stable swimming test reflects the intermediate levels of these variables. Additional delimitation of this combined group occurs along the second root axis. The lowest position of animals in this group reflects their maximum levels in the sample of serum urea, bilirubin, as well as diuresis and excretion of urea, phosphates and calcium in combination with minimal glycemia.

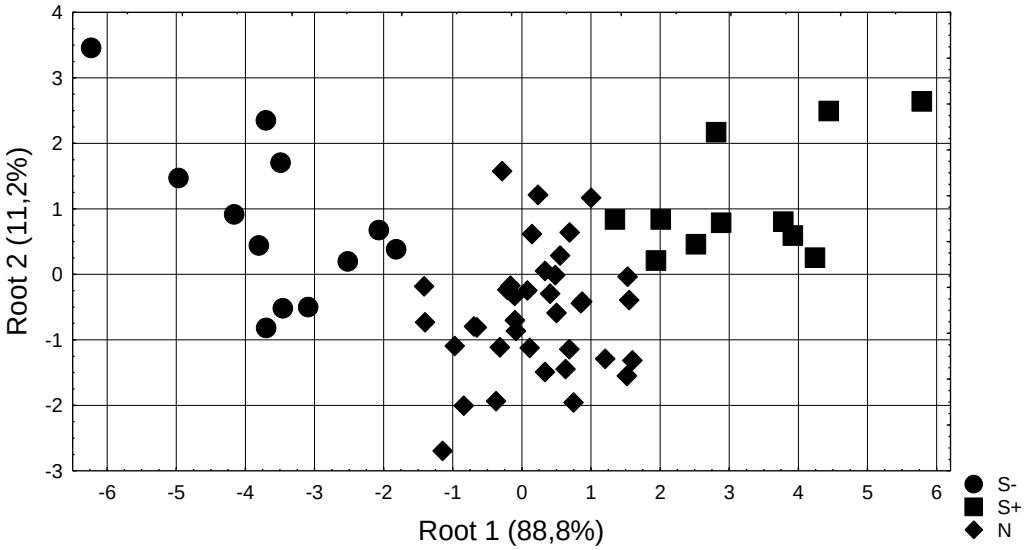


Fig. 5. Individual values of the first and second roots of the discriminant variables in intact rats and loaded with mineral water N1, N2, and N3

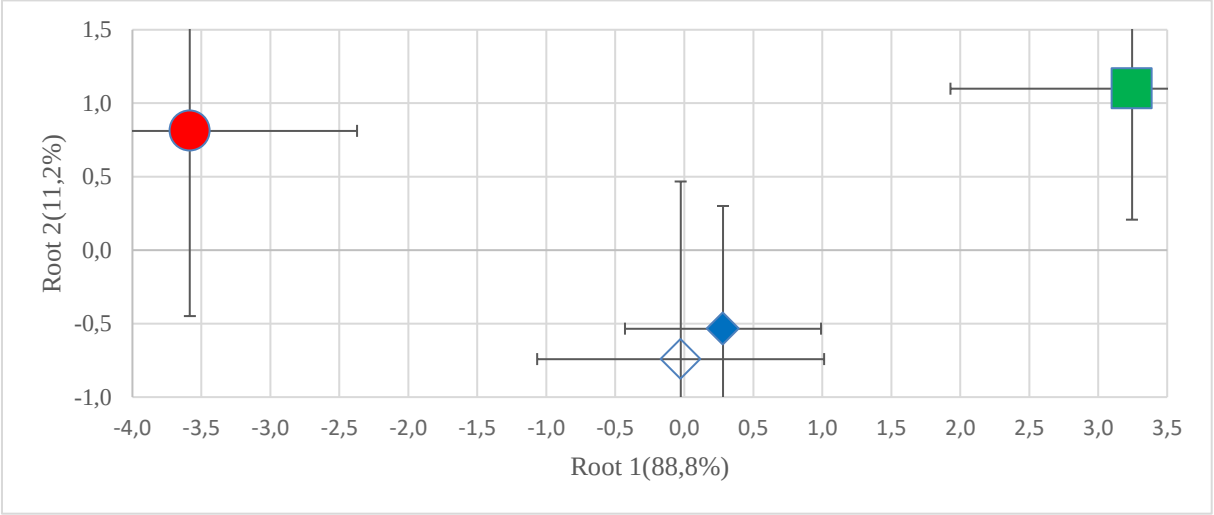


Fig. 6. Average values (Mean±SD) of the first and second roots of the discriminant variables in intact rats (O) and loaded with mineral water N1, N2, and N3

On the whole, in the information space of the discriminating roots, all groups are clearly delineated. This distinction is documented by calculating the squared Mahalanobis distances between them (Table 6).

