

The effect of mineral-based alkaline water on hydration status and the metabolic response to short-term anaerobic exercise

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ABSTRACT: Previously it was demonstrated that mineralization and alkalization properties of mineral water are important factors influencing acid-base balance and hydration in athletes. The purpose of this study was to investigate the effects of drinking different types of water on urine pH, specific urine gravity, and post-exercise lactate utilization in response to strenuous exercise. Thirty-six male soccer players were divided into three intervention groups, consuming around 4.0 l/day of different types of water for 7 days: HM (n=12; highly mineralized water), LM (n=12; low mineralized water), and CON (n=12; table water). The athletes performed an exercise protocol on two occasions (before and after intervention). The exercise protocol consisted of 5 bouts of intensive 60-s (120% $\text{VO}_{2\text{max}}$) cycling separated by 60 s of passive rest. Body composition, urinalysis and lactate concentration were evaluated – before (t0), immediately after (t1), 5' (t2), and 30' (t3) after exercise. Total body water and its active transport (TBW – total body water / ICW – intracellular water / ECW – extracellular water) showed no significant differences in all groups, at both occasions. In the post-hydration state we found a significant decrease of specific urine gravity in HM (1021 ± 4.2 vs 1015 ± 3.8 g/L) and LM (1022 ± 3.1 vs 1008 ± 4.2 g/L). We also found a significant increase of pH and lactate utilization rate in LM. In conclusion, the athletes hydrated with alkaline, low mineralized water demonstrated favourable changes in hydration status in response to high-intensity interval exercise with a significant decrease of specific urine gravity, increased urine pH and more efficient utilization of lactate after supramaximal exercise.

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INTRODUCTION

A good hydration state, regardless of the sport discipline and training intensity, provides the opportunity to achieve an optimal physical and mental state [1]. Hydration protocols during training and competition are a basic part of athletic training [2]. The hydration strategy should consider in detail the type and properties of administered fluids, and their volume in relation to the type of physical activity, its intensity and duration [3]. The American College of Sports Medicine, The National Athletic Trainer's Association and other institutions of sport and science present their recommendations to optimize performance, and to reduce the probability of injuries and overtraining due to dehydration [4]. The optimal hydration state is determined by monitoring urine specific gravity [5].

Acid-base equilibrium within the body is tightly maintained through the interaction of three complementary mechanisms: blood and tissue buffering systems (e.g., bicarbonate), the diffusion of carbon

dioxide from the blood to the lungs via respiration, and the excretion of hydrogen ions from the blood to the urine by the kidneys [6].

The most widely used fluid during exercise includes water. Different properties and especially the mineral content, the proportions between SO_4^{2-} and HCO_3^- as well as the pH determine hydration status and other therapeutic properties. This has been documented in numerous experiments and clinical trials [7, 8]. Mineral water has a significant impact on acid-base balance, which determines anaerobic exercise capacity [9]. Many researchers have suggested that water rich in Ca is characterized by this specific quality [10]. Even subtle changes in blood and tissue pH have significant metabolic consequences, including the response to oxidative stress [11, 12]. During supramaximal exercise, there is a significant increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS). In trained individuals the antioxidant system is more efficient due to

the adaptation to exercise [13, 14, 15, 16]. The neutralization of ROS may promote the use of water rich in hydrogen ions. This action is explained by the stimulation of numerous antioxidant proteins [17, 18]. The results of long-term research show that the use of hydrogen-rich water is helpful in preventing metabolic diseases [19], including diabetes [20]. Alkaline mineral water with a high pH, through the effect on acid-base balance can increase the rate of lactate utilization following anaerobic exercise [10].

Many studies have shown that the consumption of alkalizing supplements can have a significant effect on the body's acid-base balance using surrogate markers of urine and blood pH [21]. It is possible that the regular consumption of alkaline water could have an effect similar to nutritional supplements on markers of acid-base balance, yet this has not been evaluated in a controlled manner.

The aim of this study was to investigate the effects of water intake with different mineralization and alkalization properties on the state of hydration and the rate of lactate utilization in athletes following high-intensity interval exercise.

