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Acute ingestion of hydrogen-rich water does not improve incremental treadmill running performance in endurance-trained athletes

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ABSTRACT

There is emerging evidence that hydrogen-rich water (H₂-water) has beneficial effects on the physiological responses to exercise. However, few studies investigate its ergogenic potential. This randomized, controlled trial examined the effects of H₂-water ingestion on physiological responses and exercise performance during incremental treadmill running. In a double-blind crossover design, fourteen endurance-trained male runners (34±4 years old; 63.1±7.2 kg; 1.72±0.05 m) were randomly assigned to ingest two doses of 290-mL H₂-water or placebo on each occasion. The first bolus was given before six four-minute submaximal running bouts, and the second bolus was consumed before the maximal incremental running test. Expired gas, heart rate (HR), and ratings of perceived exertion (RPE) were recorded; blood samples were collected at the end of each submaximal stage and post maximal running test. Cardiorespiratory responses, RPE, and blood gas indices were not significantly different at each submaximal running intensity (range: 34−91%VO₂max) between H₂-water and placebo trials. No statistical difference was observed in running time to exhaustion (618±126 vs. 619±113 s), VO₂max (56.9±4.4 vs. 57.1±4.7 mL·kg⁻¹·min⁻¹), maximal HR (184±7 vs. 184±7 beat·min⁻¹) and RPE (19±1 vs. 19±1) in the runners between the trials. The results suggest that the ingestion of 290 mL of H₂-water before submaximal treadmill running, and an additional dose before the subsequent incremental running to exhaustion was not sufficiently ergogenic in endurance-trained athletes.

Novelty bullets:

- Acute ingestion of H₂-water does not seem to be ergogenic for endurance performance.
- A small dose of H₂-water does not modulate buffering capacity during intense endurance exercise in athletes.
Keywords:
molecular hydrogen, running economy, lactate threshold, maximal oxygen uptake, running to exhaustion, exercise capacity, ergogenic aid

INTRODUCTION

Hydrogen (H) is a chemical element that consists of one proton and one unpaired electron. The atomic hydrogen rarely exists on its own because of its propensity to form paired electrons in the form of diatomic molecules, also known as molecular hydrogen (H₂). H₂ is the most common form of hydrogen. It is a colorless, odorless, tasteless, non-toxic, non-metallic, lightweight, inert gas that stable with a neutral charge. The landmark study of Ohsawa et al. (2007) provided crucial evidence that H₂ has antioxidant and anti-apoptotic effects that protect the brain against ischemia-reperfusion injury as experimented on the rat. Since the breakthrough in 2007, the beneficial effects of H₂ have been documented for at least 166 disease models, including several human clinical studies, with a predominance of oxidative stress-mediated and inflammatory diseases (Ichihara et al. 2015).

In the light of its clinical application, recent reviews suggest that hydrogen therapy might be an effective and specific innovative treatment for exercise-induced oxidative stress and myofibrillar injury, as well as a potential ergogenic aid in exercise performance (Ostojic 2015; Nicolson et al. 2016; LeBaron et al. 2019a). The rationale for the use of hydrogen therapy in sports science is primarily due to its antioxidant and anti-inflammatory properties, its role related to cell signaling-modulation, cytoprotection (LeBaron et al. 2019a), and buffering capacity in exercise-induced acidosis (Ostojic 2015). The physiological effects of
H₂ in the form of H₂-water (i.e., water with H₂ dissolved in it) ingestion, in response to various exercises in humans, include a reduction in blood lactate (Aoki et al. 2012; Drid et al. 2016; Botek et al. 2019) and an increase in blood pH (Drid et al. 2016) and/or bicarbonate (HCO₃⁻) levels (Ostojic 2012; Ostojic and Stojanovic 2014). It also prevents exercise-induced muscle (Aoki et al. 2012) and psychometric (Botek et al. 2019; Mikami et al. 2019) fatigue, enhances recovery of range of motion in injured limbs (Ostojic et al. 2014), and suppresses exercise-induced oxidative stress (Shin et al. 2018). A recent study has also shown that the submaximal exercise HR was lower after ingesting the H₂-producing tablets with water (LeBaron et al. 2019b).

