

Role of organic substances of Naftussya bioactive water in its effects on dynamic and static fitness in rats

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Published online: November 30, 2022

(Accepted for publication November 15, 2022)

DOI:10.7752/jpes.2022.11347

Abstract:

Background. We have previously demonstrated the role of innate muscular endurance in reactions to acute stress of neuro-endocrine-immune complex in rats. Physical performance and stress resistance of the body are considered one of the main objects of influence of adaptogens. The stress-limiting effect of bioactive Naftussya water is known. At the same time, the data on its effect on muscle performance are ambiguous. Therefore, it is interesting to find out how the bioactive Naftussya water affects this important component of the body's general resistance. **Material and methods.** The experiment have been carried out at 61 male rats Wistar line weighing 180-220 g. Rats of the control group loaded through a tube with tap water (2 mL once) for 7 days, while the animals of the other groups received bioactive Naftussya water from 8 different wells of the Truskavetsian deposit. After that, diuresis and excretion of 17-ketosteroids, mineralocorticoid activity as well as static and dynamic fitness were determined. **Results.** In 23,8% of rats, neither dynamic nor static fitness did not change significantly. In 38,8% of animals, dynamic fitness also did not change, but static fitness increased by 33%. In 28,6% of rats, an increase in static fitness by 83% combined with a decrease in dynamic fitness by 14% was noted. In 11,9% of animals, a 35% decrease in dynamic fitness was found in the absence of changes in static fitness. **Conclusion.** Weekly use of Naftussya bioactive water caused ambiguous changes in the fitness and the secretion of steroids associated with amines and phenols present in the composition of water.

Keywords: Naftussya bioactive water, organic substances, swimming and static retention tests, 17-ketosteroids, mineralocorticoids, relationships, rats.

Introduction

We have previously demonstrated the role of **innate** muscular endurance in reactions to acute stress of neuro-endocrine-immune complex in rats (Fil et al., 2021; Zukow et al., 2022). Physical performance and stress resistance of the body are considered one of the main objects of influence of adaptogens and are used for quantitative assessment of adaptogenic activity (Brekhman, 1968; Panossian et al., 2021). The stress-limiting effect of bioactive Naftussya water is known (Popovych, 2011). At the same time, the data on its effect on muscle performance are ambiguous (Ruzhylo et al., 2003). Therefore, it is interesting to find out how the bioactive Naftussya water affects this important component of the body's general resistance.

Naftussya bioactive water is the main therapeutic factor of the international spa of Truskavets' (Ukraine). Unlike classic mineral waters, its physiological and therapeutic activity is determined not by salts and gases, but by organic substances and autochthonous microbes (Yessypenko, 1978; Yessypenko, 1981; Yaremenko et al., 1989; Ivassivka, 1997; Ivassivka et al., 1999; Bilas & Popovych, 2009; Popovych et al., 2009; Popovych, 2011; Popovych et al., 2018; Zukow et al., 2020; Popovych et al., 2021; Popovych et al., 2022).

Approximately 2/3 of the mass of organic substances in Naftussya water is leached from water-bearing petroleum rock, which is reflected in its name (naphta/ναφθα means petroleum in Greek), and 1/3 are the products of their biotransformation by hydrocarbon-oxidizing, sulfate-reducing and thione microbes (Yaremenko et al., 1989; Ivassivka, 1997). Another source of organic substances, in particular phenols, are fallen leaves on the surface of the deposit (Ivassivka et al., 1994).

We adduce data by Dats'ko et al., (2008) about organic compounds (in mg/L) Naftussya water obtained by Solid Phase Extraction method and mass-spectroscopy by using as Sorbents Tenacle GC 60/80 and Polysorb-2. Paraffins 4,10 and 4,20; monoolefins 1,67 and 1,75; dienes and monocycloolefins 0,84 and 0,85; alkylbenzene

1,55 and 1,54; alkenylbenzene 0,47 and 0,46; esters of aromatic acids 1,32 and 1,33; alkyl phenols 1,14 and 1,14; carboxylic acids 1,13 and 1,14, polyaromatic hydrocarbons 0,077 and 0,059; oxygene-containing connections (acids) 1,12 and 1,14; sulfur-containing connections 0,30 and 0,31; alkylnaphthalenes 0,53 and 0,53; unidentified polyaromatic hydrocarbons 0,19 and 0,19; connections required subsequent identification 0,48 and 0,50 correspondingly.

It is known that the organic component of the composition of Naftussya water fluctuates significantly even during the three-month monitoring (Ivassivka et al., 1999; Ivassivka et al., 2010). This is accompanied by significant fluctuations in the severity of its effects on the activity of tubular secretion of phenolroth and microsomal hydroxylation of nembital (Ivassivka et al., 1990; Ivassivka et al., 1999). It is significant that the latter effect is implemented precisely through aryl hydrocarbon receptors (Esser & Rannug, 2015), primarily of hepatocytes.

Based on the above, we set ourselves the goal of finding out the role of the quantitative and qualitative composition of organic substances in Naftussya water on dynamic and static muscle performance in rats.

Material and methods

Participants. The experimentt have been carried out at 61 male rats Wistar line weighing 180-220 g 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 OO Bohomolets' Institute of Physiology. 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).

Procedure / Test protocol / Skill test trial / Measure / Instruments. Rats of the control group (n=19) loaded through a tube with tap water (2 mL once) for 7 days, while the animals of the other groups (n=5÷6) received bioactive Naftussya water from 8 different wells of the deposit according to a similar scheme. The content of gross organic carbon, carboxylic acids, amines, phenols, bitumen, as well as the oxidability of organic substances was determined in water samples (Methods of chemical analysis, 1985).