MATERIALS AND METHODS

Study participants

The study included 36 male, well-trained soccer players with an average age of 21.3 ± 1.8 years (Table 1). The selected group of athletes was homogeneous with strictly defined criteria for research – age (19-23 years), anthropometric indices (BM, %TBW, ECW, BF), and chosen variables of aerobic and anaerobic capacity (VO_{2max} , P_{max}). All players had valid medical examinations and showed no contraindications to participate in the experiment.

The athletes ($n = 36$) were randomly assigned to 3 groups – two experimental groups (HM; $n = 12$), (LM; $n = 12$) and a control group (CON; $n = 12$). All participants were submitted to different hydration protocols, ingesting water with specific biochemical properties for 7 days. Group I ingested highly mineralized water (Table 2), group II drank low mineralized high alkaline water, while the control group hydrated with table water. The volume of water intake was individualized based on the recommendation of the National Athletic Trainers Association and averaged 4.2 litres per day. All study

TABLE 1. Basic anthropometric characteristics of the experimental and control groups.

Variables	High mineral (n=12)	Low mineral (n=12)	Control (n=12)
AGE	21.0 ± 3.0	20.0 ± 3.0	21.0 ± 2.0
BM (kg)	69.9 ± 8.6	76.1 ± 6.8	71.5 ± 4.8
FFM (kg)	62.1 ± 7.2	67.8 ± 6.5	64.3 ± 4.6
FM (kg)	7.2 ± 2.3	8.4 ± 0.9	7.2 ± 2.7
FM (%)	10.2 ± 2.9	11.0 ± 1.3	9.8 ± 3.3
TBW (l)	45.6 ± 5.5	49.6 ± 4.3	47.1 ± 2.1
ICW (l)	28.8 ± 3.5	31.4 ± 2.8	29.9 ± 1.4
ECW (l)	16.8 ± 2.0	18.1 ± 1.6	17.2 ± 0.7

Note: Data are expressed as mean ± SD, $n=12$; BM (kg) - total body mass, FFM (kg) - fat free mass, FM (kg/%) - fat mass, TBW (l) - total body water, ICW (l) - intracellular water, ECW (l) - extracellular water

TABLE 2. Chemical properties of mineral water used in the study.

Variable	Measurement unit	High mineral	Low mineral	Control
pH	pH	6.1 ± 0.04	8.0	5.00 ± 0.08
CO ₂	mg/L	92.2 ± 6.2	11.23 ± 2.3	14.98 ± 0.66
HCO ₃ ⁻	mg/l	1326 ± 11.3	260 ± 6.14	3.62 ± 0.12
Cl ⁻	mg/l	8.4 ± 0.3	7.9 ± 1.3	0.41 ± 0.03
SO ₄ ²⁻	mg/l	28.7 ± 2.0	68.0 ± 3.6	1.60 ± 0.09
Na ⁺	mg/l	82.7 ± 6.2	8.24 ± 1.1	1.21 ± 0.05
K ⁺	mg/l	7.41 ± 0.05	1.83 ± 0.5	0.30 ± 0.03
Ca ⁺⁺	mg/l	177 ± 5.2	89.6 ± 4.6	1.21 ± 0.05
Mg ⁺⁺	mg/l	151 ± 4.1	11.4 ± 2.7	0.40 ± 0.04

Note: Data show the mean ± SD of three analyses for each water; pH - potential of hydrogen, CO₂ - carbon dioxide, HCO₃⁻ - bicarbonate, Cl⁻ - chlorine, SO₄²⁻ - sulphates, Na⁺ - sodium, K⁺ - potassium, Ca⁺⁺ - calcium, Mg⁺⁺ - magnesium

participants abstained from intensive exercise 3 days prior to initial evaluations and testing.

The study participants consumed an isocaloric, mixed diet, both before and during the experiment (55% carbohydrates, 20% protein, 25% fat). The players participating in the experiment did not take any medications or ergogenic substances, 2 weeks before and during the study. In addition, all athletes taking part in the experiment adhered to the requirement of 8 hours of sleep daily, and refrained from consuming alcohol and supplements during the experiment.

