INFLUENCE OF "SNEZNIK-1/79" MINERAL WATER ON ANTHROPOMETRIC, FUNCTIONAL AND BIOCHEMICAL PARAMETERS OF PROFESSIONAL BASKETBALL PLAYERS: ROLE OF OXIDATIVE STRESS

Dijana Lalovic[1], Aleksandra Vranic[2], Jovana Jeremic[2], Dejan Stanojevic[3], Bolevich Sergey[4], Bolevich Stefani[5], Jelena Ristic[6], Nikola Cikiriz[7], Deniel Pesic[7], Zagor Zagorac[8] ,Vladimir Zivkovic[9] and Vladimir Jakovljevic[9,10]
[1]*Medical High School "Nadežda Petrović", Zemun, Belgrade, Serbia*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia*
[3]*Special Hospital Merkur, Vrnjacka Banja, Serbia*
[4]*I.M. Sechenov First Moscow State Medical University, Department of Human Pathology, Laboratory of Navigational Redox Lipidomics, Moscow, Russian Federation*
[5]*I.M. Sechenov First Moscow State Medical University, Institute of Molecular Medicine, Moscow, Russian Federation*
[6]*Representative Office Richter Gedeon Serbia, Belgrade, Serbia*
[7]*Department of Exercise Physiology, Institute of Hygiene, Military Medical Academy, Belgrade, Serbia*
[8]*Clinic for Surgery, Clinical Hospital Center "Dr Dragisa Misovic-Dedinje" Belgrade, Serbia*
[9]*University of Kragujevac, Faculty of Medical Sciences, Department of Physiology, Kragujevac, Serbia*
[10]*I.M. Sechenov First Moscow State Medical University, Department of Human Pathology, Moscow, Russian Federation*

UTICAJ MINERALNE VODE "SNEŽNIK-1/79" NA ANTROPOMETRIJSKE, FUNKCIONALNE I BIOHEMIJSKE PARAMETRE PROFESIONALNIH KOŠARKAŠA: ULOGA OKSIDACIONOG STRESA

Dijana Lalović[1], Aleksandra Vranić[2], Jovana Jeremić[2], Dejan Stanojević[3], Bolevich Sergey[4], Bolevich Stefani[5], Jelena Ristić[6], 9Nikola Cikiriz[7], Deniel Pesić[7], Zagor Zagorac[8], Vladimir Zivković[9] i Vladimir Jakovljević[9,10]
[1]*Srednja medicinska škola "Nadežda Petrović", Zemun, Beograd, Srbija*
[2]*Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za farmaciju, Kragujevac, Srbija*
[3]*Specijalna bolnica Merkur, Vrnjacka Banja, Srbija*
[4]*Prvi moskovski državni medicinski univerzitet I.M. Sechenov, Katedra za humanu patologiju, Laboratorija za navigacijsku redoks lipidomiku, Moskva, Ruska Federacija*
[5]*Prvi moskovski državni medicinski univerzitet I.M. Sechenov, Institut za molekulsku medicinu, Moskva, Ruska Federacija*
[6]*Predstavnik Richter Gedeon Srbija, Beograd, Srbija*
[7]*Odsek za praktičnu fiziologiju, Institut za medicinsku higijenu, Vojno-medicinska akademija, Beograd, Srbija*
[8]*Klinika za hirurgiju, Kliničko-bolnički centar „Dr Dragiša Mišović-Dedinje" Beograd, Srbija*
[9]*Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za fiziologiju, Kragujevac, Srbija*
[10]*Prvi moskovski državni medicinski univerzitet I.M. Sechenov, Katedra za humanu patologiju, Moskva, Ruska Federacija*

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ABSTRACT

Adequate hydration represents the balance between the water intake and loss and has an unambiguous significance for public health and it is essential to sustain life. The changes in electrolyte balance which occur during and after training affect on athletes health and performance. Therefore, fluid replacement with adequate mineral composition is of utmost importance. The aim of the present study was to examine the influence of low mineral water from the well Sneznik-1/79 on anthropometric, functional, biochemical parameters and redox status of professional basketball players. In total, 17 male basketball players were included, during the pre-competitive mesocycle, and after the initial testing, they were randomly divided into two groups: group 1 – consumed the commercial drinking water for four weeks (n = 7), and group 2 – consumed water from the well Sneznik-1/79 for four weeks (n = 10).

SAŽETAK

Adekvatna hidratacija predstavlja ravnotežu između unosa i gubitka vode i ima nedvosmislen značaj za javno zdravlje i esencijalna je za održavanje života. Promene u ravnoteži elektrolita koje nastaju tokom i nakon treninga utiču na zdravlje i učinak sportista. Zbog toga je nadoknada tečnošću sa adekvatnim mineralnim sastavom od izuzetne važnosti. Cilj ove studije bio je ispitivanje uticaja slabo mineralne vode sa izvora Snežnik-1/79 na antropometrijske, funkcionalne, biohemijske parametre i redoks status profesionalnih košarkaša. U istraživanje je uključeno 17 košarkaša tokom pred-takmičarskog mezociklusa, pri čemu su nakon inicijalnog testiranja, nasumično podeljeni u dve grupe: grupa 1 - konzumirala je komercijalnu vodu četiri nedelje (n = 7) i grupa 2 - konzumirala je vodu iz bunara Snežnik-1/79 tokom četiri nedelje (n = 10). Izvršeno je određivanje antropometrijskih, funkcionalnih, biohemijskih parametara i redoks statusa.

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Corresponding author:
Vladimir Zivkovic, MD, PhD
University of Kragujevac, Faculty of Medical Sciences,
Department of Physiology, Serbia
69 Svetozara Markovica Street, 34000 Kragujevac, Serbia
Tel: +381 34 306 800
E-mail: vladimirziv@gmail.com

Determination of the anthropometric, functional, biochemical parameters and redox status was performed. Our results pointed out that consumption of mineral water from the well Sneznik is completely safe from the aspect of affecting various anthropometric, functional and biochemical parameters as well as systemic oxidative stress of professional athletes. In addition, existence of discretely better effects over commercial drinking water indicates that a long period of monitoring may certainly be of interest for further investigation.

