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The Effects of Consuming Mineral Water from the Spring „Topla voda" on the Body Composition, Functional and Biochemical Parameters of Professional Male Handball Athletes: A Pilot Study

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Abstract: Adequate hydration is crucial for an athlete's health and performance. There is some evidence that different compositions of various mineral waters may improve exercise performance and affect different biomarkers. The aim was to investigate the consumption of mineral water from the spring „Toplavoda" in terms of safety profile and its effect on body composition and functional and biochemical parameters in professional athletes. During the preparation phase of their mesocycle, 14 male professional handball players underwent a complete sports medical screening exam with cardiopulmonary stress test (CPET), blood gas analysis, and oxidative stress markers dynamics at four points during the CPET. The athletes were then randomized into two equal groups where the first group consumed mineral water, and the second group consumed tap water. After four weeks, biochemical analysis and CPET were repeated. Routine analyses showed that the "mineral water" group increased the mean corpuscular hemoglobin (ANCOVA=0,05) and mean corpuscular hemoglobin concentration (ANCOVA=0,001), a greater metabolic equivalent of task (MET) at the end of the test (ANCOVA=0,49), with no significant changes in other measured parameters. Consuming "mineral water" appears safe, with some potential positive effects compared to tap water, mostly on hemoglobin parameters, fatigue perception, and exercise tolerance.

Keywords: hydration; athlete; mineral water; CPET; hemoglobin; fatigue

1. Introduction

Water is the principal chemical constituent of the human body. For an average young adult male, total body water represents 50% to 70% of body weight [1]. Variability in total body water is primarily due to differences in body composition, and various techniques can measure hydration [2]. Net body water balance is regulated by thirst and hunger drives, coupled with *ad libitum* access to food and fluids that offset water losses. Among the greatest challenges to body water homeostasis are exercise

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and exercise-heat stress. Normal hydration can be achieved with a wide range of water intake by sedentary and active people across the lifespan [1]. Adequate fluid intake can be defined as a volume of fluid (from water, beverages, and food) sufficient to replace water losses and provide for solute excretion. A wide range of fluid intakes is compatible with normal hydration, whereby total body water varies narrowly from day to day by 600 to 900 mL (<1% body mass) [3].

Rehydration during exercise should meet the primary goal of preventing more than 2% body weight loss from water deficit to avoid performance deterioration and negative health outcomes [4]. The effects of hypohydration include reduced blood volume, notably in hot environments, increased skin blood flow, and increased sweat rate, subsequently increased core temperature causing further cardiovascular strain, decreased venous return, preload, and compensatory increased heart rate (HR) [5]. Considering the importance of specific ions for the cell membrane stability and conductivity, especially in excitable tissues responsible for exercise and adequate physical exertion, not only the amount but the fluid composition has great importance during exercise. Sodium is the main electrolyte lost in sweat (20-70 mEq/L). Sodium supplementation during exercise is often required for heavy and "salty" sweaters to maintain plasma volume and plasma sodium balance [6]. A precise refueling strategy during exercise should be taken into account when considering the type, duration, and level of exercise [7].

On the other hand, exercise-induced oxidative stress has been researched for a long time. It is still unclear whether the increases in reactive oxygen species are detrimental to health and performance [8]. As shown in studies investigating adaptation in altitude training, there is not enough evidence to recommend high-dose single antioxidant supplementation, as this may actually impair endurance and altitude-based training adaptations. However, ingesting ample amounts of antioxidant-rich foods into athletes' diets does not produce this detrimental effect [9]. To the authors' knowledge, no investigation has been done into the ingestion of various water characteristics and its effect on redox parameters.

There is some evidence that alkaline (hydrogen-rich) water, in some cases, improves exercise performance [10], and even affect blood pH in physically active men [11]. The ability to attenuate the rate of muscle hydrogen ion (H^+) accumulation during exercise and/or enhance its removal from the muscle during recovery may affect the extent of exercise-induced disruption to excitation-contraction coupling, glycolytic flux, and phosphocreatine recovery and permit increased performance during continuous and intermittent high-intensity exercise [12].

Another important factor that influences performance and fatigue in athletes is iron status. Although iron deficiency occurs more often in females, 5-11% of male athletes exhibit it. Iron deficiency can lead to reduced hemoglobin concentration and iron-deficient anemia in the later stages [13]. Based on their iron status, athletes should check their hemoglobin concentrations (and other relevant red blood cell parameters), serum ferritin, and transferrin concentrations at least annually.

Regarding all the factors mentioned affecting exercise performance, the aim of our study was to investigate the consumption of mineral water from the spring „Toplavoda" in terms of safety profile and its effect on body composition and functional and biochemical parameters in professional athletes.

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2. Materials and Methods

Participants

The study included 14 male professional handball players aged 23,7±4,9 years with long-term experience in this sport. The study was conducted during the preparation phase of their mesocycle.

Experiment Design

This was a prospective, randomized-controlled study.

Water characteristics

Table 1 shows the chemical and mineral properties of the mineral water from the spring "Toplavoda." The control group consumed tap water.

Table 1. Mineral water properties.