Despite a growing body of evidence supporting the beneficial effects of H₂-water ingestion in physiological responses to exercise, only three studies examined its ergogenic potential in human exercise performance (Drid et al. 2016; Da Ponte et al. 2018; Mikami et al. 2019). First, Drid et al. (2016) observed no difference in judo-specific fitness performance between the H₂-water and placebo trials. Then, Da Ponte et al. (2018) subjected experienced cyclists to a 16-second maximal sprint cycling every three minutes, repeatedly for ten times. They found that peak power output significantly decreased in the placebo trial from the sixth sprint onward but not in the H₂-water trial. Da Ponte et al. (2018) concluded that two weeks of H₂-water intake (2 L·day⁻¹) might help to maintain peak power output in repetitive intermittent sprints to exhaustion over 30 minutes. Recently, Mikami et al. (2019) reported an enhanced endurance capacity (as judged by the automated estimation of maximal oxygen uptake (VO₂max) by using an ergometry program during submaximal cycling) in a cohort of fitness club members following acute ingestion of 500 mL H₂-water. However, they provided no clear performance benefits, such as total cycling time to fatigue in their volunteers.
Well-controlled experimental trials are relatively rare when it comes to those that have examined the ergogenic potential of H\textsubscript{2}-water ingestion on endurance performance, such as exercise tolerance to an incremental running to exhaustion in well-trained individuals. While the research of the ergogenic effect of H\textsubscript{2}-water on athletic performance is still in its infancy, H\textsubscript{2}-containing beverages have been marketed worldwide as “energy drinks” (Park 2017), “nutritional aids” (Ostojic 2012), or “the metabolic beverages” (e.g., https://htwo.com) for health and performance. These beverages are sold in a 230 to 500-mL pouch, and their efficacy as an ergogenic aid in endurance performance has not been validated. The emergence of exaggerative claims and false advertisement from the flourishing industry of hydrogen products have alerted the leading scientists in the field to call for research to be focused on clinical evidence of hydrogen medicine (Iida et al. 2016; Zeng et al. 2019). The increasing interest in H\textsubscript{2}-water consumption, coupled with anecdotal accounts of performance boosts despite insufficient scientific evidence has created a need to examine the potential benefits of H\textsubscript{2}-water ingestion on athletic performance.

Therefore, we conducted this study to determine whether prior ingestion of H\textsubscript{2}-water would be ergogenic for submaximal (oxygen cost of running, cardiovascular strain, lactate threshold, self-perceived exertion) and maximal incremental treadmill running performance (running time to exhaustion, VO\textsubscript{2}\textsubscript{max}) in endurance-trained athletes. We hypothesized that acute ingestion of H\textsubscript{2}-water would improve submaximal and maximal incremental treadmill running performance in endurance-trained athletes.

**MATERIALS AND METHODS**

**Participants**
Fourteen endurance-trained male runners/triathletes consented to participate in this study, which was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (Study Protocol Approval Code: USM/JEPeM/15100446). Means ± standard deviation (SD) for age, body mass, standing height, body mass index, and percentage of body fat of the participants were: 34 ± 4 years old, 63.1 ± 7.2 kg, 1.72 ± 0.05 m, 21.4 ± 1.9 kg·m\(^{-2}\), and 12.9 ± 3.8%, respectively. The participants reported to be physically active 5 ± 1 day·week\(^{-1}\) and engaged in endurance exercise training for 9.5 ± 4.6 hour·week\(^{-1}\) during the study.

**Study Design**

Participants visited our laboratory for a total of four occasions with at least seven days apart. The first visit consisted of medical examination and health screening, followed by a familiarization trial. The familiarization trial was repeated in the second visit to minimize the carry-over of any practice effects on the actual experimental trials. For the third and fourth visits (the experiment trials), participants ingested an acute dose of 290 mL of either H\(_2\)-water or placebo within 5 to 10 minutes before submaximal running test and once more before maximal treadmill running in a randomized, double-blind, crossover design (Figure 1A). Participants were asked to prepare themselves for the experimental trials as if they were preparing for their race events. Seven-day intervals were allowed between trials for the participants to plan for recovery and training days. The single-dose H\(_2\)-water ingestion before and during exercise was substantiated by the previous studies (e.g., 1 500 mL in Aoki *et al.* 2012; 300 mL in Drid *et al.* 2016; 1 000 mL in Shin *et al.* 2018; 600 mL in Botek *et al.* 2019; and 500 mL in Mikami *et al.* 2019). This supplementation strategy denotes practicality during sporting events in a real-life situation. Many ready-to-drink beverages were acclaimed to
have contained as high as 2.60 ppm of dissolved H$_2$ and are marketed as a health care product as well as an ergogenic aid. These H$_2$-water are sold in a 230 to 500-mL pouch, and their efficacy as an ergogenic aid in endurance performance is still questionable.

