Hydrogen Rich Water Consumption Positively Affects Muscle Performance, Lactate Response, and Alleviates Delayed Onset of Muscle Soreness After Resistance Training

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Abstract

Botek, M, Krejčí, J, McKune, A, Valenta, M, and Sládečková, B. Hydrogen rich water consumption positively affects muscle performance, lactate response, and alleviates delayed onset of muscle soreness after resistance training. *J Strength Cond Res* 36(10): 2792–2799, 2022—Positive outcomes of hydrogen rich water (HRW) supplementation on endurance performance have been shown, but the effects of HRW in resistance training are unclear. The aim of this study was to assess the effects of 1,260 ml of HRW intake on physiological, perceptual, and performance responses to a resistance training and after 24 hours of recovery. This randomized, double-blinded placebo-controlled cross-over study included 12 men aged 23.8 ± 1.9 years. Subjects performed a half squat, knee flexion, and extension exercises with the load set at 70% of 1 repetition maximum for 3 sets (10 reps/set). Lunges were performed with a load of 30% of body mass for 3 sets (20 reps/set). Time of each set, lactate, and ratings of perceived exertion were assessed mid-way through exercise and immediately after the exercise. Creatine kinase, muscle soreness visual analog scale ratings, countermovement jump, and heart rate variability were evaluated before the training and at 30 minutes, 6, and 24 hours of recovery. Lunges were performed faster with HRW compared with placebo ($p < 0.001$). Hydrogen rich water reduced lactate at mid-way and immediately after the exercise (HRW: 5.3 ± 2.1 and 5.1 ± 2.2, placebo: 6.5 ± 1.8 and 6.3 ± 2.2 mmol·L^{-1}, $p \leq 0.008$). Visual analog scale ratings were significantly lower with HRW (26 ± 11 vs. 41 ± 20 mm, $p = 0.002$) after 24 hours of recovery. In conclusion, an acute intermittent HRW hydration improved muscle function, reduced the lactate response, and alleviated delayed onset of muscle soreness.

Key Words: molecular hydrogen, recovery, exercise, antifatigue, muscle pain

Introduction

Intense resistance training that includes eccentric muscle contractions and unaccustomed exercises, induces muscle tissue damage, and delayed onset muscle soreness (DOMS) (24). Traditionally, DOMS is characterized by a sensation of discomfort which is the most apparent in skeletal muscle between 24 and 48 hours after exercise (38), and it commonly disappears within 5–7 days after exercise (3). Delayed onset muscle soreness can be noninvasively evaluated e.g., using a visual analog scale (VAS) (18), whereas invasive indirect blood biomarkers of muscle cell damage, such as creatine kinase (CK), have also been used (25). Creatine kinase usually peaks 24–72 hours after resistance training (5). Heart rate variability (HRV) as a noninvasive index of autonomic cardiac regulation (16) has been used to reflect homeostatic activity or adaptations in response to both acute aerobic/endurance exercise bouts and chronic training effects (11). However, the results of autonomic cardiac response to resistance training (39) and its relation to DOMS are still unclear (23). It was shown that the underlying mechanism that causes DOMS after resistance training is more likely multifactorial, including effect of connective tissue damage, local inflammation response involving leukocyte accumulation in damage muscle tissue, apoptosis (17), and a potential role of the reactive oxygen species (ROS) (15). Generally, a rise in level of ROS, as a result of disbalance between production and removal of ROS, has been associated with pathological outcomes that include mitochondrial dysfunction and cellular damage (13). Consequently, a magnitude of metabolic ROS production during resistance training seems to be depended on exercise intensity, and it may contribute to the impairment of muscle function (12), fatigue, and likely also with delayed recovery in athletes (35).

Molecular hydrogen (H_2) has been shown to be a strong and selective antioxidant with high scavenger affinity to cytotoxic hydroxyl free radicals (31,32). Besides its antioxidative property, H_2 has recently been shown to have anti-inflammatory (2), antiapoptotic (29), and antifatigue (1,2,7,16) properties. Hydrogen rich water (HRW), where H_2 is dissolved in the aqueous medium, is currently a very popular method to apply H_2 in daily practice (20). Because of its beneficial effects on endurance (2,7,27), repeated sprint ability (16), and maximal isokinetic muscle strength (1) performance, HRW supplementation has become increasingly popular in elite and nonelite athletes (20). However, to the best of our knowledge, there is a lack in data about the effect of H_2 in resistance training. From a metabolic

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perspective, HRW consumption before exercise lowered blood lactate during cycling at higher exercise intensities (8), and immediately after exercise (1). In addition, HRW intake attenuated rating of perceived exertion (RPE) (8) during exercise and VAS after endurance exercise (27). However, others found no changes in RPE or lactate response during submaximal and maximal exercise tests (33). Regarding DOMS after exercise-induced muscle damage, Kawamura et al. (19) showed that H_2 bathing for 20 minutes after 30 minutes downhill running (−8% slope at intensity of 75% $\dot{V}O_2$max) significantly reduced DOMS in the first 2 days after running. However, according to this experiment, H_2 bathing had no effect on oxidative stress markers and muscle damage. Taken together, H_2 intake may alter biochemical, performance, physiological, and psychometric variables when "an appropriate" dose of H_2 and administration time is maintained. In this context, Kawamura et al. (20) recently discussed the future directions of H_2 application in sport sciences and recommended HRW intake immediately before and during exercise to increase H_2 efficacy because of the short half-life of H_2 (peaks at 10–15 minutes) in the body (32).