Oxygen saturation (%)	**33,4**
Dry residue at 180°C (mg/L)	1996
pH	6,7
Nitrates (mg/L)	<1,0
Nitrites (mg/L)	<0,005
Fluorides (mg/L)	3,45
Chlorides (mg/L)	38
Sulphates (mg/L)	8,6
Sulphites (mg/L)	0,022
Cyanides (mg/L)	<0,01
Bicarbonates (mg/L)	2135,0
Dissolved carbon-dioxide (mg/L)	666
Phenols (mg/L)	<0,003
Water hardness (oN)	33,6
Sodium (mg/L)	532
Potassium(mg/L)	69,3
Calcium (mg/L)	66,1
Magnesium (mg/L)	56,3
Iron (mg/L)	0,75
Manganese (mg/L)	0,2
Copper (mg/L)	<0,03
Arsenic (mg/L)	<0,01
Barium (mg/L)	<0,05
Cadmium (mg/L)	<0,002
Lead (mg/L)	<0,005
Mercury (mg/L)	<0,0005
Selenium (mg/L)	<0,002
Antimony (mg/L)	<0,002
Chromium (mg/L)	<0,02

Study Protocol

The athletes underwent a complete sports medical screening exam that included a physical exam, ECG at rest (*Cardiovit* AT-102 G2, *Schiller*, Switzerland), body composition determination, routine laboratory analyses, heart echocardiogram (*CX50*, *Philips*, Nederlands and *Acuson Juniper*, *Siemens*, Germany), and cardiopulmonary stress test (CPET). Additionally, fingertip blood gas analysis was performed, and oxidative stress markers were measured.

A complete examination was performed during the first visit. Following at least a 3-hour fasting period, a sample for the basic biochemical panel was taken at rest. Given the dynamic changes during

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the test, the blood gas analysis and oxidative stress parameters were sampled four times during the test: at 8 AM (basal), before the CPET (point 1), during maximal exertion/end of CPET (point 2), 5 minutes into the rest phase (point 3), and 10 minutes into rest phase (point 4).

After the initial test, the athletes were randomized into two groups by simple randomization. The first group (n=7) consumed mineral water, "*Toplavoda*", while the second group (n=7) consumed tap water.

During that period, each athlete received his own drinking bottle, and his water intake was carefully monitored. Water intake was controlled before, during, and after training (or friendly games). The intake averaged 2,1±0,5 L during that period.

After four weeks of consuming mineral water or tap water, biochemical analysis and CPET were repeated during the second (final) visit. Testing was performed each day at the same time (10 a.m.), and athletes fasted at least three hours before the test. Blood was collected from the cubital vein in adequate frozen vacutainers until analyzed.

Body Composition Parameters

The bioelectrical impedance analysis (BIA) method (*InBody 370,* South Korea) was used to obtain the following anthropometric parameters: body height, body weight, and body composition indicators (bone mass, soft tissue mass, total fat mass, skeletal muscle mass (SMM), fat percentage, total body water, and body mass index (BMI)).

Functional Parameters

The functional parameters during CPET (*Quark* CPET metabolic cart and *h/p/cosmos* pulsar treadmill, *Cosmed,* Italy) examined were the absolute and relative maximum oxygen consumption (VO2max), respiratory exchange ratio (RER), maximal heart rate (HRmax), respiratory reserve, anaerobic threshold, and anaerobic threshold. The modified Borg rating of perceived exertion (RPE) scale of 0-10 was used after the test to evaluate the subjective feel of maximal exertion.

Routine Laboratory Analyses

The laboratory analyses performed on the *Mythic 18* analyzer (*Orphee,* Switzerland) included complete blood count of leukocytes (WBC), erythrocytes (RBC), platelets (PLT), lymphocytes (Lymph), granulocytes (Gran), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC); mean platelet volume (MPV); plateletcrit (PCT). The biochemical parameters sodium (Na), potassium (K), glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, AST, ALT, total proteins, iron, total bilirubin, direct bilirubin, and ferritin were determined in serum samples on the Aries analyzer *(Instrumentation Laboratory,* Italy*)*.

Blood Gas Analysis

The pH, blood gases, electrolytes, glucose, lactate, and oximetry in heparinized whole blood were measured using The *ABL90 FLEX* automated analyzer (*Radiometer Medical ApS,* Denmark). Four different measuring principles employed in sensors in the *ABL 90 FLEX PLUS* analyzer were used: potentiometry (pH, pCO2, K+, Na+, Ca2+, Cl-), amperometry (cGlu, cLac), optical pO2 (pO2), and spectrophotometry (ctHb, sO2, FO2Hb, FCOHb, FHHb, FMetHb). Derived parameters calculated or estimated on the basis of measured and keyed-in data were: anion gap, the concentration of total carbon dioxide in plasma (ctCO2(P)), the concentration of total carbon dioxide in whole blood (CO2 content (ctCO2(B)), concentration of hydrogen carbonate (HCO3-), standard bicarbonate (SBC), Actual Base Excess (ABE), Standard Base Excess (SBE), ABE of fully oxygenated blood, partial pressure of oxygen at half saturation (50 %) in blood (p50), partial pressure of oxygen in alveolar air (pO2(A)), ratio of the partial pressure (of oxygen in arterial blood and alveolar air (pO2(a/A)),

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respiratory index (RI), difference in the partial pressure of oxygen in alveolar air and arterial blood (pO2(A-a)).

Evaluation of Systemic Redox State

The redox status was evaluated spectrophotometrically on the *UV-1800 (Shimadzu*, Japan) by measuring the levels of prooxidative parameters, hydrogen peroxide (H2O2), superoxide anion radical (O2-), nitrites (NO2-), and index of lipid peroxidation (TBARS) in plasma. Activities of the corresponding antioxidative enzymes superoxide dismutase (SOD), catalase (CAT), glutathione s-transferase(s) (GST(s)), glutathione peroxidase (GPx), 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 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 0.8 ml plasma and 0.4 ml TCA (trichloroacetic acid). After that, the samples were put on ice for 10 min and centrifuged for 15 min at 6000 rpm [14]. 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) [15]. 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 gl K2CO3 was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe. The level of O2- 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 [16]. The determination of H2O2 concentration is based on the oxidation of phenol red using hydrogen peroxide in the reaction catalyzed by the enzyme peroxidase from horse radish (POD) [17]. 200 gl samples with 800 gl PRS (phenol red solution) and 10 gl POD were combined (1:20) and measured at 610 nm.

