© 2017 EDIZIONI MINERVA MEDICA
Online version at http://www.minervamedica.it

The Journal of Sports Medicine and Physical Fitness 2018 May;58(5):612-21
DOI: 10.23736/S0022-4707.17.06883-9

ORIGINAL ARTICLE
EXERCISE PHYSIOLOGY AND BIOMECHANICS

Effects of hydrogen rich water on prolonged intermittent exercise

Alessandro DA PONTE [1, 2] *, Nicola GIOVANELLI [1, 3], Daniele NIGRIS [4], Stefano LAZZER [1, 3]

[1]Department of Medical and Biological Sciences, University of Udine, Udine, Italy; [2]School of Sports Medicine, University of Udine, Udine, Italy; [3]School of Sport Sciences, University of Udine, Udine, Italy; [4]Department of Laboratory Medicine, University of Udine, Udine, Italy

*Corresponding author: Alessandro Da Ponte, Department of Medical and Biological Sciences School of Sport Sciences, University of Udine, P.le Kolbe 4, 33100 Udine, Italy. E-mail: dott.daponte@gmail.com

ABSTRACT

BACKGROUND: Recent studies showed a positive effect of hydrogen rich water (HRW) intake on acid-base homeostasis at rest. We investigated 2-weeks of HRW intake on repeated sprint performance and acid-base status during prolonged intermittent cycling exercise.
METHODS: In a cross over single-blind protocol, 8 trained male cyclists (age [mean±SD] 41±7 years, body mass 72.3±4.4 kg, height 1.77±0.04 m, maximal oxygen uptake [$\dot{V}O_{2max}$] 52.6±4.4 $mL\cdot kg^{-1}\cdot min^{-1}$) were provided daily with 2 liters of placebo normal water (PLA, pH 7.6, oxidation/reduction potential [ORP] +230 mV, free hydrogen content 0 ppb) or HRW (pH 9.8, ORP -180 mV, free Hydrogen 450 ppb). Tests were performed at baseline and after each period of 2 weeks of treatment. The treatments were counter-balanced and the sequence randomized. The 30-minute intermittent cycling trial consisted in 10 3-minute blocks, each one composed by 90 seconds at 40% $\dot{V}O_{2max}$, 60 seconds at 60% $\dot{V}O_{2max}$, 16 seconds all out sprint, and 14 seconds active recovery. Oxygen uptake ($\dot{V}O_2$), heart rate and power output were measured during the whole test, while mean and peak power output (PPO), time to peak power and Fatigue Index (FI) were determined during all the 16 seconds sprints. Lactate, pH and bicarbonate (HCO_3^-) concentrations were determined at rest and after each sprint on blood obtained by an antecubital vein indwelling catheter.
RESULTS: In the PLA group, PPO in absolute values decreased significantly at the 8th and 9th of 10 sprints and in relative values, ΔPPO, decreased significantly at 6th, 8th and 9th of 10 sprints (by mean: -12±5%, $P<0.006$), while it remained unchanged in HRW group. Mean power, FI, time to peak power and total work showed no differences between groups. In both conditions lactate levels increased while pH and HCO_3^- decreased progressively as a function of the number of sprints.
CONCLUSIONS: Two weeks of HRW intake may help to maintain PPO in repetitive sprints to exhaustion over 30 minutes.

(*Cite this article as:* Da Ponte A, Giovanelli N, Nigris D, Lazzer S. Effects of hydrogen rich water on prolonged intermittent exercise. J Sports Med Phys Fitness 2018;58:612-21. DOI: 10.23736/S0022-4707.17.06883-9)

Key words: Acidosis - Hydrogen-ion concentration - Bicycling.

It is common practice for athletes to take supplements;[1] typically sodium bicarbonate is ingested between 1 and 3 hours before competition to increase the body's alkaline reserve and to enhance the anaerobic performance. While the first studies on this topic started in 1930s,[2] it was in 1980s that a more scientific approach hinted at a positive effect of buffer "substances" on athletic performance, in particular for track and field 400 and 800 meters.[3, 4] To date, many studies have investigated the influence of buffer substances on various types of exercise. Authors reported positive or no effects [5, 6] in many sports: cycling,[7-10] running,[11, 12] swimming,[13, 14] rowing,[15, 16] basket,[17] boxing,[18] cross country skiing,[19] tennis [20] and rugby.[21] It has been suggested that the ideal amount of buffer to be ingested is commonly 300 $mg\cdot kg^{-1}$ body weight (BW) about 60 minutes before exercise for bicarbonate and ammonium chloride and 500 $mg\cdot kg^{-1}$ BW for citrate, the latter recommended to be ingested about 2 hours before exercise. However, bicarbonate ingestion, which is the most common buffer used, is often associated with gastrointestinal discomfort.[22] Moreover, an important factor to consider is that 27% of sodium bicarbonate is sodium: since the "ergogenic" acute bicarbonate load is about 21 grams for an adult subject, the amount of sodium ingested (in this case almost 6 grams) by far exceeds the maximum recommended intake of 2.3 grams per day.[22]

Recently, devices that can produce hydrogen rich water (HRW) with a pH higher than normal water have become commercially available. Theoretically, consumption of HRW could replace ingestion of bicarbonate to influence the body's alkaline reserve and exercise performance. Animal studies suggest that HRW may act as a neutralizing agent and scavenger of active oxygen species.[23-25] Furthermore, recent basic and clinical research revealed that hydrogen is an important physiological regulatory factor with antioxidant, anti-inflammatory and antiapoptotic protective effects on cells and organs.[26, 27] Moreover, hydrogen may also have positive effects in limiting metabolic acidosis.[28] However, to our knowledge, only a few studies have analyzed the effects of hydrogen rich and/or alkaline water intake in humans [29-32] and no one of these have measured its effect on physical performance. Brancaccio *et al.*[33] studied calcium bicarbonate rich water (with pH 6.1) supplementation on intermittent exercise (5x60 seconds at 85% max power with 60 seconds rest between cycling sprint) and showed a positive effect on pH. Other authors [34] found that hydrogen rich water was associated with lower torque decrease and decreased lactate level in prolonged isokinetic exercise. Further, a preliminary pre-post design study without placebo [35] showed a significant pH increase after one week of HRW (obtained from tap water adding magnesium) intake in healthy men both at rest or after exercise. Also, Ostojic & Stojanovic [36] confirmed a significant pH and HCO_3^- increase at rest and post exercise after two weeks of HRW intake compared to control.