**Preparation of H$_2$-water and Placebo**

An investigator who was not involved in the data collection and analysis prepared fresh H$_2$-water before the experiments by using an electrolysis device (SUSO Pure Hydrogen Maker, Seoul, Korea), at a room temperature of ~23°C and a barometric pressure of ~756 mmHg. In brief, the hydrogen maker was filled with approximately one liter of distilled water, and the device was switched on to run the electrolysis for 30 minutes. The distilled water (i.e., H$_2$-water) in the hydrogen maker was then slowly poured into two 290-mL glass bottles until they were full, and the lids were tightly closed immediately. The concentration of H$_2$ was measured by immersing the sensor of a portable H$_2$ meter (Trustlex ENH-1000, Tokyo, Japan) into ~50 mL of H$_2$-water separately, and the reading was recorded after one minute. The placebo consisted of the same distilled water used in preparing the H$_2$-water without the 30-minute electrolysis process. Both H$_2$-water and placebo are identical in appearance, texture, and taste in the glass bottles.

**Preliminary Screening and Familiarization Trials**

During the first two visits, we familiarized the participants with the testing equipment and experimental protocol. Upon arrival at the laboratory, participants were void, and standing height was recorded. Body mass and percentage of body fat were determined by using an air displacement plethysmography (Bod Pod® Body Composition Tracking System, California,
USA). After that, participants completed a six-stage submaximal running test with a starting speed of 6 km∙h\(^{-1}\) and a slope of 0% on a treadmill (h/p/cosmos pulsar® 3p, München, Germany). Treadmill speed was increased by 2 km∙h\(^{-1}\) after every four minutes and interspersed by one-minute recovery at 4 km∙h\(^{-1}\). The test was then followed by 5 to 10 minutes of rest interval. Then, participants undertook a maximal incremental treadmill running to exhaustion test at a constant treadmill speed of 12 km∙h\(^{-1}\). The slope of the treadmill, however, was increased by 2% every two minutes until the participants were unable to continue. The total time of volitional running to exhaustion for each participant was recorded to the nearest second.

Expired gas of the participants was collected continuously throughout the treadmill running tests for the analysis of minute ventilation (\(V_E\)), oxygen uptake (\(V_O_2\)), and carbon dioxide production (\(V_CO_2\)) via a face mask (Hans Rudolph Inc., Kansas, USA) connected to a metabolic measurement system (Parvo Medics TrueOne 2400, Utah, USA). During the final 30 seconds of each submaximal running stage, HR was recorded by a heart rate monitor (Polar RS800CX, Kempele, Finland) and RPE was determined by Borg’s category rating scale (Borg 1982). \(V_O_2_{max}\) was taken as the highest 60-second average values attained before the participants’ volitional exhaustion.

**Experimental Trials and Procedures**

On the third and fourth visits, exercise training and dietary control were implemented by requiring the participants to keep their training schedule consistent for the last three days and dietary intake for the last 24 hours, as indicated in the pre-test questionnaire. Participants were reminded to prepare themselves for the treadmill running to exhaustion tests as if they...
were physically and mentally preparing for race events. They were also asked to refrain from strenuous exercise training and consumption of alcohol 24 hours before the trials. After the compliance check, participants completed the body composition measurements and were cannulated via a left antecubital vein.

Approximately 5 to 10 minutes before the commencement of submaximal and the subsequent maximal treadmill running test, participants ingested 290 mL of either \( \text{H}_2 \)-water (1.02 ± 0.05 ppm) or placebo (distilled water, the concentration of \( \text{H}_2 \) undetected) within one to two minutes to minimize the reduction in \( \text{H}_2 \) dissolution after opening the glass bottles (Figure 1B). This procedure ensured that the level of \( \text{H}_2 \) peaked within 5 to 10 minutes and remained above baseline in the body for 30 to 40 minutes after drinking the \( \text{H}_2 \)-water (Ito et al. 2012; Shimouchi et al. 2012; Mikami et al. 2019). Both participants and the investigator were not aware of whether the drinks prepared in the bottles were \( \text{H}_2 \)-water or placebo until the participants had completed all the experimental trials. Participants then performed the treadmill running bouts to which they have been familiarized with during the first two visits.