***Keywords**: alkaline water, anthropometry, function, oxidative stress, Sneznik*

Rezultati istraživanja ukazuju da je upotreba mineralne vode iz izvora Snežnik potpuno sigurna sa aspekta uticaja na različite antropometrijske, funkcionalne i biohemijske parametre, kao i na sistemski oksidacioni stres profesionalnih sportista. Pored toga, postojanje diskretno boljih efekata u poređenju sa komercijalnom vodom, ukazuje da bi duži period praćenja mogao biti od interesa za dalja istraživanja.

***Ključne reči:** alkalna voda, antropometrija, funkcija, oksidacioni stres, Snežnik*

INTRODUCTION

Adequate hydration represents the balance between the water intake and loss and has an unambiguous significance for public health and it is essential to sustain life. This balance has a very important role in general population, during the changed physiological conditions, disease, physical activities, in work environment (1).

During exercise, losing of the body water has a big impact on physiological and perceptual strain. This condition can impair endurance performance, especially in hot environments, leading to the increased exertional heat illness risk, whereby the fluid loss up to 4% of the body mass does not compromise performance (2). Consuming fluid during the exercise has a very important role in decreasing the perceived exertion and thirst sensation as well as reducing the cardiovascular and thermal stress and it mostly depends on the type of exercise (3).

Strategies for the fluid replacement during the exercise include a large spectrum of different thirst sensations, amount consumption and electrolyte compensation with a well-planned and structured intake (4). According to pH values, two different types of water can be used as supplementation and rehydration in athletes, acidic and alkaline water. Today, acidic rich water is suggested to be used in healthy people and some medical conditions (5, 6), while alkaline water is intended for general public. Recent study suggest that acidic water can be associated with maintained peak power output trained male cyclists (7).

On the other hand, metabolic acidosis that occurs during the high intensity training,represents metabolic alteration, emerging due to the lactate acid production in a working muscle. A part of the produced lactate can be realised in the blood leading to the acid-base disbalance and decreased pH blood values. In this state, cells are forced to rely on anaerobic ATP shifting that leads to proton releasing and the blood pH decreasing that can impair performance (8). Moreover, lactate produced during the exercise, generally remains in plasma while hydrogen ions are largely buffered in red blood cells due to the fact that red blood cells have a higher buffering capacity than the blood plasma (8). Although sodium bicarbonate has shown a positive effect on the strength and speed endurance, it has a lot of limitations such as gastrointestinal disorders including nausea, stomach pain, diarrhea, vomiting, metabolic alkalosis, even edema due to sodium overload (9). However, the newest data showed that treatment with acidic water can decrease blood lactate levels and enhance ventilatory efficiency in healthy males (10).

THE AIM OF THE PAPER

Having in mind all mentioned above it is assumed that the daily intake of low mineral content water Sneznik-1/79 could be important and useful for maintaining the physiological function of professional athletes in terms of influencing both sports performance and recovery from the training processes. Therefore, the aim of the present study was to examine the influence of low mineral water from the well Sneznik-1/79 on the anthropometric, functional, biochemical parameters and redox status of professional basketball players.

SUBJECTS AND METHODS

Study design

In accordance with the aim of our study, we included male basketball players (n = 17), 21 ± 3 years of age with years of professional basketball experience, members of the senior Basketball Club Borac , Cacak. The basketball players were put to the test during the pre-competitive mesocycle, from August 15th to September 15th, 2019.

After the initial testing, the participants were randomly divided into two groups: group 1 – consumed commercial drinking water for four weeks (n = 7), and group 2 – consumed water from the well Sneznik-1/79 for four weeks (n = 10).

During the aforementioned monitoring period, each participant received his own drinking bottle while the water intake was carefully monitored and recorded. Water was controlled immediately before, during and after the training or preparation matches. The total of 2 ± 0.5 liters/day was the average of consumed water during the indicated period.

Following at least a 3-hour fasting period, the testing began at the same time at 10:00 am. Blood was collected from the cubital vein in Vacutainer tubes (BD Vacutainer Blood Collection System) in a quiet, air-conditioned, temperature-controlled room (22-24°C). Sweat was collected from the back skin in 1.5 ml mini plastic tubes and stored in the freezer until the appropriate electrolytes were measured.

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and principles of Good Clinical Practice (GCP). The written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee, prior to the onset of the study.

Anthropometric parameters

The following anthropometric parameters were examined: the body height, body weight, body composition indicators (bone mass, soft tissue mass, total fat mass, skeletal muscle mass (SMM), fat percentage, total body water, basal metabolism, body mass index (BMI), intracellular water (ICW), extracellular water (ECW)). The basic anthropometric indicators were determined during the standard sports-medical examination. The body composition parameters were measured by the bioelectrical impedance analysis (BIA) (before and immediately after Ergospirometry). BIA uses the electrical properties of the human body to alternate the current flow and measures resistance values to estimate the body water content and composition.

Functional parameters

The functional parameters were determined during the cardiopulmonary exercise test (CPET) – the ergospirometric test on a treadmill (Trackmaster TMX428 Stress Treadmill), conducted at the beginning of the monitoring period (the initial, first measurement) and after four weeks of drinking commercial or Sneznik drinking water (the final, second measurement). This test provides an assessment of the integrative exercise responses involving the cardiovascular, pulmonary, neuropsychological, and skeletal muscle systems, which are not adequately reflected through the measurement of the individual organ system functions. The following functional parameters were examined: the absolute and relative maximum oxygen consumption (VO_{2max}), respiratory exchange ratio (RER), heart rate (HRmax), respiratory reserve, anaerobic threshold, aerobic efficiency).

Evaluation of Systemic Redox State

The redox status was evaluated spectrophotometrically by measuring the levels of prooxidative parameters, hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), nitrites (NO_2^-) and index of lipid peroxidation (TBARS) in plasma. Activities of the corresponding antioxidative enzymes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured in erythrocytes in the same manner.