The athletes were informed about the purpose and the procedure of the research and signed an informed consent form before participating in the study. They were also informed about the potential risks and benefits associated with participating in the research project. The study received the approval of the Bioethics Committee at the Academy of Physical Education in Katowice, Poland. The test protocol was presented in detail to each player during the interview. Each participant was informed about the possibility to withdraw from the research at any stage of the experiment.

Procedures

The experiment lasted 7 days. Three phases were distinguished – diagnostic, therapeutic and control. Subjects involved in the diagnostic stage were qualified based on previously described criteria.

The diagnostic and control stage involved the following tests and assays (Fig. 1):

- Determination of body mass and body composition based on the method of electrical impedance (BM, TBW, ECW, ICW).
- Determination of urine pH and its specific gravity.
- The measurement of body temperature.
- Interval test protocol with evaluation of acid-base balance and lactate concentration.

The following variables were assessed:

- At rest, before the stress test (t0) – TBW, ECW, ICW, body temperature, urine specific gravity, urine pH.
- Immediately after the stress test (t1) – body temperature.
- 5 minutes after the stress test (t2) – TBW, ECW, ICW.
- 30 minutes after the stress test (t3) – specific gravity of urine, urine pH.

The therapeutic hydration phase involved the daily intake of 4-4.5 l of a particular type of water. The athletes drank either alkaline water, highly mineralized water or base mineral water as a control. The duration of the hydration procedure was 7 days.

Laboratory tests

Two sets of laboratory analysis were performed in this study. Stress tests were carried out at the beginning and at the end of the experiment. All biochemical variables were determined within 24 h, in both the diagnostic and control phases. The research was carried out at the Human Performance Laboratory of the Academy of Physical Education in Katowice.

Body mass and body composition

The measurements of body mass and body composition were conducted in the morning between 8.00 and 9.00. The day before the measurements, the participants had the last meal at 20.00 and hydrated with 1.5 litres of water between 20.00 and 22.00. The subjects were informed about the need to standardize measurement conditions. They reported to the laboratory after an overnight fast, refraining from exercise for 24 h and not consuming alcohol or liquids containing caffeine and carbohydrates before the diagnostic measurements. Body mass and body composition were determined based on

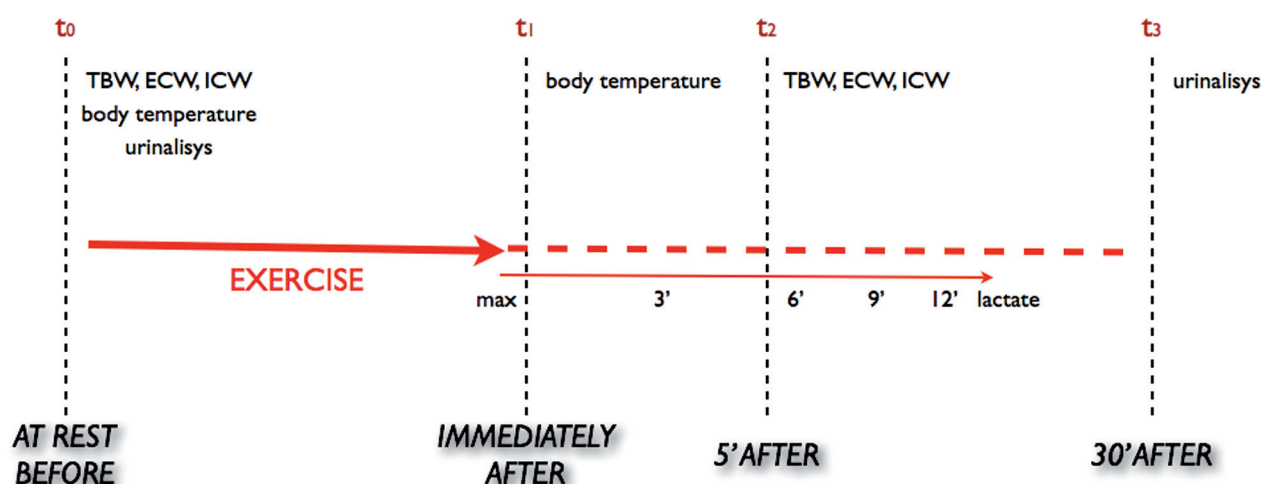


FIG. 1. Temporal structure of examinations.