Determination of Antioxidative Enzyme Activity

Isolated RBCs were washed three times with 3 volumes of ice-cold 0.9mmol/l NaCl, and hemolysates containing about 50g Hb/l, prepared according to McCord and Fridovich [18] were used for the determination of catalase (CAT) activity. The determination of CAT activity was done according to Beutler[19]. Lysates were diluted with distilled water (1:7v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove hemoglobin. Then 50 gl CAT buffer, 100 gl sample, and 1ml 10mM H2O2 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 the epinephrine method of Misra and Fridovich[20]. 100gl ly- sate and 1 ml carbonate buffer were mixed, and then epinephrine was added in a volume of 100 gl. The detection was performed at 470 nm. This method belongs to the 'negative' type group of methods since it monitors the decrease of autoxidation speed in an alkaline medium, which is dependent on O2. The level of GSH concentration was determined based on GSH oxidation with 5.5-dithiobis-6.2-nitrobenzoic acid using the Beutler method [21]. The measurement of absorbance is carried out at a wavelength of the maximum absorption of 420nm.

Statistical Analysis

Depending on the type of variables, data description is presented as n (%) and mean±standard deviation. For testing the differences in means between two independent samples (mineral and tap water), an independent samples t-test was used. To test differences in means between two repeated measurements (pre-post), a paired samples t-test was applied. For testing differences in multiple

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repeated measurements for each type of water separately, a repeated measures ANOVA was used. The Bonferroni procedure was applied for multiple comparisons of repeated measures data. In the analysis of modeling the relationship of outcome numerical variables in relation to the type of water, adjusted for baseline values, ANCOVA was performed. For modeling the relationship of dependent variables in repeated measurements in relation to the type of water and measurement time, a linear mixed model was used. Statistical hypotheses were tested using a 0.05 (alpha) statistical significance level. All data were processed using the IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY, USA) and R-4.0.0 software (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Body Composition Parameters

As shown in Table 2, the two groups of athletes did not significantly differ in all body composition parameters, including blood pressure and HR at rest.

Table 2. Subject characteristics.

Parameter	Measurement	Mineral water	Tap water	*p-value*
Age		24,14 ± 6,26	23,29±5,74	*0,794*
Height (cm)		186,43±6,26	192,07±3,76	*0,063*
Weight (kg)	Before	94,80±9,56	99,06±10,11	*0,434*
	After	94,91±9,15	99,76±10,02	
BMI (kg/m^2)	Before	27,36±3,19	26,83±2,25	*0,726*
	After	27,39±3,16	27,03±2,27	
Body fat percentage (%)	Before	14,44±4,61	14,33±4,17	*0,962*
	After	14,33±4,56	13,71±3,69	
Musle mass (kg)	Before	46,76±3,80	49,98±4,99	*0,213*
	After	46,99±3,65	49,05±5,37	
Muscle mass percentage (%)	Before	49,46±2,50	50,26±2,73	*0,578*
	After	49,63±2,40	49,71±2,40	
Total body water (kg)	Before	59,17±4,58	63,52±6,33	*0,181*
	After	59,30±4,31	62,91±6,32	
Free fat mass (kg)	Before	80,86±6,32	86,90±8,53	*0,170*
	After	81,09±5,99	86,01±8,59	

Results are expressed as mean ± standard deviation.

Biochemical Parameters

Table 3 shows the hematological and biochemical parameters. Hemoglobin (Hb) concentration increased in both groups, almost reaching statistical significance in the "mineral water group." Two parameters related to Hb and iron metabolism that increased to statistical significance in the "mineral water" group after four weeks are the mean corpuscular hemoglobin, MCH (*ANCOVA* 0,05), and mean corpuscular hemoglobin concentration, MCHC (*ANCOVA* 0,001). Other parameters did not show a statistically significant difference.

Table 3. Routine laboratory analyses.

Parameter	Measurement	Mineral water	Tap water	*p-value*	*ANCOVA*
Leukocytes (*10^9/L)	Before	5,44±1,09	6,01±0,63	*0,252*	*0,540*
	After	5,41±0,84	5,80±1,77	*0,611*	
Lymphocytes (%)	Before	33,79±10,58	32,67±8,92	*0,835*	*0,495*