Based on the literature (1) and our previous research using HRW (7,8), we expected that HRW administration compared with placebo would have a positive effect on physiological, biochemical, perceptual, and performance variables during resistance training and during up to 24 hours of recovery. In this regard, we hypothesized that there would be a significant reduction in lactate and CK concentration, reduction in RPE and VAS, reduction in lunge time, improvement in vertical jump height, and an increase in the cardiac vagal activity.

Methods

Experimental Approach to the Problem

The study was a double-blind, cross-over design with administration of HRW and placebo randomized and counterbalanced. The experimental study protocol consisted of 4 laboratory sessions (Figure 1). The first session included detailed instructions relating to the experimental procedure and familiarization with the testing equipment. The second session occurred the following day and included anthropometric measurements and determination of individualized 1 repetition maximum (1RM). The subjects were advised to avoid drinking coffee, tea, and any other substance potentially affecting the selected physiological, biochemical, and perceptual responses to exercise, for at least 2 hours before sessions 2–4. In addition, subjects were also asked to avoid vigorous physical activity and alcohol for 72 hours before all testing. A small standardized meal (1 banana) was ingested by all subjects at least 60 minutes before each exercise. To avoid possible diurnal variations, all exercise testing was scheduled between 8:30 and 11:00 AM in a faculty facility (room temperature 20–22° C). The third session took place 2 weeks after the second session. In this session, subjects were randomly divided into 2 groups: HRW or placebo (n = 6 each). Randomization was performed by means of lots, using an equal number of 2 colored strips (red and blue). Subjects drew only one strip while blinded. The fourth session took place after a 1 week washout, and the subjects performed the same resistance training protocol as in the third session, except that the beverages were exchanged (HRW for placebo and vice versa).

Subjects

This study included 12 male students of the Faculty of Physical Culture with the following characteristics (mean ± *SD*): age: 23.8 ± 1.9 years (range 21–27 years), body mass: 78.2 ± 6.0 kg, body fat: 12.1 ± 3.6%, and height: 180.0 ± 5.0 cm). All subjects were healthy, nonsmokers, were physically active, and had resistance training experience. They were not on any medication or using dietary supplements and were free of any known (self-reported) cardiovascular, pulmonary, and metabolic conditions. The study was approved by the Ethics Committee of the Faculty of Physical Culture, Palacký University Olomouc (reference number 75/2017) in accordance with the Declaration of Helsinki. All subjects were informed of the benefits and risks of the investigation before signing an approved written informed consent document to participate in the study. In addition, to the best of our knowledge, no side effects during or after the HRW administration have been reported (29) or were reported in this study.

Procedures

Anthropometric Measurement. Body height (to the nearest 1 cm) and body mass (to the nearest 0.1 kg) were measured using a digital weighing scale SOEHNLE 7307 (Leifheit, Nassau, Germany). Percent body fat was determined using bioimpedance analysis (Tanita BC-418 MA, Tanita, Tokyo, Japan).

Determination of One Repetition Maximum. Although the subjects had resistance training and 1RM testing experience, they performed a 1RM test to obtain current baseline data. Before 1RM testing, they performed a 5-minute light warm-up on a bicycle ergometer which was followed by standard static stretching exercise set focused on the lower limb muscles. They then performed a specific warm-up consisting of 8–10 repetitions at approximately 50% of anticipated 1RM. After a 3-minute rest, they performed 4 repetitions at 70–75% of anticipated 1RM. After another 3-minute rest, subjects performed a further 2–3 repetitions at 85–90% of the anticipated 1RM. After a 4-minute rest, the information received from the third set was then used to determine the final mass for individual 1RM determination (4). One repetition maximum was determined in the following order: half-squat, knee extension, and knee flexion. Between each exercise, there was a passive recovery period set at 5 minutes. Passive recovery involved sitting on a jump box. The half-squat was included as part of the strength test.