On these premises, the primary aim of our study was to assess whether two weeks of HRW ingestion could influence exercise performance. A secondary aim of the study was to assess the body's alkaline reserve. We hypothesized that ingestion of HRW could improve sprint performance during prolonged intermittent cycling and could reduce exercise-related metabolic acidosis, delaying fatigue onset. We reproduced the study of Price *et al.*[37] where the authors studied the effects of bicarbonate ingestion on prolonged intermittent exercise, with the exception that in our research the buffering bicarbonate pre-exercise load was replaced with two weeks of HRW intake.

Materials and methods

Participants

Eight well-trained, healthy male cyclist (mean±SD: age 41±7 years, Body Mass Index [BMI] 22.6±1.1 kg·m^{-2}, I) participated in this study. The experimental protocol was approved by the Ethics Committee of the University of Udine. Before starting the research, the purpose and objectives of the study were carefully explained to each subject and written informed consent was obtained from all of them. The participants were recruited among experienced cyclists who filled out questionnaires on physical exercise activity, demographics, medical history and lifestyle.[38] They reported aerobic exercise 5 times a week for a mean of 8.8±3.8 hours·week^{-1} (range: 6-14 hours·week^{-1}). The subjects were classified of performance level 2-3 according to De Pauw.[39]

Experimental protocol

The protocol consisted in evaluating ergometric, cardiorespiratory and hematological parameters during prolonged intermittent exercise performed in two different counter balanced trials: once during hydration with control water (PLA) and once during hydration with HRW.

The crossover study consisted in three sessions. During the first session, after a familiarization with all the equipment, participants completed a graded cycling exercise test to exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}) and maximal power output (P_{max}, Watt).

Then, the participants were randomized in two groups of 4 and 4 people and provided daily with water for 4 weeks. The athletes were unaware if they were consuming PLA or HRW while investigators were aware of the study phase (single blind trial). The first group (4 athletes) received PLA for the first two weeks and HRW for the following two weeks, while the second (4 athletes) group received the opposite treatment without wash out after HRW period. Each athlete was daily provided by a researcher with 2 liters of water in anonymous bottles and instructed to keep it refrigerated in the dark at 4 °C. Moreover, athletes were required to minimize any other beverage consumption (alcohol, fruit juice etc.).

The HRW (pH 9.8, free hydrogen content 450 ppb) was obtained using a "Miracle max" water ionizer (Chanson Water Company Taiwan, provided by NERO' H_2O, Chanson Water Italy). This device, starting from normal tap water, filters and then modifies water pH through electrolytic process involving medical titanium plates coated with noble metal like platinum. This process has a long history in the chemical industry and is considered a reliable industrial process validated and approved worldwide. Our device was ISO 9001 certified and in compliance with CE regulations.

During the second and third visit to the laboratory, after two weeks of PLA and after two week of HRW hydration according to randomization, each subject undertook 30 minutes of intermittent cycle exercise (Figure 1) following the protocol suggested by Price *et al.*[37] All

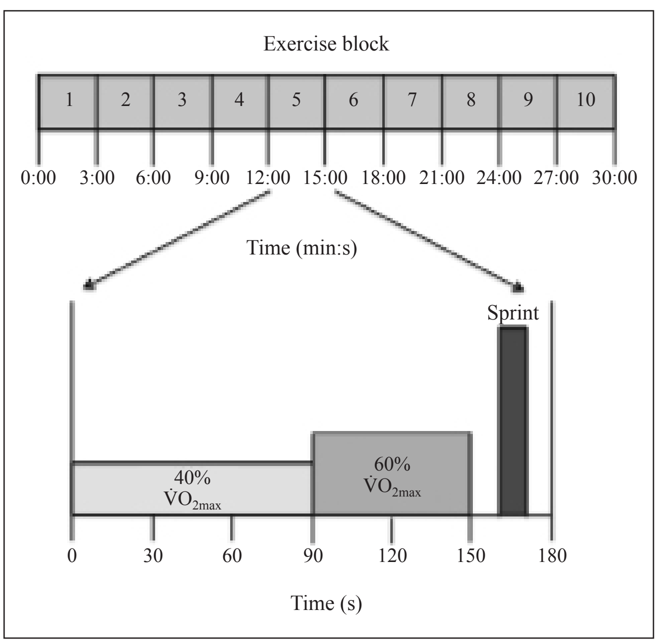

Figure 1.—Intermittent protocol representation.
Each exercise block was 3 min in duration (top section) with each 3-min block comprising 90 seconds at 40% $\dot{V}O_{2max}$, 60 sec at 60% $\dot{V}O_{2max}$, 16 seconds maximal sprint, and 14 sec active rest (bottom section). Adapted from Price *et al.*[37]

tests were performed at the same time in the morning without changing breakfast habits and after two days of easy training.

During the period of the study athletes did not change their training habits (expressed as training volume and intensity). Moreover, each athlete filled a training diary reporting the daily training load during the study. For macro and micronutrients analysis each participant recorded the consumed meals for a week, and oxygen radical absorbance capacity (ORAC) and potential renal acid load (PRAL) were calculated using Metadieta software (Meteda, San Benedetto del Tronto, Ascoli Piceno, Italy).

Physiological measurements

Anthropometric characteristics and body composition.

