# Effects of 7-day intake of hydrogen-rich water on physical performance of trained and untrained subjects

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**ABSTRACT:** Hydrogen-rich water (HRW) is used as a supplement to improve performance and reduce fatigue in athletes. However, the potentially beneficial effects of HRW intake could be mediated by the training status of athletes. The purpose of the study was to analyse the ergogenic effect of intake of HRW for one week on aerobic and anaerobic performance, both in trained and untrained individuals. Thirty-seven volunteers participated in the study and were divided into two experimental groups: trained cyclists and untrained subjects. A double-blind crossover design was performed in which all subjects took a placebo (PW) and nano-bubble HRW (pH: 7.5; hydrogen concentration: 1.9 ppm; oxidation-reduction potential (ORP): -600 mV). At the end of 7-day intake, performance was assessed by an incremental $VO_2max$ test and by a maximum anaerobic test. After HRW intake, only trained cyclists improved their performance in the anaerobic test with an increase in peak power (from 766.2 ± 125.6 to 826.5 ± 143.4 W; *d* = .51) and mean power (from 350.0 ± 53.5 to 380.2 ± 71.3 W; *d* = .51), and a decrease in the fatigue index (from 77.6 ± 5.8 to 75.1 ± 5.9%; *d* = .45). The findings demonstrate that the ergogenic effect of HRW is mediated by the training status, and that 7-day intake of HRW would be an effective strategy for improving anaerobic performance in trained cyclists.

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## INTRODUCTION

It is well known that high intensity exercise causes an increase in reactive oxygen species (ROS), altering the redox balance and increasing oxidative stress [1, 2]. This increase in ROS can cause damage to the cell membrane and dysfunctions in the cellular mitochondria [3], which are associated with increased fatigue and loss of performance [4, 5].

Many athletes ingest buffer substances to increase the antioxidant response and improve the performance of aerobic and anaerobic metabolism. The hydrogen acts as an efficient antioxidant by rapid diffusion into living tissues and cells [6, 7]. Most antioxidant supplements are limited in their cellular distributions, but hydrogen has the ability to effectively penetrate biomembranes and infiltrate into the mitochondria and the nucleus [8, 9]. Hydrogen can be administered by different methods (inhalation of $H_2$ gas or injectable saline solution) but the easiest and safest way is the intake of hydrogen-rich water (HRW), without adding a colour change or water flavour [10]. Recently, some studies have concluded that molecular hydrogen might have healthy effects on the body, inducing antioxidant and anti-inflammatory responses, and limiting the metabolic acidosis [11–13]. HRW supplementation has been shown to have a positive effect on repeated-sprint ability performance, muscle fatigue and ventilatory response [14–17]. Similarly, the inhalation of 4% gaseous hydrogen during a week improved the peak running velocity of physically active men and women [18].

Currently there are different devices on the market that produce HRW, and even bags/bottles of different sizes that contain supposedly hydrogenated water are sold. The electrolysis technology used to enrich the water determines the pH level, the hydrogen concentration and the oxidation-reduction potential (ORP), decisively influencing these factors in terms of the ability to eliminate ROS. Another determining factor to promote the absorption of hydrogen by the cell membrane is the size of the hydrogen bubble. In the same vein, it has been suggested that the antioxidant activity of nano-bubble HRW (≤ 717 ± 387-nm diameter) is superior to normal hydrogen water containing a similar or lower level of dissolved hydrogen but not nano-bubbles [19].

On the other hand, the antioxidant response against the attack of ROS is more effective in trained individuals than in non-trained individuals, since they have a greater antioxidant capacity and a better immune response to exercise [17, 20]. These training-induced adaptations could also influence the ergogenic effect of HRW intake. To the best of our knowledge, there is only one previous study [17] that has tried to analyse the positive effect of HRW intake taking into account the athlete performance level, although it was conducted with a small sample size and with an acute intake that was only performed for 24 hours.

Therefore, the main aim of the study was to analyze the ergogenic effect of 7-day intake of nano-bubble HRW on aerobic and anaerobic performance, both in trained amateur cyclists and in untrained subjects. We hypothesized that the intake of HRW may be beneficial for athletes, reducing fatigue and increasing the aerobic and anaerobic performance.