Determination of prooxidative parameters

The degree of lipid peroxidation in plasma was estimated by measuring of thiobarbituric acid reactive substances (TBARS) using 0.4 ml 1% of thiobarbituric acid (TBA) in 0.05 NaOH mixed with 0.8 ml of plasma, incubated at 100 °C for 15 min and measured at 530 nm. Distilled water was used as a blank probe. The TBA extract was obtained by combining 0.8ml plasma and 0.4ml TCA (trichloroacetic acid). Thereafter, the samples were put on ice for 10 min, and centrifuged for 15 min at 6000 rpm (11). Nitric oxide (NO) decomposes rapidly to form the stable metabolite nitrite/nitrate products. The method for detection of the plasma nitrite levels is based on the *Griess* reaction. Nitrites were determined as an index of NO production with the *Griess* reagent (forms purple diazocomplex) (12). 0.1 ml 3 N PCA (perchloric acid), 0.4 ml 20mM EDTA (ethylenediaminetetraacetic acid), and 0.2 ml plasma were put on ice for 15 min, then centrifuged for 15 min at 6000 rpm. After pouring off the supernatant, 220 μl K_2CO_3 were added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe. The level of O_2^- was measured using Nitro Blue Tetrazolium (NBT) reaction in TRIS-buffer with plasma and read at 550 nm. Distilled water was used as a blank probe (13). The determination of H_2O_2 concentration is based on oxidation of phenol red using hydrogen peroxide, in the reaction catalyzed by the enzyme peroxidase from horse radish (POD) (14). 200 μl sample with 800 μl PRS (phenol red solution) and 10 μl POD were combined (1:20) and measured at 610 nm.

Determination of antioxidative enzymes activity

Isolated RBCs were washed three times with 3 volumes ice-cold 0.9mmol/l NaCl and hemolysates containing about 50g Hb/l, prepared according to McCord and Fridovich (15) were used for the determination of catalase (CAT) activity. The determination of CAT activity was done according to Beutler (16). Lysates were diluted with distilled water (1:7

v/v) and treated with chloroform–ethanol (0.6:1 v/v) to remove hemoglobin. Then 50 µl CAT buffer, 100 µl sample, and 1ml 10mM H_2O_2 were added to the samples. The detection was performed at 360nm. Distilled water was used as a blank probe. The determination of SOD activity is based on epinephrine method of Misra and Fridovich (17). 100µl lysate and 1 ml carbonate buffer were mixed, and then epinephrine in a volume of 100 µl was added. The detection was performed at470 nm. This method belongs to the 'negative' type group of methods, since it monitors the decrease of autoxidation speed in alkaline medium, which is dependent of $O_2^{\cdot -}$. The level of GSH concentration was determined based on GSH oxidation with 5.5-dithiobis-6.2-nitrobenzoic acid, using Beutler method (18). The measurement of absorbance is carried out at a wavelength of the maximum absorption of 420 nm.

Laboratory analyses

The laboratory analyses of leukocytes (WBC), erythrocytes (RBC), platelets (PLT), lymphocytes (Lymph), granulocytes (Gran) (Beckman Coulter LH780 analyzer), hemoglobin (HBG, Cyanmethemoglobin method, Beckman Coulter LH780 analyzer), hematocrit (HCT, MCV(fl) x number of erythrocytes (10^{12})), mean corpuscular volume (MCV, Beckman Coulter LH780), mean corpuscular hemoglobin (MCH, hemoglobin (g/L)/erythrocyte count (10^{12})); mean corpuscular hemoglobin concentration (MCHC, hemoglobin (g/L)/hematocrit value); mean platelet volume (MPV, Beckman Coulter LH780); plateletcrit (PCT, Beckman Coulter LH780); Na, K, Cl (Ion-selective electrodes ISE method, biochemical analyser Beckman Coulter AU680); Ca (Colorimetric method - Arsenazo III, biochemical analyser Beckman Coulter AU680); P (Colorimetric method - modification of Daly and Ertingshausen method, biochemical analyser Beckman Coulter AU680) were performed.

Points of interest

All mentioned anthropometric, functional and oxidative stress parameters were determined at the beginning of the monitoring period (the initial, first measurement) and after four weeks of consumption of Sneznik drinking water (the final, second measurement) and compared between the groups which consumed different types of water as well as within the groups. In addition, electrolytes from blood and sweat as well as the haematological parameters, due to their dynamics during the initial and final measurements, were determined at three time points of interest: 1. at rest (before the ergospirometric test); 2. during the maximum effort (immediately after completion of the ergospirometric test); 3. in the 15^{th} minute of recovery and compared between the groups that consumed different types of water as well as within the groups.

Statistics

IBM SPSS Statistics 20.0 Desktop for Windows was used for the statistical analysis. Distribution of the data was checked by Shapiro–Wilk test. Where distribution between the groups was normal, statistical comparisons were performed using the one-way analysis of variance (ANOVA) tests with a Tukey's post hoc test for multiple comparisons. Kruskal–Wallis was used for comparison between the groups where distribution of the data was different than normal. The values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Anthropometric parameters

Body height and age

The studied groups did not differ statistically by the age and height (Table 1). There was also no difference in the body height between the first and second measurements, i.e. after the follow-up period. These data are important to ensure adequate homogeneity of the study sample, which will allow for the subsequent relevance of other results.

Body composition

The results of the body composition parameters are shown in Table 2. It can be observed that there was no statistically significant difference in the body weight of basketball players at the beginning and at the end of the study, both between the groups and within groups. In addition, the subjects did not differ in terms of the amount of total body water in both body compartments (extracellular and intracellular). The proportion of muscle mass in both groups was no significantly higher after four weeks, while at the same time the percentage of body fat decreased. The body mass index and basal metabolism were almost identical at the first and second measurements in both groups with no differences between the groups. These results indicate that there were no changes in the body composition between the groups after four weeks of the follow-up period, as it was expected. Improvements that exist discreetly in both groups (the fat and protein content) support a good training process carried out during the preparatory period.

Ergospirometry

The results of the ergospirometry parameters are shown in Table 3 and Figure 1. Based on the findings obtained, it can be observed that the absolute and relative maximum oxygen consumption was higher after the follow-up period in both basketball groups with a discreetly higher increase in the group that consumed Sneznik water. The results related to the maximum heart rate and anaerobic threshold showed no significant differences within as well as between the groups.