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany), stature was measured to the nearest 0.001 m on a standardized wall-mounted height board. BMI was calculated as BM (kg) · $stature^{-2}$ (m). Body composition was measured by bioelectrical impedance (BIA 101, Akern, Florence, Italy) according to the method of Lukaski *et al.*[40] Body composition including fat-free mass (FFM), fat mass (FM) and total body water (TBW) was obtained from the software provided by the manufacturer.

Water characteristics

PLA and HRW were measured with "Ph 55" Martini Instruments pH-meter and "Redox MW500" Wilwakee ORP-meter, while for free hydrogen measure was used an ENH-1000 (Trastlex, Japan).

Graded exercise test to exhaustion

Maximal oxygen uptake ($\dot{V}O_{2max}$) and maximal heart rate (HR_{max}) were determined by a graded exercise test on an SRM cycle ergometer (SRM, Germany) under medical supervision. During the experiment, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Pavona di Albano, Rome, Italy). The volume and gas analyzers were calibrated using a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2), respectively. During the tests, HR was measured with a dedicated device (Polar, Oulu, Finland). The tests were performed one week before the beginning of protocol study and comprised a 5-minute rest period followed by cycling at 2W·kg^{-1}BM for 5 minutes; the power was then increased by 25 W every minute until volitional exhaustion. Subjects were required to maintain a cadence of 90 rpm throughout the test. A leveling off of oxygen uptake (defined as an increase of no more than 1 mL·kg^{-1}·min^{-1}) was observed in all subjects during the last one or two minutes of the exercise test indicating that $\dot{V}O_{2max}$ had been attained. $\dot{V}O_{2max}$ and HR_{max} were calculated as the average oxygen uptake and HR of the last 20 sec of the test. Respiratory Exchange Ratio (RER) was calculated as $\dot{V}CO_2 \cdot \dot{V}O_2^{-1}$.

Intermittent protocol

Intermittent protocol consisted of 30 min exercise (Figure 1): each 3-minute block consisted of 90 sec at power (W) corresponding to 40% $\dot{V}O_{2max}$, 60 sec at power (W) corresponding 60% $\dot{V}O_{2max}$, 16 seconds maximal sprint, and 14 secomds active recovery as proposed by Price *et al.*[37]

The maximal sprint was undertaken using a computer controlled cycle ergometer (SRM, Jülich, Germany), with continuous power measurement during the 4 phases of each block. Athletes were required to maintain a cadence of 90 rpm throughout the 30-minute protocol except for the maximal sprint in which subjects were encouraged to sprint as fast as possible in isokinetic modality at 100 rpm. For each sprint, peak power output (PPO), time to peak power output, minimum power output (MinPO) and mean power output (MPO) were recorded. Average peak power, total work (W) and fatigue index (FI; %) were calculated for each sprint; FI was determined from the difference between PPO and MinPO, expressed as a percentage of PPO for that sprint. To overcome individual variation in sprint performance, PPO data for each subject was also expressed relative to the initial sprint (PPOrel).

During the whole intermittent exercise, expired air was measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy) and ratings of perceived exertion (RPE, 10 point Borg Scale) was recorded after each sprint.[41]

Blood sampling

An indwelling catheter (BD Venflon Pro Safety 21G, Becton Dickinson Italy) was inserted in an antecubital vein of subjects before beginning sprint test. The first blood sample was taken with tourniquet before exercise with subjects in a supine position while the following ten samples were taken without tourniquet with subjects sitting on the cycle ergometer. Each 1 mL venous sample, collected with a heparin containing syringe, was kept refrigerated at 4 °C and analyzed within one hour using an ABL 700 analyzer (Radiometer Medical ApS, Copenaghen, Denmark).

Statistical analysis

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $P<0.05$. All results are expressed as mean±SD or mean±95 % confidence intervals when changes or percent changes are presented. Normal distribution of the data was tested using the Kolmogorov-Smirnov Test. Sphericity (homogeneity of covariance) was verified by the Mauchly's Test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse-Geisser procedure. Changes of all tested variables were studied with General Linear Model repeated measures with two factors considering group (G, PLA *vs.* HRW) and time (T), and the interaction (group x time). Multiple comparisons between "Time" within each group were achieved using the paired *t*-test with Bonferroni correction for multiple comparisons.

Results

Characteristics of subjects

The anthropometric characteristics of the 8 athletes who participated the study are reported in Table I. Their average $\dot{V}O_{2max}$ and maximal power reached during the incremental test were 52.6±4.4 mL·min^{-1}·kg^{-1} and 380±41 W, respectively.

The athletes' percentage of body water was not significantly different during the two trials (61.8±4.4% and 62.1±3.4% of BM during PLA and HRW hydration, respectively). Regarding dietary habits, there was no correlation between either ORAC or PRAL and blood

TABLE I.—*Physical characteristics of the subjects (N.=8).*

Age (year)	41±7	[29-51]
Body mass (kg)	72.3±4.4	[66-80]
Stature (m)	1.77±0.04	[1.73-1.87]
Body mass index ($kg\cdot m^{-2}$)	22.6±1.1	[21-24]
Fat mass (%)	14.6±5.1	[9-21]
$\dot{V}O_{2max}$ ($mL\cdot min^{-1}\cdot kg^{-1}$)	52.6±4.4	[48-60]
HR_{max} (bpm)	176±11	[151-187]
P_{max} (W)	380±41	[320-460]

All values are mean±standard deviation. Range in square brackets.
$\dot{V}O_{2max}$: maximal oxygen uptake; HR: heart rate; P_{max}: maximal power attained during the incremental test.

acid-base status at rest and during exercise, as no correlation was seen with respect to power output. ORAC values were positive (7743±4681 Trolox equivalents, μmol/100g) in all athletes while mean PRAL was negative (-6.4±14.0 mEq/d) but with great interpersonal variability.