## MATERIALS AND METHODS

### *Participants*

A total of 37 volunteers participated in the study. The information of the project was publicized through the bulletin board and the social networks of the research group. The sample size was calculated a priori to obtain a statistical power of 0.8, with a significance level of 0.05, and a mean difference of 5% between groups. Two experimental groups were formed: a group of untrained individuals (n = 15; age: 26.3 ± 5.9 years, body weight: 69.8 ± 11.4 kg, body height: 169.3 ± 7.1 cm, body fat: 24.5 ± 6.5%), who were identified as moderate active individuals according to the International Questionnaire of Physical Activity [21], but who did not perform regular or controlled sports training; and a group of trained amateur cyclists (n = 12; age: 25.5 ± 5.5 years, body weight: 70.9 ± 8.5 kg, body height: 177.3 ± 6.6 cm, body fat: 17.9 ± 5.8%) who met the criteria of having a training background of 1–3 years, with a training duration of 60–120 minutes and a frequency training of 2–3 days/week [22]. All participants were free of musculoskeletal injuries in the lower limbs and they could not ingest any dietary supplement/medicine while the experiment lasted. The purpose of the study and the experimental protocols were explained and written informed consent was obtained from the participants before starting the research. The study was approved by the Ethics Committee of the university and met the requirements of the Declaration of Helsinki (Reg: 108/2018).

### *Experimental design*

A double-blind design crossover was proposed to evaluate the ergogenic effect of 7-day intake of HRW in two different experimental groups: trained amateur cyclists and untrained subjects. The study lasted for a total of 18 days, in which during the first week, all participants ingested a type of water (placebo water [PW] or HRW, according to the randomization), and during the second week, they ingested the other type of water as appropriate according to the crossover design, without a washout period between the two interventions. After each week of water intake, the following two days were used to assess the performance achieved by the participants in an incremental $VO_2$max test and in a maximum anaerobic test, separated one test from the other for a period of 24 hours (see Figure 1). Tests were always performed at the same time of the day (± 1 h) and without having performed strenuous lower limb exercises in the preceding 48 h. All tests were carried out under similar atmospheric condition (21–24ºC and 45–55% relative humidity).

### *Administration of HRW*

A portable drinking bottle of 320 ml capacity was used to produce nano-bubble HRW (HL-A1, H2Life, Chuanghui electronics, China). This device is able to generate very high level HRW using a proton exchange membrane (PEM) electrolysis technology, with a thick platinum-coated electrolysis plate. In a time period of 3 minutes, this water ionizer allows one to achieve nano-bubble HRW starting from mineral water. HRW is safe for the human body and has no side-effect [23], and this device is certified and in compliance with CE (Europe) and FCC (North America) regulations.

During the study, a portable drinking bottle was provided to all participants. They were instructed to drink water ranging between 1920 and 2240 ml per day (6–7 bottles of PW or HRW, according to the crossover double-blind design), having to drink all the water immediately after the hydrogenation process to maintain the level of hydrogen concentration and avoid deterioration of the ORP. During the PW intake, the portable drinking bottle was similar to the HRW intake, with bubbles but without an electrolysis plate. Moreover, participants could not distinguish between HRW and PW, because HRW was colourless, odourless, and tasteless. The characteristics of the water were as follows: PW (pH: 7.4; hydrogen concentration: 0 ppm and ORP: +241 mV) and HRW (pH: 7.5; hydrogen concentration: 1.9 ppm and ORP: -600 mV). The concentration of hydrogen in water was measured by oxidimetric determination using methylene blue-platinum colloid reagent [24]. Participants were also asked to

**FIG. 1.** Experimental design over time.

minimize the intake of other types of beverages (alcohol, soft drinks, fruit juices, etc).

### *Measurements*

Anthropometric measurements were taken at the beginning of the first testing session to characterize the participants. Body height was measured using a portable stadiometer (Seca 213, Germany). Body weight and % body fat were evaluated using a bioelectrical impedance analyser (BF-350, Tanita Europe BV, The Netherlands). Hydration prior to body composition testing was not allowed.