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	After	33,61±8,76	36,97±7,59	*0,458*	
Monocytes (%)	Before	5,03±0,95	5,99±0,92	*0,079*	*0,418*
	After	5,36±1,10	6,27±1,02	*0,133*	
Granulocytes (%)	Before	61,19±10,54	61,34±8,64	*0,976*	*0,384*
	After	61,11±9,38	56,76±7,88	*0,365*	
Erythrocytes (*10^{12}/L)	Before	5,01±0,28	4,72±0,21	*0,051*	*0,473*
	After	5,06±0,24	4,86±0,14	*0,077*	
Hemoglobin (g/L)	Before	142,86±5,21	135,14±9,21	*0,078*	*0,321*
	After	150,29±9,43	141,57±5,35	*0,055*	
Hematocrit (l/L)	Before	0,43±0,01	0,41±0,03	*0,059*	*0,896*
	After	0,44±0,02	0,02	*0,215*	
Mean corpuscular volume, MCV (fL)	Before	85,61±3,71	86,17±1,72	*0,725*	*0,432*
	After	89,03±5,54	88,43±1,79	*0,790*	
Mean corpuscular hemoglobin, MCH (pg)	Before	28,60±1,49	28,60±0,96	*1,000*	*0,050**
	After	29,69±1,54	29,14±0,52	*0,394*	
Mean corpuscular hemoglobin concentration, MCHC (g/L)	Before	333,86±6,62	332,00±7,81	*0,640*	*0,001**
	After	338,29±4,46	329,57±2,76	*0,001**	
Thrombocytes (*10^{9}/L)	Before	249,86±24,76	233,86±42,89	*0,409*	*0,254*
	After	241,57±31,25	244,00±36,30	*0,896*	
Sedimentation rate, SE (mm/h)	Before	2,57±0,79	2,57±0,98	*1,000*	*0,174*
	After	2,71±0,76	4,71±3,45	*0,181*	
Potassium (mmol/L)	Before	4,29±0,32	4,11±0,30	*0,284*	*0,501*
	After	4,34±0,29	4,39±0,32	*0,871*	
Soidum (mmol/L)	Before	142,01±3,31	144,13±1,20	*0,138*	*0,436*
	After	141,96±2,38	140,47±1,65	*0,199*	
Glucose (mmol/L)	Before	4,94±0,43	4,97±0,28	*0,886*	*0,368*
	After	5,17±0,23	5,27±0,14	*0,342*	
Total cholesterol (mmol/L)	Before	4,63±0,51	4,11±1,28	*0,344*	*0,385*
	After	4,37±0,30	4,17±0,96	*0,608*	
HDL (mmol/L)	Before	1,59±0,24	1,55±0,21	*0,757*	*0,223*
	After	1,47±0,20	1,53±0,17	*0,557*	
LDL (mmol/L)	Before	2,79±0,55	2,36±1,08	*0,373*	*0,408*
	After	2,45±0,27	2,37±0,75	*0,795*	
Triglycerides (mmol/L)	Before	0,53±0,07	0,48±0,25	*0,619*	*0,530*
	After	0,69±0,18	0,70±0,42	*0,974*	
Urea / Blood urea nitrogen (mmol/L)	Before	8,24±1,73	7,67±2,10	*0,589*	*0,567*
	After	6,79±1,34	6,77±1,57	*0,986*	
Creatinine (µmol/L)	Before	106,14±7,95	100,57±10,66	*0,289*	*0,182*
	After	96,00±5,77	88,43±9,27	*0,092*	
AST (U/L)	Before	47,71±29,85	37,43±16,92	*0,443*	*0,372*
	After	26,71±6,21	30,14±4,06	*0,245*	
ALT (U/L)	Before	35,14±18,77	26,29±2,36	*0,239*	*0,386*
	After	24,86±7,63	20,71±4,15	*0,231*	
Proteins (g/L)	Before	70,86±2,73	73,14±2,41	*0,123*	*0,973*
	After	67,86±3,72	69,14±2,48	*0,461*	
Iron (µmol/L)	Before	23,54±6,70	21,92±6,85	*0,662*	*0,363*
	After	14,77±6,21	17,37±6,82	*0,470*	
	Before	17,64±3,40	15,88±5,47	*0,484*	*0,927*

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Total bilirubin (µmol/L)	After	13,48±4,90	12,04±4,83	*0,589*	
Direct bilirubin (µmol/L)	Before	3,58±0,51	3,38±0,97	*0,639*	*0,592*
	After	3,03±1,21	2,65±0,65	*0,479*	
Ferritin (ng/mL)	Before	124,97±54,28	84,13±21,51	*0,089*	*0,325*
	After	107,80±70,16	72,64±15,61	*0,220*	

Results are expressed as mean ± standard deviation.

CPET Parameters

CPET parameters are shown in Table 4. Relative VO2 uptake was greater after four weeks in both groups. The increase was somewhat more in the "mineral water" group, although not statistically significant. The athletes consuming "mineral water" showed a greater metabolic equivalent of task (MET) at the end of the test (*ANCOVA*=0,49), and the increase was greater in the same group after four weeks compared to the control group (*p=0,04)*. These athletes reached higher HRmax in the second visit at the end of the task, compared to the control group (*p=0,04),* also when expressed as the percentage of theoretical HRmax (*p=0,01*). The RPE scale showed lower values in the "control" group at the first visit (*p=0,001*), but the RPE did not differ during the second visit. Other parameters did not show a statistically significant difference.

Table 4. CPET parameters.