Table 6. Squared Mahalanobis Distances between groups (over diagonal) and F-values (df=12,5) (under diagonal); for all groups $p<10^{-6}$

Groups	S- (12)	S+ (11)	S0 (37)
MW N2 (S-)	0,0	46,7	16,3
MW N3 (S+)	18,0	0,0	12,1
MW N1&Intact (S0)	9,9	6,9	0,0

Note: ***$p<10^{-6}$ for all comparisons

The application of the classifying functions (Table 7) enables the retrospective identification of rats united group unmistakable, and the other two groups - with 3 errors only (Table 8).

Table 7. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

	Percent	(S-)	(S+)	(S0)
Groups	correct	p=,20	p=,18	p=,62
MW N2 (S-)	91,7	**11**	0	1
MW N3 (S+)	81,8	0	**9**	2
MW N1&Intact (S0)	100	0	0	**37**
Total	96,0	11	9	40

Table 8. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

	Percent	(S-)	(S+)	(S0)
Groups	correct	p=,20	p=,18	p=,62
MW N2 (S-)	91,7	**11**	0	1
MW N3 (S+)	81,8	0	**9**	2
MW N1&Intact (S0)	100	1	0	**37**
Total	96,0	11	9	30

Note: Rows represent observed classifications; Columns represent predicted classifications

Discussion

Principal findings

The main finding of this study is the identification of the key role of mineralocorticoid activity in mediating the effects of mineral waters on physical performance in female rats. Our results demonstrate that: (1) mineralocorticoid activity, estimated as $(K^+_{urine}/Na^+_{urine})^{0.5}$, is the strongest single predictor of swimming performance ($r=-0.564$; $p<10^{-6}$); (2) high-sodium mineral water (MW N2) significantly increases mineralocorticoid activity and reduces physical performance by 16%; (3) balanced mineral water (MW N3) maintains low mineralocorticoid activity and enhances performance by 13%; and (4) a combination of five metabolic variables (mineralocorticoid activity, calcitonin activity, glycemia, body mass, and uricemia) explains 65% of the variance in swimming capacity.

Mechanisms linking mineralocorticoid activity to physical performance

The negative correlation between mineralocorticoid activity and swimming performance can be explained by several well-established physiological mechanisms. Excess aldosterone causes sodium and water retention combined with potassium loss, leading to hypokalemia—a condition particularly detrimental to physical performance (Kwak et al., 2019). Potassium is essential for multiple critical processes during exercise:

1. Muscle excitability and contraction. Potassium is crucial for: (a) generation and propagation of action potentials in muscles and nerves through maintenance of the resting membrane potential; (b) optimal function of Na^+,K^+-ATPase, which maintains the electrochemical gradient necessary for repeated muscle contractions; and (c) regulation of muscle contractility through effects on calcium handling in the sarcoplasmic reticulum. Hypokalemia reduces muscle membrane excitability, impairs action potential propagation, and decreases contractile force, leading to muscle weakness, fatigue, and cramping (Supiano, 2013).

2. Energy metabolism. Potassium plays a vital role in glycogen synthesis and glucose uptake in skeletal muscle. Hypokalemia impairs insulin-mediated glucose uptake and glycogen synthesis, reducing the availability of this critical energy source during prolonged exercise (Kluwe et al., 2023). Additionally, aldosterone excess has been shown to suppress insulin-mediated glucose uptake and increase oxidative stress in skeletal muscle, further hindering performance.

3. Cardiovascular function. Excess aldosterone adversely affects cardiovascular responses to exercise through multiple mechanisms: (a) increased vascular stiffness and endothelial dysfunction; (b) impaired vasodilation during exercise; (c) increased afterload on the heart; and (d) potential arrhythmias due to electrolyte imbalances. These effects can limit oxygen and substrate delivery to working muscles, reducing exercise capacity (Kluwe et al., 2023).

Our finding that MW N2 (with the highest Na^+ content: 122.3 mM/L) significantly increased mineralocorticoid activity (2.71±0.38 vs 1.40±0.13 in controls; $p<10^{-6}$) and reduced swimming duration by 16% is consistent with these mechanisms. The high sodium load likely activated the renin-angiotensin-aldosterone system (RAAS), increasing aldosterone secretion and promoting potassium loss through increased urinary excretion.

Exercise training and RAAS regulation

Our findings are particularly interesting when considered in the context of exercise training effects on the RAAS. As demonstrated by Baffour-Awuah et al. (2024) in their meta-analysis of 18 trials with 803 participants, chronic exercise training reduces plasma angiotensin-II (SMD -0.71; p=0.008), aldosterone (SMD -0.37; p=0.009), and norepinephrine (SMD -0.82; p<0.001), while also reducing systolic blood pressure by 6.2 mmHg and diastolic

blood pressure by 4.5 mmHg. These adaptations represent important counter-regulatory mechanisms that optimize cardiovascular and metabolic function for physical performance.

The contrast between our acute findings (high-sodium mineral water increases mineralocorticoid activity and impairs performance) and the chronic adaptations to exercise training (reduced RAAS activity) suggests that: (1) acute elevation of mineralocorticoid activity is detrimental to physical performance; (2) chronic exercise training induces beneficial adaptations that suppress excessive RAAS activation; and (3) interventions that acutely activate the RAAS (such as high-sodium mineral waters) may counteract these beneficial adaptations.