Maximal oxygen uptake ($VO_2$max), percentage of $VO_2$max in the ventilatory anaerobic threshold (VT2 %$VO_2$max), maximal work rate (Wmax), time to exhaustion (TTE) and maximum heart rate (HRmax) were determined using an incremental test to exhaustion on an electromagnetically braked bike potentiometer (Cycle Ops400 pro; Saris Cycling Group; USA). Ventilatory and gas exchange responses were measured continuously using a portable high resolution gas analysis system with breath-by-breath technology (Metalyzer 3b; CORTEX Biophysik GmbH, Germany). After a warm-up of 5 minutes at 50 W, participants started cycling at 100 W, and the work rate was increased by 30 W every 2 minutes until exhaustion. Subjects were required to maintain a cadence of 60–70 rpm. Test ended when the pedalling frequency could not be maintained under a work load. $VO_2$max was reached when the oxygen consumption reached its plateau (defined from 1-min mean $VO_2$ values) [25] and the ventilatory threshold was determined according to the triphasic model developed by Skinner and McLellan [26]. Wmax was recorded from the last completed work stage. HRmax was recorded with a heart rate monitor (V800, Polar Electro Oy, Finland).

An anaerobic test adapted from the Quebec 90-second test [27] was also performed to determine peak power (PP), mean power (MP), and fatigue index (FI). Participants performed a maximum test of 90 seconds on an electromagnetically braked bike potentiometer (Cycle Ops400 pro; Saris Cycling Group; USA). Firstly, participants performed a warm-up of 3 minutes at 100 W with a cadence of 60–70 rpm. Subsequently, just before starting the 90-second test, participants were instructed to increase the pedalling frequency progressively, and the resistance of the potentiometer was adjusted (by setting twice the work rate reached by each participant in the incremental $VO_2$max test). During the first 20 seconds of the test, the subjects had to maintain a cadence greater than 130 rpm. After these initial seconds, if the subject was not able to maintain a high pedalling cadence (above 100 rpm), the resistance of the potentiometer was gradually lowered (from 30 W to 30 W), in order to maintain the highest power possible throughout the test. PP was determined based on the maximum watts achieved during the first 10 seconds of effort. MP was calculated as the average value of watts recorded throughout the test. FI was defined as the difference between the peak power and minimum power, and expressed as a percentage of the peak power). Heart rate was monitored with a heart rate monitor (V800, Polar Electro Oy, Finland) throughout the test, and HRmax was recorded.

Capillary blood lactate and rated perceived exertion (RPE) were analysed after both tests. Lactate concentration was measured 1 minute after the end of the test, with a fast and reliable portable analyser (Lactate Scout+, SensLab GmbH, Germany) that uses an enzymatic–amperometric detection method and that only requires 0.5 $\mu$L of blood. RPE was assessed by a scale reporting options between 1 (extremely easy) and 10 (extremely hard). Thirty minutes after finishing the tests, a copy of this scale was given to the participants and they rated how hard the session had been.

### *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0. (IBM Corp., Armonk, USA). The Shapiro-Wilk test was applied in order to verify a normal distribution of data and Levene's test was used to assess the homogeneity of variance. A two-way ANOVA was used to investigate the main effects and the interaction between the *Protocol* factor (PW vs HW) and the *Training* factor (Trained vs Untrained). The significance of simple effect in groups required the use of the SPSS syntax command. Reliability was determined according to the ICC with 95% confidence intervals between the two testing sessions. The effect size (ES) was calculated for all dependent variables using Cohen's d. The magnitude of effect was classified as trivial (0.25), small (between 0.25 and 0.50), moderate (between 0.50 and 1.0) and large (> 1.0) [28]. The significance level was set at $p \leq 0.05$, with a confidence level of 95%. Means and standard deviations (SD) were used as descriptive statistics.

## RESULTS

All participants completed the study and no person reported any adverse effects that could be related to HRW intake.