Parameter	Measurement	Mineral water	Tap water	*p-value*	*ANCOVA*
Maximal speed (km/h)	Before	11,71±0,49	10,57±0,79	0,008	0,686
	After	11,43±0,54	10,86±0,90	0,174	
Maximal incline (%)	Before	12,00±0,00	10,86±1,07	0,030	*1,000*
	After	12,00±0,00	11,14±1,07	0,055	
Test duration (s)	Before	601,29±58,07	541,71±61,85	0,088	*0,480*
	After	579,86±30,88	566,43±84,58	0,700	
METs	Before	14,89±0,96	14,53±1,28	0,564	*0,490**
	After	16,00±1,02	14,60±1,23	0,040*	
HRmax (beats per min)	Before	189,29±4,57	180,14±11,34	0,071	*0,109*
	After	185,57±3,65	174,71±10,10	0,030*	
Predicted/theoretical HRmax (%)	Before	96,71±3,30	91,79±3,83	0,024	*0,236*
	After	94,86±3,67	89,14±3,34	0,010*	
Heart rate recovery 1st minute	Before	164,86±8,75	151,71±8,20	0,013*	*0,822*
	After	163,00±6,76	152,00±12,00	0,056	
Heart rate recovery 3rd minute	Before	104,29±11,91	98,14±12,59	0,367	*0,157*
	After	112,14±13,84	100,71±8,46	0,087	
Maximal systolic blood pressure (mmHg)	Before	193,57±16,51	182,86±13,80	0,212	*0,529*
	After	184,29±19,02	187,14±20,59	0,792	
Maximal diastolic blood pressure (mmHg)	Before	65,71±18,13	51,43±6,90	0,075	*0,509*
	After	54,29±5,35	48,57±6,90	0,109	
Ventilatory anaerobic threshold, VAT (HR)	Before	184,00±5,69	175,86±10,43	0,095	*0,151*
	After	178,43±4,54	168,29±9,41	0,031*	
Aerobic threshold, AT (HR)	Before	173,43±9,27	162,29±10,10	0,053	*0,573*
	After	165,29±4,42	157,00±9,26	0,054	
Respiratory exchange ratio, RER	Before	1,021±0,01	1,026±0,02	0,570	*0,430*
	After	1,076±0,06	1,047±0,05	0,330	
Maximal VO2 uptake, VO2max (ml/kg/min)	Before	53,69±2,81	51,97±4,77	0,429	*0,159*
	After	55,80±2,55	52,57±4,48	0,123	

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Rating of perceived exertion, RPE	Before	8,71±0,91	6,71±0,91	0,001*	*0,941*
	After	8,14±0,85	8,14±1,11	1,000	

Results are expressed as mean ± standard deviation.

Blood Gas Analyses

The blood gas analyses are shown in Table 5. The statistical analyses compared parameters between two groups and inside each group (between the first and second measurements). The arterial and alveolar PaO_2 (a/ApO_2 (%)) and the gradient PO_2 between alveolar and arterial blood (AaDpO2 (kPa)) did not show statistical differences between four measurements during each visit, nor did comparing the first to second visit between groups and inside each group. Actual base excess (ABE (mmol/L)) showed negative values (the buffer deficit, bicarbonates most of all). ABE values were greater in both groups during the second visit. The anion gap increased in all three measurements compared to the rest, with no significant difference between the groups. Bicarbonate ion concentration correlated with the anion gap dynamic. Other electrolytes did not show a statistically significant difference in all measurements.

Table 5. Blood gas analysis results.

Parameter	**Measurement**		**Mineral water**	**Tap water**	***p-value***	***mixed effect interaction***
pO2(a/A) (%)	**Before**	**1.**	68,81±3,94	68,97±6,33	*0,956*	*0,756*
		2.	95,01±20,51	88,79±7,51	*0,465*	
		3.	91,46±5,40	83,77±7,72	*0,052*	
		4.	86,40±5,58	79,89±4,59	*0,035*	
	After	**1.**	69,92±6,10	72,20±3,97	*0,425*	
		2.	88,89±4,05	90,51±8,08	*0,642*	
		3.	89,55±6,44	90,47±1,78	*0,723*	
		4.	85,51±6,67	85,22±3,96	*0,924*	
ABE (mmol/L)	**Before**	**1.**	-0,03±1,21	0,23±0,45	0,607	*0,981*
		2.	-13,50±2,82	-9,13±1,73	0,006	
		3.	-14,81±2,70	-9,03±3,02	0,003	
		4.	-13,10±2,87	-6,51±2,67	0,001	
	After	**1.**	0,36±1,20	0,30±1,04	*0,926*	
		2.	-15,21±3,01	-11,57±2,35	*0,028*	
		3.	-16,81±4,03	-11,93±2,53	*0,022*	
		4.	-14,79±3,81	-9,76±2,57	*0,015*	
Aniongap (mmol/L)	**Before**	**1.**	10,91±1,93	10,81±1,41	*0,914*	*0,721*
		2.	21,47±2,64	18,50±1,38	*0,027*	
		3.	23,49±2,20	18,51±3,28	*0,007*	
		4.	21,54±2,99	16,73±2,68	*0,008*	
	After	**1.**	10,09±1,61	9,64±1,13	*0,561*	
		2.	22,43±2,87	18,44±1,67	*0,010*	
		3.	23,59±3,53	19,77±2,68	*0,044*	
		4.	22,34±2,83	18,37±2,36	*0,015*	
HCO_3^- (mmol/L)	**Before**	**1.**	25,16±1,45	25,70±0,85	*0,408*	*0,991*
		2.	13,99±2,21	17,51±1,15	*0,005*	
		3.	12,24±2,12	17,16±2,38	*0,002*	
		4.	13,31±2,68	18,81±2,10	*0,001*	
	After	**1.**	25,34±1,19	25,50±0,72	0,771	
		2.	14,14±2,63	16,99±1,52	0,034	
		3.	11,99±1,86	15,66±1,88	0,017	
		4.	12,80±2,87	16,77±1,88	0,011	

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pO2(a/A)**:** theratio of the partial pressure of oxygen in arterial blood and alveolar air; ABE**:**actual Base Excess; HCO_3^- **:**the concentration of hydrogen carbonate. Results are expressed as mean ± standard deviation.