The day after completion the course of water loads the animal were placed in individual chambers with perforated bottom for collecting for 10 hours urine, in which determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). Then the animals were loaded with distilled water (6 mL) through a tube and placed in individual Plexiglas machines to collect two-hour urine, in which the concentration of potassium and sodium was determined (by flaming photometry) in order to assess mineralocorticoid activity (MCA) by the K/Na ratio. The next day, static muscle fitness was first tested (by the time of holding on a vertical wooden pole until falling on a soft bed), and then dynamic muscle fitness (by the time of swimming to exhaustion in the water t⁰ 26⁰C).

Data collection and analysis / Statistical analysis. Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6 StatSoft Inc".

Results

A preliminary analysis revealed differences both in the composition of organic substances in Naftussya water of different wells, primarily amines and phenols, and in its physiological activity, primarily in relation to dynamic muscle performance. To simplify further analysis, four clusters were created based on these three parameters (Aldenderfer & Blashfield, 1989). In the first cluster there were animals that received Naftussya water from three wells, in the second and third - from two, and in the fourth - from only one well. At the next stage, the characteristic physiological manifestations of Naftussya water activity and the peculiarities of its organic component (Tables 1 and 2) were revealed by the method of discriminant analysis (Klecka, 1989).

Table 1. Discriminant Function Analysis Summary for Variables as well as their Norm and Variability
 Step 10, N of vars in model: 10; Grouping: 4 grps; Wilks' Λ : 0,0126; approx. $F_{(3,1)}=9,8$; $p<10^{-6}$

Variables currently in the model	Clusters of Entropy (n)				Parameters of Wilk's Statistics						
	II (10)	I (15)	III (12)	IV (5)	Wilks Λ	Partial Λ	F-re-move (3,3)	p-level	Tolerance	Norm DW (19)	Cv SD
Swimming test, min	207 2	188 1	172 2	130 4	0,104	0,121	70,6	10 ⁻⁶	0,291	201 8	0,165
Static retention test, sec	47 2	64 7	88 15	49 3	0,018	0,715	3,85	0,020	0,447	48 6	0,503
Diuresis stimulated, mL/2h	5,42 0,15	5,21 0,03	5,15 0,06	4,52 0,12	0,017	0,746	3,28	0,035	0,514	5,02 0,09	0,077
Ku/Nau as MCA	1,99 0,05	2,01 0,05	2,11 0,09	2,95 0,08	0,015	0,837	1,88	0,156	0,552	2,06 0,07	0,140
Diuresis spon-tan., mL/10h	5,21 0,19	5,32 0,12	5,60 0,16	5,61 0,58	0,017	0,742	3,36	0,032	0,608	5,35 0,14	0,113

Amines, $\mu\text{g/L}$	238 113	211 85	392 102	508 224	0,016	0,802	2,39	0,089	0,568	20 6	0,367
Phenols, $\mu\text{g/L}$	39 23	166 91	147 50	280 140	0,014	0,868	1,48	0,242	0,478	0	3,175
Carboxylic acids, $\mu\text{eqv/L}$	50 6	44 5	53 5	51 6	0,015	0,855	1,64	0,201	0,663	41 6	0,377
Carbon orga-nic, mg/L	13,6 3,1	12,2 0,9	11,9 0,8	15,5 1,9	0,014	0,901	1,06	0,382	0,833	5,60 0,88	0,428
Oxidizability, $\text{mg O}_2/\text{L}$	0,92 0,04	0,89 0,07	0,66 0,09	0,99 0,37	0,018	0,699	4,15	0,015	0,401	0,99 0,02	0,438
Variables currently not in model	II (10)	I (15)	III (12)	IV (5)	Wilks Λ	Par- tial Λ	F to enter	p- le- vel	Tole- ran- cy	Norm (19)	Cv
17-Ketostero- ids, $\text{nM}/10\text{h}$	84 2	82 1	80 2	63 3	0,012	0,950	0,49	0,689	0,026	68 5	0,305
Bitumen, mg/L	1,44 0,26	1,34 0,23	1,31 0,21	1,38 0,36	0,011	0,905	0,98	0,415	0,618	0,75 0,15	0,587

Notes. In each column, the first line is the average, the second – SE for variables and Cv or **SD** for Norm. The control group and daily water are not subject to discriminant analysis

Table 2. Summary of Stepwise Analysis for physiological and chemical Variables. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Swimming test, min	190,7	10^{-6}	0,062	190,7	10^{-6}
Ku/Nau as Mineralocorticoid activity	4,496	0,009	0,046	45,39	10^{-6}
Oxidizability of organic substances, $\text{mg O}_2/\text{L}$	3,254	0,033	0,036	28,51	10^{-6}
Diuresis stimulated by water load, $\text{mL}/2\text{h}$	1,817	0,162	0,031	21,01	10^{-6}
Diuresis spontaneous, $\text{mL}/10\text{h}$	1,832	0,160	0,027	17,05	10^{-6}
Carboxylic acids, $\mu\text{eqv/L}$	1,518	0,228	0,024	14,42	10^{-6}
Static retention test, sec	1,948	0,142	0,020	12,83	10^{-6}
Amines, $\mu\text{g/L}$	2,547	0,074	0,016	11,94	10^{-6}
Phenols, $\mu\text{g/L}$	1,440	0,251	0,014	10,85	10^{-6}
Carbon organic, mg/L	1,058	0,382	0,012	9,845	10^{-6}

The identifying information contained in the 10 discriminant variables is condensed into three roots. The major root contains 96,1% of discriminatory opportunities ($r^*=0,984$; Wilks' $\Lambda=0,013$; $\chi^2_{(30)}=149$; $p<10^{-6}$), while minor root – 3,3% only ($r^*=0,716$; Wilks' $\Lambda=0,404$; $\chi^2_{(18)}=31$; $p=0,031$), and the third is not worth paying attention to (0,6%).