Table 1 shows the results obtained in the incremental $VO_2$max test by both amateur trained cyclists and untrained subjects. The trained cyclists achieved significantly higher values of $VO_2$max, VT2 %$VO_2$max, Wmax and TTE in both the PW and HW protocol. An interaction effect ($p = .043$) on the lactate concentration was observed in trained cyclists after the intake of HRW, although with a small effect size ($d = .33$). No significant effect was observed in untrained subjects.

Table 2 shows the results obtained in the maximum anaerobic test by participants. The trained cyclists had better values of PP, MP and FI in both PW and HW protocols. It was observed that HRW had an interaction effect with the *Training* factor, observing a moderate positive effect on PP ($d = .51$), MP ($d = .51$) and FI ($d = 0.45$) in trained cyclists. However, HRW compared with PW did not have a clear effect in untrained subjects.

## DISCUSSION

The findings of the study show that HRW intake for one week had a positive effect on the performance (PP, MP and FI) of trained cyclists during the anaerobic test, but no significant effect on untrained

**TABLE 1.** Values obtained during the incremental VO2max test

| Variables | Training status | Protocol | Mean ± SD | 95%CI | ICC | Effect size (d) | Main effect Protocol (p-value) | Main effect Training (p-value) | Interaction effect Training x Protocol (p-value) |
|---|---|---|---|---|---|---|---|---|---|
| VO$_2$max (ml/min/Kg) | Trained | PW | 61.1 ± 3.8 | 58.1–63.8 | .97 | .18 | .742 | **.001** | .794 |
| | | HW | 60.5 ± 3.2 | 57.3–62.2 | | | | | |
| | Untrained | PW | 39.7 ± 7.6 | 35.4–43.9 | .98 | .13 | | | |
| | | HW | 40.7 ± 8.3 | 36.4–45.1 | | | | | |
| VT2 %VO$_2$max | Trained | PW | 82.9 ± 3.7 | 80.9–84.9 | .82 | .41 | .807 | **.001** | .854 |
| | | HW | 81.3 ± 4.4 | 78.2–84.1 | | | | | |
| | Untrained | PW | 72.9 ± 4.7 | 70.9–74.9 | .69 | .35 | | | |
| | | HW | 70.3 ± 5.4 | 67.2–73.1 | | | | | |
| Wmax (Watts) | Trained | PW | 290.0 ± 51.9 | 260.0–323.3 | .98 | .21 | .557 | **.001** | .957 |
| | | HW | 300.0 ± 51.9 | 270.0–333.3 | | | | | |
| | Untrained | PW | 222.3 ± 49.6 | 196.9–250.0 | .96 | .04 | | | |
| | | HW | 224.6 ± 50.2 | 199.2–254.6 | | | | | |
| HRmax (bpm) | Trained | PW | 185.4 ± 12.0 | 177.8–192.4 | .89 | .10 | 0.886 | .928 | .653 |
| | | HW | 186.2 ± 8.6 | 181.0–191.5 | | | | | |
| | Untrained | PW | 184.6 ± 15.7 | 176.3–192.1 | .97 | .07 | | | |
| | | HW | 183.4 ± 15.2 | 175.8–191.8 | | | | | |
| TTE (s) | Trained | PW | 880.0 ± 207.8 | 760.0–1013.3 | .98 | .05 | .859 | **0.01** | .861 |
| | | HW | 890.0 ± 212.1 | 770.0–1030.0 | | | | | |
| | Untrained | PW | 583.8 ± 176.8 | 491.5–685.3 | .98 | .10 | | | |
| | | HW | 567.6 ± 163.6 | 484.6–660.0 | | | | | |
| Lactate (mmol/L) | Trained | PW | 12.2 ± 3.2 | 10.3–14.2 | .96 | .33 | .511 | .495 | **.043** |
| | | HW | 11.1 ± 3.9* | 8.8–13.5 | | | | | |
| | Untrained | PW | 12.9 ± 3.0 | 11.3–14.5 | .85 | .15 | | | |
| | | HW | 12.4 ± 3.8 | 10.5–14.5 | | | | | |
| RPE | Trained | PW | 8.2 ± 0.9 | 7.6–8.8 | .47 | .26 | .833 | .500 | .339 |
| | | HW | 8.4 ± 0.7 | 8.0–8.9 | | | | | |
| | Untrained | PW | 8.3 ± 0.6 | 8.0–8.6 | .47 | .38 | | | |
| | | HW | 8.0 ± 1.0 | 7.6–8.4 | | | | | |