Experimental Resistance Training Protocol. Before each work out, all subjects performed a 5-minute warm-up on a bicycle ergometer (ER 900, Ergoline, Bitz, Germany) at a load 0.5 $W \cdot kg^{-1}$ and with cadence 60 rounds per minute, that was followed by 5 minutes of self-selected static stretching exercises. They then performed 1 set (6–8 repetitions with load 50% of 1RM) each of half-squats, knee flexion, and extension drills, that were interspersed by 1 minute of passive recovery. The resistance training protocol included exercises in the following order: half-squat, knee extension, and knee flexion, each consisting of 3 sets of 10 repetitions at 70% 1RM. The recovery period was 3 minutes between each set and 4 minutes between the different exercises. The half-squats were performed using a Multipress Station 0223 (GRÜN SPORT, Horní Bříza, Czech Republic). The subjects executed the half-squat from a fully extended position, with their feet a little wider than hip width apart and the barbell held across the top of the shoulders and upper back. They then squatted to a depth that resulted in a 90° knee angle and then returned to the starting position. The appropriate execution of each half-squat

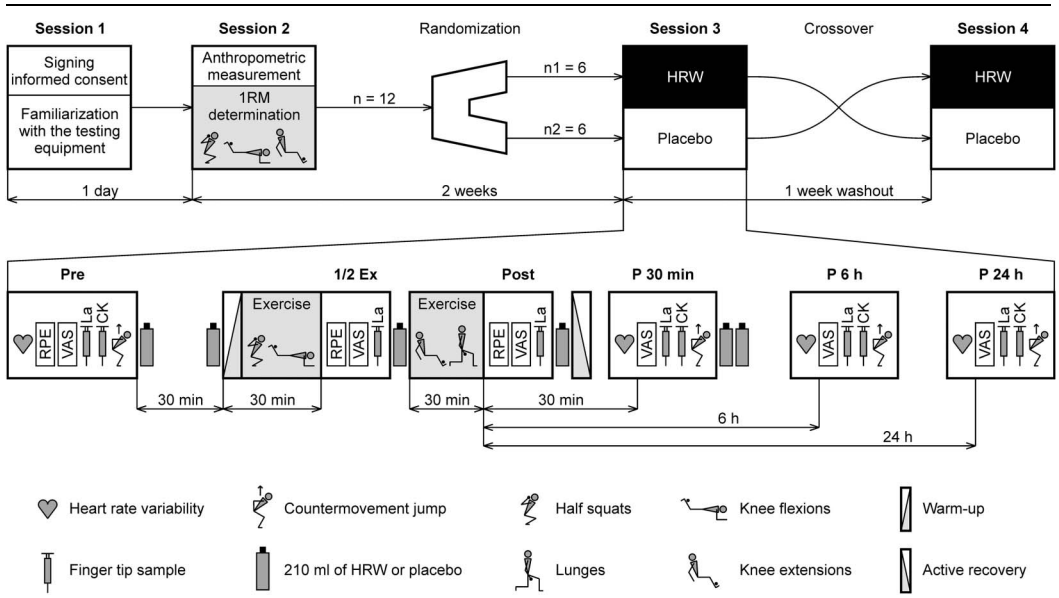

Figure 1. Overview of the study protocol and labeling of sessions. The time axis is not proportional with time values to capture large differences in time values. 1RM = 1 repetition maximum; HRW = hydrogen rich water; La = blood lactate concentration; CK = creatine kinase; RPE = rate of perceived exertion; VAS = visual analog scale; pre = preliminary; 1/2 Ex = half of exercise session; post = immediately postexercise; p 30 minutes = 30 minutes postexercise; p 6 hours = 6 hours postexercise; p 24 hours = 24 hours postexercise.

and safety of the subjects was ensured by 2 fitness trainers positioned at each end of the barbell. Knee flexion and extension was performed on a Leg Extension/Curl 5530 machine (HUR, Kokkola, Finland). Before the subjects started with the exercise, they manually adjusted the seat, support roller and distal lever arm roller position. Subjects performed the knee flexion and extension in a seated position, with their waist and thigh held in position using specific belts. The chest belt ensured that the back was positioned firmly against the back pad. The legs were always positioned parallel to each other. Before the exercise was initiated, subjects grasped the handles on the side of the seat. Bilateral knee extension involved raising a roller pad (connected to the load), that was placed anteriorly on the shin, moving from a 90° to 180° knee angle. When the 180° knee angle was reached, the equipment sent out a control signal. After that, legs then moved downward back to the starting position. Knee flexion involved moving the roller pad (placed posteriorly on the calf muscle) from the 180° to 90° knee angle. After the control signal, the legs moved back to the starting position. The last exercise consisted of lunges, 3 sets of 20 repetitions, performed at a load of 30% of body mass, with sets interspersed by a 3-minute recovery. The lunges were executed using the following technique: (a) start by standing up tall and with a dumbbell held in each hand, (b) lunge forward with right foot until your thigh is at a 90° knee angle to the floor, (c) lift the front lunging leg to return to the starting position, and then repeat these movements using the opposite leg. Subjects were requested to perform each repetition with maximal voluntary effort, ensuring that a proper technique was maintained. To prevent any health issues during the strength drills, subjects were monitored by 2 fitness trainers. The time of each set was manually measured by using a digital timer (HS80, Casio, Shibuya, Japan).