On the other hand, it is interesting that the athletes who consumed Sneznik water after four weeks of monitoring, had almost an unchanged respiratory reserve, unlike the group of athletes who consumed commercial drinking water and that had a decrease in this parameter during the same period. In addition, a more pronounced increase in the aerobic efficiency was observed in the group that used Sneznik water. Although there was no statistical significance between the groups, the unambiguous difference would probably be more noticeable after a longer follow-up period.

Biochemical parameters

Oxidative stress parameters

The results of the redox status are shown in Figure 2 and 3. It can be observed that the values of NO2–, in both groups, were not significantly higher after the follow-up period. On the other hand, the values of O2– were insignificantly higher in the group that consumed commercial water, after four weeks, while in the group of athletes who consumed Sneznik water, decreasing of this parameter was recorded (without the statistical significance) (Figure 2). Furthermore, H2O2 was significantly decreased after the four-week consumption of both, commercial and Sneznik water, while TBARS was significantly increased (Figure 2).

The activity ofantioxidative enzymes was insignificantly higher in both groups of participants after the follow-up period, which again goes in a favour of the physiological adaptation to the training process. There were no statistical differences in the achieved dynamics between the groups (Figure 3).

Electrolytes from blood and sweat

Table 4 represents the effects of the four-week Sneznik water consumption on the electrolyte values at different points of interest in blood and sweat. The obtained results showed that there were no statistically significant differences in ionograms from the blood samples at all measurement times, both within and between the groups. Interestingly, the concentration of sodium, potassium and chlorine in sweat of both groups was higher at the end of the study with minor deviations in the group that consumed Sneznik drinking water. All these differences were statistically insignificant (Table 4).

Laboratory analysis

The effects of the four-week water consumption from the well Sneznik on the values of blood parameters of interest are shown in Table 5. The results of total leukocytes as well as the absolute and relative leukocyte formulas show that there was no statistically significant difference between and within the groups. The parameters of erythrocytes (the total erythrocyte count, hematocrit and hematological indexes) did not also change during the follow-up period within the groups, with no differences between them. The same trend was observed in the parameters related to platelets (the total number as well as distribution and volume of platelets). On the other hand, it is interesting that the hemoglobin values in both groups were higher at the end of the study with no statistical significance (Table 5).

Table 1. Characteristics of study participants.

	Age (years)	**Height (cm)**	
		1st measurement	**2nd measurement**
Commercial water	20.14 ± 4.60	195.17 ± 9.15	195.50 ± 8.95
Sneznik water	22.00 ± 3.06	195.78 ± 7.87	196.33 ± 7.68

Results are expressed as mean ± standard error.

Table 2. Effects of four-week water consumption from the well Sneznik on body composition.

	Commercial water	Sneznik water						
	1^{st} measurement	2^{nd} measurement	1^{st} measurement	2^{nd} measurement				
	before	after	before	after	before	after	before	after
ICW	36.40 ± 4.88	36.33 ± 4.91	37.85 ± 5.40	38.98 ± 5.30	37.70 ± 3.46	37.52 ± 3.44	39.04 ± 3.36	38.73 ± 3.40
ECW	21.87 ± 2.91	21.86 ± 3.00	22.35 ± 3.21	23.06 ± 3.14	22.41 ± 2.38	22.20 ± 2.45	22.97 ± 2.22	22.77 ± 2.22
Proteins	15.73 ± 2.14	15.73 ± 2.14	16.35 ± 2.33	16.84 ± 2.30	16.31 ± 1.48	16.21 ± 1.49	16.88 ± 1.45	16.74 ± 1.47
Minerals	5.29 ± 0.66	5.25 ± 0.69	5.54 ± 0.75	5.62 ± 0.74	5.64 ± 0.65	5.50 ± 0.66	5.80 ± 0.66	5.74 ± 0.60
Bone mass	4.34 ± 0.54	4.31 ± 0.56	4.52 ± 0.61	4.59 ± 0.60	4.66 ± 0.55	4.51 ± 0.58	4.80 ± 0.58	4.73 ± 0.51
Total fat mass	6.81 ± 3.13	6.44 ± 3.16	5.30 ± 2.04	4.82 ± 1.81	8.31 ± 3.16	7.85 ± 3.27	6.43 ± 2.90	6.60 ± 2.74
Total body water	58.27 ± 7.79	58.19 ± 7.91	60.20 ± 8.58	62.04 ± 8.43	60.11 ± 5.80	59.72 ± 5.86	62.01 ± 5.56	61.50 ± 5.61
Soft tissue mass	74.91 ± 9.90	74.84 ± 10.16	77.57 ± 11.0	79.92 ± 10.9	77.38 ± 7.36	76.91 ± 7.46	79.90 ± 7.13	79.24 ± 7.19
Fat free mass	79.33 ± 10.57	79.14 ± 10.71	82.12 ± 11.7	84.52 ± 11.5	82.03 ± 7.91	81.43 ± 8.02	84.69 ± 7.69	83.98 ± 7.69
Weight	86.14 ± 10.22	85.59 ± 10.34	87.42 ± 11.1	89.34 ± 10.7	90.34 ± 10.68	89.28± 11.05	91.12 ± 10.31	90.58± 10.12
SMM	45.49 ± 6.36	45.41 ± 6.38	47.33 ± 7.05	48.80 ± 6.92	47.16 ± 4.49	46.94 ± 4.52	48.91 ± 4.38	48.48 ± 4.44
BMI (kg/m^2)	22.39 ± 1.75	22.23 ± 1.74	22.82 ± 1.72	23.00 ± 1.73	23.55 ± 1.68	23.12 ± 1.89	23.60 ± 1.58	23.46 ± 1.54
Fat percentage (%)	8.00 ± 3.62	7.43 ± 3.89	6.20 ± 2.55	5.56 ± 2.41	8.96 ± 2.52	8.54 ± 2.59	6.83 ± 2.50	7.08 ± 2.30
Basal metabolism	2082.71±228.3	2079.14 ± 231	2143 ± 251	2194.6 ± 247	2141.8 ± 171	2128.40 ± 173	2195.89 ± 161	2183.3 ± 166

Results are expressed as mean ± standard error. Before – before ergospirometric test; After – after ergospirometric test. ICW – intracellular water; ECW – extracellular water; SMM – skeletal muscle mass; BMI – body mass index

Table 3. Effects of four-week water consumption from the well Sneznik on ergospirometric parameters.