Blood samples were drawn while participants were at rest on the treadmill before the ingestion of \( \text{H}_2 \)-water or placebo, during the one-minute recovery period of the submaximal running stages, and within five minutes at the exhaustion/termination of the maximal running test. Whole blood samples of ~0.5 mL were collected into a heparinized syringe (Smiths Medical, Minnesota, USA); blood lactate, glucose, \( \text{HCO}_3^- \), and pH were determined immediately by using a blood gas analyzer (GEM Premier 3000 Instrumentation Laboratory, Massachusetts, USA). Another ~1 mL of blood samples were allowed to clot for an hour at room temperature prior to centrifuging at 2 700 rpm at 4°C for 10 minutes (Dynamica Velocity 18R Refrigerated Centrifuge, Livingston, UK). The serum were then stored at
−20°C for subsequent analysis of post maximal exercise lactate concentration in duplicate (YSI 1500 Sport L-Lactate Analyzer, Ohio, USA).

Running economy was considered to be the steady-state VO$_2$ for a treadmill running speed of 14 km·h$^{-1}$ expressed in mL·kg$^{-1}$·km$^{-1}$, and was calculated as “VO$_2$ (mL·kg$^{-1}$·min$^{-1}$) × Pace (min·km$^{-1}$)”, with the pace at 14 km·h$^{-1}$ was determined as 4.29 min·km$^{-1}$ (Lucia et al. 2006). By plotting each participant’s blood lactate against submaximal treadmill running speeds on the third-order polynomial curve, the lactate threshold was determined as the speed at the onset of blood lactate accumulation (OBLA) of 4 mmol·L$^{-1}$ (Sjödin and Jacobs 1981). The corresponding %VO$_{2}$max and %HR$_{max}$ of running economy and OBLA were determined from the linear regression relationship between the participant’s VO$_2$ and HR against submaximal treadmill running speeds.

All experimental trials were conducted at the same time (± 1 hour) of the day, approximately 2 to 4 hours postprandial (i.e., after breakfast or lunch), in a climate chamber that was maintained at 22°C and 40% relative humidity. At the completion of each trial, participants were asked whether they experienced any adverse effects, such as headaches, diarrhea or stomach upsets, or a feeling of vomiting.

**Statistical Analysis**

Statistical analysis was carried out using IBM SPSS Statistics Version 24 software (IBM, Illinois, USA). A two-way repeated-measures ANOVA (treatment × time as factors) was used to compare the differences of all submaximal treadmill running physiological measures and blood variables between the trials over time. All data were assessed by the Shapiro-
Wilk’s test for normality on the studentized residuals ($p > 0.05$). The sphericity of the data was verified by the Mauchly’s test. The Greenhouse-Geisser analysis was applied when the assumption of sphericity was not met. A paired $t$-test was used to compare the differences in data collected during the maximal treadmill running test. Within-subject typical error of measurement as a coefficient of variation (CV, expressed as means and 95% confidence limit) for total time to exhaustion and VO$_{2\text{max}}$ between the trials was estimated by using log-log modelling (Hinckson and Hopkins 2005). All data are presented as mean ± SD, the level of significance for all analyses was set at $p < 0.05$, and $n = 14$ unless stated otherwise.

**RESULTS**

None of the participants were able to distinguish the difference between H$_2$-water and placebo drinks. They also reported no side effects with the ingestion of H$_2$-water. There was no apparent difference in the pre-trial 72-h training schedules and 24-h dietary intake between the experimental conditions. There was also no difference in the changes in body mass before the treadmill running bouts (H$_2$-water: 63.0 ± 7.1 kg vs. placebo: 63.2 ± 7.6 kg), indicating that the participants were adequately hydrated before the trials.