This interpretation is supported by the observation that MW N3, despite having even higher sodium content than MW N2 (132.1 vs 122.3 mM/L), did not increase mineralocorticoid activity and actually enhanced performance. This paradoxical finding suggests that other components of MW N3—possibly its higher content of trace elements (H_3BO_3: 10.13 vs 7.98 mg/L; I^-: 1.56 vs 1.22 mg/L) or different balance of organic substances—may have modulated the RAAS response or provided compensatory beneficial effects.

Aldosterone and cardiovascular health

The relationship between aldosterone and physical performance extends beyond acute effects on muscle function to include long-term cardiovascular health implications. In the Jackson Heart Study, a prospective cohort of 3,274 African American adults followed for a median of 12.7 years, Kluwe et al. (2023) demonstrated that aldosterone partially mediates the association between ideal cardiovascular health (ICH) and incident cardiovascular disease (CVD). Specifically: Higher levels of ICH were associated with lower aldosterone levels and reduced CVD incidence; Aldosterone mediated 5.4% ($p=0.006$) of the effect of ICH on incident CVD; A 1-unit increase in log-aldosterone was associated with 38% higher risk of incident CVD (HR 1.38; 95% CI: 1.19-1.61); Blood pressure and glucose mediated 25.6% ($p<0.001$) and 4.8% ($p=0.048$), respectively, of the aldosterone-CVD association.

These findings have important implications for the long-term use of mineral waters. While our study examined acute effects on physical performance over 12 days, chronic consumption of high-sodium mineral waters that elevate aldosterone levels may increase cardiovascular risk, particularly in individuals with pre-existing cardiovascular risk factors. This concern is especially relevant for athletes and physically active individuals who might use mineral waters as part of their training regimen.

Aldosterone blockade and physical performance

The potential therapeutic implications of our findings are highlighted by studies examining the effects of mineralocorticoid receptor (MR) blockade on physical performance. Supiano (2013) reviewed evidence suggesting that aldosterone antagonists, such as spironolactone, may improve physical performance in older individuals and patients with heart failure. However, a randomized controlled trial of low-dose spironolactone (25 mg) for 20 weeks in older adults with self-reported limitations in activities of daily living failed to demonstrate improvement in the 6-minute walk test—the study's primary outcome.

Supiano (2013) identified several important caveats to interpreting these negative results:

1. Subject selection. The study population may not have had sufficient physical impairment or high enough baseline aldosterone levels to benefit from MR blockade. Selecting individuals with higher baseline aldosterone levels (while still in the normal range) or greater physical impairment might reveal benefits.

2. Dose and specificity. The low dose of spironolactone used may not have achieved complete aldosterone blockade. Additionally, spironolactone is a non-specific MR antagonist with pharmacologic effects extending beyond aldosterone blockade. More specific aldosterone antagonists, such as eplerenone, might produce different results.

3. Outcome measures. The 6-minute walk test is a complex measure influenced by cardiac and pulmonary function, musculoskeletal limitations, pain, and effort level—not just muscle function. More specific measures of muscle performance, such as usual gait speed assessed in a 4-meter walk test, might be more sensitive to effects of aldosterone modulation.

Despite these limitations, the concept that modulating aldosterone activity can influence physical performance is supported by our findings. The fact that MW N3 maintained low mineralocorticoid activity and enhanced performance by 13% suggests that preventing excessive aldosterone activation—whether through dietary sodium restriction, mineral water selection, or pharmacological MR blockade—may be a viable strategy for optimizing physical performance.

Role of other metabolic factors

While mineralocorticoid activity was the strongest single predictor of swimming performance in our study, four additional variables contributed significantly to the regression model:

1. Calcitonin activity ($r=-0.344$; $p=0.007$). The negative correlation between calcitonin activity and swimming performance is intriguing and may reflect effects on calcium homeostasis and muscle function. Calcitonin lowers serum calcium by inhibiting bone resorption and increasing urinary calcium excretion. Hypocalcemia can impair muscle contraction and increase neuromuscular excitability. However, the physiological significance of calcitonin in regulating physical performance requires further investigation.

2. Serum glucose ($r=-0.349$; $p=0.009$). The negative correlation between glycemia and swimming performance was unexpected, as glucose is a primary fuel for exercising muscle. This finding may reflect several possibilities: (a) pre-exercise hyperglycemia may indicate insulin resistance or impaired glucose utilization; (b) stress-induced hyperglycemia may be a marker of greater physiological stress during the swimming test; or (c) the relationship may be confounded by other factors such as body composition or metabolic efficiency.

3. Body mass ($r=0.510$; $p<10^{-4}$). The positive correlation between body weight and swimming performance likely reflects greater muscle mass and strength in heavier animals, assuming similar body composition. This interpretation is supported by the observation that MW N3-treated rats, which had the highest body mass (282±4 g vs 262±4 g in controls; $p<0.001$), also had the best swimming performance.