the method of electrical impedance using the 370 InBody apparatus (InBody 370, USA). Body mass (BM), body fat (FAT), fat-free mass (FFM) and the compartmental and total body water (TBW, ICW, ECW) were determined, while BMI was calculated from BM and height. The t0 test was conducted with the above standards, while the t2 test was conducted following supervised hydration.

Body temperature

Body temperature was measured with a Braun thermometer (Thermo Scan Pro 6000, Germany) according to the timetable of the experiment.

Urine test

Urine samples were placed in a plastic container and mixed with 5 ml/L of a 5% solution of isopropyl alcohol and thymol to secure the properties. Material was stored at 5°C. Urine samples were assayed for the presence of blood and proteins. Specific gravity of urine was determined using the Atago Digital refractometer (Atago Digital, USA). Urine pH was determined based on the standardized Mettler Toledo potentiometer (Mettler Toledo, Germany).

Biochemical assays

To determine lactate concentration (LA) and acid-base equilibrium variables: pH, pCO₂, pO₂, serum bicarbonate (SB), base excess (BE), oxygen saturation (O₂SAT) and total bicarbonate (ctCO₂), capillary

blood samples were collected. Blood was taken from the fingertip in a volume of 1 ml. Determination of lactate was based on an enzymatic method using a commercial test from Boehringer Mannheim using the Shimadzu UV1201 spectrophotometer (Shimadzu UV 1201, Japan).

Stress tests

The exercise stress test included an interval test protocol, performed under control conditions (CT) and after hydration (HT). The exercise protocol consisted of 5 sets of 60 s exercise at an individualized load of 120% VO₂max and a cadence within 75-80 rpm. The rest interval between bouts of exercise equalled 60 s. Before the stress test, each athlete performed a 5-min warm-up with a resistance of 100 W and cadence within 70-80 rpm. After the general warm-up on the ergo-cycle, the athletes stretched the lower limbs, and proceeded with the stress test. To avoid the orthostatic effect, the participants were advised to rest for 3 min in the supine position. In order to determine the intensity of glycolysis, the concentration of lactate (LA) and acid-base equilibrium variables were determined. Finger-tip blood samples were drawn at rest, immediately after the last set of the stress test and in the 3rd, 6th, 9th and 12th minute of recovery. The obtained values were used to determine the rate of post-exercise lactate utilization.

The test was performed on an Excalibur Sport bicycle ergometer (Lode, Netherlands) with electromagnetic adjustable resistance of