The oxygen cost of the running or running economy at various submaximal treadmill speeds did not change with the H$_2$-water treatment compared to placebo (**Figure 2A**). The six different submaximal running speeds corresponded to 34 ± 7% and 35 ± 6%, 47 ± 6% and 47 ± 4%, 57 ± 6% and 57 ± 6%, 68 ± 8% and 69 ±6%, 80 ± 8% and 81 ± 7%, 91 ± 6% and 90 ± 6% of the participants’ VO$_{2\text{max}}$, in H$_2$-water and placebo trials, respectively. Other submaximal exercise responses such as respiratory exchange ratio (RER), HR, and RPE were also similar between H$_2$-water and placebo trials (**Figure 2B–D**). Blood lactate, glucose,
HCO$_3^-$, and pH responses to each submaximal running intensity did not differ between the H$_2$-water and placebo trials (Figure 3A–D). The blood samples of one participant were not collected during one of the trials due to technical difficulty and time constraints. Consequently, the data analysis of submaximal running blood gas responses in Figure 3 and Table 1 included only 13 participants.

Acute H$_2$-water ingestion has no significant effect on incremental treadmill running to exhaustion performance (H$_2$-water: 618 ± 126 s vs. placebo: 619 ± 113, Figure 4). The CV for total time to exhaustion between the H$_2$-water and placebo trials was 5.2% (95% confidence limit: 3.7–8.5%). Running economy, lactate threshold, maximal aerobic capacity, and blood gas indices of these endurance-trained athletes during the incremental treadmill running tests were similar for both trials (Table 1). The serum samples of two participants were excluded from lactate analysis due to technical constraints. Therefore, only 12 participants were included for data analysis of post maximal running serum lactate concentration in Table 1. The CV for $\dot{V}O_{2max}$ between the H$_2$-water and placebo trials was 1.4% (95% confidence limit: 1.0–2.3%).

DISCUSSION

In this study, we demonstrated that two doses of 290-mL H$_2$-water each consumed before submaximal and the subsequent maximal exercise, did not have an ergogenic effect on the physiological factors that played the determinant roles in human endurance exercise performance. Contrary to the initial hypothesis, we observed no performance enhancement in incremental treadmill running time to exhaustion, following the ingestion of H$_2$-water compared with a similar amount of placebo in endurance-trained athletes. While some of the
previous studies (Ostojic 2012; Ostojic and Stojanovic 2014; Da Ponte et al. 2018) used 7 to
14 days of supplementation regime, our study explored only the ergogenic potential of an
acute dose of H2-water ingestion.

**Effects of H2-water on Steady-state and Maximal Exercise Response**

Ingestion of H2-water did not reduce the O2 cost of submaximal running, and this was
corroborated by no changes in RER, HR, or RPE throughout the exercise. These data of VO2
and RER, except RPE and HR, are in line with the previous studies of acute H2-water (Botek
et al. 2019; Mikami et al. 2019) and 5-mg H2-producing tablets (LeBaron et al. 2019b)
ingestion. However, these studies did not provide clear and convincing evidence for the RPE
and HR-lowering effect of H2-water ingestion during submaximal exercise. Mikami et al.
(2019) only reported a graphical representation of a slight but significant difference in the
change of post-exercise HR (500-mL H2-water: −1 ± 0.6 vs. placebo: +1 ± 0.6 bpm) between
the trials. Meanwhile, LeBaron et al. (2019b) reported mean treadmill exercise HR during the
H2-treated trial was 4 and 5 bpm lower (p < 0.001) than the baseline test and placebo trial,
respectively. Interestingly, mean exercise HR during the baseline test was 2 bpm lower (p =
0.008) than the placebo trial. On the other hand, Botek et al. (2019) reported a lower RPE
with acute 600-mL H2-water ingestion (18.5 ± 0.8 vs. 17.8 ± 1.2). However, they
acknowledged that practical consideration of the differences in RPE at ~91% of VO2max
cycling intensity in their study should not be overestimated. These findings were recorded in
the recreationally active men and women, which does not accurately reflect the demographics
in our study of endurance-trained male athletes.
In contrast to our results on the submaximal running blood lactate following H\textsubscript{2}-water ingestion, Aoki et al. (2012) reported reduced blood lactate at the end of 30-minute fixed-load cycling at 75% of VO\textsubscript{2max}. Botek et al. (2019) also observed lower blood lactate during the 8\textsuperscript{th} minute of cycling at 73% and 91% of VO\textsubscript{2max}. The participants in our study, however, ran for a total of 24 minutes across six different submaximal treadmill speeds that corresponded to an average of ~63% (range: 34–91%) of their VO\textsubscript{2max} during the trials. Aoki et al. (2012) supplemented their soccer players (VO\textsubscript{2max} = 53.2 ± 4.9 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}) with 1500 mL of H\textsubscript{2}-water (1.86–2.06 ppm) within 8 hours before the exercise trial, while we gave our runners (VO\textsubscript{2max} = 57.9 ± 4.1 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}) 290 mL of H\textsubscript{2}-water (1.02 ± 0.05 ppm) 5 to 10 minutes before each submaximal and maximal exercise bout for a total of 580 mL in volume before exercise. Similarly, Botek et al. (2019) gave their student participants (VO\textsubscript{2max} = 51.1 ± 6.1 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}) 600 mL of H\textsubscript{2}-water before the stepwise incremental cycling test (300 mL 30 minutes before and 300 mL one minute before; 0.5 ppm). Therefore, it is unlikely that the much higher dosage of H\textsubscript{2}-water supplementation in the study of Aoki et al. (2012) could have contributed to this discrepancy in steady-state exercise blood lactate response. Further investigation is required to determine the dose-response of the H\textsubscript{2}-water consumption on blood lactate response during steady-state exercise, in relation to the physical training history of the participants.