Hydrogen Rich Water and Placebo Preparation. A total volume of 1,260 ml of HRW (Aquastamina HRW, Nutristamina, Ostrava, Czech Republic) or placebo (Aquastamina H_2 free, Nutristamina, Ostrava, Czech Republic) was administered in 5 doses, specifically 210 ml at 30 minutes and at 1 minute before training, 210 ml in the middle of exercise session, then another 210 ml immediately after the end of exercise session, and 420 ml of HRW at 30 minutes of recovery. This HRW hydration protocol included a 1-week washout period similarly to previous HRW studies (1,8). According to manufacturer information, HRW was produced by the infusing H_2 under high pressure directly into water. Both drinks were served in visually identical plastic-aluminum packages. Subjects could not distinguish between HRW and placebo because H_2 is colorless, odorless, and tasteless (29). The chemical characteristic of both HRW and placebo (Table 1) were determined using the pH/ORP/temperature meter (AD14, Adwa Instruments, Szeged, Hungary). The dissolved hydrogen concentration was determined using H_2Blue reagent (H2 Sciences, Henderson, NV) according to the manufacturer instructions.

Blood Sampling and Analyses. Capillary (fingertip) blood samples were collected to assess blood lactate and CK concentration. Before collecting the sample, the finger was cleaned using an alcohol wipe to make the area clean and free of sweat. The skin was punctured with a lancet, and the first blood drop was wiped away. For lactate sampling, the second drop was analyzed using a blood analyzer Lactate Scout+ (EKF Diagnostics, Cardiff, United

Table 1
Chemical characteristic of hydrogen rich water (HRW) and placebo.*

Property	HRW	Placebo
pH	7.8	7.6
ORP (mV)	−652	+170
Temperature (°C)	22	22
H_2 concentration (ppm)	0.9	0.0

*ORP = oxidation reduction potential.

Kingdom). The instrument's accuracy was checked before sampling according to the manufacturer's guidelines. For CK concentration measurement, approximately 32 μL of capillary blood was collected from a finger by a prick made with a spring-loaded lancet (Accu-Chek, Roche Diagnostics, Rotkreuz, Switzerland) set at 2.3 mm depth. A Reflotron applicator with a 32 μL disposable pipette tip was used to extract a 32 μL sample of blood and place it on a CK assay strip (Reflotron CK strips, Roche Diagnostics, Rotkreuz, Switzerland). The blood sample was immediately analyzed using a spectrophotometer (Reflotron Plus, Roche Diagnostics, Rotkreuz, Switzerland) for plasma CK concentration.

Rate of Perceived Exertion. At predetermined times, the subjects were asked to score subjective RPE, using the scale developed by Borg (6). All subjects were familiarized with the Borg scale before testing. The RPE scale ranged from 6 (no exertion at all) to 20 (maximum exertion). The degree of subjective exertion was expressed in numbers only.

Visual Analog Scale. The VAS was used to determine lower limb muscle pain before the experimental resistance protocol, and in 30 minutes, 6 hours, and 24 hours postexercise. The VAS was a horizontal 100 mm length line, marked with 0, indicating "no pain" and 100 indicating the "worst imaginable pain" (18). The VAS rating was scored for both legs together immediately after a countermovement jump (CMJ) attempt.

Countermovement Jump Test. Before the test, each subject completed an individual warm-up procedure which consisted of running at an intensity of 50% of their perceived maximal speed for 3 minutes, 10 squats, and 1 submaximal CMJ. After 1 minute of rest, each subject performed 3 single maximum effort countermovement CMJs with 30 seconds of rest between each jump. The starting position for the CMJ was an upright posture with the hands placed on the hips. Exclusion criteria for CMJ were as follows: (a) slow speed from the upright posture to squat, (b) knee flexion not 90°, and (c) failure to keep hands on hips. All subjects had the opportunity to familiarize with the CMJ testing on the familiarization day (session 1). On the experimental days (sessions 3 and 4), no jump was observed that would meet the exclusion criteria. Vertical ground reaction force was measured on 2 parallel force platforms (AMTI OR6-7-1000, Advanced Mechanical Technology, Watertown, MA) with a sampling frequency of 1,000 Hz. A quiet standing period of 2 seconds was recorded before the initiation of each CMJ to ensure an initial velocity of zero and to calculate the body mass. The jump height was calculated from the force-time curve, and the maximal value of 3 CMJ repetitions was considered and used for statistical analysis.

Heart Rate Variability Analysis. To determine the resting heart rate and HRV variables, the electrocardiograph signal was measured at a sampling frequency of 1,000 Hz using a DiANS PF8 (DIMEA Group, Olomouc, Czech Republic). Each record lasted approximately 12 minutes while subjects performed an orthoclinostatics maneuver, specifically 1-minute supine (not analyzed), 5-minute standing, and 5-minute supine. The record was examined, and all premature ventricular contractions, missing beats, and any artefacts were manually filtered. A spectral analysis was performed using the fast Fourier transform with a sliding 256 points Hanning window. The power spectra were quantified by integrating the area under the power spectral density curve. Two frequency bands were used: low frequency (LF) from 0.05 to 0.15 Hz and high frequency (HF) from 0.15 to 0.50 Hz. The HF power is solely modulated by cardiac vagal activity, whereas LF power is associated with baroreflex activity and the bilateral effect of sympathetic and vagal activity on the sinus node, and the LF/HF ratio reflects the sympathovagal balance (16). A time domain variable, the square root of the mean of the squares of the successive differences (RMSSD) as index of vagal activity was also included because RMSSD is suggested to be resistant to the effects of breathing frequency (11).