Oxidative Stress Markers

The oxidative stress markers and antioxidative defense results are shown in Tables 6 and 7. The statistical analyses compared parameters between two groups and inside each group (between the first and second measurements). The values of lipid peroxidation, nitrite, and superoxide anion radicals showed no statistically significant difference in all measurements. The same trend is shown for hydrogen peroxide concentration. The activity of a great/er part of enzyme antioxidative protection is shown to be greater in both groups in the second measurement. Although not statistically significant, greater superoxide dismutase, reduced glutathione, and glutathione peroxidase activity were noted during the second visit in the "mineral water" group.

Table 6. The dynamics of oxidative stress parameters.

Parameter	Measurement		Mineral water	Tap water	*p-value*	*mixed effect interaction*
TBARS (µmol/mL)	**Before**	**1.**	0,99±0,23	0,96±0,24	*0,807*	*0,886*
		2.	1,02±0,34	0,98±0,26	*0,801*	
		3.	1,04±0,32	0,96±0,25	*0,646*	
		4.	0,98±0,22	0,98±0,23	*1,000*	
	After	**1.**	1,18±0,05	1,14±0,16	*0,515*	
		2.	1,13±0,15	1,17±0,14	*0,647*	
		3.	1,11±0,20	1,18±0,14	*0,472*	
		4.	1,16±0,17	1,17±0,13	*0,906*	
NO_2^- (nmol/mL)	**Before**	**1.**	4,25±0,41	4,29±0,38	*0,848*	*0,389*
		2.	4,48±0,58	4,24±0,39	*0,383*	
		3.	4,09±1,23	4,24±0,33	*0,778*	
		4.	4,33±0,38	4,16±0,41	*0,429*	
	After	**1.**	5,66±0,76	5,51±0,58	*0,680*	
		2.	5,88±0,88	5,52±0,55	*0,377*	
		3.	5,80±0,80	5,58±0,59	*0,567*	
		4.	5,61±0,76	5,84±0,64	*0,546*	
$O2^-$ (nmol/mL)	**Before**	**1.**	1,51±0,46	3,30±1,34	*0,012*	*0,075*
		2.	3,39±1,37	2,64±1,29	*0,311*	
		3.	1,70±0,72	1,16±0,58	*0,169*	
		4.	2,64±1,36	1,98±1,59	*0,421*	
	After	**1.**	1,13±0,42	1,08±0,59	*0,867*	
		2.	1,46±0,57	1,74±1,04	*0,539*	
		3.	3,77±1,04	3,34±1,01	*0,453*	
		4.	2,54±0,65	3,06±0,78	*0,201*	
H_2O_2 (nmol/mL)	**Before**	**1.**	2,59±0,37	2,05±0,54	*0,053*	*0,008**
		2.	2,48±0,60	2,14±0,44	*0,250*	
		3.	2,03±0,30	1,99±0,39	*0,801*	
		4.	2,17±0,45	2,15±0,39	*0,951*	
	After	**1.**	3,59±0,24	3,83±0,49	*0,257*	
		2.	3,60±0,39	4,00±0,24	*0,063*	
		3.	3,80±0,50	3,86±0,39	*0,806*	
		4.	3,48±0,33	3,78±0,45	*0,197*	

TBARS: the index of lipid peroxidation measuring thiobarbituric acid reactive substances; NO_2^- : nitrites; $O2^-$: superoxide anion radical; H_2O_2: hydrogen peroxide. Results are expressed as mean ± standard deviation.

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Table 7. The dynamics of antioxidant defense.

Parameter	Measurement		Mineral water	Tap water	*p-value*	*mixed effect interaction*
SOD (U/g Hg * 10³)	**Before**	**1.**	16,28±8,14	18,61±13,05	*0,696*	*0,411*
		2.	9,30±3,08	16,28±9,40	*0,087*	
		3.	17,44±7,32	16,28±7,28	*0,780*	
		4.	13,95±7,74	13,95±6,15	*1,000*	
	After	**1.**	23,26±18,46	25,58±5,62	*0,755*	
		2.	34,89±13,87	24,42±16,94	*0,230*	
		3.	20,93±9,23	22,09±10,20	*0,827*	
		4.	26,75±18,03	26,75±12,18	*1,000*	
CAT (U/g Hg * 10³)	**Before**	**1.**	7,04±3,76	3,84±2,41	*0,083*	*0,148*
		2.	5,11±3,92	6,82±5,14	*0,496*	
		3.	3,71±3,78	5,33±3,11	*0,422*	
		4.	4,64±2,79	4,25±3,62	*0,824*	
	After	**1.**	2,14±1,60	3,57±2,31	*0,203*	
		2.	1,75±1,63	3,04±2,67	*0,299*	
		3.	2,54±2,38	3,07±2,00	*0,657*	
		4.	3,00±1,62	3,54±1,98	*0,589*	
GSH (nmol/mL RBC * 10³)	**Before**	**1.**	68448,33±4681,62	68725,90±4375,96	*0,911*	*0,375*
		2.	76608,84±8794,14	72833,92±4914,65	*0,341*	
		3.	78718,36±11797,06	77848,64±8423,36	*0,883*	
		4.	77552,57±4640,73	82160,21±9861,09	*0,285*	
	After	**1.**	100701,77±16809,37	91875,09±12860,61	*0,291*	
		2.	105031,83±105031,83	91875,10±91875,10	*0,054*	
		3.	11467,88±11467,88	11527,30±11527,30	*0,085*	
		4.	102700,26±102700,26	92763,31±92763,31	*0,201*	
GPx (nmol/min/mL)	**Before**	**1.**	24,03±23,73	39,62±20,47	*0,213*	*0,288*
		2.	35,92±13,04	31,11±9,48	*0,445*	
		3.	22,21±18,59	39,97±17,73	*0,107*	
		4.	22,38±19,76	24,68±20,37	*0,834*	
	After	**1.**	30,55±14,71	31,53±15,72	*0,906*	
		2.	28,68±18,98	32,55±12,11	*0,657*	
		3.	38,94±14,07	23,96±12,49	*0,057*	
		4.	27,67±13,16	42,43±12,82	*0,055*	
GST (mmol/mL/min)	**Before**	**1.**	1,94±0,89	2,46±0,65	0,232	*0,907*
		2.	2,69±1,04	1,86±1,11	0,174	
		3.	2,03±0,53	2,28±0,52	0,399	
		4.	2,06±0,98	1,64±0,93	0,428	
	After	**1.**	2,23±0,22	2,20±0,74	0,922	
		2.	2,05±0,57	2,31±0,81	0,496	
		3.	2,22±0,81	2,51±0,77	0,498	
		4.	2,15±1,68	1,91±1,43	0,777	