	Commercial water		Sneznik water	
	1st measurement	2nd measurement	1st measurement	2nd measurement
VO_2 (ml/min)	4348.86 ± 435.15	4723.00 ± 528.56	4282.70 ± 497.69	4552.44 ± 453.66
RER	1.15 ± 0.08	1.08 ± 0.03	1.18 ± 0.15	1.09 ± 0.04
HR max	184.00 ± 5.83	184.80 ± 5.31	181.60 ± 5.56	184.00 ± 8.44
Vt BTPS (L)	130.03 ± 28.53	141.94 ± 28.53	126.56 ± 19.55	135.04 ± 20.77
Respiratory reserve	39.14 ± 18.85	35.60 ± 16.59	41.60 ± 9.56	41.67 ± 8.03
RR (number/min)	46.14 ± 12.76	46.80 ± 13.18	45.40 ± 7.76	43.56 ± 12.14
ANP	1.00 ± 0.00	1.00 ± 0.00	0.99 ± 0.01	1.00 ± 0.00

Results are expressed as mean ± standard error. Before - before ergospirometric test; After - after ergospirometric test. VO_2 – (absolute and relative maximum oxygen consumption); RER – respiratory exchange ratio; HR – heart rate; Vt BTPS – tidal volume at body temperature and pressure saturated; RR – respiratory rate; ANP – anaerobic threshold

Table 4. Effects of four-week water consumption from the well Sneznik on electrolyte values in blood and sweat at different points of interest.

	Commercial water		Sneznik water	
	1st measurement	2nd measurement	1st measurement	2nd measurement
Ca (Peace)	2.38 ± 0.19	2.29 ± 0.04	2.39 ± 0.18	2.28 ± 0.13
Ca (Max)	2.43 ± 0.15	2.43 ± 0.07	2.47 ± 0.20	2.45 ± 0.10
Ca (Rest)	2.36 ± 0.16	2.30 ± 0.04	2.33 ± 0.19	2.33 ± 0.13
Ca (Sweat)	5.06 ± 2.92	3.34 ± 1.09	4.40 ± 2.36	3.26 ± 1.40
Na (Peace)	139.08 ± 1.93	137.64 ± 2.17	139.20 ± 2.19	136.99 ± 2.19
Na (Max.)	139.52 ± 2.06	139.12 ± 1.84	139.06 ± 2.34	138.99 ± 2.04
Na (Rest)	139.65 ± 2.59	138.30 ± 1.00	138.63 ± 2.40	138.29 ± 1.79
Na (Sweat)	111.95 ± 17.17	138.44 ± 34.94	100.06 ± 28.38	115.01 ± 25.99
K (Peace)	4.64 ± 0.59	4.17 ± 0.34	4.71 ± 0.51	4.32 ± 0.21
K (Max)	4.50 ± 0.57	4.81 ± 0.46	4.76 ± 0.39	4.69 ± 0.41
K (Rest)	4.31 ± 0.54	4.46 ± 0.37	4.53 ± 0.34	4.39 ± 0.33
K (Sweat)	18.64 ± 9.48	21.46 ± 2.42	20.31 ± 6.06	25.06 ± 3.61
Cl (Peace)	99.03 ± 5.11	97.43 ± 1.00	98.37 ± 3.89	97.71 ± 4.02
Cl (Max)	101.92 ± 3.21	98.83 ± 3.82	102.03 ± 4.50	101.47 ± 3.24
Cl (Rest)	100.68 ± 3.63	98.14 ± 3.26	99.95 ± 3.83	100.06 ± 3.84
Cl (Sweat)	102.71 ± 42.50	130.69 ± 53.55	75.48 ± 30.34	96.25 ± 32.85
P (Peace)	1.14 ± 0.08	1.23 ± 0.16	1.19 ± 0.09	1.19 ± 0.11
P (Max)	1.40 ± 0.10	1.66 ± 0.10	1.39 ± 0.16	1.50 ± 0.06
P (Rest)	1.23 ± 0.06	1.28 ± 0.10	1.21 ± 0.06	1.26 ± 0.03
P (Sweat)	0.60 ± 0.37	0.47 ± 0.05	0.48 ± 0.10	0.44 ± 0.02

Results are expressed as mean ± standard error. Peace - before ergospirometric test; Max - immediately after ergospirometric test; Rest - 15 minutes after ergospirometric test.

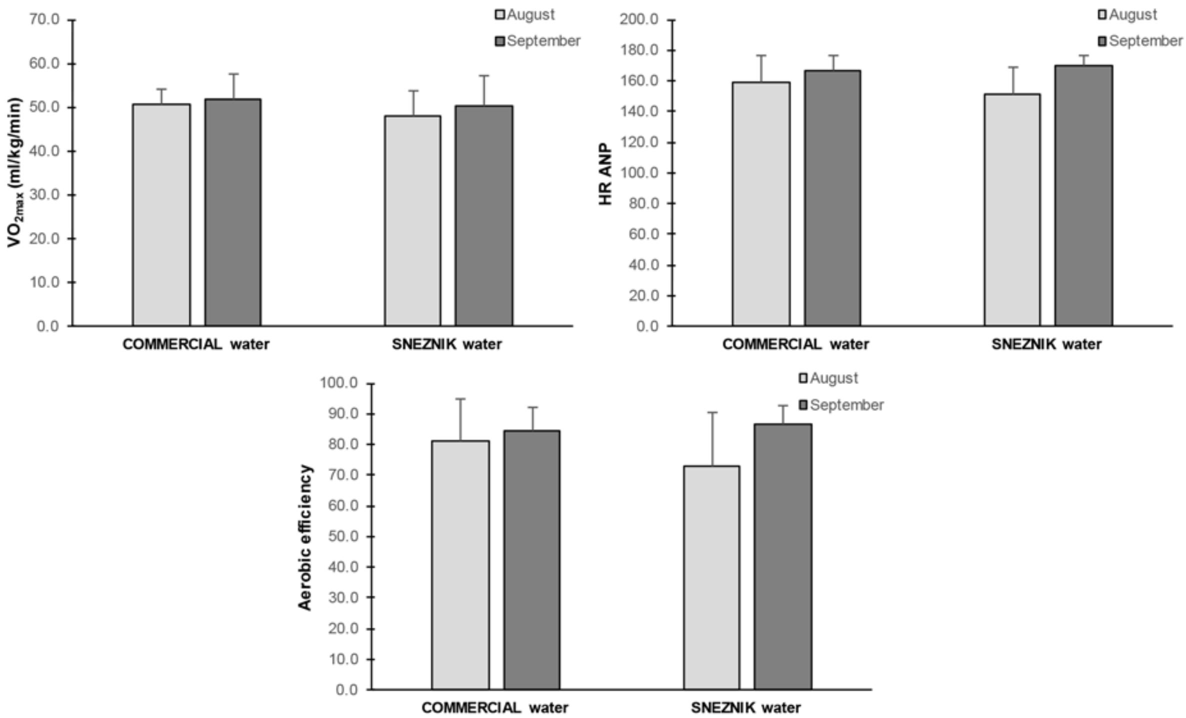
Table 5. Effects of four-week water consumption from the well Sneznik on values of parameters of interest in blood picture