Statistical Analyses

Data are presented as arithmetic mean ± *SD*. Normality of data was checked using the Kolmogorov-Smirnov test. The effect of HRW on dependent variables was evaluated using a linear mixed-effects model with 1 random factor (subject), 2 fixed factors (administered water and time), and interaction (water × time). Because neither HRW nor placebo was administered before the preliminary stage, water as a factor was not considered at this stage. When any factor or interaction was statistically significant, pairwise comparisons were performed using the Fisher's least significant difference test. Differences in means were also expressed using 95% confidence intervals (CIs). For all tests, $p < 0.05$ was considered statistically significant. In addition to the statistical significance, effect size measures were also used. The partial eta-squared (η^2) was used for linear model factor, and Cohen's standardized difference (d_z) was used for pairwise comparison. Cohen's d_z was calculated according the formula $d_z = \frac{m_1 - m_2}{SD_{\text{diff}}}$ where m_1, m_2 are means to compare. The standard deviation of the difference scores was calculated as $SD_{\text{diff}} = \sqrt{2\text{MS}_{\text{error}}}$ where MS_{error} is the mean square error obtained from the linear model. The magnitude of the effect size measures was interpreted according following thresholds: trivial ($\eta^2 < 0.01$, $d_z < 0.2$), small ($\eta^2 \geq 0.01$, $d_z \geq 0.2$), medium ($\eta^2 \geq 0.06$, $d_z \geq 0.6$), and large ($\eta^2 \geq 0.14$, $d_z \geq 1.2$). Statistical analyses were performed using MATLAB 8.4 with Statistics Toolbox 9.1 (MathWorks, Natick, MA). A sensitivity analysis was performed using G*Power version 3.1.9.7. The calculation was performed for a 2-sample 2-tailed *t*-test for a statistical significance of 0.05, power of 0.80, and sample size of 12. The result was that the minimal detectable effect size would be $d_z = 0.89$.

Results

The normal distribution was not rejected for all dependent variables except RPE and Ln $\text{RMSSD}_{\text{standing}}$ (time of lunges: $p = 0.65$, lactate: $p = 0.074$, CK: $p = 0.17$, VAS: $p = 0.69$, CMJ: $p = 0.86$, Ln $\text{RMSSD}_{\text{supine}}$: $p = 0.52$, Ln $\text{HF}_{\text{supine}}$: $p = 0.22$, Ln $\text{HF}_{\text{standing}}$: $p = 0.51$, Ln $\text{LF}_{\text{supine}}$: $p = 0.78$, Ln $\text{LF}_{\text{standing}}$: $p = 0.40$, Ln $\text{LF/HF}_{\text{supine}}$: $p = 0.51$, and Ln $\text{LF/HF}_{\text{standing}}$: $p = 0.25$). Rating of perceived exertion ($p = 0.020$) and Ln $\text{RMSSD}_{\text{standing}}$ ($p = 0.046$) were not normally distributed. After visual inspection of the data distribution, the variables were processed untransformed as F-statistics testing is considered a robust test against such violations of normality.

Statistically significant water factors were found for time of lunges and lactate (Table 2). Significant time factors were found for CK, lactate, RPE, VAS, CMJ, and Ln $\text{RMSSD}_{\text{supine}}$ (Table 2). Significant interaction was found for VAS (Table 2). Post-hoc comparisons are displayed in Figure 2.

Table 2
Results from the linear mixed-effects model.*

Variable	Water factor		Time factor		Interaction	
	p	η^2	*p*	η^2	*p*	η^2
Time of lunges (s)	<0.001	0.41	0.66	0.02	0.19	0.06
Creatine kinase ($U \cdot L^{-1}$)	0.16	0.02	<0.001	0.57	0.90	0.00
Lactate ($mmol \cdot L^{-1}$)	0.003	0.07	<0.001	0.83	0.18	0.05
RPE (points)	0.48	0.01	<0.001	0.87	0.38	0.01
VAS (mm)	0.45	0.00	<0.001	0.62	0.043	0.08
CMJ (cm)	0.44	0.01	<0.001	0.28	0.94	0.00
Ln $RMSSD_{standing}$ (ms)	0.27	0.02	0.30	0.05	0.80	0.01
Ln $RMSSD_{supine}$ (ms)	0.88	0.00	0.035	0.10	0.85	0.00
Ln $LF_{standing}$ (ms^2)	0.43	0.01	0.74	0.02	0.47	0.02
Ln LF_{supine} (ms^2)	0.75	0.00	0.83	0.01	0.59	0.01
Ln $HF_{standing}$ (ms^2)	0.26	0.02	0.60	0.02	0.88	0.00
Ln HF_{supine} (ms^2)	0.46	0.01	0.094	0.08	0.50	0.02
Ln $LF/HF_{standing}$	0.44	0.01	0.80	0.01	0.17	0.04
Ln LF/HF_{supine}	0.82	0.00	0.48	0.03	0.24	0.04

*p = statistical significance; η^2 = partial eta-squared effect size; RPE = rate of perceived exertion; VAS = visual analog scale; CMJ = height of countermovement jump; Ln = natural logarithm; RMSSD = square root of the mean of the squares of the successive differences; HF = high-frequency power; LF = low-frequency power; LF/HF = low-frequency/high-frequency ratio.