SOD: the activity of superoxide dismutase; CAT: the activity ofcatalase; GSH: reduced glutathione; GPx: glutathione peroxidase; GST: the activity of glutathione s-transferase. Results are expressed as mean ± standard deviation.

4. Discussion

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This pilot experiment aimed to investigate the influence of consuming "mineral water" on body composition and various biochemical and CPET parameters in professional male handball athletes compared to athletes consuming tap water.

The data from BIA showed no difference between the two groups during the first and second visits, enabling a relevant comparison of these two groups. The slightly favorable changes (muscle mass and fat mass) can be attributed to the adequately periodized and planned training in this period of four weeks. Generally, each method for body composition measurement has its own strengths and weaknesses [22], but all of the athletes in this experiment were measured in the same conditions during both visits. After four weeks of intensive training preparation, consuming mineral water did not negatively affect body composition. However, a longer consumption period is needed to investigate potential positive effects on these parameters.

The two groups generally had no significant basic biochemical panel changes in the two visits. The mineral water group had a slightly greater increase in Hb, although not statistically significant, probably due to the small sample size. However, MCV and MCHC showed a statistically significant increase in the mineral water group compared to the control group. Given the role of hemoglobin in oxygen transport, better performance and recovery can be expected. Looking at the changes between the training type, volume, and sport, there are no consistent findings regarding which athletes will experience the greatest increase in Hb parameters [23]. All athletes in this experiment showed optimal iron reserves in starting serum ferritin being above 50 ng/mL, allowing for adequate erythropoiesis in response to training. No significant differences were shown in the serum ferritin during the second visit. The beneficial effect in Hb parameters is probably reflected during CPET, where the athletes in the mineral water group reached greater MET values. Athletes in the control group reported lower RPE during the first visit, but there was no difference between groups during the second visit. The greater HRmax achieved in the mineral water group can suggest that these athletes better adapted to the training protocol, as blunted HRmax can be a sign of "overtraining" or greater fatigue [24]. These facts can suggest that the group consuming mineral water better responded to the training protocol or developed a higher fatigue threshold for higher-intensity work than the control group. Again, it should be noted that athletes underwent the same training protocol and the same standardized meals. To our knowledge, no study has investigated the correlation of hydration, various water characteristics, and iron status in athletes. Recent data from our laboratory [25] investigated water with low mineral content in basketball players and showed no improvement in Hb status. The recommended iron concentration for athletes [26]is not expected to be found in any drinking water, but through other mechanisms that positively affect homeostasis, athletes may benefit from drinking water with similar characteristics as mineral water. This hypothesis would require further investigation with more participants, preferably in different sports.

A study by Chiron et al. [27]investigated how the bicarbonate-rich water affects various parameters when combined with either an "alkalizing" or "acidizing" diet. The results show that bicarbonate-rich water can alter the acid-base balance during warm-up and after high-intensity exercise, potentiating possible beneficial effects of an alkalizing diet on the acid-base and reducing the acid load induced by an acidifying diet. No beneficial effect was observed regarding maximal exercise. It should be noted that the water was consumed for only one week in a crossover design.

Specific rehydration protocols with various oral solutions containing various electrolytes and carbohydrates are suggested around training, especially as a strategy during and after longer duration activity [28]. This was not the aim of our study, where we investigated the "habitual" water intake as a baseline, in addition to proper nutrition and rehydration strategies. As shown by [29], where muscle cramping was investigated, solely mineral water intake is not a viable strategy after dehydration in hot conditions. Richard et al. [30] investigated the "acute" ingestion of different types of water around an exercise test, and they noted the lowest pH and muscular fatigue with the bicarbonate water. Another experiment by Harris et al. [31] showed that mineral water with different properties could aid better rehydration following exercise, as measured by serum osmolarity.

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Capillary blood gas sampling (from the fingertip) was used to evaluate this study's acid-base and ventilation status. Given the sampling during CPET, performing arterial blood sampling was neither safe nor viable. For most blood gas parameters, the results do not differ when using these two methods [32]. The a/ApO2almost reached statistical significance, favoring the mineral water group, potentially showing the better efficacy of O2 gas exchange through the alveolar membrane; however, as mentioned by Zavorsky et al., arterial oxygen pressure can differ using this method, so this result should be interpreted with caution. ABE values were higher at the second visit, which the intense training protocol can mostly explain. Blood pH values were lower in the mineral water group. This suggests that four weeks was insufficient for the mineral water to express its buffer capacity, given the expected effect of its characteristics, primarily bicarbonate concentration. An expected increased anion gap resulted from metabolic acidosis at the expense of bicarbonate and its role as a buffer. As mentioned earlier, that is one of mineral water's main expected beneficial effects, and the effect on the ion dynamic is probably expected in a longer period than investigated. The role of Hb in buffering pH changes in blood by the combined transport of carbon dioxide and hydrogen ions (H^+) in the form of bicarbonate ions [33]. The previously mentioned beneficial increase in Hb parameters may also have an additional effect on better exercise tolerance and a higher fatigue threshold.