Water characteristics

HRW pH produced by ionizer was quite stable during the first 24 hours decreasing from 9.8 to 9.2, while Oxidation-reduction potential (ORP) deteriorated (from -180 mV to +100 mV) as free hydrogen (from 450 to 150 ppb), even keeping water in the dark at 4°C as recommended by ionizer producer. As shown in Table II no differences were recorded in PLA characteristics during 24 hours storage.

Oxygen consumption, RER, heart rate and ratings of perceived exertion changes

No significant differences between trials were observed regarding mean oxygen consumption either considering the whole 30-minute trials or only the sprint phases. For the whole 30-minute trials mean $\dot{V}O_2$ was 2.54±0.19 $L\cdot min^{-1}$ and 2.56±0.25 $L\cdot min^{-1}$ for PLA and HRW condition respectively. These intensities corresponded to 66±4% of $\dot{V}O_{2\ max}$ for both trials. Analysing only the sprint phases, $\dot{V}O_2$ was 2.98±0.21 and 2.95±0.25 $L\cdot min^{-1}$ for PLA and HRW condition respectively, equivalent to 77±5% of $\dot{V}O_{2max}$.

TABLE II.—*Water quality characteristics.*

	Control water PLA	Hydrogen rich water HRW	
pH (at 24 h)	7.6 (7.6)	9.8 (9.2)	P<0.001
ORP mV (at 24 h)	230 (228)	-180 (100)	P<0.001
Free Hydrogen ppb (at 24 h)	0 (0)	450 (150)	P<0.001
TDS mg/L	180	180	

All values are mean±standard deviation. Range in square brackets.
ORP: oxidation reduction potential in millivolts; Free Hydrogen in parts per billion; TDS: total dissolved solids.

A GLM repeated measures multivariate test shows significant differences for Time (P<0.001) for RER, HR and RPE, while not significant differences in "time x group" interaction. Particularly, before the exercise RER was not different between trials (0.78±0.07 and 0.75±0.04, for the PLA and HRW condition, respectively, Table III). It increased (P<0.004) to 1.24±0.07 and 1.24±0.09 at the 3rd minute of exercise, for the PLA and HRW condition, respectively, although not differences were observed between groups. After the 3rd minute of exercise RER decreased progressively till the 21st minute and then remained constant until the end of the test protocol (1.06±0.05 and 1.06±0.07, for the PLA and HRW respectively, Table III). Heart rates were not significantly different between groups (Table III). After the 1st sprint heart rates increased (P<0.001) to 152±16 and 149±9 and then to 164±13 and 162±9 beats·min^{-1} after 10th sprint, respectively.

Rating of perceived exertion (Figure 2, Table III) increased significantly (P<0.003) compared with the 1st

TABLE III.—*Metabolic, ergometric and blood results of General Linear Model repeated measures with two factors considering group (G, PLA* vs. *HRW) and time (T), and the interaction (group x time).*

	P Group effect	P Time effect	P Group x time
Respiratory Exchange Ratio	0.425	0.004	0.125
Heart rate (bpm)	0.635	0.001	0.352
Rating of perceived exertion	0.534	0.003	0.267
Ergometric parameter			
Peak power output (W)	0.944	0.001	0.106
Changes in Peak power output (%)	0.158	0.001	0.101
Mean power (W)	0.864	0.001	0.144
Time to peak power (s)	0.432	0.012	0.334
Fatigue index (%)	0.655	0.007	0.372
Blood parameters			
pH	0.386	0.001	0.341
Bicarbonate [HCO_3^-] ($mml\cdot L^{-1}$)	0.226	0.001	0.363
Base excess ($mEq\cdot L^{-1}$)	0.123	0.001	0.880
Lactate ($mml\cdot L^{-1}$)	0.184	0.001	0.611
pO_2 (mmHg)	0.642	0.001	0.258
pCO_2 (mmHg)	0.716	0.001	0.921
Haemoglobin Sat (%)	0.740	0.001	0.479
Haemoglobin ($g\cdot dL^{-1}$)	0.588	0.001	0.738
Glucose ($mg\cdot dL^{-1}$)	0.371	0.001	0.860

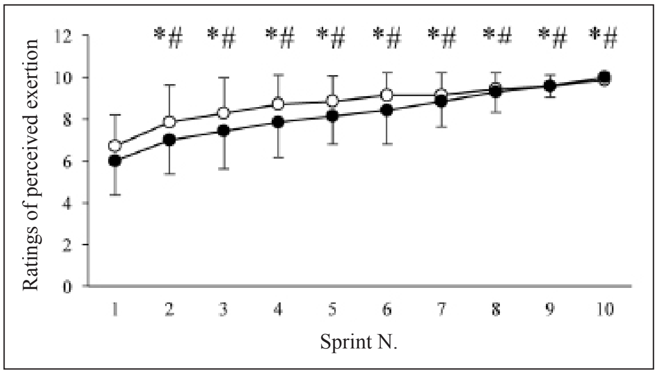

Figure 2.—Ratings of perceived exertion (RPE) values during the intermittent exercise in the PLA (O) and HRW (●) groups.
All values are mean±standard deviation;
*P<0.006 significantly different from 1st sprint in the PLA group; #P<0.006 significantly different from 1st sprint in the HRW group.