Hydrogen rich water administration, compared with placebo, significantly reduced the time of lunges for the first repetition (HRW: 43.4 ± 5.3 seconds, placebo: 45.6 ± 4.1 seconds, CI: −4.2 to −0.1 seconds, $p = 0.037$, $d_z = -0.62$, medium effect), at the second repetition (HRW: 42.7 ± 3.2 seconds, placebo: 46.6 ± 2.8 seconds, CI: −5.9 to −1.9 seconds, $p < 0.001$, $d_z = -1.12$, medium effect), and at the third repetition (HRW: 42.8 ± 2.9 seconds, placebo: 47.5 ± 3.8 seconds, CI: −6.8 to −2.7 seconds, $p < 0.001$, $d_z = -1.35$, large effect). Hydrogen rich water significantly reduced lactate concentration during the middle of the exercise session (HRW: 5.3 ± 2.1 $mmol \cdot L^{-1}$, placebo: 6.5 ± 1.8 $mmol \cdot L^{-1}$, CI: −2.1 to −0.3 $mmol \cdot L^{-1}$, $p = 0.008$, $d_z = -0.78$, medium effect) and immediately after the end of the exercise session (HRW: 5.1 ± 2.2 $mmol \cdot L^{-1}$, placebo: 6.3 ± 2.2 $mmol \cdot L^{-1}$, CI: −2.1 to −0.4 $mmol \cdot L^{-1}$, $p = 0.006$, $d_z = -0.81$, medium effect). However, the effect of HRW on lactate concentration at all 3 recovery times was not significant (all $p \geq 0.60$, all d_z in range −0.09 to −0.15, trivial effects). Hydrogen rich water effect on the VAS was significant only at 24 hours of recovery (HRW: 26 ± 14 mm, placebo: 42 ± 20 mm, CI: −26 to −6 mm, $p = 0.002$, $d_z = -0.90$, medium effect), the effects were not statistically significant at other time points (all $p \geq 0.45$, all d_z in range −0.02 to 0.22, trivial to small effects).

Discussion

The aim of this study was to assess the effects of 1,260 ml of HRW supplementation on physiological, perceptual, and performance responses to a resistance training session and after 24 hours of recovery. The primary findings of the study were as follows: (a) a significantly lower blood lactate was found with HRW compared with placebo halfway through and immediately after the session, (b) all sets of lunges were performed significantly faster when HRW was applied, and (c) perceived muscle soreness assessed by the VAS was scored significantly lower with HRW compared with placebo at 24 hours of recovery. There were no significant differences between HRW and placebo for blood CK concentration, explosive muscle power, autonomic cardiac regulation, and RPE.

In our experimental resistance training protocol, all exercise sets were performed by the subjects for longer than 20 seconds, at maximal voluntary effort, interspersed by 3 minutes of passive recovery. In the case of high-intensity exercise, after phosphocreatine (PC) system withdrawal within the first few seconds, anaerobic glycolysis starts to become the dominant metabolic pathway, and the onset of blood lactate accumulation and fatigue causing H^+ ions are typically observed (9,10). However, HRW supplementation was associated with a significant reduction in exercise and postexercise blood lactate in this study. This finding is in line with recently published research (8), where authors detected lower lactate at an exercise intensity of 3 and 4 $W \cdot kg^{-1}$ during 8 minutes of bicycling after acute pre-exercise supplementation with 600 ml of HRW. A significantly lower postexercise lactate concentration was also observed in soccer players, after 30 minutes of cycling at 75% of $\dot{V}O_2max$ and 100 repetitions of maximal isokinetic extensions (1). The mechanism underlying the lactate lowering effect of H_2 was reported in an in vitro study, where application of H_2 caused stimulation of mitochondrial oxidative phosphorylation and mitochondrial adenosine triphosphate (ATP) production (28). By contrast, Ooi et al. (33) found no changes in lactate response after acute intake of 290 ml of HRW (H_2 = 1.0 ppm) before either a submaximal or maximal exercise test, and concluded that the given dose of HRW was more than likely not sufficient to elicit positive metabolic and performance responses in endurance-trained runners.