Various oxidative stress biomarkers are shown in exercise studies, showing oxidants, antioxidants, oxidative damage markers, and redox balance measurements [34].Antioxidative parameters did not significantly differ in the two groups. Given the intense training protocol, the antioxidative enzyme activity was, as expected, increased in the second visit, with slightly higher activity of superoxide dismutase (SOD), reduced glutathione (GSH), and glutathione peroxidase (GPx) in the second visit in the mineral water group. This result shows that mineral water slightly improved antioxidative capacities in the first and last line of defense against oxidative stress. However, a longer follow-up would be needed for a valid conclusion with more participants. A similar observation is noticed in oxidative stress biomarkers, where only H2O2 values were statistically significant compared to the control. Of course, that can be the starting point for further investigation, specifically concerning oxidative stress.

Based on this study, we can conclude that this type of mineral water did not significantly alter body composition. The appropriate training strategy for athletes can lead to positive outcomes in terms of body composition, which was also the case with routine biochemical parameters. On the other hand, markers of Hb metabolism showed more positive outcomes compared to the control group, which can potentially lead to better training adaptation, fatigue tolerance, improved performance, and recovery. Furthermore, consuming the "mineral water" led to better CPET parameters and their dynamic compared to the control group. This is based on the values of relative VO2max and METs suggesting better exercise tolerance. The effects may be more evident if this water is consumed for a longer period of time (longer than four weeks).

Furthermore, blood gas parameters showed greater metabolic acidosis in all athletes, suggesting inadequate bicarbonate production. Consuming mineral water can potentially positively influence this ion dynamic and create a potentially positive medium. Additionally, oxidative stress parameters did not show any negative changes in the "mineral water" group; therefore, there was no greater oxidative damage. Slightly greater activity of antioxidative enzymes was noted compared to the control group.

Four weeks was not enough time for "mineral water" and its increased bicarbonate concentration to show any significant buffer activity, especially in the athletes with elevated blood lactate values (and lower blood pH values).

In general, during this phase of investigating the influence of mineral water on all mentioned parameters in professional male handball athletes, it can be concluded that consuming "mineral water" is safe, with some potential positive effects compared to tap water, mostly on Hb concentration parameters and fatigue perception and exercise tolerance. All mentioned effects might be more evident if this water is consumed for a longer period of time (longer than four weeks).

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Therefore, future research will aim to investigate the effects of consuming "mineral water" for a longer duration with more athletes included.

Limitations

The authors acknowledge the limitations of this study. The small sample size and short experiment duration are inadequate for solid conclusions. Regarding nutrition, although the athletes were served the standard meals during the preparation phase, meal composition was not tracked. The sweat rate was not measured, and some athletes potentially required more water than was provided. This pilot study investigates the effects of mineral water consumption, and the authors will address these limitations in further experiments.

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Abbreviations

The following abbreviations are used in this manuscript:

HR	heart rate
HRmax	maximal heart rate
H^+	hydrogen ion
GCP	good clinical practice
CPET	cardiopulmonary stress test
BIA	bioelectrical impedance analysis
SMM	skeletal muscle mass
BMI	body mass index
VO2max	relative maximum oxygen consumption
RER	respiratory exchange ratio
RPE	rating of perceived exertion
ANCOVA	analysis of covariance
ANOVA	analysis of variance
MET	metabolic equivalent
ECG	electrocardiogram
WBC	leukocytes
RBC	erythrocytes
PLT	platelets
Lymph	lymphocytes

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Gran	granulocytes
Hb	hemoglobin
HCT	hematocrit
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MPV	mean platelet volume
PCT	plateletcrit
Na	Sodium
K	potassium
AST	aspartate transaminase
ALT	alanine transaminase
ctCO2(P)	concentration of total carbon dioxide in plasma
ctCO2(B)	concentration of total carbon dioxide in whole blood
HCO3-	concentration of hydrogen carbonate
SBC	standard bicarbonate
ABE	Actual Base Excess
SBE	Standard Base Excess
p50	partial pressure of oxygen at half saturation
pO2(A)	partial pressure of oxygen in alveolar air
pO2(a/A)	ratio of the partial pressure (of oxygen in arterial blood and alveolar air)
RI	respiratory index
pO2(A-a)	difference in the partial pressure of oxygen in alveolar air and arterial blood
H2O2	hydrogen peroxide
O2-	superoxide anion radical
NO2-	Nitrites
TBARS	index of lipid peroxidation
SOD	superoxide dismutase
CAT	the activity ofcatalase
GST(s)	glutathione s-transferase
GPx	glutathione peroxidase
GSH	reduced glutathione
TBA	thiobarbituric acid
TCA	trichloroacetic acid
NO	nitric oxide
PCA	perchloric acid
EDTA	ethylenediaminetetraacetic acid
NBT	Nitro Blue Tetrazolium
POD	peroxidase
PRS	phenol red solution
CAT	catalase
GSH	reduced glutathione
pO2(a/A)	theratio of the partial pressure of oxygen in arterial blood and alveolar air
ABE	actual Base Excess
HCO_3^-	the concentration of hydrogen carbonate

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