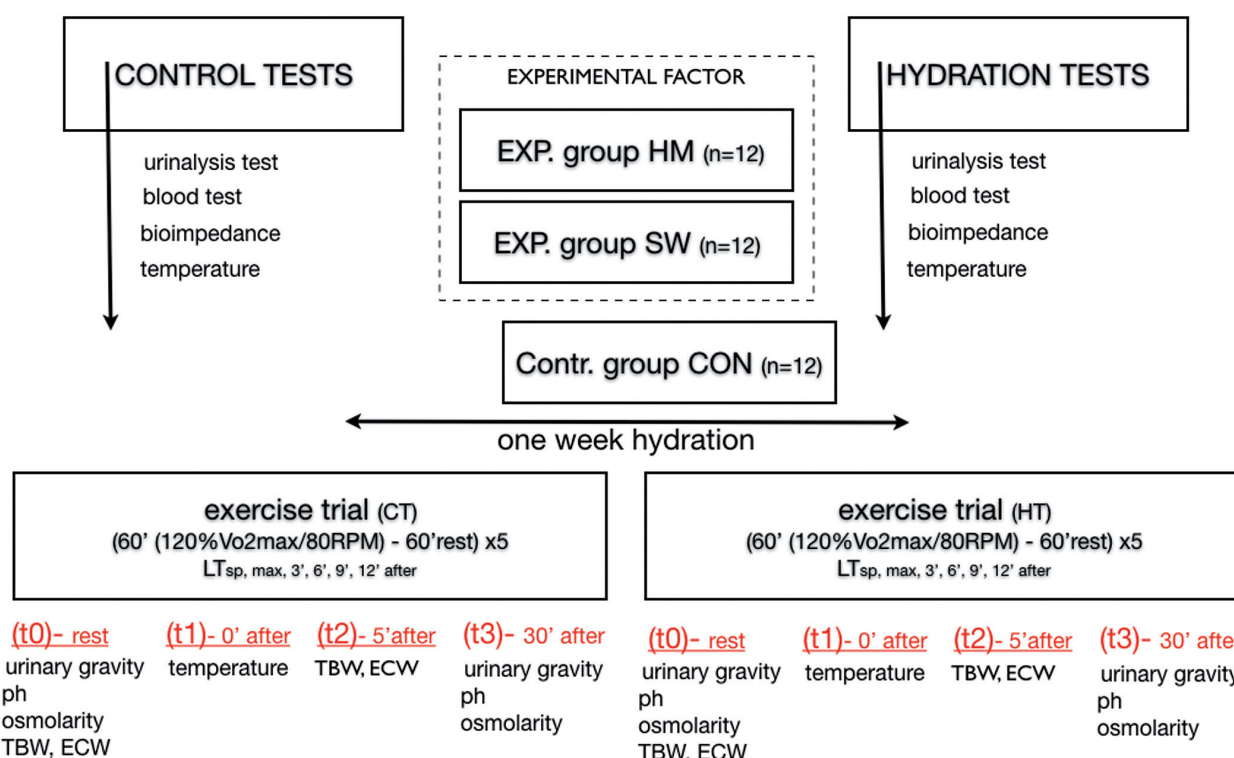


FIG. 2. Experimental procedure.

the flywheel. The generated power (W), cadence (RPM) and total work performed (J) were recorded with the Lode ergometer manager software.

The progressive test during which the 120% VO_{2max} load was determined was conducted a week before the start of the experiment. Each participant performed a ramp ergo-cycle test (T20x1) (20 W/1 min) with the work load increasing linearly (0.33 W per 1 s). The test evaluating VO_{2max} started with a resistance of 40 W and lasted until volitional exhaustion. The cadence was maintained with 60-65 RPM. Heart rate (HR), minute ventilation (VE), oxygen uptake (VO₂), expired carbon dioxide (CO₂), respiratory ratio (RER) and breath frequency (BF) were constantly monitored (Meta Lyzer 3B-2R, Cortex, Germany). Maximal oxygen uptake (VO_{2max}) and maximal work rate (Wr_{max} – W) were registered using the Lode Ergometry Manager (LEM Software, Germany).

Statistical analysis

Normality of distribution was verified using the Shapiro-Wilk test. All data are presented as mean ± SD. Verifications of the differences between analyzed variables and groups were carried out using ANOVA with repeated measures. Statistical significance was set at

TABLE 3. Changes in urine pH in the initial and hydration state before and after exercise.

TEST _{pre} HYDRATION	T ₀	T ₃
HM	6.8 ± 0.3	6.3 ± 0.7
LM	6.0 ± 0.8	5.7 ± 0.5
CON	6.4 ± 0.4	5.3 ± 0.4
TEST _{post} HYDRATION	T ₀	T ₃
HM	6.1 ± 1.0	6.3 ± 0.8
LM	6.0 ± 1.0	6.5 ± 0.5
CON	6.4 ± 1.1	6.3 ± 1.3

Note: Data are expressed as mean ± SD, n=12. HM - high mineral water, LM - low mineral water, CON - control group (table water).

p<0.05. All statistical analyses were performed using Statistica 9.1 with the neural network module, and Microsoft Office Excel 2010.