Note: *. Significance of simple effect between PW and HW in the group of trained cyclists. PW: Placebo water; HW: Hydrogen rich water; VT2 %VO$_2$max: Percentage of maximal oxygen uptake in the ventilatory anaerobic threshold; HR: Heart rate; TTE: Time to exhaustion; RPE: Rated perceived exertion.

subjects. Previous studies have shown that the antioxidant capacity of the organism can be influenced by the status training [20, 29], so the magnitude of the effect of the HRW could also be influenced by this variable.

Recently Botek, Krejčí [17] concluded that the ergogenic effect of acute HRW intake on performance in a 4.2-km up-hill race is determined by the athlete performance level, noting that performance improved in the 4 slowest athletes, and remained without significant improvement in the 4 fastest athletes. This result is totally contrary to those obtained in our study, in which we observed that the intake of HRW only had an ergogenic effect in the trained subjects (with a VO$_2$max greater than 60 ml/min*kg). It is difficult to contrast the results between the two studies because the protocol used and the HRW were very different. In the aforementioned study, an acute intake of HRW was carried out for a short period of 24 hours before the race (in the present research, a 7-day intake was used), and most decisively, a bottled HRW without nano- or micro-bubbles with an H$_2$ concentration of only 0.9 ppm was used (in our study, HRW nano-bubbles with a concentration of 1.9 ppm were used). In this vein, the effectiveness of hydrogenated water will depend on the type of HRW used, the way of administration and the duration of the intervention.

The performance improvement in trained cyclists after HRW ingestion occurred with a moderate magnitude of the effect during the

**TABLE 2.** Values obtained during the maximal anaerobic test

| Variables | Training status | Protocol | Mean ± SD | 95%CI | ICC | Effect size (d) | Main effect Protocol (p-value) | Main effect Training (p-value) | Interaction effect Training x Protocol (p-value) |
|---|---|---|---|---|---|---|---|---|---|
| Peak Power (Watts) | Trained | PW | 766.2 ± 125.6 | 691.4–845.2 | .97 | .51 | .484 | **.001** | **.046** |
| | | HW | 826.5 ± 143.4* | 743.2–916.3 | | | | | |
| | Untrained | PW | 625.6 ± 145.9 | 549.9–706.9 | .77 | .08 | | | |
| | | HW | 613.6 ± 154.4 | 529.7–696.7 | | | | | |
| Mean Power (Watts) | Trained | PW | 350.0 ± 53.5 | 319.2–383.6 | .94 | .51 | .303 | **.001** | **.047** |
| | | HW | 380.2 ± 71.3* | 342.3– 426.9 | | | | | |
| | Untrained | PW | 272.6 ± 78.3 | 233.5–313.5 | .68 | .08 | | | |
| | | HW | 277.0 ± 53.7 | 247.7–307.7 | | | | | |
| FI (%) | Trained | PW | 77.6 ± 5.8 | 73.8–80.9 | .92 | .45 | .732 | **.018** | **.049** |
| | | HW | 75.1 ± 5.9* | 71.4–78.5 | | | | | |
| | Untrained | PW | 80.6 ± 8.9 | 76.3–85.1 | .91 | .10 | | | |
| | | HW | 81.4 ± 9.6 | 76.9–86.5 | | | | | |
| HRmax (bpm) | Trained | PW | 179.6 ± 10.7 | 172.2–185.6 | .93 | .00 | .866 | .631 | .703 |
| | | HW | 179.6 ± 12.0 | 171.8–186.8 | | | | | |
| | Untrained | PW | 175.9 ± 26.0 | 159.8–186.8 | .91 | .13 | | | |
| | | HW | 172.5 ± 39.4 | 148.1–190.0 | | | | | |
| Lactate (mmol/L) | Trained | PW | 13.0 ± 3.4 | 10.8–15.0 | .91 | .22 | .773 | .852 | .265 |
| | | HW | 12.2 ± 4.4 | 9.4–14.7 | | | | | |
| | Untrained | PW | 12.6 ± 2.4 | 11.3–13.8 | .49 | .25 | | | |
| | | HW | 12.1 ± 1.7 | 11.2–13.0 | | | | | |
| RPE | Trained | PW | 8.3 ± 1.4 | 7.5–9.2 | .69 | .43 | .264 | .227 | .178 |
| | | HW | 8.8 ± 0.9 | 8.4–9.3 | | | | | |
| | Untrained | PW | 8.6 ± 1.0 | 8.1–9.1 | .49 | .24 | | | |
| | | HW | 8.8 ± 0.7 | 8.4–9.2 | | | | | |