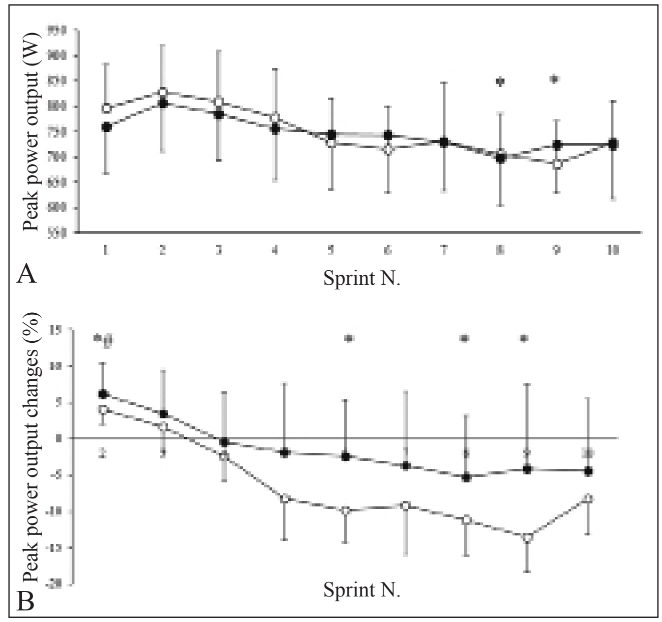

Figure 3.—Peak power output (A: PPO, W) and PPO changes (B: ΔPPO, %) during the intermittent exercise in the PLA (O) and HRW (●) groups PPO values are mean±standard deviation; ΔPPO values are mean±confidence interval.
*P<0.006 significantly different from 1st sprint in the PLA group; #P<0.006 significantly different from 1st sprint in the HRW group.

sprint in both trials but there were not significantly differences between PLA and HRW hydration.

Ergometric parameter

PPO reached maximum power at the 2nd sprint in both groups, decreasing in the following sprints (Figure 3A, Table III). In particular, PPO decreased significantly at the 8th and 9th sprint ($P<0.006$) in the PLA group, while did not change significantly in the HRW group.

Relative changes in PPO decrease significantly ($P<0.006$) at 6th, 8th and 9th sprint in PLA group only (Figure 3B, Table III). Regarding absolute PPO and ΔPPO, no significant differences were observed between groups during the entire exercise (Table III). Mean power, time to peak power and fatigue index showed no differences between PLA and HRW group (Table III). Finally, average peak power and total work over the 10 sprints showed no significant differences between PLA and HRW group.

Blood parameters

Resting pH was similar in both trials (7.33±0.03 *vs.* 7.33±0.04, for PLA and HRW respectively, Figure 4A, Table III). The main pH decrease ($P<0.006$) occurred during the first three sprints and then remained quite stable during the rest of the trials, reaching the value of 7.18±0.05 and 7.20±0.05 respectively for PLA and HRW group and the end of exercise. Similarly, HCO_3^- values (Figure 4B, Table III) at rest were not significantly different between groups and then decreased significantly ($P<0.006$) in the first nine minutes (three sprint) in both groups reaching lowest value at the 10th sprint (12.9±2.0 *vs* 14.9±2.4 mmol·L^{-1}, in PLA and HRW group respectively). Base excess (BE, Figure 4C, Table III) presented similar values at rest whilst they changed (although not significantly) from the 1st sprint in favour of HRW group till the end of exercise. As well, values for lactate were not different at rest between groups (1.6±0.9 *vs.* 1.4±0.8 mmol·L^{-1} for PLA and HRW, respectively) (Figure 4D, Table III), whilst they increased significantly ($P<0.001$) from the 3rd sprint for both groups reaching maximal values at the end (16.7±2.9 *vs.* 14.5±2.8 mmol·L^{-1} for PLA and HRW, respectively).

Venous blood gas analysis showed similar values at rest and a similar pattern during the 30 min exercise in both trials: pO_2 (Figure 4E, Table III) decreased till the 2nd sprint and then increased progressively and from 8th sprint was significantly different ($P<0.001$) from 1st sprint. Regarding pCO_2 (Figure 4F, Table III), it increased till the 2nd sprint then decreased, and from 6th sprint was significantly lower ($P<0.001$) than after the

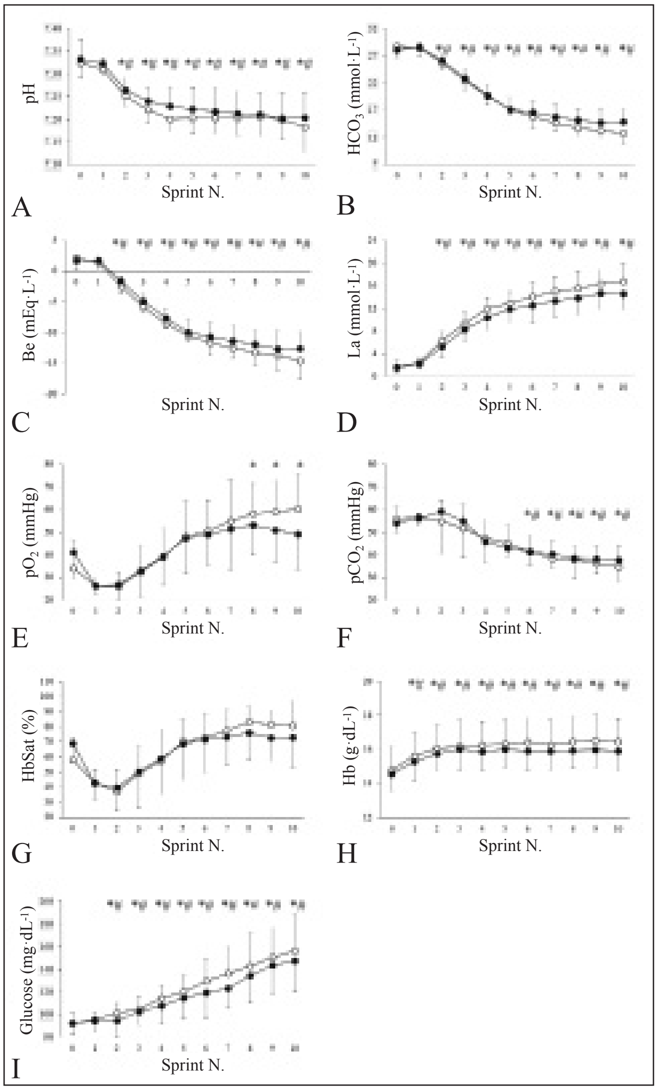

Figure 4.—pH (A), Lactate (B), HCO_3^- (C), PO_2 (D), BE (E), PCO_2 (F), HbSat (G), Hb (H), Glucose (I) values during the intermittent exercise in the PLA (O) and HRW (●) groups
All values are mean±standard deviation.
*P<0.006 significantly different from 1st sprint in the PLA group; #P<0.006 significantly different from 1st sprint in the HRW group.