RESULTS

All participants completed the described testing protocol. The procedure was carried out in identical environmental conditions with the air temperature of 19.20 ± 0.34°C and humidity of 57.92 ± 0.46%.

The analysis of urine in the pre-hydration state, in accordance with the anticipated acute adaptation to anaerobic exercise, showed a decrease in pH and a reduction in specific gravity. Intergroup comparative analysis with respect to pH and the specific gravity of urine, carried out before and after exercise, showed no significant difference (HM vs. LM vs. Con.).

After the hydration procedure the athletes consuming alkaline water with specific mineralization (LM) showed significantly increased urine pH. In all groups of athletes, after exercise, the specific gravity of urine decreased (HM, LM, Con.). Intergroup analysis showed that the gradation of urine specific gravity was lowest in the LM group, consuming low mineralized water.

The total body water and its active transport (TBW / ICW / ECW) showed no significant differences in all investigated groups, at both the pre- and post-hydration state.

There were no changes in the value of LT_{max} in all groups (HM, LM and Con). The largest statistically significant differences related to the rate of lactate utilization ΔrestLT were observed after the stage of hydration. Lactate utilization was most efficient in the group of athletes using low mineralized water (LM).

DISCUSSION

Many studies have focused on the maintenance of proper hydration during prolonged aerobic exercise [1], whereas inadequate data have been presented regarding rehydration procedures and benefits during short-term anaerobic exercise. During high-intensity exercise water loss is minimal and other aspects of recovery should be taken into account.

TABLE 4. Exercise-induced changes in the concentration of lactate in the pre-hydration and post-hydration state

pre HYDRATION	rest	max	3'	6'	9'	12'	Δ	Δ res
HM	1.20±0.33	10.10±1.96	8.60±2.01	7.52±2.27	6.52±2.34	5.75±2.47	8.90±2.02	4.35±0.32
LM	1.38 ±0.36	8.94 ±0.95	7.90 ±1.18	7.17 ±0.91	5.59 ±0.94	4.83 ±0.80	7.56 ±1.08	4.11 ±0.12
Con.	1.19 ±0.13	10.80 ±1.08	9.31 ±1.29	7.90 ±1.38	7.14 ±1.01	6.28 ±1.24	9.60 ±1.18	4.52 ± 1.12
post HYDRATION	rest	max	3'	6'	9'	12'	Δ	Δ res
HM	1.25 ±0.31	10.09 ±1.52	9.00 ±1.89	7.76 ±1.63	6.49 ±2.06	5.69 ±2.10	8.85 ±1.45	4.40 ±0.34
LM	1.12±0.41	9.34±0.93	6.43±1.01	5.57±1.04	4.23±1.06	3.51±0.90	8.22±0.81	5.83±0.25
Con.	1.34±0.45	9.74±1.18	9.07±1.43	8.26±1.40	7.01±1.33	6.33±1.26	8.40±1.17	3.41±0.18

Note: Data are expressed as mean ± SD, n=12. HM - high mineral water, LM - low mineral water, CON - control group (table water).

Fluid-electrolyte balance and hydration affect the mental and physical state of athletes. Hydration can have a significant influence on both aerobic and anaerobic performance [1]. The results of our study indicate that the use of water with alkalizing properties exhibits a significant potential for hydration. It reduces fluid-electrolyte disturbances and accelerates the rate of lactate utilization following intensive anaerobic interval exercises.

Skeletal muscle fatigue is caused by numerous mechanisms, including accumulation of metabolites, such as potassium, or H^+ [20]. To a large extent, the structural damage to the myocytes and inflammation depend on the exercise ROS production [22].