	Commercial water		Sneznik water	
	1st measurement	2nd measurement	1st measurement	2nd measurement
WBC – Peace (10^9/l)	5.06 ± 1.60	5.22 ± 1.40	5.32 ± 1.17	5.17 ± 1.19
WBC - Max (10^9/l)	7.87 ± 2.28	8.92 ± 2.05	8.17 ± 1.52	8.96 ± 1.58
WBC - Rest (10^9/l)	4.97 ± 1.55	5.40 ± 1.68	4.95 ± 1.11	5.12 ± 0.96
Lymph - Peace (10^9/l)	1.66 ± 0.40	1.88 ± 1.88	1.75 ± 0.44	1.80 ± 0.46
Lymph - Max (10^9/l)	3.30 ± 0.82	3.90 ± 1.67	3.35 ± 0.68	4.03 ± 0.90
Lymph - Rest (10^9/l)	1.60 ± 0.54	2.02 ± 0.61	1.50 ± 0.34	1.43 ± 0.44
Gran - Peace (10^9/l)	3.00 ± 1.20	2.92 ± 0.92	3.20 ± 0.78	2.97 ± 0.79
Gran - Max (10^9/l)	3.77 ± 1.37	3.88 ± 1.43	4.08 ± 0.94	4.09 ± 1.06
Gran - Rest (10^9/l)	2.94 ± 1.07	2.90 ± 1.05	3.11 ± 0.77	3.06 ± 0.77
Lymph - Peace %	34.04 ± 8.06	36.55 ± 6.35	32.90 ± 4.73	34.82 ± 5.94
Lymph - Max %	42.83 ± 7.37	46.60 ± 6.95	41.44 ± 4.98	45.07 ± 7.51
Lymph - Rest %	32.91 ± 8.21	37.78 ± 5.13	30.18 ± 2.92	33.12 ± 7.82
Gran - Peace %	57.80 ± 7.94	54.72 ± 7.06	59.61 ± 4.82	56.80 ± 6.14
Gran - Max %	47.36 ± 6.78	44.54 ± 10.15	49.57 ± 5.15	45.42 ± 8.42
Gran - Rest %	58.07 ± 7.97	52.50 ± 5.81	62.33 ± 3.05	59.06 ± 7.24
HGB - Peace (g/l)	138.43 ± 8.50	146.33 ± 9.97	145.20 ± 9.80	148.44 ± 6.54
HGB - Max (g/l)	145.86 ± 9.53	156.80 ± 10.87	154.30 ± 8.87	160.44 ± 6.97
HGB - Rest (g/l)	136.71 ± 6.92	146.00 ± 9.22	146.00 ± 9.10	148.89 ± 7.24
RBC - Peace (10^{12}/l)	4.51 ± 0.19	4.61 ± 0.23	4.69 ± 0.33	4.65 ± 0.25
RBC - Max (10^{12}/l)	4.73 ± 0.26	4.99 ± 0.30	4.94 ± 0.32	5.01 ± 0.32
RBC - Rest (10^{12}/l)	4.43 ± 0.15	4.66 ± 0.20	4.64 ± 0.35	4.73 ± 0.27
HCT - Peace %	41.79 ± 2.34	42.60 ± 2.75	43.79 ± 3.04	43.66 ± 1.78
HCT - Max %	44.16 ± 1.72	46.32 ± 3.18	46.38 ± 3.03	46.54 ± 2.30
HCT - Rest %	40.96 ± 1.61	42.78 ± 2.18	43.29 ± 3.21	43.84 ± 2.16
MCV – Peace (fL)	92.66 ± 2.98	92.52 ± 3.14	93.52 ± 2.04	93.98 ± 2.26
MCV – Max (fL)	93.47 ± 3.18	92.92 ± 3.15	94.06 ± 2.12	94.58 ± 1.97
MCV – Rest (fL)	92.50 ± 2.26	91.96 ± 2.88	93.48 ± 2.12	93.68 ± 2.20
MCH – Peace (pg)	30.60 ± 1.11	31.68 ± 1.14	30.92 ± 0.75	31.87 ± 0.82
MCH – Max (pg)	30.63 ± 1.29	31.36 ± 1.02	31.23 ± 0.66	31.76 ± 0.85
MCH – Rest (pg)	30.77 ± 1.23	31.30 ± 1.26	31.23 ± 0.87	31.73 ± 0.60
MCHC – Peace (g/L)	330.57 ± 5.41	343.00 ± 1.67	331.20 ± 7.00	340.56 ± 6.04
MCHC – Max (g/L)	329.71 ± 5.35	338.00 ± 2.55	332.40 ± 4.48	337.33 ± 3.35
MCHC – Rest (g/L)	332.86 ± 6.99	340.60 ± 4.39	334.60 ± 5.27	339.00 ± 4.18