Calculating the values of discriminant roots for each rat by the raw coefficients and the constant (Table 3) allows visualization of each animal in the information space of roots.

Table 3. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Variables currently in the model						
Swimming test	-1,758	-0,121	0,299	-0,293	-0,020	0,050
Ku/Nau as Mineralocorticoid activity	-0,186	-0,507	0,870	-0,825	-2,244	3,850
Oxidizability of organic substances	0,347	-1,100	-0,270	0,961	-3,048	-0,747
Diuresis stimulated by water load	0,702	0,024	0,300	2,625	0,089	1,122
Diuresis spontaneous	-0,637	0,033	0,431	-0,975	0,050	0,660
Carboxylic acids	-0,430	-0,1978	0,3412	-23,21	-10,69	18,46
Static retention test	-0,636	0,566	0,693	-0,019	0,017	0,020
Amines	-0,460	0,370	0,659	-1,262	1,016	1,810
Phenols	0,125	0,713	-0,094	0,039	0,222	-0,030
Carbon organic	-0,005	-0,450	0,292	-0,001	-0,085	0,055
			Constants	48,42	10,16	-29,42
			Eigenvalues	31,2	1,05	0,21
			Cumulative Proportions	0,961	0,994	1

Calculating Z-scores allows us to correctly compare variables expressed in different units on the same scale (Table 4).

Table 4. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables	Correlations Variables-Roots		II (10)	I (15)	III (12)	IV (5)
	Root 1	Root 2				
Root 1 (96,1%)	Root 1	Root 2	-6,2	-1,5	+1,5	+12,5
Swimming test	-0,693	-0,189	+0,18	-0,38	-0,86	-2,13
Diuresis stimulated by water load	-0,177	0,139	+1,04	+0,50	+0,33	-1,29
17-Ketosteroids excretion	Currently not		+0,77	+0,68	+0,58	-0,25
Ku/Nau as Mineralocorticoid activity	0,228	-0,492	-0,24	-0,16	+0,19	+3,07
Diuresis spontaneous	0,037	0,133	-0,23	-0,05	+0,41	+0,43
Amines of Naftussya water	0,047	0,036	+0,59	+0,52	+1,04	+1,33
Phenols of Naftussya water	0,036	0,017	+0,10	+0,44	+0,39	+0,75
Root 2 (3,3%)	Root 1	Root 2	-1,1	+0,1	+1,3	-1,3
Static retention test	0,011	0,469	-0,04	+0,67	+1,65	+0,05
Carboxylic acids of Naftussya water	0,011	0,038	+0,59	+0,18	+0,77	+0,64
Oxidizability of organic substances	0,002	-0,314	-0,17	-0,23	-0,77	+0,01
Carbon organic of Naftussya water	0,023	-0,202	+3,29	+2,61	+2,57	+4,09
Bitumen of Naftussya water	Currently not		+1,58	+1,35	+1,27	+1,43

Judging by the structural coefficient, the major discriminant root reflects, first of all, the swimming test. The extreme left localization of the members of the second cluster (Fig. 1) reflects the duration of swimming, which is the maximum for the sample and practically does not differ from that in the control.

This is accompanied by the same normal levels of mineralocorticoid activity and spontaneous diuresis, as well as maximally elevated levels of water-load-stimulated diuresis and 17-ketosteroids excretion. Rats of the fourth cluster are located at the opposite pole of the radical axis. This reflects their maximally reduced dynamic fitness and aqueous diuresis in combination with increased mineralocorticoid activity, maximal spontaneous diuresis, and minimal for the sample excretion of 17-ketosteroids, which does not differ from the control.

On the other hand, the extreme levels of physiological effects of Naftussya water are accompanied by significant differences in the content of amines and phenols in its composition. It seems that they are responsible for the decrease in dynamic fitness.