1st sprint. Haemoglobin saturation (Figure 4G, Table III) followed a pattern like pO_2 with an initial reduction during the first two sprints and then a progressive increase. Hemoglobin concentration (Figure 4H, Table III), an indirect measurement of hydration, was not significantly different at rest between groups (14.9±1.0 and 14.7±1.4 g/dL for PLA and HRW, respectively), then increased significantly (P<0.006) from the 1st sprint to 16.4±1.1 and 16.0±1.3 g/dL at the end of exercise for PLA and HRW, respectively. No differences emerged from comparison between the two trials.

Glucose level (Figure 4I, Table III) increased (P<0.006) considerably from normal resting range (92±8.1 mg/dL for PLA water *vs.* 91±10.2 for HRW) toward higher level continuing the exercise. Even if values were lower during HRW trial they were not statistically different from PLA.

Discussion

The main result of this study, based on hypothesis that HRW intake could modify body acid-base status and anaerobic performance, is that two weeks of HRW assumption does not influence significantly acid-base balance even if it may delay fatigue onset during a prolonged intermittent cycling exercise.

One primary outcome was that peak power output values were similar between trials (no between group significance) but showed a significant decrease (P<0.006) after the 8th sprint in the PLA group while it did not change in HRW group. A secondary outcome was that the PPO expressed relative to the first sprint had a significant reduction in the PLA group from the 6th sprint onward while it did not decrease in the HRW group.

Based on our findings we reject our hypothesis that two weeks of HRW intake could influence rest and exercise acidic-base status. While we did observe a pattern for a higher pH and HCO_3^- and a reduction in lactate, no statistical differences were observed between groups.

Power results, on the other side, are partially consistent with the hypothesis of a possible influence on anaerobic performance. The apparent reduction in fatigue during HRW consumption is difficult to explain physiologically as it is also difficult to compare our findings to others using HRW, due to differences in protocol and HRW production. A previous study of Brancaccio *et al.* considered the effects of ionized water on human performance:[33] their athletes performed a modified Wingate test either after a week of controlled hydration with bicarbonate calcic mineral water or after a week of controlled hydration with minimally mineralized water. They measured higher urinary pH and lower urine specific gravity after hydration with bicarbonate calcic mineral water but did not report power and hematic parameters and for this reason it is difficult to compare our data. Our results are in agreement with another investigation [34] in which Aoki *et al.* studied the effects of HRW intake on elite athletes. Indeed, these authors showed lower torque decrease and lower lactate level in prolonged isokinetic exercise during HRW, even if the study design and exercise protocol were rather different from ours. When our study is compared with the works of Ostojic *et al.*[35, 36] there are some factors to consider. In all these studies authors used HRW obtained from tap water with magnesium supplementation and they reported data at rest and after exercise, but the study protocols were in both cases rather different from ours. The first research,[35] investigating the possible effects of one weeks of HRW intake, was a preliminary pre-post design study without a placebo, while the second study [36] was a double blind one regarding the effects of two weeks of HRW intake. Authors adopted endurance [35] or incremental [36] test and not a prolonged intermittent protocol, thus a less intensive effort (post exercise arterial blood pH was 7.39 and 7.46 respectively for pre and post HRW hydration) [36] and they do not provide data about lactate and power output or work. Our results are in contrast with these studies because we did not measure differences at rest; furthermore, during exercise pH and HCO_3^- could suggest possible reduced metabolic lactic acidosis but data were not statistically significant. With regard to others ergometric parameters, we recorded no differences in absolute PPO, mean power, time to peak power and FI that were similar in both trials, as previously reported.[37]

In our research, cardiopulmonary data during intermittent trials were not different between HRW and PLA group: due to the recorded pH and HCO_3^- values and the intrinsic instrumentation accuracy limits, we noticed differences neither regarding O_2 consume nor CO_2 production, RER, ventilation and heart rate. Therefore, our results suggest that the reduction in progressive power decrement during HRW trials is not due to different cardiopulmonary performance.

Regarding the other blood parameters investigated, we observed lower glucose level during this strenuous exercise during HRW trial. This observation is in accordance with Oopik *et al.*[42] who recorded lower glucose level 5 minutes after a 5-km running trial in athletes treated with sodium citrate. In contrast with this study,[42] in which lower glucose level were considered related to the performance improvement (and more energy consumed), the subjects' performances (and work) we analyzed were similar; furthermore, we investigated an intermittent and not constant exercise and recorded glucose level throughout the whole exercise and not only 5 minutes post.

In our study RPE was not correlated with pH and HCO_3^-, showing higher but not significantly different values for PLA compared with HRW in the first phase of exercise when there is the main pH decrease. Continuing the exercise, the intensity of effort was so high, proved also by the high lactate level reached, that we observed no differences between groups. According to previous studies [34, 36] no side effects were reported during HRW ingestion which was completely unrecognizable from normal water. This could be a major advantage in favor of HRW over bicarbonate consumption for trying to improve buffer capacity, reduce metabolic acidosis, delay fatigue effects and improve performance during intense anaerobic exercise without the reported side effects due bicarbonate ingestion.[22, 43, 44]

To our knowledge this is the first study exploring the effects of HRW produced by an ionizer device on prolonged intermittent exercise, while the few other studies [33-36] on this topic used naturally rich calcium water or normal water added with magnesium.