	Commercial water		Sneznik water	
	1st measurement	2nd measurement	1st measurement	2nd measurement
PLT - Peace (10^9/l)	173.71 ± 36.89	182.67 ± 27.38	191.88 ± 73.93	223.67 ± 39.84
PLT - Max (10^9/l)	204.86 ± 37.87	220.60 ± 31.91	217.44 ± 85.11	264.89 ± 36.89
PLT - Rest (10^9/l)	173.71 ± 47.95	188.40 ± 23.41	191.89 ± 75.55	226.33 ± 40.51
MPV - Peace (fl)	9.04 ± 0.98	9.35 ± 0.44	8.98 ± 0.55	9.19 ± 0.51
MPV - Max (fl)	9.21 ± 0.99	9.56 ± 0.52	9.06 ± 0.61	9.33 ± 0.55
MPV - Rest (fl)	9.01 ± 0.76	9.46 ± 0.40	8.87 ± 0.50	9.12 ± 0.48

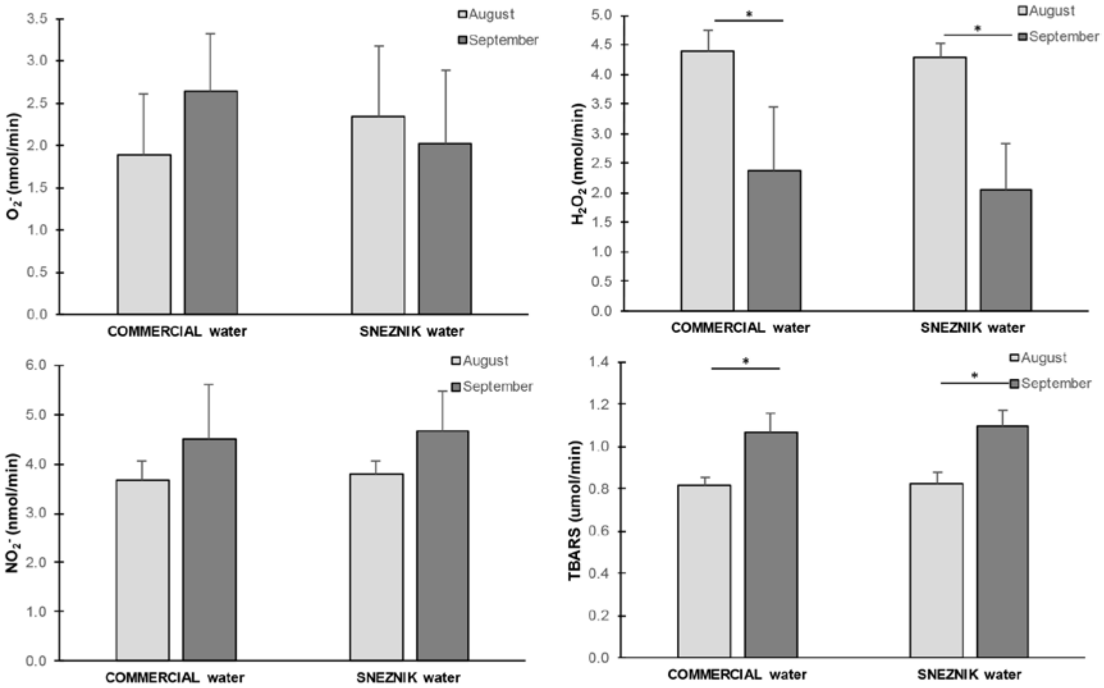
Results are expressed as mean ± standard error. Peace - before ergospirometric test; Max - immediately after ergospirometric test; Rest - 15 minutes after ergospirometric test. WBC – leukocytes, RBC – erythrocytes, PLT – platelets, Lymph – lymphocytes, Gran – granulocytes, HBG –hemoglobin, HCT – hematocrit (MCV(fl) x number of erythrocytes (10^{12})), MCV – mean corpuscular volume, MCH – mean corpuscular, MCHC – mean corpuscular hemoglobin concentration; MPV – mean platelet volume, PCT –plateletcrit

Figure 1. Effects of four-week water consumption from the well Sneznik on selected ergospirometric parameters.



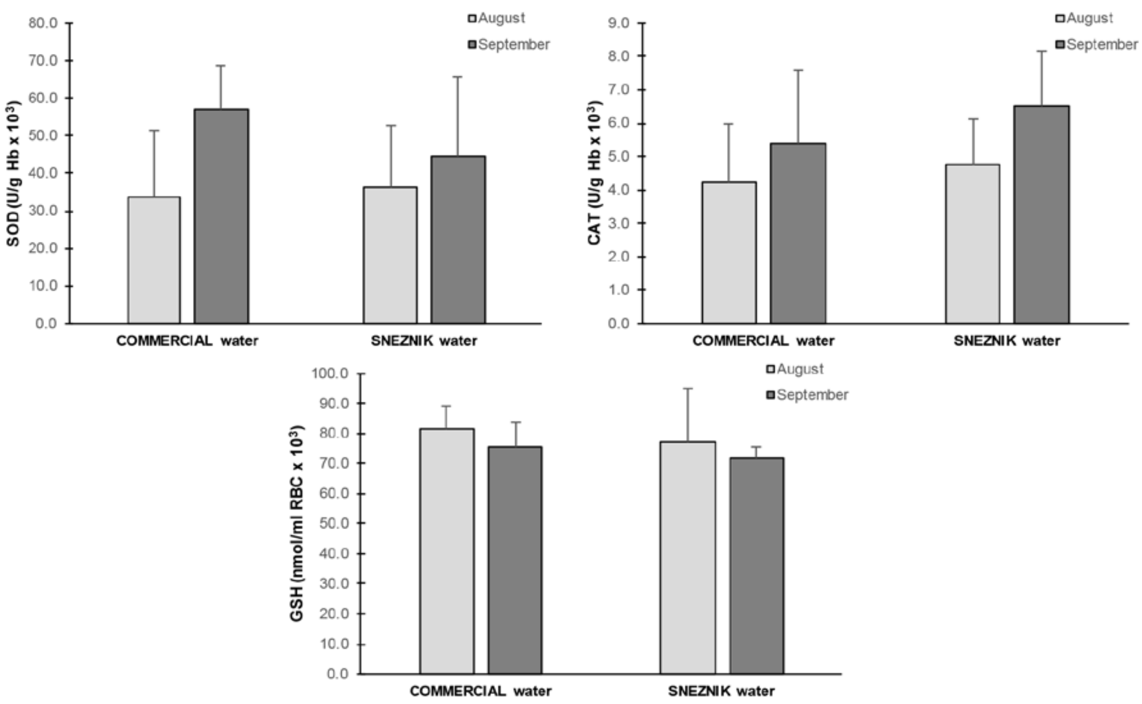
Results are expressed as mean ± standard error.
VO_{2max} – absolute and relative maximum oxygen consumption,
HR ANP– heart rate at anaerobic threshold.