From a performance perspective, the results demonstrated that after 45 minutes of heavy resistance training, HRW supplemented subjects performed the last 20 repetitions of lunges significantly faster (~8%) compared with when supplemented with placebo. An antifatigue effect of HRW across different modes of exercise has been well documented in the literature. For instance, Aoki et al. (1) demonstrated an attenuated decrease (3.7%) in peak torque after 20 maximal isokinetic knee extensions, after HRW ingestion (1.5 L of HRW, H_2 = 0.9–1.0 ppm, within 8 hours preexercise) in 10 soccer players. Hydrogen rich water supplementation also had an antifatigue effect during intermittent cycling. Da Ponte et al. (16) found an attenuation in decline of the peak power output by 7.4% after supplementation with alkaline HRW (2 L per day for 2 weeks pre exercise, H_2 = 0.2–0.5 ppm). In this study, better performance in the lunges was accompanied by a lower postexercise lactate of ~1 $mmol \cdot L^{-1}$ when supplemented with HRW compared with placebo. In this regard, a decrease in blood lactate concentration could be explained by H_2-induced stimulation of mitochondrial oxidative phosphorylation (29) resulting in the oxidation of lactate and reduction of the associated H^+ ions, attenuating the rising tissue acidity (37). Typically, a rise in muscle H^+ ions is associated with the inhibition of ATP resynthesis, lower muscle responsiveness to given Ca^{2+} (10), and, subsequently, with impairment in muscle contractions (9). Cakir-Atabek et al. (12) recently demonstrated an increase in oxidative stress and muscle damage indices after acute 60 maximal eccentric actions of the elbow flexors at a constant velocity of $60° \cdot s^{-1}$, whereas only indices of oxidative stress damage were significantly related, particularly, to the strength loss of flexors. In addition, we found no ergogenic effect of acute HRW administration on explosive-strength performance in our study. The CMJ performance, unlike the longer lasting lunge test (performed for ~42 seconds), lasts for 1–2 seconds duration, depending primarily on energy from the ATP-PC system and the elastic potential of the muscle (stretch-shortening exercise). Although HRW application exerted no ergogenic effect on single CMJ performance, we feel that administration of 1,260 ml HRW, acutely before, as well as