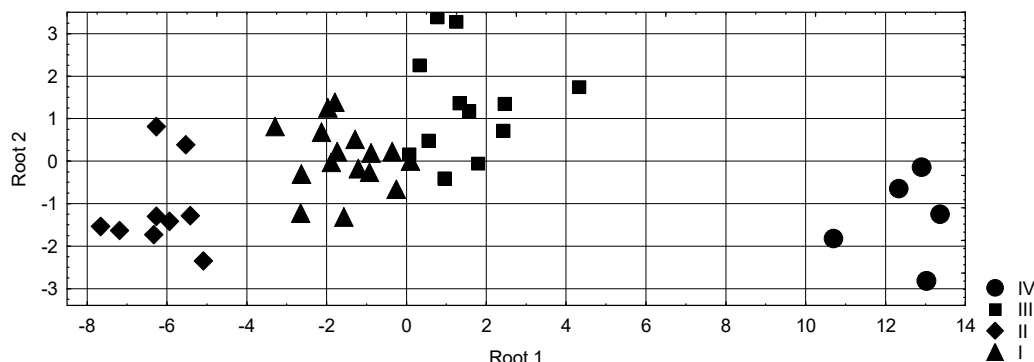


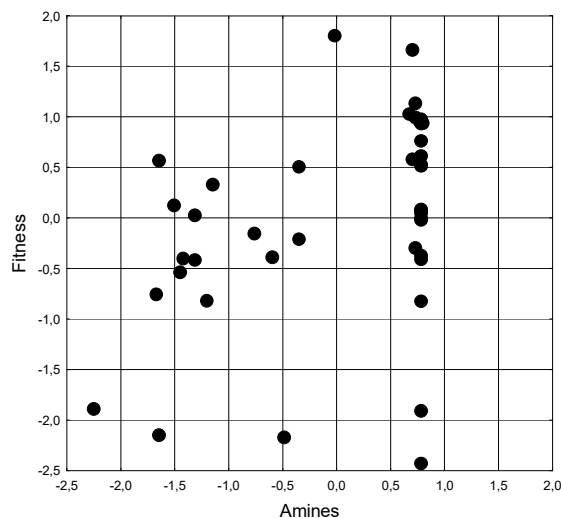
Fig. 1. Diagram of scattering of individual values of first and second Roots of rats of different clusters

The intermediate position of the other two clusters reflects the intermediate values of the physiological and chemical parameters of their members.

All four clusters are quite clearly demarcated along the axis of even one root. Additional separation occurs along the axis of the second root, which contains information about the static fitness, as well as the content of other organic substances in the water.

The top position of the third cluster reflects the maximally increased level of static fitness, which is accompanied by the maximum content of carboxylic acids in water in combination with the maximally reduced oxidizability of organic substances, as well as the minimum for sampling concentrations in water of organic carbon in general and bitumen in particular.

One gets the impression that the static fitness is upregulated by carboxylic acids, but downregulated by bitumen and the oxidizability of organic substances in the used water. However, the correlation analysis did not confirm this impression. Instead, a weak but statistically significant canonical correlation was found between the content of amines in Naftussya water and both fitness elements of water-loaded rats (Fig. 2).



$R=0,390$; $R^2=0,152$; $\chi^2_{(2)}=6,4$; $p=0,040$; Λ Prime=0,848

Fig. 2. Scatterplot of canonical correlation between the water amines level (X-line) and the swimming and static retention tests (Y-line) in rats

In order to find out the role of steroid hormones in the effect of Naftussya water on fitness, a correlation analysis was conducted. A strong direct correlation was found between the swimming test and the daily excretion of 17-ketosteroids (Fig. 3). The latter are known to be metabolites of glucocorticoids (2/3) and androgens (1/3). On the other hand, mineralocorticoids **downregulate** the maximum duration of swimming to the same extent (Fig. 4).

It is interesting that the level of diuresis stimulated by the load with distilled water correlates strongly directly with the swimming test (Fig. 5), whereas spontaneous daytime diuresis is weakly inversely correlated ($r=-0,30$).

It is well known that water load causes a decrease in the level of antidiuretic hormone/arginine vasopressin in the blood. Hence, we assume that dynamic fitness is upregulated by reactivity of source of this hormone. This source are parvocellular neurons of the paraventricular nuclei of the hypothalamus. Some parvocellular neurons contain and secrete both arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) that in turn stimulates the secretion of ACTH. AVP alone has very little ACTH secretagogue activity but is apotent synergistic factor with CRH. AVP and CRH may act synergistically on other target tissue with AVP and CRH receptors in the CNS and perhaps the periphery (Chrousos, 2000), including, let's add, in skeletal muscles.

Therefore, our assumption is consistent with the existing fundamental provisions.

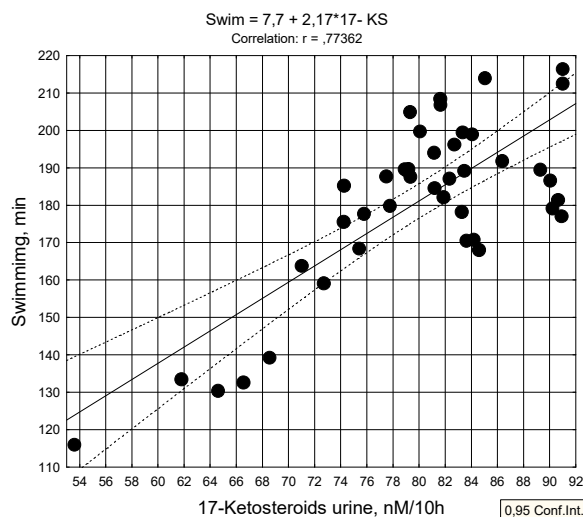


Fig. 3. Scatterplot of correlation between urine excretion of 17-Ketosteroids (X-line) and the swimming test (Y-line) in rats

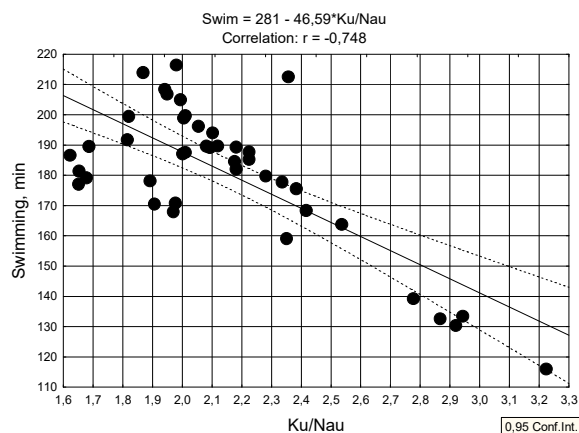


Fig. 4. Scatterplot of correlation between urine K/Na ratio (X-line) and the swimming test (Y-line) in rats