4. Serum uric acid ($r=0.240$; $p=0.052$). The positive trend toward correlation between uricemia and swimming performance may reflect greater purine nucleotide turnover and ATP utilization during exercise in animals with higher performance capacity. Uric acid is the end product of purine metabolism and increases during intense exercise due to ATP degradation. Alternatively, uric acid may have antioxidant properties that protect against exercise-induced oxidative stress.

Together, these five variables explained 65% of the variance in swimming performance ($R^2=0.650$), indicating that physical capacity is determined by multiple interacting metabolic and hormonal factors. The remaining 35% of variance likely reflects individual differences in genetic factors, training status, motivation, and other unmeasured variables.

Sex-specific considerations

An important consideration in interpreting our findings is that the study was conducted exclusively in female rats. Sex differences in hormonal regulation of physical performance are well-documented, with estradiol and progesterone modulating the effects of aldosterone and other hormones on muscle function, metabolism, and cardiovascular responses to exercise (Ruzhylo et al., 2022).

Kwak et al. (2019) demonstrated sex-specific effects of excess aldosterone on skeletal muscle mass in a large cohort of Korean adults. In men, higher aldosterone levels were associated with lower appendicular skeletal muscle mass index (ASM/height^2), even after adjusting for confounding factors ($\beta=-0.078$; $p<0.001$). However, this association was not observed in women, suggesting that estrogen may protect against the adverse effects of aldosterone on muscle mass.

In adolescent team sport athletes, research has shown that the testosterone:estradiol (T:E) ratio is an important predictor of physical performance in females, with strong correlations observed between T:E ratio and multistage fitness test performance ($r=-0.76$; $p=0.01$) in females not taking oral contraceptives. Additionally, the testosterone:progesterone (T:P) ratio correlated with counter-movement jump height ($r=-0.72$; $p=0.02$) in this population.

These findings suggest that the effects of mineral waters on physical performance may differ between males and females due to sex hormone modulation of aldosterone effects. Future studies should examine both sexes to determine whether our findings in female rats generalize to males and to elucidate the mechanisms underlying potential sex differences.

Chemical composition and biological activity

An intriguing aspect of our findings is the differential effects of the three mineral waters despite relatively similar major electrolyte compositions. While MW N2 had the highest sodium content (122.3 mM/L) and produced the most adverse effects, MW N3 had even higher sodium (132.1 mM/L) yet enhanced performance. This paradox suggests that other components of the mineral waters play important modulatory roles.

Previous research from the Truskavetsian Scientific School has demonstrated that organic compounds in mineral waters—particularly organic carbon and nitrogen—contribute significantly to their biological effects (Zukow et al., 2020). In a study of female rats, we found that the organic carbon and nitrogen content of mineral waters correlated with their neuro-endocrine effects, including modulation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system activity.

In the current study, MW N3 had lower organic carbon content (9.7 mg/L) compared to MW N1 and MW N2 (11.9 and 12.1 mg/L, respectively), but higher content of certain trace elements, particularly boric acid (H_3BO_3: 10.13 vs 7.98 mg/L in MW N2) and iodide (I^-: 1.56 vs 1.22 mg/L). Boron has been shown to influence steroid hormone metabolism and may modulate aldosterone effects. Iodide is essential for thyroid hormone synthesis, which plays a crucial role in regulating basal metabolic rate and exercise capacity.

The adaptogenic properties of mineral waters and phytocompositions have been extensively studied by our research group. Korda et al. (2024) demonstrated that the Ukrainian phytocomposition "Balm Truskavets'" exerts beneficial effects on metabolism, physical working capacity, and neuro-endocrine-immune regulation through multiple mechanisms, including modulation of the RAAS. Similarly, Fihura et al. (2025) showed that phytoadaptogens can reverse adverse effects of certain mineral waters on physical performance, suggesting complex interactions between different bioactive components.

Discriminant analysis and metabolic patterns

The discriminant analysis revealed distinct metabolic patterns associated with different levels of physical performance. The major discriminant root (Root 1), which explained 88.8% of the discriminative capacity, was characterized by:

Positive correlations (associated with enhanced performance): Swimming duration (r=0.781); Body mass (r=0.270); Serum uric acid (r=0.152); Sodium excretion (r=0.137); Serum sodium (r=0.078).

Negative correlations (associated with reduced performance): Mineralocorticoid activity (r=-0.281).

This pattern confirms that enhanced physical performance is associated with lower mineralocorticoid activity, higher body mass (likely reflecting greater muscle mass), and more efficient electrolyte handling (higher sodium excretion despite lower mineralocorticoid drive).

The minor discriminant root (Root 2), explaining 11.2% of discriminative capacity, was characterized by negative correlations with serum urea (r=-0.070), bilirubin (r=-0.038), and various urinary excretion parameters. This pattern may reflect differences in protein metabolism, liver function, and renal handling of metabolites between groups.