In our study, physiological responses at the termination of the maximal incremental running did not differ between the H\textsubscript{2}-water and placebo trials (Table 1). We are not alone in failing to observe the effect of acute H\textsubscript{2}-water ingestion on maximal exercise response. Other studies also reported no significant changes in VO\textsubscript{2max} (LeBaron et al. 2019b; Mikami et al. 2019), HR\textsubscript{max} (Drid et al. 2016), RER (LeBaron et al. 2019b), and RPE (Mikami et al. 2019) following maximal all-out intermittent or continuous exercises. However, Aoki et al. (2012)
reported a reduction in blood lactate following submaximal cycling and 100 repetitions of maximal isokinetic knee extensions at a constant velocity of $90^\circ \text{s}^{-1}$ (for ~3.5 minutes) with H$_2$-water ingestion. On the other hand, Drid et al. (2016) showed lower post-exercise blood lactate after ~75 seconds of maximal intermittent throwing in the judo-specific fitness test in the H$_2$-water trial. The discrepancy between these results is not entirely clear, and further investigation is warranted. Although some reporting and interpretation of the maximal aerobic capacity (LeBaron et al. 2019b; Mikami et al. 2019) were equivocal, the overall results suggest that H$_2$-water or H$_2$-producing tablets (when consumed with water) might not be an effective supplementation in improving the maximal aerobic capacity and mechanical efficiency in human.

**Effects of H$_2$-water on Endurance Exercise Performance**

Performance enhancement was not observed in incremental treadmill running to exhaustion following acute H$_2$-water ingestion. This observation is supported by our findings that acute H$_2$-water ingestion did not improve the key physiological determinants of endurance exercise performance, namely VO$_{2\text{max}}$, running economy, and lactate threshold. Hence, it is rational to find that H$_2$-water ingestion did not prolong the total time to exhaustion in the present study. To our knowledge, only one study had indicated the ergogenic potential of acute H$_2$-water ingestion. Mikami et al. (2019) reported an improvement in endurance capacity, as judged by estimated VO$_{2\text{max}}$ following 500 mL of H$_2$-water ingestion. It should be noted that the VO$_{2\text{max}}$ was “automatically” estimated by an in-built program of a cycle ergometer. This took place during the 11-minute submaximal cycling, based on age-predicted maximal HR (measured by the ear sensor attached to the ergometer) of the volunteers. Interestingly, when the peak VO$_2$ was determined by a metabolic measurement system in a graded treadmill exercise to
exhaustion using Bruce Protocol, no difference was observed between the H\textsubscript{2}-producing tablets and placebo-treated volunteers (LeBaron \textit{et al.} 2019\textit{b}). These findings of endurance capacity, as judged by the VO\textsubscript{2max}, are challenging to interpret due to the ambiguous reporting of the data (i.e., only estimated marginal means statistics or relative changes were provided), as well as its reliability. Although intriguing, these findings warranted a more robust and rigorous validation of the efficacy of H\textsubscript{2}-water on endurance exercise performance. However, our data do not seem to support the use of acute H\textsubscript{2}-water ingestion as an ergogenic aid.