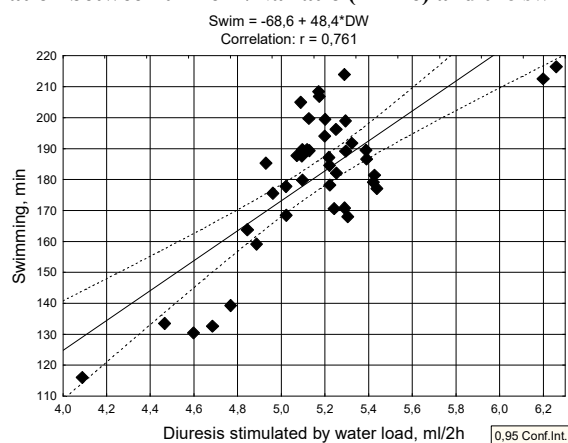


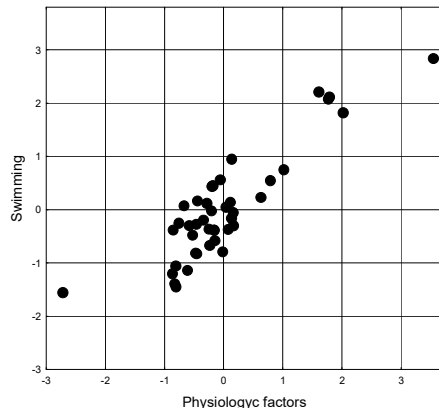
Fig. 5. Scatterplot of correlation between diuresis stimulated by water load (X-line) and the swimming test (Y-line) in rats

Taken together, these hormonal factors determine dynamic fitness by 78.9% (Table 6 and Fig. 6).

Table 6. Regression Summary for swimming test

R=0,888; R²=0,789; Adjusted R²=0,766; F_(4,4)=35; p<10⁻⁶; SE of estimate: 11 min

N=42		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₇₎	p-level
Variables	r		Intercept	382,0	85,0	4,49	10 ⁻⁴
17-Ketosteroides, nM/10h	0,77	-1,832	0,545	-5,14	1,53	-3,36	0,00182
Diuresis stimulated, ml/2h	0,76	1,364	0,303	86,72	19,26	4,50	0,00006
Ku/Nau as MC activity	-0,75	-1,479	0,322	-92,15	20,04	-4,60	0,00005
Diuresis spontaneous, ml/10h	-0,30	-0,210	0,077	-7,48	2,74	-2,73	0,00958



R=0,888; R²=0,789; $\chi^2_{(4)}=59$; p<10⁻⁶; Λ Prime=0,211

Fig. 6. Scatterplot of canonical correlation between some physiological factors (X-line) and the swimming test (Y-line) in rats

At the same time, static fitness is very weakly related to the physiological effects of Naftussya bioactive water (Table 7).

Table 7. Regression Summary for static retention test

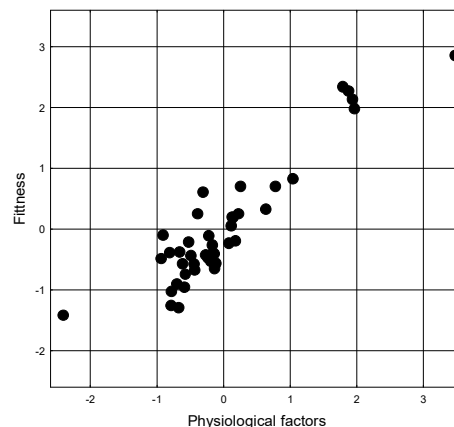
R=0,378; R²=0,143; Adjusted R²=0,075; F_(3,4)=2,1; p=0,115; SE of estimate: 35 sec

N=42		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₈₎	p-level
Variables	r		Intercept	6	108	0,06	0,956
17-Ketosteroides, nM/10h	0,25	0,667	0,356	2,95	1,57	1,88	0,068
Diuresis spontaneous, ml/10h	0,19	0,177	0,153	9,93	8,58	1,16	0,254
Diuresis stimulated, ml/2h	0,14	-0,444	0,358	-44,52	35,89	-1,24	0,222

Finally, an analysis of the canonical correlation between endocrine parameters, on the one hand, and parameters of muscle performance, on the other hand, was performed. It was established that the hormonal effects of Naftussya water determine its actotropic effects by 84,8% (Table 8 and Fig. 7).