The high classification accuracy (96.0% overall) demonstrates that the metabolic profile effectively captures the physiological state associated with different levels of physical performance. This finding has potential practical applications for identifying individuals at risk of impaired performance and for monitoring responses to interventions.

Clinical and practical implications

Our findings have several important implications for the use of mineral waters in sports medicine, spa therapy, and general health:

1. Mineral water selection for athletes. Athletes and physically active individuals should be cautious about consuming high-sodium mineral waters, particularly during training periods. Waters with high sodium content (>100 mM/L) may acutely impair physical performance through mineralocorticoid activation and potassium loss. Waters with moderate sodium content and balanced trace element composition may be preferable.

2. Cardiovascular risk considerations. Individuals with cardiovascular risk factors, hypertension, or heart failure should be particularly cautious about chronic consumption of high-sodium mineral waters, as these may elevate aldosterone levels and increase cardiovascular risk (Kluwe et al., 2023).

3. Electrolyte monitoring. When mineral waters are used therapeutically or as part of a training regimen, periodic monitoring of serum and urinary electrolytes may be advisable to detect and correct imbalances before they impair performance or health.

4. Individualized recommendations. The optimal mineral water composition may vary between individuals based on baseline aldosterone levels, cardiovascular health, training status, and other factors. Personalized recommendations based on individual metabolic and hormonal profiles may be more effective than one-size-fits-all approaches.

5. Integration with other interventions. The effects of mineral waters should be considered in the context of other dietary and pharmacological interventions that affect the RAAS, including sodium intake, potassium supplementation, and use of antihypertensive medications.

Limitations and future directions

Several limitations of this study should be acknowledged:

1. Sex specificity. The study was conducted exclusively in female rats. As discussed above, sex hormones modulate aldosterone effects, and findings may not generalize to males. Future studies should examine both sexes.

2. Duration of exposure. The 12-day exposure period examined acute effects of mineral water consumption. Longer-term studies are needed to determine whether chronic consumption produces different effects through adaptive mechanisms.

3. Indirect assessment of aldosterone. We estimated mineralocorticoid activity based on electrolyte ratios rather than directly measuring aldosterone levels. While this approach has been validated (Popovych et al., 2022) and correlates with aldosterone activity, direct hormone measurements would strengthen the conclusions.

4. Single performance test. We used swimming until exhaustion as the sole measure of physical performance. Additional tests of strength, power, and anaerobic capacity would provide a more comprehensive assessment.

5. Mechanism elucidation. While we identified mineralocorticoid activity as a key predictor of performance, the specific molecular mechanisms linking mineral water composition to RAAS activation require further investigation.

Future research should address these limitations and explore several additional questions: Do the effects of mineral waters on physical performance differ between trained and untrained individuals? Can pharmacological MR blockade prevent the adverse effects of high-sodium mineral waters? What is the optimal mineral water composition for enhancing performance in different types of exercise (endurance vs. strength vs. power)? Do genetic polymorphisms in RAAS components (e.g., ACE, aldosterone synthase) influence individual responses to

mineral waters?Can biomarkers identified in this study (mineralocorticoid activity, calcitonin activity, etc.) predict responses to training or other interventions?

Conclusion

This study demonstrates that mineralocorticoid activity is the primary determinant of physical performance in female rats consuming mineral waters with different chemical compositions. High-sodium mineral water (MW N2) significantly increased mineralocorticoid activity and reduced swimming performance by 16%, while balanced mineral water (MW N3) maintained low mineralocorticoid activity and enhanced performance by 13%. These effects are mediated through well-established physiological mechanisms involving potassium homeostasis, muscle excitability, energy metabolism, and cardiovascular function.

The findings have important implications for mineral water selection in sports medicine and spa therapy, particularly for athletes, physically active individuals, and those with cardiovascular risk factors. Mineral waters with high sodium content should be used cautiously, as they may acutely impair physical performance and potentially increase cardiovascular risk with chronic consumption. Waters with balanced electrolyte composition and appropriate trace element content may be preferable for optimizing physical performance and health.

The study also highlights the importance of considering hormonal regulation—particularly the RAAS—when evaluating the effects of dietary and environmental interventions on physical performance. The strong predictive value of mineralocorticoid activity, combined with other metabolic variables, provides a framework for understanding individual differences in performance capacity and for developing personalized recommendations.

Acknowledgments

We express sincere gratitude to Volodymyra R. Bilas, PhD, for help in carry out of metabolic analyses.

Declarations

Funding

No funding

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author (Kovalchuk G.Y., galynakovalchuk5@gmail.com) upon reasonable request.

Author contributions: W.Z.: Conceptualization, Methodology, Statistical Analysis, Writing - Review & Editing, Supervision; I.V.S.: Investigation, Resources, Project Administration; H.Y.K.: Formal Analysis, Data Curation, Writing - Original Draft, Visualization; O.I.M.: Investigation, Methodology, Validation.