Figure 2. Effects of four-week water consumption
from the well Sneznik on prooxidative parameters.



Results are expressed as mean ± standard error. O_2^- – superoxide anion radical;
H_2O_2 – hydrogen peroxide; NO_2^- – nitrites; TBARS – index of lipid peroxidation. * $p < 0.05$.

Figure 3. Effects of four-week water consumption
from the well Sneznik on parameters of antioxidant protection.



Results are expressed as mean ± standard error.
SOD –superoxide dismutase; CAT – catalase; GSH – reduced glutathione.

DISCUSSION

Present study aimed to estimate the potential benefits of alkaline water consumption from the Sneznik well and its potentially positive effects on the body composition, anthropometric and laboratory values as well as oxidative stress in professional sports. In order to examine these effects, we compared the results before and after consumption of commercial and Sneznik water, where we found out that Sneznik water is completely safe from the aspect of affecting various anthropometric, functional and biochemical parameters as well as systemic oxidative stress of professional basketball players.

Evaluation of the body composition is required in order to monitor the training outcomes, nutrition and general health. The body composition among athletes varies across different types of sport. This is very important in order to develop specific dietary regimes as well as adequate and optimal trainings. The fat mass and fat-free mass are mostly used to monitor the nutrition needs and energy consumption. Moreover, an increase in the body fat can impair the physical performances (19). Furthermore, the total body bioimpedance can predict the body composition compartments due to the fact that the body's fluid is equally distributed and body segmental lengths are proportional to segmental, which is very important for the appropriate hydration (20).

The results of the previous study which indicate that there is no statistical difference in the basic anthropometric characteristics, body mass, bioimpedence, including total body water and its active transport (TBW – total body water / ICW – intracellular water / ECW – extracellular water) in athletes who consumed alkaline high or low as well as commercial water, correlate with our results (21, 22). We haven't noticed any significance between and within the followed groups in terms of the body composition.

According to our results, from the aspect of the water Sneznik influence on the body composition, we might say that consumption of water from the mentioned well did not disturb the parameters of the body composition and with an efficient and healthy training process, it can be associated with the trend of positive effects on the body composition, which is supposed to be more obvious during longer basketball follow-up time (more than four weeks).

The exchange of ions, CO_2, and water between the intracellular and extracellular compartments helps to restore the acid-base balance following the intensive exercise. During the exercise, well trained athletes can decrease muscle pH from 7.0at rest to the values as low as 6.4–6.5 during the exercise (23). There are some data indicating that supplementation with acidic water cane have an alkalizing effect in young physically active men. (24). In correlation with these results, the ergospirometric test showed that consumption of water from the well Sneznik was associated with a slightly better functional capacity of the tested athletes, and it did not disturb the parameters of the ergospirometry. In addition, the positive trend achieved after consuming Sneznik water would be statistically confirmed over a longer monitoring period, indicating that further research would certainly be of interest.

It is believed that water rich in hydrogen ions may improve neutralization of ROS by stimulation of various antioxidant proteins, whereby hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals (25). Moreover, hydrogen-rich water can be mainly beneficial in different metabolic disorders (26). On the other hand, alkaline water, with high pH values, mainly has an important role in increasing the lactate utilization through the effect on the acid-base balance, following anaerobic exercise (21). Furthermore, the fluid replacement following dehydration reduces oxidative stress during the recovery (27). We wanted to examine whether weakly mineralized and weakly acid might have an antioxidative potential.

Our results showed significantly lower values of H_2O_2, after four weeks of different water consumptions which can point out that water from Sneznik potentially has an antioxidative role and it doesn't disturb the redox status in athletes. On the other hand, in trained individuals, the antioxidant system is more efficient due to the adaptation to exercise (21) which supports our unchanged values of antioxidative enzymes.

Interestingly, the level of TBARS was significantly increased after fourweeks in both groups, which goes in favor of an ongoing training process and micro-injuries that occur physiologically due to the intense physical activity. The previous studies indicate that prooxidative markers can be recorded in a higher concentration in athletes during the supramaximal exercise (21).

Based on these results, it can be observed that consumption of water from the well Sneznik was not associated with negative changes in the redox balance of the athletes and thus greater oxidative damage. In addition, as in the previous cases, it can be assumed that discrete positive changes would be more noticeable after a long-time exposure to this water.

Regarding the effect of water on the blood parameters, the previous study showed a significant difference in the whole blood viscosity when assessing high-pH, electrolyte water versus acceptable standard purified water (22). In our study, initiation of the training process did not affect changes of the most important blood parameters i.e., it was not associated with any hematological disorders. From the aspect of the water Sneznik influence on the examined blood parameters and electrolytes, we might say that consumption of water from the mentioned well is completely safe. This can indicate that low mineral and weakly acid water might maintain the adequate hydration. Also, although statistically unconfirmed, a smaller deviation in the concentration of the most important sweat ions between the first and second measurements compared to the group that used commercial drinking water speaks in favor of potentially beneficial effects whose validation requires further research. A longer follow-up time is suggested for confirmation and deeper analysis of these results.

CONCLUSION

In summary, our results pointed out that consumption of low mineral water from the well Sneznik is completely safe from the aspect of affecting various anthropometric, functional and biochemical parameters as well as systemic oxidative stress of professional basketball players.

In addition, existence of discretely better effects over commercial drinking water indicates that a long period of monitoring may certainly be of interest for further investigation.

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