Table 7. Factor structure of Endocrine and Fitness canonical roots

Left set	R
17-Ketosteroides	-0,911
Diuresis stimulated	-0,861
Ku/Nau as MCA	0,894
Diuresis spontaneous	0,260
Right set	R
Swimming test	-0,959
Static test	-0,176



R=0,921; R²=0,848; $\chi^2_{(8)}=75$; p<10⁻⁶; Λ Prime=0,134

Fig. 7. Scatterplot of canonical correlation between some physiological factors (X-line) and the swimming and static retention tests (Y-line) in rats

Discussion

Data on the influence of balneotherapy at the Truskavets' spa on the muscular performance of patients (adults and children), assessed by veloergometric or step tests, are ambiguous too (Ruzhylo et al., 2003; Popovych et al., 2005; Zukow et al., 2020; Zukow et al., 2021). Since the balneotherapy complex includes, in addition to drinking Naftussya water, ozokerite applications and mineral baths (Popovych et al., 2003), a controlled experiment was conducted on female rats that received only Naftussya water for 3 weeks. But even under these conditions, the results of the test with swimming to exhaustion turned out to be ambiguous: in 33,3% of the animals, a significant increase in working capacity was noted, in another 44,4% - a moderate increase, while in 22,2% it decreased (Ruzhylo et al., 2003; Popovych et al., 2005). The authors explain such polyvariance of actotropic effects by the individual reactivity of the body.

In this study, it was found that the effect of Naftussya bioactive water on dynamic (to a greater extent) and static (to a lesser extent) fitness is carried out through steroid hormones, which, in turn, are regulated by amines and phenols present in the composition of water.

Unfortunately, we did not have the opportunity to monitor the composition of organic substances in the water according to the full program. However, it is quite likely that amines and phenols reflect previously identified groups of substances.

At least some of the listed organic substances (alkylbenzene, alkenylbenzene, alkyl-naphthalenes, alkyl phenols, esters of aromatic acids, polyaromatic hydrocarbons) are, obviously, agonists of aryl hydrocarbon receptors (AhR), which are expressed by almost all types of cells of living organisms, starting from unicellular. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants, the activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the endocrine and immune response (Quintana & Sherr, 2013; Esser & Rannug, 2015). The known modulating effects of drinking medicinal waters (Naftussya, Sofiya, Hertsa et al) on the neuro-endocrine-immune complex of both rats and humans (Popovych et al., 2003; Kostyuk et al., 2006; Popovych, 2011; Kozyavkina et al., 2015; Popovych, 2018; Popovych, 2019; Popodynets' et al., 2020; Hrytsak et al., 2022; Popovych, 2022) are explained by the effect of their organic substances on AhR of neurons, endocrinocytes and immunocytes as well as of autochthonous microflora on Toll-like receptors (TLR) of immunocytes of GALT (Ruzhylo et al., 2021; Popovych et al., 2022). It is interesting that the expression of Ah and TL receptors is interconnected (Murray & Perdew, 2020).

It seems that the overall effect of Naftussya water on fitness, depending on the content of organic substances in it, is neutral or even unfavorable. We regard this as a "physiological payment" for the generally favorable effect of Naftussya bioactive water on the neuro-endocrine-immune complex. The mirror situation occurs under conditions of intensive endurance training: the increase in fitness is combined with the suppression of immunity.

By the way, a similar constellation of organic substances was found in the composition of other medicinal waters of Ukrainian Carpathians and Podolia (Shestopalov et al., 2016) as well as Siberia (Khutoryansky et al., 2013). This gives reason to predict their effects, similar to those of Naftussya bioactive water. Physical rehabilitation, insufficient attention is paid to the mineral metabolism and metabolic function of a bone tissue, taking into account gender characteristics of the patients with arthrosis and periods of hormonal restructuring of women (Savchenko et al., 2020). Young people of all age groups and genders before developing swimming skills will determine the degree of any proper and reliable level of dominance in men's swimming over women (Ganchar et al., 2022a). It is necessary for the theory and practice of training specialists in physical education and sports undergoing professional training in the field of swimming at many faculties of pedagogical institutes, academies and universities of Ukraine, CIS countries and swimmers from different continents to realize their sports interests and opportunities at the most prestigious competitions of our time (Ganchar et al., 2022b). Developed method for evaluation of swimming training level of water polo players 13, 14, 15 years old that makes it possible to identify weaknesses in the level of swimming training of each player and to compare the level of swimming readiness of different players among themselves (Chaplins'kyi et al., 2018).

Conclusion

Thus, weekly use of Naftussya bioactive water caused ambiguous changes in the fitness. In 23,8% of rats, neither dynamic nor static fitness did not change significantly. In 38,8% of animals, dynamic fitness also did not change, but static fitness increased by 33%. In 28,6% of rats, an increase in static fitness by 83% combined with a decrease in dynamic fitness by 14% was noted. In 11,9% of animals, a 35% decrease in dynamic fitness was found in the absence of changes in static fitness.

Compliance with Ethical Standards

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

Conformity to ethical standards. All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study. All subjects of the institutional survey gave consent for anonymized data to be used for publication purposes.

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 carry out 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).

References

- Aldenderfer, MS, & Blashfield, RK. (1989). Cluster analysis (Second printing, 1985) [trans. from English in Russian]. In: *Factor, Discriminant and Cluster Analysis*. Moskva, *Finansy i Statistika*, 139-214.
- Bilas, VR, & Popovych, IL. (2009). Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. *Medical Hydrology and Rehabilitation*, 7(1), 68-102.