AI USAGE DISCLOSURE CLAUSE

Transparency Statement on Artificial Intelligence Assistance

In accordance with emerging standards for research transparency and the ethical use of artificial intelligence in scientific writing, the authors declare the following regarding AI assistance in manuscript preparation:

AI Tools Used: This manuscript was developed with assistance from Claude (Anthropic, version Sonnet 4.5), a large language model artificial intelligence system, specifically for: (1) Literature Integration - synthesizing findings from provided research articles (Baffour-Awuah et al., 2024; Kluwe et al., 2023; Supiano, 2013; Kwak et al., 2019) into the Discussion section and integrating them with the study's empirical findings, (2) Structural Organization - organizing complex statistical results into coherent sections and creating logical flow between Introduction, Methods, Results, and Discussion, (3) Language Enhancement - improving clarity, grammar, academic writing style, and ensuring consistent terminology throughout the manuscript, (4) Methodological Descriptions - elaborating standard statistical procedures, their assumptions, and interpretation guidelines in accessible language, (5) Hypothesis Formulation - assisting in the formal articulation of five research problems, five general hypotheses, five research hypotheses, and five pairs of statistical hypotheses with appropriate operationalization and testing procedures, and (6) Expansion and Elaboration - developing concise bullet points and preliminary drafts provided by human authors into fully developed paragraphs with appropriate scientific context and citations.

Human Oversight and Intellectual Contribution: All research design, data collection, statistical analyses, and primary interpretations were conducted entirely by the human authors without AI involvement; all numerical results, tables, figures, and statistical outputs represent original data from the actual experiment conducted by the authors; critical scientific judgments, theoretical frameworks, hypotheses, and conclusions were formulated by the human research team based on their domain expertise and knowledge of the literature; AI-generated text was thoroughly reviewed, edited, fact-checked, and validated by all authors for accuracy, appropriateness, scientific validity, and alignment with the actual research findings; the AI tool did not have access to raw data and did not perform any statistical analyses or data manipulations; all interpretations of statistical results and their biological/clinical significance were provided by the human authors; and the decision to accept, modify, or reject AI-generated content rested entirely with the human authors.

Limitations of AI Assistance: AI-generated content may contain inaccuracies, misinterpretations, or inappropriate extrapolations that were identified and corrected during human review; the AI tool cannot replace expert scientific judgment, domain-specific knowledge, or critical evaluation of research findings; references and citations were verified independently by authors against original sources to ensure accuracy and appropriate context; statistical interpretations were validated against actual analytical outputs from STATISTICA software; the AI tool may have introduced stylistic or organizational preferences that do not reflect the authors' original intent, which were modified during editing; and AI-generated hypotheses and theoretical frameworks were evaluated for biological plausibility and consistency with established scientific principles before inclusion.

Ethical Considerations: The use of AI assistance does not diminish the authors' full responsibility for the accuracy, integrity, and scientific validity of all content in the manuscript; all authors have read, critically evaluated, and approved the final manuscript and take full responsibility for its content; no confidential, proprietary, or unpublished data was shared with AI systems during manuscript preparation; the AI tool was used as a writing assistant to improve clarity and organization, not as a co-author, independent researcher, or source of scientific knowledge; all scientific content, interpretations, and conclusions originate from the human authors' expertise and analysis of their experimental data; and the authors confirm that AI assistance was limited to language and organization, not scientific substance.

Compliance: This disclosure complies with: International Committee of Medical Journal Editors (ICMJE) recommendations on the use of AI in scientific writing (updated 2023), Committee on Publication Ethics (COPE) guidelines on AI-assisted writing and authorship, Journal of Physical Education and Sport editorial policies regarding AI use (if applicable), and general principles of research transparency and scientific integrity.

Corresponding Author Statement: The corresponding author (Halyna Y. Kovalchuk, PhD, galynakovalchuk5@gmail.com) takes full responsibility for ensuring that all AI-assisted content has been appropriately reviewed, validated, and attributed, that this disclosure accurately represents the extent and nature of AI involvement in manuscript preparation, that all co-authors are aware of and approve the use of AI assistance as described, and that the manuscript meets all ethical and scientific standards of the journal regardless of AI involvement in its preparation.

Date of AI Assistance: February 7-8, 2026 **AI System:** Claude Sonnet 4.5 (Anthropic) **Human Authors Responsible for Validation:** Walery Zukow, Ivan V. Savytskyi, Halyna Y. Kovalchuk, Oksana I. Melnyk

References

Babelyuk, V. Y., Dubkowa, G. I., Korolyshyn, T. A., Holubinka, S. M., Dobrovolskyi, Y. G., Zukow, W., & Popovych, I. L. (2017). Operator of Kyokushin Karate via Kates increases synaptic efficacy in the rat hippocampus, decreases C3-θ-rhythm SPD and HRV vagal markers, increases virtual chakras energy in the healthy humans as well as luminosity of distilled water in vitro: Preliminary communication. *Journal of Physical Education and Sport, 17*(1), 383–393. https://doi.org/10.7752/jpes.2017.01057

Baffour-Awuah, B., Man, M., Goessler, K. F., Hinton, P. S., Moe, S. M., Kraus, M. A., Gassman, J. J., Chan, W., Perwad, F., & Ix, J. H. (2024). Effect of exercise training on the renin–angiotensin–aldosterone system: A meta-analysis. *Journal of Human Hypertension, 38*, 89–101. https://doi.org/10.1038/s41371-023-00872-4

Chebanenko, O. I., Flyunt, I. S., Popovych, I. L., Balanovs'kyi, V. P., & Lakhin, P. V. (1997). *Water Naftussya and water-salt exchange*. Naukova dumka.