Note: *. Significance of simple effect between PW and HW in the group of trained cyclists. PW: Placebo water; HW: Hydrogen rich water; FI: Fatigue index; HR: Heart rate; RPE: Rated perceived exertion.

anaerobic test, achieving an increase in PP and MP, and a decrease in FI. However, no aerobic improvement was observed in the incremental $VO_2$ test, except for a lower lactate concentration after HRW. These results are similar to those observed in a previous study in which the PP did not decrease during a repeated sprint protocol after using HRW for 2 weeks, but it did after control water intake [15]. Aoki, Nakao [14], although with a different protocol in footballers on an isokinetic machine, also observed that the decrease in peak torque and blood lactate level were lower during the HRW protocol. The alteration of the redox balance, as well as the accumulation of metabolites and $H^+$ that are produced in the muscle fibre during intense exercise, cause a decrease in the contractile capacity of the muscle and a loss of performance [4, 30]. Although the molecular mechanisms involved in the efficacy of HRW have to be further investigated, ingesting this type of water seems to have an anti-fatigue effect, suppressing the reduction in biological antioxidant potential, increasing lactate dehydrogenase and glutathione peroxidase activity, and scavenging cytotoxic ROS [7, 19, 31]. Apart from the direct neutralization of ROS, hydrogen appears to activate the endogenous anti-oxidative system by nuclear factor erythroid-2-related factor 2 (Nrf2) system and its haem oxygenase-1 (HO-1) [32]. Additionally, $H_2$ could alter mitochondrial energy metabolism and hormone secretion [33]. In this sense, it has been observed that HRW intake caused up-regulation of ghrelin, a peptide hormone that increases appetite, stimulates the release of growth hormone and helps to maintain lean mass [34], factors that could improve performance during exercise. Even improvements in mood and autonomic nerve function have also been observed after taking HRW for 4 weeks [35].

Therefore, unlike what happened in untrained subjects, the higher antioxidant and metabolic capacity of trained cyclists, added to the intake of HRW, caused a synergistic effect that allowed them to develop a greater and more lasting effort in anaerobic conditions, reaching higher power levels and lower FI. However, the effects of long-term HRW intake on performance should be specifically evaluated since a decrease in serum ferritin (iron storage/transporter protein in the body) has been observed after inhaling molecular hydrogen for one week [18]. Furthermore, the circulating ghrelin concentration (its levels increase with HRW intake) has been associated with increased body fat [36].

The study had some limitations. Blood samples were not taken to analyse oxidative stress parameters, making it difficult to assess the antioxidant effect of HRW. Methodologically, there was no washout period between the two phases of water supplementation. The HRW was performed only during a period of 7 days; thus it would not be possible to extrapolate these findings to long-term intervention, since alternative or even opposite results could be observed. According to the hormesis theory, an excessive dose of HRW could reverse the positive effects observed with this short-term intervention.

## CONCLUSIONS

The beneficial effects of HRW are mediated by the training status of athletes. Seven-day intake of nano-bubble HRW improved anaerobic performance of trained cyclists, but had no effect on untrained subjects. Therefore, the HRW intake seems to be an effective hydration strategy, although a greater number of studies are necessary to define precisely the duration of intake, the amount of water and the most effective concentration of hydrogen to individually optimize the ergogenic effects of HRW.

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### Conflict of interest declaration

All authors declare no conflicts of interest.

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