- Brekhman, II. (1968). Eleutherococcus [in Russian]. *Leningrad. Nauka*, 186.
- Chaplins'kyi, M., Briskin, Y., Ostrov'ska, N., Sydorko, O., Ostrov'skyi, M., Pityn, M., & Polehoiko, M. (2018). Evaluation of the training level of water polo swimming players (13-15 years old). *Journal of Physical Education and Sport*, 18(Suppl.1), 356-362. doi: 10.7752/jpes.2018.s148.
- Chrousos, GP. (2000). The stress response and immune function: Clinical implications. In: *Neuroimmunomodulation. Perspectives at the new millennium / Ed by A Conti et al. Ann NYAS*, 917
- Dats'ko, OR, Bubnyak, AB, & Ivassivka SV. (2008). The organic part in mineral water Naftussya. Development of knowledges about its composition and origination [in Ukrainian]. *Medical Hydrology and Rehabilitation*, 6(1), 168-174.
- Esser, C, & Rannug, A. (2015). The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacol Rev.*, 67(2), 259-279.
- Fil, V, Zukow, W, Kovalchuk, G, Voloshyn, O, Kopko, I, Lupak, O, & Stets V. (2021). 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. *Journal of Physical Education and Sport*, 21(Suppl. 5), 3030-3039.
- Ganchar, A., Ganchar, I., Chernyavskiy, O., Ciorba, C., Medynskiy, S., Pylypko, O., & Arkhypov, O. (2022a). Monitoring the formation of swimming skills among men and women in the world Universiade program (at stages I-II from 1959 to 2019). *Journal of Physical Education and Sport*, 22(1), 130-137. doi: 10.7752/jpes.2022.01016.
- Ganchar, I, Ganchar, O, Ciorba, C, Medynskiy, S, Pylypko, O, Bliznyuk, Y, Pylypko, A, Lyashenko, A (2022b). **Monitoring the assessment of the swimming skills formation among swimmers-prize-winners at stages I-II-III of the Olympic Games (1896-2021)**. *Journal of Physical Education and Sport*, 22(8), 1869–1877. doi:10.7752/jpes.2022.08236.
- Hrytsak, MV, Popovych, DV, Badiuk, NS, Hrytsan, II, & Zukow, X. (2022). Comparative study of the effect on the neuroendocrine-immune complex and metabolism of drinking monotherapy with Naftussya water and therapy supplemented with “Myroslava” and “Khrystyna” mineral waters. *Journal of Education, Health and Sport*, 12(1), 355-367.
- Ivassivka, SV, Bubnyak, AB, Kovbasnyuk, MM, & Popovych, IL. (1994). Genesis and role of phenols in waters from Naftussya layer [in Ukrainian]. In: Problems of pathology in experiment and clinic. Scientific works of Drohobych Medical Institute. Vol XV. Drohobych. 6-11.
- Ivassivka, SV, Popovych, IL, Aksentiychuk, BI, & Bilas, VR. (1999). The Nature of Naftussya Water Balneofactors and the Essence of its Therapeutic and Preventive Action [in Ukrainian]. *Truskavets' Truskavets'kurort*; 125.
- Ivassivka, SV, Popovych, IL, Yaremenko, MS, & Kovbasnyuk, MM. (1990). Mineral water Naftussya as a xenobiotic [in Russian]. *Fiziol Zhurn*, 36(3), 40-45.
- Ivassivka SV. (1997). Biologically Active Substances of Naftussya Water, their Genesis and Mechanisms of Physiological Action [in Ukrainian]. Kyiv. Naukova dumka; 110.
- Ivassivka, SV, Kovbasnyuk, MM, Bubnyak, AB, & Sovyak, DG. (2010). Monitoring of the activity of the autochthonous microflora of the Naftussya water of the Truskavets' field and its relationship with the organic factors of this water and the intensity of precipitation [in Ukrainian]. *Medical hydrology and rehabilitation*, 8(2), 57-78.
- Khutoryansky, VA, Smirnov, AI, Matveev, DA, Ogarcova, LA, & Gambudgapova, LB. (2013). Extraction and chromate-mass-spectrometry investigation of organic components from mineral water “Munoc” [in Russian]. *Medical hydrology and rehabilitation*. 11(1), 88-96.
- Klecka, WR. (1989). Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: *Factor, Discriminant and Cluster Analysis. Moskwa: Finansy i Statistika*. 78-138.
- Kostyuk, PG, Popovych, IL, & Ivassivka, SV (editors). (2006). Chornobyl', Adaptive and Defensive Systems, Rehabilitation [in Ukrainian]. Kyiv. Computerpress; 348.
- Kozyavkina, OV, Kozyavkina, NV, Gozhenko, OA, Gozhenko, AI, Barylyak, LG, & Popovych, IL. (2015). Bioactive Water Naftussya and Neuroendocrine-Immune Complex [in Ukrainian]. Kyiv. UNESCO-SOCIO; 349.
- Murray, IA, Perdeu, GH. (2020). How Ah receptor ligand specificity became important in understanding its physiological function. *Int J Mol Sci*, 21(24), 9614.
- Panossian, AG, Efferth, T, Shikov, AN, Pozharitskaya, ON, Kuchta, K, Mukherjee, PK, Banerjee, S, Heinrich, M, Wu, W, Guo, DA, & Wagner, H. (2021). Evolution of the adaptogenic concept from traditional use to medical systems: Pharmacology of stress- and aging-related diseases. *Med Res Rev*, 41(1),
- Popadynets', O, Gozhenko, A, Badyuk, N, Popovych, I, Skaliy, A, Hagner-Derengowska, M, Napierata, M, Muszkieta, R, Sokołowski, D, Zukow, W, & Rybałko, L. (2020). Interpersonal differences caused by adaptogen changes in entropies of EEG, HRV, immunocytogram, and leukocytogram. *Journal of Physical Education and Sport*, 20(Suppl. 2), 982-999.