Fihura, O. A., & Klishch, I. M. (2025). Comparative study of adaptogenic effects of Ukrainian phytocompositions "Balm Truskavets'" and tea ATINE®. *Prospects and Innovations of Science (Series "Medicine"), 9*(55), 1710–1734. https://doi.org/10.5208/2786-4952-2025-9(55)-1710-1734

Fihura, O. A., Ruzhylo, S. V., & Zakalyak, N. R. (2022). Phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance in healthy rats. *Quality in Sport, 8*(2), 45–55. https://doi.org/10.12775/QS.2022.08.02.004

Gozhenko, A. I. (2016). Functional-metabolic continuum [in Russian]. *Journal of the National Academy of Medical Sciences of Ukraine, 22*(1), 3–8.

Gozhenko, A. I., Smagliy, V. S., Korda, I. V., Badiuk, N. S., Zukow, W., & Popovych, I. L. (2019). Functional relationships between parameters of uric acid exchange and immunity in female rats. *Actual Problems of Transport Medicine, 4*(58), 123–131.

Goryachkovskiy, A. (1998). *Clinical biochemistry* [in Russian]. Astroprint.

Ivassivka, S. V., Bubnyak, A. B., Kovbasnyuk, M. M., & Popovych, I. L. (1994). Genesis and role of phenols in waters from Naftussya layer. *Problems of Pathology in Experiment and Clinic. Scientific Works of Drohobych Medical Institute, 15*, 6–11.

Ivassivka, S. V., Popovych, I. L., Aksentiychuk, B. I., & Bilas, V. R. (1999). *The nature of Naftussya water balneofactors and the essence of its therapeutic and preventive action*. Truskavets'kurort.

Klecka, W. R. (1980). *Discriminant analysis* (Sage University Paper Series on Quantitative Applications in the Social Sciences, 07-019). Sage Publications. https://doi.org/10.4135/9781412983938

Kluwe, B., Pohlman, N., Kesireddy, V., Zhao, S., Tan, Y., Kline, D., Brock, G., Odei, J. B., Effoe, V. S., Echouffo Tcheugui, J. B., Kalyani, R. R., Sims, M., Taylor, H. A., Mongraw-Chaffin, M., Akhabue, E., & Joseph, J. J. (2023). The role of aldosterone and ideal cardiovascular health in incident cardiovascular disease: The Jackson Heart Study. *American Journal of Preventive Cardiology, 14*, Article 100494. https://doi.org/10.1016/j.ajpc.2023.100494

Korda, M. M., Fihura, O. A., Melnyk, O. I., Klishch, I. M., Yanchij, R. I., Zukow, W., Ruzhylo, S. V., Popovych, D. V., & Popovych, I. L. (2024). *Ukrainian phytocomposition "Balm Truskavets'", metabolism, physical working capacity and neuro-endocrine-immune complex*. Svit. https://doi.org/10.5281/zenodo.14640273

Kwak, M. K., Lee, S. E., Cho, Y. Y., Suh, S., Kim, B. J., Song, K. H., Koh, J. M., Kim, J. H., & Lee, S. H. (2019). The differential effect of excess aldosterone on skeletal muscle mass by sex. *Frontiers in Endocrinology, 10*, Article 195. https://doi.org/10.3389/fendo.2019.00195

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proceedings of the National Institute of Sciences of India, 2*(1), 49–55.

Mullur, R., Liu, Y. Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews, 94*(2), 355–382. https://doi.org/10.1152/physrev.00030.2013

Nielsen, F. H. (2014). Update on human health effects of boron. *Journal of Trace Elements in Medicine and Biology, 28*(4), 383–387. https://doi.org/10.1016/j.jtemb.2014.06.023

Panasiuk, I. M., Levkut, L. H., Popovych, I. L., Barylyak, L. G., Kovalchuk, H. Y., Zukow, W., & Kovbasnyuk, M. M. (1994). Experimental research on the adaptogenic properties of Kryms'kyĭ balsam [in Ukrainian]. *Fiziolohichnyi Zhurnal (Kiev, Ukraine: 1994), 40*(3–4), 25–30. https://pubmed.ncbi.nlm.nih.gov/7621951/

Panossian, A. G., Efferth, T., Shikov, A. N., Pozharitskaya, O. N., Kuchta, K., Mukherjee, P. K., Banerjee, S., Heinrich, M., Wu, W., Guo, D. A., & Wagner, H. (2021). Evolution of the adaptogenic concept from traditional use to medical systems: Pharmacology of stress- and aging-related diseases. *Medical Research Reviews, 41*(1), 630–703. https://doi.org/10.1002/med.21743

Popovych, I. L. (2007). 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. *Medical Hydrology and Rehabilitation, 5*(2), 68–80.