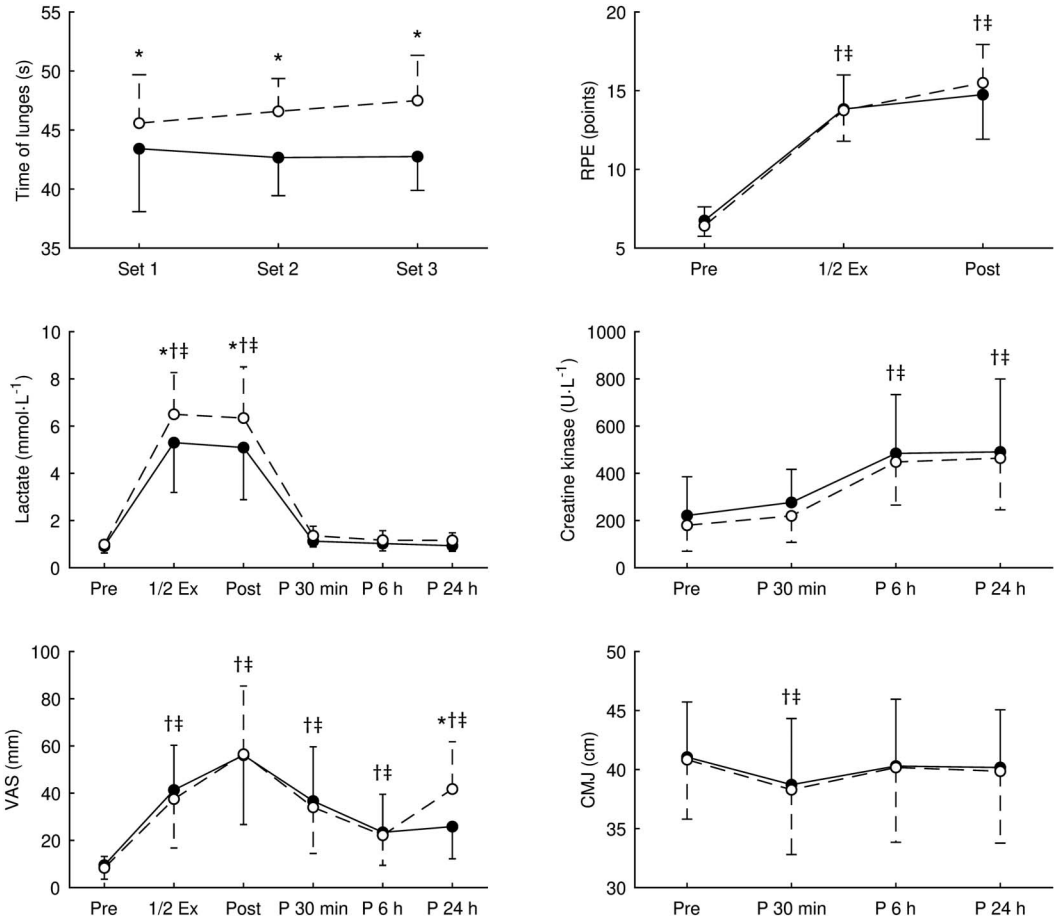

Figure 2. Effect of hydrogen rich water on performance, psychometric, and physiological variables. Values are presented as the mean and standard deviation. RPE = rate of perceived exertion; VAS = visual analog scale; CMJ = height of countermovement jump; pre = preliminary; 1/2 Ex = half of exercise session; post = immediately postexercise; p 30 minutes = 30 minutes postexercise; p 6 hours = 6 hours postexercise; p 24 hours = 24 hours postexercise; ● = hydrogen rich water; ○ = placebo; ⋆ = statistically significant ($p < 0.05$) difference between hydrogen rich water and placebo at the same time; † = statistically significant ($p < 0.05$) difference between this time and the preliminary stage when hydrogen rich water was administered; ‡ = statistically significant ($p < 0.05$) difference between this time and the preliminary stage when placebo was administered.

during exercise, may enhance the endogenous antioxidant capacity to respond to the intensity dependent, mitochondrial production of ROS (36), reduce oxidative stress (12), and enhance mitochondrial ATP production (29), lactate clearance (8), and H^+ removal. Through these mechanisms, it is felt that H_2 could enhance strength-endurance performance during resistance training. Interestingly, it has been shown that ROS and nitric oxide, during exercise in mice, can act by exercise-induced nuclear factor erythroid 2-related factor 2, to functionally regulate skeletal muscle mitochondrial biogenesis and antioxidant defense gene expression (26). This same molecular pathway seems to be stimulated by exposure to H_2 (40). Thus, chronic (4 weeks or more) H_2 intake during resistance training may possibly help to increase mitochondrial biogenesis, endogenous antioxidative system, and enhance strength performance.

There were no significant differences between HRW and placebo during the 24-hour postexercise recovery for the muscle damage biomarker, blood CK concentration. However, there was a significant increase in CK concentration from baseline at 6 hours ($p < 0.001$) and 24 hours ($p < 0.001$) postexercise. This increase suggested the presence of exercise-induced muscle damage that is associated with DOMS (15). However, at 24 hours postexercise, there was a significantly alleviated perception (assessed by VAS) of muscle pain in the HRW compared with placebo. Based on these findings, there was an apparent discrepancy between postexercise VAS and the CK concentration response to HRW administration. This may be related to intersubject variability in CK concentration (22) with some previous studies reporting no relationship between CK concentration and muscle soreness after eccentric exercise (30). However, other studies have shown that CK variability after eccentric exercise is closely related to DOMS (22), and the development of DOMS may be a more complex phenomenon, involving inflammation (14). For instance, DOMS development after eccentric exercise was recently associated with several key factors such as the inflammatory response, oxidative stress, and phagocyte activation (21). Therefore, it is feasible that H_2, as a strong selective antioxidant and anti-inflammatory agent (32), exerts "an analgesic effect" on muscle pain perception at 24 hours postexercise in this study, irrespective of the elevated CK concentration. A similar finding was reported by Kawamura et al. (19) who found that a 1-week bathing procedure in HRW for 20 minutes, significantly reduced perceived DOMS at 24 and 48 hours after a 30-minute bout at intensity of 75% $\dot{V}O_2$max of

down-hill running with −8% slope. However, the authors mentioned that this alternative recovery procedure is not likely an effective approach for reducing inflammation and oxidative stress after downhill running (21). Mikami et al. (27) reported a significant reduction in a VAS rating of fatigue after mild exercise in nontrained subjects who consumed 500 ml of HRW (H_2 = 0.8 ppm) 30 minutes before cycling. Interestingly, the subjects who felt more fatigue after exercise were classified as more sensitive to the effects of H_2. Similarly, Botek et al. (7) reported that the magnitude of effect of H_2 depended on the individual adaptation level, with faster athletes seeming to be less sensitive to acute H_2 supplementation compared with slower athletes who exhibit higher benefits from acute H_2 intake.

There are some limitations and issues regarding the HRW application. First, the dosage of H_2 was constant per subject for logistical reasons and was not adjusted to body mass. The immune system activity was not determined in this study. It seems that this information may be helpful for a deeper understanding of how H_2 may alter the immune system response and the subsequent effects on DOMS. In addition, this study did not examine the possible, separate, antifatigue effects of H_2 on concentric versus eccentric phases of muscle contraction.

Practical Applications

Acute intake of HRW shows promise as a beneficial hydration strategy for athletes who are involved in resistance training because of its ability to lower blood lactate concentration, enhance lower-limb muscular endurance performance, and alleviate muscle pain perception after heavy resistance training. Based on our findings, HRW ingestion could also be recommended before, as well as during, circuit training that generally combines endurance, resistance, and high-intensity aerobics drills. On the other hand, H_2 could not be considered as suitable ergogenic aid for improving explosive-strength performance. Determining the optimal HRW dosing strategy remains challenging as the dose-response curve is not yet known.

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