\textit{Buffering Capacity of H\textsubscript{2}-water during Exercise}

It has been reported that H\textsubscript{2} functions as an alkalizing agent with a blood buffering effect during exercise in physically active and non-athletic individuals (Ostojic 2012; Ostojic and Stojanovic 2014; Drid \textit{et al.} 2016). However, no significant effects of H\textsubscript{2}-water on blood HCO\textsubscript{3}– and pH level were seen in our study, although we adopted a similar incremental running protocol (in duration and intensity) to the previous study (Ostojic and Stojanovic 2014). The aerobic fitness of the participants may have a contributing effect on the efficacy of H\textsubscript{2}-water ingestion. In fact, the VO\textsubscript{2max} of the participants in our study was \textasciitilde{}9\% higher than those of male athletes (VO\textsubscript{2max} = 53.2 \pm 6.4 \text{mL\cdot kg\textsuperscript{-1}\cdot min\textsuperscript{-1}}) recruited in the study of Ostojic and Stojanovic (2014). The lack of efficacy of H\textsubscript{2}-water ingestion on blood pH in our study could not be caused by the insufficient loading of H\textsubscript{2} from acute H\textsubscript{2}-water ingestion. The athletes in the study of Ostojic and Stojanovic (2014) received 2 L\textsuperscript{-1} of H\textsubscript{2}-water for 14 days. Their study incorporated a total of 28 000 mL of H\textsubscript{2}-water. In comparison, 580 mL of H\textsubscript{2}-water was used in our study. However, a 300-mL H\textsubscript{2}-water intake significantly increased post-exercise blood pH of the female athletes, in contrast to the results of the
placebo (Drid et al. 2016). Future study should establish an optimal loading protocol for H₂ in the form of H₂-water supplementation as well as its potential application in exercise-induced metabolic acidosis in relation to sex differences.

**Experimental Considerations and Future Directions**

H₂ levels in the blood, urine, and breath samples of the participants were not measured in our study due to financial and technical constraints. Therefore, even though measures were taken to control the dietary intake of the participants, which may influence the H₂ production, e.g., ingestion of milk (Shimouchi et al. 2009), we were unable to control the endogenous production of H₂ in the digestive system. However, it has been shown in a mouse model the process of drinking H₂-water was more effective than the restitution of hydrogenase-positive bacteria that produced H₂ in intestines in ameliorating hepatitis (Kajiya et al. 2009). Future studies examining the effects of H₂-water on exercise response should include the measurement of H₂ level in the breath, urine, blood, or tissue sample of the participants.

Further, the effectiveness of the acute H₂-water ingestion may be dependent on the timing of H₂-water administration (Botek et al. 2019; Mikami et al. 2019). Participants in the previous studies (Drid et al. 2016; Botek et al. 2019; Mikami et al. 2019) started the exercise 10 to 30 minutes after drinking H₂-water, whereas participants in our study started both submaximal and maximal running tests 5 to 10 minutes after the H₂-water ingestion. It could be argued that before producing any effects, the H₂-water needs to be emptied by the stomach and absorbed by the intestine, but exercise slows both of these processes. It is possible that not all H₂-water was integrated into the body of participants during maximal exercise, consequently reducing the effectiveness of acute H₂-water ingestion in our study. Lastly, our study did not incorporate a chronic supplementation strategy in addition to the acute administration of H₂-water.
water. We cannot rule out the ergogenic effects of chronic H₂-water administration (of > 2 weeks), and its relationship to the training stress and adaptation of elite endurance athletes.

**Conclusion**

Prior ingestion of H₂-water in a small amount (i.e., two doses of 290 mL at 1.02 ± 0.05 ppm, each before exercise) did not affect physiological responses of submaximal and maximal incremental running in endurance-trained individuals. Consequently, this acute H₂-water drinking strategy did not improve the performance of incremental treadmill running to exhaustion. The data of our study do not support acute H₂-water ingestion as an ergogenic aid for intense, exhaustive endurance running performance. Hence, consuming H₂-water before or during the endurance events (of ~ 40 minutes) might be of limited practical relevance in well-trained endurance athletes. Future research should strive to establish the optimal dose-response relationship of H₂-water ingestion for mitigating metabolic disturbance during intense exercise, the interaction of long-term H₂-water supplementation (of > 2 weeks) and aerobic fitness on antioxidant capacity and training adaptation in endurance athletes.

**ACKNOWLEDGMENTS**

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES


Park, A. 2017. Is hydrogen water actually good for you [online]? Available from:


Table 1: Running economy, lactate threshold, maximal exercise aerobic capacity, and post maximal exercise blood gas indices of the participants following H$_2$-water and placebo ingestion.