- Popovych, AI. (2018). Features of the immunotropic effects of partial components of the balneotherapeutic complex of spa Truskavets'. *Journal of Education, Health and Sport*, 8(12), 919-935.
- Popovych, AI. (2019). Features of the neurotropic effects of partial components of the balneotherapeutic complex of spa Truskavets'. *Journal of Education, Health and Sport*, 9(1), 396-409.
- Popovych, IL. (2011). Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya [in Ukrainian]. Kyiv. Computerpress; 300.
- Popovych, IL. (2022). Similarity effects of two types of adaptogens (transcutaneous electrical stimulation with the device "VEB"® and using of bioactive Naftussya water) on the human body. *Journal of Education, Health and Sport*, 12(4), 213-258.
- Popovych, IL, Flyunt, IS, Alyeksyeyev, OI, Barylyak, LG, & Bilas, VR. (2003). Sanogenetic Bases of Rehabilitation on Spa Truskavets' Urological Patients from Chornobylian Contingent [in Ukrainian]. Kyiv. Computerpress; 192.
- Popovych, IL, Gozhenko, AI, Korda, MM, Klishch, IM, Popovych, DV, & Zukow, W (editors). (2022). Mineral Waters, Metabolism, Neuro-Endocrine-Immune Complex. Odesa. Feniks; 252.
- Popovych, IL, & Ivassivka, SV. (2009). The role of organic matter in Naftussya water in its physiological activity [in Ukrainian]. *Medical hydrology and rehabilitation*, 7(2), 6-26.
- Popovych, IL, Ruzhlylo, SV, Ivassivka, SV, Aksentiychuk, BI, Bilas, VR et al. (2005). Balneocardioangiology. The impact of balneotherapy in the spa Truskavets' on the cardiovascular system and physical performance [in Ukrainian]. Kyiv. Computerpress; 239.
- Popovych, IL, Zavidnyuk, YV, Korda, MM, Mysula, IR, Klishch, IM, & Zukow, W. (2018). Role of organic carbon and nitrogen of mineral waters in their metabolic effects at female rats. *Journal of Education, Health and Sport*, 8(12), 793-802.
- Quintana, FJ, & Sherr, DH. (2013). Aryl hydrocarbon receptor control of adaptive immunity. *Pharmacol Rev.*, 65(4), 1148-1161.
- Ruzhlylo, SV, Tserkovnyuk, AV, & Popovych, IL. (2003). Actotropic Effects of Balneotherapeutic Complex of Truskavets spa [in Ukrainian]. Kyiv. Computerpress; 131.
- Ruzhlylo, SV, Popovych, AI, Zakalyak, NR, Chopyk, RV, Fihura, OA, Bilas, VR, Badiuk, NS, Gozhenko, AI, Popovych, IL, & 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.
- Savchenko, V., Maykova, T., Afanasiev, S., Kashuba, V., Afanasieva, A., Moskalenko, N., ... & Savlyuk, S. (2020). Disorders of the mineral exchange and metabolism of bone tissue as a pathogenetic basis of physical rehabilitation patients with coxartrosis. *Journal of Physical Education and Sport*, 20(1), 447-451. doi: 10.7752/jpes.2020.s1065.
- Shestopalov, VM, Moiseeva, NP, Ishchenko, AP, Kondratiuk, YeI, Usov, VYu, Moiseev, AY et al. (2006). "Naftusia" medicinal waters of Ukrainian Carpathians and Podolia [in Russian]. Chernivtsi. Bukrek; 600.
- Methods of chemical analysis during the desalination of salt water. (1985). [in Russian]. Moskva. Izdatelstvo standartov; 49.
- Yaremenko, MS, Ivassivka, SV, Popovych, IL, Bilas, VR, Yassevych, HP, Zahorodnyuk, VP et al. (1989). Physiological Bases of Curative Effect of Water Naftussya [in Russian]. Kyiv. Naukova dumka; 144
- Yessypenko, BYe. (1978). Physiological properties of Naftussya mineral water. In: Composition and properties of mineral water "Naftussya" [in Russian]. Kyiv. Naukova dumka; 93-123.
- Yessypenko, BYe. (1981). Physiological action of "Naftussya" mineral water [in Russian]. Kyiv. Naukova dumka; 216.
- Zukow, W, Flyunt, I-S S, Ponomarenko, RB, Rybak, NY, Fil', VM, Kovalchuk, HY, Sarancha, SM, & Nahurna, YV. (2020). Polyvariant change of step-test under the influence of natural adaptogens and their accompaniments. *Pedagogy and Psychology of Sport*, 6(2), 74-84.
- Zukow, W, Gozhenko, OA, Zavidnyuk, YV, Korda, MM, Mysula, IR, Klishch, IM, Zhulkevych, IV, Popovych, IL, 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 J of Applied Exercise Physiology*, 9(4), 20-25.
- Zukow, W, Flyunt, I-S S, Ruzhlylo, SV, Kovalchuk, HY, Nahurna, YV, Popovych, DV, Sarancha, SM. (2021). Forecasting of multivariant changes in step test under the influence of natural adaptogens. *Pedagogy and Psychology of Sport*, 7(1), 85-93.
- Zukow, W, Fil, VM, Kovalchuk, HY, Voloshyn, OR, Kopko, IY, Lupak, OM, Ivassivka, AS, Musiyenko, OV, Bilas, VR, Popovych, IL. (2022). The role of innate muscular endurance and resistance to hypoxia in reactions to acute stress of immunity in rats. *Journal of Physical Education and Sport*, 21(7), 1608-1617.