Analysis of urine osmolality, specific urine gravity and colour may suggest the state of hydration [23]. Consumption of alkaline water following a dehydrating bout of cycling exercise has previously been shown to rehydrate cyclists faster and more completely than the consumption of placebo water. Following the consumption of alkaline water, the cyclists demonstrated lower total urine output, their urine was more concentrated (higher specific gravity), and total blood protein concentration was lower, all of which are expected observations for improved hydration status [24]. Heil [6] reported that water retention at the end of a 3-hour recovery period was $79.2 \pm 3.9\%$ when subjects drank alkaline water versus $62.5 \pm 5.4\%$ when drinking a placebo ($p < 0.05$). Thus, the present study has shown that the habitual consumption of mineralized bottled water can actually improve indicators of hydration status. The test procedures included in the study determined urine specific gravity and urine pH before and after exercise, in the pre- and post-hydration state. In both pre-hydration and the post-hydration states, the urine specific gravity decreased. However, in the LM group after hydration the changes were more significant (HM 1015 ± 3.8 g/L vs LM 1008 ± 4.2 g/L, LM 1008 ± 4.2 g/L vs Con. 1014 ± 4.1 ; $p < 0.001$).

The specific gravity of urine is dependent on the amount and weight of the solutes, including electrolytes. Improved water absorption causes a lower concentration of soluble particles and suggests stronger water retention, as observed in the LM group.

Simultaneously, we recorded an exercise-induced increase in urinary pH. This change could be the result of drinking large amounts of alkaline water with aforementioned mineralization properties. Consumption of alkaline water in the present study was associated with an increase in urine pH, while the dietary composition remained stable. Previous research by Welch *et al.* [25] demonstrated that urinary pH from 24-hour collection samples could function as an effective surrogate marker for changes in acid-base balance when evaluating differences in dietary intake.

A study conducted by Konig *et al.* [11] suggested that drinking water rich in minerals causes an increase in urine pH (5.94 to 6.57). Numerous experiments confirm the benefits of alkalizing additives in water. Heil [26] described the effects of highly alkalized water on the state of hydration and improved acid-base balance. Similarly, Berardi *et al.* [27] reported that urinary pH increased from 6.07 to

6.21 and 6.27 following one and two weeks of ingestion, respectively, of a plant-based supplement. The observations from these studies [6, 11] are consistent with the changes in urine pH (6.00 to 6.51) observed by the present study for group 2. Our study confirms the possible effect on hydration and accelerated recovery. Group 2 hydrating with alkaline water had much more efficient lactate utilization following the high-intensity interval training protocol (5.83 ± 0.25 $p < 0.05$). This result can be attributed to the specific properties of the alkaline water used for hydration in this group of athletes.

There are a few well-recognized activating factors at the cellular level: ATP, inorganic phosphate and H^+ ions. Skeletal muscle has a large capacity for ammonia production, which is usually revealed by its high blood accumulation during exercise above $60 \text{ VO}_{2\text{max}}$. It is clear that an increase in lactate production mirrors recruitment and activity of type II muscle fibres, and this process starts at exercise intensity reaching the anaerobic threshold. Rapid adenosine triphosphate hydrolysis during high-intensity exercise builds up adenosine di-phosphate and adenosine monophosphate (AMP). In the further metabolic degeneration cascade known as the purine nucleotide cycle, AMP is deaminated into inosinomono-phosphate, with the parallel formation of ammonia (NH_3). Because ammonia is correlated with the number of fast switch muscle fibres, an increase in lactate and efficacy of oxidative metabolism, this may suggest that ammonia might be an important player in modulation of central fatigue [28].

Dehydration in athletes may also lead to fatigue, poor performance, decreased coordination, and muscle cramping. Although further studies are absolutely warranted, drinking highly alkaline water seems an effective fluid hydration strategy for high-intensity interval training.

CONCLUSIONS

Drinking alkaline water in amounts of 4.0 l per day shows a positive effect on hydration status after anaerobic exercise with a significant decrease of specific urine gravity.

Intake of alkaline water also shows a positive effect on urine pH during the anaerobic test protocol, and much more efficient lactate utilization after the high-intensity interval exercise.

The consumption of alkaline water was associated with improved acid-base balance and hydration status. In contrast, subjects who consumed table water showed no changes over the same period of time. These results indicate that the habitual consumption of alkaline water may be a valuable nutritional vector influencing both acid-base balance and hydration status in active healthy adults.

These preliminary data demonstrated that consumption of alkaline water can improve anaerobic performance and post-exercise recovery.

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