<table>
<thead>
<tr>
<th></th>
<th>H$_2$-water</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running Economy (mL·kg$^{-1}$·km$^{-1}$)</td>
<td>197 ± 12</td>
<td>198 ± 12</td>
</tr>
<tr>
<td>- %VO$_{2\text{max}}$ (%)</td>
<td>81 ± 7</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>- %HR$_{\text{max}}$ (%)</td>
<td>89 ± 5</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>Speed at OBLA (km·h$^{-1}$)$^a$</td>
<td>12.8 ± 1.3</td>
<td>13.1 ± 0.9</td>
</tr>
<tr>
<td>- %VO$_{2\text{max}}$ (%)$^a$</td>
<td>74 ± 6</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>- %HR$_{\text{max}}$ (%)$^a$</td>
<td>84 ± 4</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>56.9 ± 4.4</td>
<td>57.1 ± 4.7</td>
</tr>
<tr>
<td>HR$_{\text{max}}$ (beat·min$^{-1}$)</td>
<td>184 ± 7</td>
<td>184 ± 7</td>
</tr>
<tr>
<td>VE$_{\text{max}}$ (L·min$^{-1}$)</td>
<td>139.5 ± 21.0</td>
<td>138.9 ± 20.6</td>
</tr>
<tr>
<td>RER at VO$_{2\text{max}}$</td>
<td>1.12 ± 0.06</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td>RPE at VO$_{2\text{max}}$</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Serum Lactate (mmol·L$^{-1}$)$^b$</td>
<td>16.6 ± 2.7</td>
<td>16.5 ± 2.6</td>
</tr>
<tr>
<td>Blood Glucose (mmol·L$^{-1}$)$^a$</td>
<td>7.1 ± 0.8</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>Blood HCO$_3^-$ (mmol·L$^{-1}$)$^a$</td>
<td>11.7 ± 2.8</td>
<td>12.3 ± 3.2</td>
</tr>
<tr>
<td>Blood pH$^a$</td>
<td>7.16 ± 0.08</td>
<td>7.16 ± 0.09</td>
</tr>
</tbody>
</table>

Values are mean ± SD; $^a$n= 13, and $^b$n=12; OBLA, onset of blood lactate accumulation at 4 mmol·L$^{-1}$; VO$_{2\text{max}}$, maximal oxygen uptake; HR$_{\text{max}}$, maximal heart rate; VE$_{\text{max}}$, maximal minute ventilation; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; HCO$_3^-$, bicarbonate.
FIGURE CAPTIONS

Figure 1: Schematic overview of (A) the study design and (B) experimental trial procedures.

Figure 2: (A) VO₂, oxygen uptake; (B) RER, respiratory exchange ratio; (C) HR, heart rate; and (D) RPE, ratings of perceived exertion of the participants during submaximal running at various treadmill speeds. Values are expressed as mean ± SD.

Figure 3: (A) Blood lactate, (B) glucose, (C) HCO₃⁻, and (D) pH of the participants during submaximal running at various treadmill speeds. Values are expressed as mean ± SD, n = 13.

Figure 4: Total time to exhaustion for maximal incremental treadmill running performance. Values are expressed as mean ± SD.
A

Endurance-trained Participants (n = 14) → Familiarization Trial 1 → Familiarization Trial 2

Randomization → 2 × 290 mL H₂-water → Crossover → 2 × 290 mL H₂-water

Experimental Trial 1

Experimental Trial 2

2 × 290 mL Placebo → 7-day apart → 2 × 290 mL Placebo

B

<table>
<thead>
<tr>
<th>Pre-Test</th>
<th>Submaximal Running at 0% Slope</th>
<th>Rest Interval</th>
<th>Run to Exhaustion at 12 km·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>6 km·h⁻¹</td>
<td>8 km·h⁻¹</td>
<td>10 km·h⁻¹</td>
</tr>
</tbody>
</table>

Legend: ⚫ = blood sampling, recording of HR and RPE

= 290 mL of H₂-water or placebo ingestion
A. \( \dot{V}O_2 \) (mL \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \))

- H\(_2\)-water
- Placebo

B. RER

C. HR (beat \( \cdot \) min\(^{-1} \))

D. RPE
Time to Exhausation (s)

H₂-water  Placebo