Limitations of the study

We acknowledge that our study has some limitations. One is that we had only one device for the production of HRW and all athletes were provided each morning with two liters of HRW or PLA. However, pH, ORP and hydrogen content changed significantly during the 24 hours conservation even if in ideal condition. This could be a pitfall because part of hydration was probably with water of quality poorer than expected and it was impossible to control every single athlete adherence to conservation protocol. The influence of this water quality deterioration, if any, could have been only against positive effects of HRW but this could in part explain the great variability among the participants regarding both blood and power parameters. Another limit of the study is that the investigators were not blind about the type of hydration because of logistic reasons. Also, in contrast to the majority of studies on exercise and acid-base status,[6, 45] our blood samples were venous and not capillary-arterial, adding a small but possibly significant different when our work is compared with others. A third limitation is that we have no data about the reactive oxygen and nitrogen species (ROS and RNS), making difficult to evaluate any antioxidant effect of HRW. Related to this issue, our data about ORAC show that all athletes had a healthy dietary habit with good value for antiossidative capacity against oxygen free radicals. Accordingly, the mean dietary PRAL was negative but with great variability among subjects and the lowest value, as expected, in a vegetarian athlete. Neither ORAC nor PRAL seem to influence acid-base status at rest and during exercise.

Regarding there was no wash out period between the two phases of water supplementation, this could be another limit even if it was not necessary between PLA and HRW period and, in the group receiving HRW in the first phase of the study, we hypothesized that the followings two weeks of PLA were actually a wash out.

We could test only few athletes and this limit of our research, a small sample size, could explain the low significance of many recorded results.

Finally, most studies on bicarbonate, especially those showing an ergogenic effect, evaluated the acute and not the chronic intake.[8, 12] Future studies should investigate whether acute ingestion of HRW is followed by blood pH alkalosis as occurs with acute bicarbonate load and measure the metabolic and ergogenic responses using only just treated, and not 24 hours stored, water. Moreover, further studies could consider measuring morning urine pH as marker of organism acid-base balance and using a standard HRW hydration during hours preceding tests.

Conclusions

Two weeks of hydrogen rich water intake may help to maintain PPO in repetitive sprints to exhaustion over 30 minutes. This research on HRW, produced by a dedicated device, suggests that HRW could substitute bicarbonate ingestion for improving performance in anaerobic lactic exercise with the advantages of no side effects, no sodium overload and of encouraging correct hydration. Since this is the first research on HRW and athletic performance to date, further studies in this area of interest are warranted.

References

1. Maughan RJ, Greenhaff PL, Hespel P. Dietary supplements for athletes: Emerging trends and recurring themes. J Sports Sci 2011;29(Suppl 1):S57-66.
2. Dennig H, Talbott JH, Edwards HT, Dill DB. Effect of acidosis and alkalosis upon capacity for work. J Clin Invest 1931;9:601-13.
3. Wilkes D, Gledhill N, Smyth R. Effect of acute induced metabolic alkalosis on 800-m racing time. Med Sci Sports Exerc 1983; 15:277-80.
4. Goldfinch J, McNaughton L, Davies P. Induced metabolic alkalosis and its effects on 400-m racing time. Eur J Appl Physiol Occup Physiol 1988;57:45-8.
5. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance: A meta-analysis. Sports Med 2011;41:801-14.
6. Mcnaughton LR, Siegler J, Midgley A. Ergogenic effects of sodium bicarbonate. Curr Sports Med Rep 2008;7:230-6.
7. Siegler JC, McNaughton LR, Midgley AW, Keatley S, Hillman A. Metabolic alkalosis, recovery and sprint performance. Int J Sports Med 2010;31:797-802.
8. Danaher J, Gerber T, Wellard RM, Stathis CG. The effect of beta-alanine and nahco3 co-ingestion on buffering capacity and exercise performance with high-intensity exercise in healthy males. Eur J Appl Physiol 2014;114:1715-24.
9. Vanhatalo A, McNaughton LR, Siegler J, Jones AM. Effect of induced alkalosis on the power-duration relationship of "all-out" exercise. Med Sci Sports Exerc 2010;42:563-70.
10. Zabala M, Peinado AB, Calderon FJ, Sampedro J, Castillo MJ, Benito PJ. Bicarbonate ingestion has no ergogenic effect on consecutive all out sprint tests in bmx elite cyclists. Eur J Appl Physiol 2011;111:3127-34.
11. Oopik V, Saaremets I, Medijainen L, Karelson K, Janson T, Timpmann S. Effects of sodium citrate ingestion before exercise on endurance performance in well trained college runners. Br J Sports Med 2003;37:485-9.
12. Ducker KJ, Dawson B, Wallman KE. Effect of beta alanine and sodium bicarbonate supplementation on repeated-sprint performance. J Strength Cond Res 2013;27:3450-60.
13. Mero AA, Hirvonen P, Saarela J, Hulmi JJ, Hoffman JR, Stout JR. Effect of sodium bicarbonate and beta-alanine supplementation on maximal sprint swimming. J Int Soc Sports Nutr 2013;10:52.
14. Joyce S, Minahan C, Anderson M, Osborne M. Acute and chronic loading of sodium bicarbonate in highly trained swimmers. Eur J Appl Physiol 2012;112:461-9.
15. Hobson RM, Harris RC, Martin D, Smith P, Macklin B, Elliott-Sale KJ, *et al.* Effect of sodium bicarbonate supplementation on 2000-m rowing performance. Int J Sports Physiol Perform 2014;9:139-44.
16. Christensen PM, Petersen MH, Friis SN, Bangsbo J. Caffeine, but not bicarbonate, improves 6 min maximal performance in elite rowers. Appl Physiol Nutr Metab 2014;39:1058-63.
17. Afman G, Garside RM, Dinan N, Gant N, Betts JA, Williams C. Effect of carbohydrate or sodium bicarbonate ingestion on performance during a validated basketball simulation test. Int J Sport Nutr Exerc Metab 2014;24:632-44.
18. Siegler JC, Hirscher K. Sodium bicarbonate ingestion and boxing performance. J Strength Cond Res 2010;24:103-8.
19. Heil DP, Jacobson EA, Howe SM. Influence of an alkalizing supplement on markers of endurance performance using a double-blind placebo-controlled design. J Int Soc Sports Nutr 2012;9:8.
20. Wu CL, Shih MC, Yang CC, Huang MH, Chang CK. Sodium bicarbonate supplementation prevents skilled tennis performance decline after a simulated match. J Int Soc Sports Nutr 2010;7:33.
21. Cameron SL, McLay-Cooke RT, Brown RC, Gray AR, Fairbairn KA. Increased blood ph but not performance with sodium bicarbonate supplementation in elite rugby union players. Int J Sport Nutr Exerc Metab 2010;20:307-21.
22. Kahle LE, Kelly PV, Eliot KA, Weiss EP. Acute sodium bicarbonate