Popovych, I. L. (2008). Functional interactions between neuroendocrine-immune complex in males rats. *Achievements of Clinical and Experimental Medicine, 2*(9), 80–87.

Popovych, I. L. (2022). Similarity of adaptogenic effects of bioactive Naftussya water and phytocomposition "Balm Truskavets'". *Journal of Education, Health and Sport, 12*(12), 344–356.

Popovych, I. L., & Barylyak, L. G. (2008). Influence of course using of bioactive water Naftussya on stress level at women with endocrine at gynecological pathology. *Medical Hydrology and Rehabilitation, 7*(3), 100–118.

Popovych, I. L., Gozhenko, A. I., Korda, M. M., Klishch, I. M., Popovych, D. V., & Zukow, W. (Eds.). (2022). *Mineral waters, metabolism, neuro-endocrine-immune complex*. Feniks. https://doi.org/10.5281/zenodo.6604298

Ruzhylo, S., Popovych, A., Zakalyak, N., Chopyk, R., Fihura, O., Bilas, V., Badiuk, N., Gozhenko, A., Popovych, I., & Zukow, W. (2021). Bioactive water Naftussya and ozokerite have the same neuro-endocrine-immune effects in male rats caused by aryl hydrocarbons. *PharmacologyOnLine, 3*, 213–226.

Ruzhylo, S. V., Fihura, O. A., Zakalyak, N. R., Kovalchuk, H. Y., Żukow, X., & Popovych, D. V. (2022). Sexual dimorphism in the neuro-endocrine regulation of bicycle ergometric test parameters in untrained individuals with dysfunction of the neuro-endocrine-immune complex. *Journal of Education, Health and Sport, 12*(9), 971–984. https://doi.org/10.12775/JEHS.2022.12.09.110

Ruzhylo, S. V., Tserkovnyuk, A. V., & Popovych, I. L. (2003). *Actotropic effects of balneotherapeutic complex of Truskavets spa*. Computerpress.

Supiano, M. A. (2013). Does aldosterone blockade improve physical performance in older individuals? *The American Journal of Medicine, 126*(7), 559–560. https://doi.org/10.1016/j.amjmed.2013.03.010

Vis'tak, H. I., & Popovych, I. L. (2011). Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats. *Medical Hydrology and Rehabilitation, 9*(2), 39–57.

Zukow, W., Fihura, O. A., Żukow, X., Muszkieta, R., Hagner-Derengowska, M., Smoleńska, O., Michalska, A., Melnyk, O. I., Ruzhylo, S. V., Zakalyak, N. R., Kondratska, H. D., Voloshyn, O. R., & Popovych, I. L. (2024). Prevention of adverse effects of balneofactors at Truskavets' Spa on gastroenterologic

patients through phytoadaptogens and therapeutic physical education: Mechanisms of rehabilitation. *Journal of Physical Education and Sport, 24*(4), 791–810. https://doi.org/10.7752/jpes.2024.04093

Zukow, W., Gozhenko, O. A., Zavidnyuk, Y. V., Korda, M. M., Mysula, I. R., Klishch, I. M., Zhulkevych, I. V., Popovych, I. L., Muszkieta, R., Napierata, M., Hagner-Derengowska, M., & Skaliy, A. (2020). Role of organic carbon and nitrogen of mineral waters in their neuro-endocrine effects at female rats. *International Journal of Applied Exercise Physiology, 9*(4), 20–25.

Zukow, W., Muszkieta, R., Hagner-Derengowska, M., Smoleńska, O., Żukow, X., Fil, V. M., Kovalchuk, H. Y., Voloshyn, O. R., Kopko, I. Y., Lupak, O. M., Ivasivka, A. S., Musiyenko, O. V., Ruzhylo, S. V., Kindrat, V., & Popovych, I. L. (2022a). Role of organic substances of Naftussya bioactive water in its effects on dynamic and static fitness in rats. *Journal of Physical Education and Sport, 22*(11), 2733–2742. https://doi.org/10.7752/jpes.2022.11345

Zukow, W., Muszkieta, R., Hagner-Derengowska, M., Smoleńska, O., Żukow, X., Melnyk, O. I., Popovych, D. V., Tserkoniuk, R. G., Hryhorenko, A. M., Yanchij, R. I., Kindrat, V., & Popovych, I. L. (2022b). Effects of rehabilitation at the Truskavets' spa on physical working capacity and its neural, metabolic, and hemato-immune accompaniments. *Journal of Physical Education and Sport, 22*(11), 2708–2722. https://doi.org/10.7752/jpes.2022.11343