loading has negligible effects on resting and exercise blood pressure but causes gastrointestinal distress. Nutr Res 2013;33:479-86.
23. Lee MY, Kim YK, Ryoo KK, Lee YB, Park EJ. Electrolyzed-reduced water protects against oxidative damage to DNA, rna, and protein. Appl Biochem Biotechnol 2006;135:133-44.
24. Luo ZL, Cheng L, Ren JD, Fang C, Xiang K, Xu HT, *et al.* Hydrogen-rich saline protects against ischemia/reperfusion injury in grafts after pancreas transplantations by reducing oxidative stress in rats. Mediators Inflamm 2015;2015:281985.
25. Guo SX, Jin YY, Fang Q, You CG, Wang XG, Hu XL, *et al.* Beneficial effects of hydrogen-rich saline on early burn-wound progression in rats. PLoS One 2015;10:e0124897.
26. Sun Q, Kang Z, Cai J, Liu W, Liu Y, Zhang JH, *et al.* Hydrogen-rich saline protects myocardium against ischemia/reperfusion injury in rats. Exp Biol Med (Maywood) 2009;234:1212-9.
27. Mao YF, Zheng XF, Cai JM, You XM, Deng XM, Zhang JH, *et al.* Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats. Biochem Biophys Res Commun 2009;381:602-5.
28. Abol-Enein H, Gheith OA, Barakat N, Nour E, Sharaf AE. Ionized alkaline water: New strategy for management of metabolic acidosis in experimental animals. Ther Apher Dial 2009;13:220-4.
29. Kang KM, Kang YN, Choi IB, Gu Y, Kawamura T, Toyoda Y, *et al.* Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors. Med Gas Res 2011;1:11.
30. Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. J Clin Biochem Nutr 2010;46:140-9.
31. Huang KC, Yang CC, Lee KT, Chien CT. Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. Kidney Int 2003;64:704-14.
32. Huang KC, Yang CC, Hsu SP, Lee KT, Liu HW, Morisawa S, *et al.* Electrolyzed-reduced water reduced hemodialysis-induced erythrocyte impairment in end-stage renal disease patients. Kidney Int 2006;70:391-8.
33. Brancaccio P, Limongelli FM, Paolillo I, D'Aponte A, Donnarumma V, Rastrelli L. Supplementation of Acqua Lete® (Bicarbonate Calcic Mineral Water) improves hydration status in athletes after short term anaerobic exercise. J Int Soc Sports Nutr 2012;9:35.
34. Aoki K, Nakao A, Adachi T, Matsui Y, Miyakawa S. Pilot study: effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes. Med Gas Res 2012;2:12.
35. Ostojic SM. Serum alkalinization and hydrogen-rich water in healthy men. Mayo Clin Proc 2012;87:501-2.
36. Ostojic SM, Stojanovic MD. Hydrogen-rich water affected blood alkalinity in physically active men. Res Sports Med 2014;22:49-60.
37. Price M, Moss P, Rance S. Effects of sodium bicarbonate ingestion on prolonged intermittent exercise. Med Sci Sports Exerc 2003;35:1303-8.
38. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, *et al.* International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381-95.
39. De Pauw K, Roelands B, Cheung SS, De Geus B, Rietjens G, Meeusen R. Guidelines to classify subject groups in sport-science research. Int J Sports Physiol Perform 2013;8:111-22.
40. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol 1986;60:1327-32.
41. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982;14:377-81.
42. Oopik V, Saaremets I, Timpmann S, Medijainen L, Karelson K. Effects of acute ingestion of sodium citrate on metabolism and 5-km running performance: A field study. Can J Appl Physiol 2004;29:691-703.
43. Carr AJ, Slater GJ, Gore CJ, Dawson B, Burke LM. Effect of sodium bicarbonate on [hco3-], ph, and gastrointestinal symptoms. Int J Sport Nutr Exerc Metab 2011;21:189-94.
44. Siegler JC, Marshall PW, Bray J, Towlson C. Sodium bicarbonate supplementation and ingestion timing: Does it matter? J Strength Cond Res 2012;26:1953-8.
45. Stephens TJ, McKenna MJ, Canny BJ, Snow RJ, McConell GK. Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. Med Sci Sports Exerc 2002;34:614-21.

Funding.—Funding for this study was provided to the University of Udine by FORSG srl via Cavour 8 33080 Puia di Prata PN Italy, that provided the Chanson miracle max ionizer and covered partly the study cost.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgements.—We are grateful to the athletes for their kind collaboration during the study, to Dr. A. Botter for helping during tests and to Prof. M. Isola for statistical support.

Article first published online: April 26, 2017. - Manuscript accepted: April 13, 2017. - Manuscript revised: March 15, 2017. - Manuscript received: July 29, 2016.