Hydrogen Rich Water Improved Ventilatory, Perceptual and Lactate Responses to Exercise

Authors
Michal Botek1, 2, Jakub Krejčí1, Andrew J. McKune2, 3, 4, Barbora Sládečková1, Nenad Naumovski2, 5

ABSTRACT
The potential anti-fatigue and performance benefits of hydrogen rich water (HRW) have resulted in increased research interest over the past 5 years. The aim of this study was to assess physiological and perceptual responses to an incremental exercise protocol after administration of 600 ml HRW within 30 min before exercise. This randomized, double blinded placebo-controlled cross over study included twelve healthy males aged 27.1 ± 4.9 years. The exercise protocol consisted of a 10 min warm-up at 1.0 W.kg\(^{-1}\), followed by 8 min at 2.0, 3.0, and 4.0 W.kg\(^{-1}\), respectively. Cardio-respiratory variables, lactate and ratings of perceived exertion (RPE) were assessed in the last minute of each step. A significantly lower blood lactate was found with HRW (4.0 ± 1.6 and 8.9 ± 2.2 mmol.l\(^{-1}\)) compared to Placebo (5.1 ± 1.9 and 10.6 ± 3.0 mmol.l\(^{-1}\)) at 3.0, and 4.0 W.kg\(^{-1}\), respectively. Ventilatory equivalent for oxygen and RPE exhibited significantly lower values with HRW (32.3 ± 7.2, and 17.8 ± 1.2 points, respectively) compared to Placebo (35.0 ± 8.4, and 18.5 ± 0.8 points, respectively) at 4 W.kg\(^{-1}\). To conclude, acute pre-exercise supplementation with HRW reduced blood lactate at higher exercise intensities, improved exercise-induced perception of effort, and ventilatory efficiency.

Introduction
Molecular hydrogen (H\(_2\)) is the lightest gas and until relatively recently it was considered to be physiologically inert and non-functional [1]. However, Ohsawa et al. [2] were the first to report that H\(_2\) has strong and selective anti-oxidative properties, particularly related to the reduction of cytotoxic hydroxyl free radicals. Additionally, Ohta [1] reported H\(_2\) to be a novel anti-oxidative element that contributes to endogenous anti-oxidative capacity by helping to neutralize both reactive oxidative species (ROS) and reactive nitrogen species (RNS). ROS and RNS are acknowledged as contributing to the development of high number of diseases including cardiovascular, neurodegenerative, metabolic and gastrointestinal diseases [3].
Additionally, the high levels of ROS and RNS are associated with pathological outcomes that include mitochondrial dysfunction and cellular damage [4, 5]. Consequently, these negative effects may contribute to fatigue and delayed recovery in athletes [6]. Several clinical studies have indicated that H₂ acts as an alkalizing [7], anti-apoptotic, anti-allergic, and anti-inflammatory agent [1, 8–10].

Due to the highly flammable property of hydrogen gas (lower flammability limit of 4.0% v/v), one of the easiest and safest methods of hydrogen delivery is via the utilization of hydrogen rich water (HRW) where H₂ is dissolved in the aqueous medium. This can be achieved via several different methods such as water electrolysis [11], infusing H₂ under high pressure directly into water [12], that changes pH of HRW very gently, or by using hydrogen forming capsules where HRW results from chemical reaction of magnesium with water (Mg + 2H₂O -> Mg(OH)₂ + H₂), and pH level of this HRW achieve up to 10 [7].

Due to the potential beneficial effects for athlete performance [13–15], fatigue [16, 17], recovery [18], mood state [19], and a vast range of other innovative exercise science applications [7], research into the utilization of HRW is increasing in popularity among sport scientists. Hydration with HRW before exercise had a positive effect on muscle resistance to exercise-induced fatigue during prolonged intermittent sprints [14] and isokinetic knee flexion [16]. Similarly, Ara et al. [17] showed that using HRW contributed to significant improvements in endurance performance in an animal model. From a scientific perspective, researchers have noted that using HRW improved or maintained a variety of physiological responses to exercise [14, 15], fatigue [16, 17], recovery [18], mood state [19], and a vast range of other innovative exercise science applications [7].

This study was approved by the Ethics Committee of the Faculty of Physical Culture, Palacky University Olomouc (reference number 75/2017) and all participants provided written informed consent in accordance with the ethical standards [23]. In addition, to the best of our knowledge, no side effects during or after the HRW administration have been previously reported [3, 22] or were reported in the present study.

### Experimental protocol

The experimental study protocol consisted of 4 laboratory sessions (Fig. 1). The first session included detailed instructions relating to the experimental procedure as well as familiarization with the testing equipment. The second session occurred the following day and included anthropometric measurements and a graded exercise test to exhaustion. The participants were advised to avoid drinking coffee, tea and/or any other substance potentially affecting the selected physiological and perceptual responses to exercise, for at least two hours before the pre-experimental evaluation and two experimental trials. In addition, participants were also asked to avoid vigorous physical activity and alcohol for 48 hours before all testing. A small standardized meal (one banana taken together with 200 ml of tap water) was ingested by all participants at least 90 min before each exercise. All exercise testing was scheduled between 8:30 and 11:00 AM in a laboratory (room temperature 22–24°C). The third session took place two weeks after the second session. In this session, participants were randomly divided into two groups; HRW or Placebo (n = 6 each). Randomization was performed by means of lots using the equal number of two coloured strips (red and blue). Participants drew only one strip while blinded. The administration of the beverage (HRW or Placebo) was done in two steps; 300 ml at 30 min, and 300 ml at 1 min before the start of the stepwise incremental exercise protocol performed on a bicycle ergometer (ER 900, Ergoline, Bitz, Germany). The fourth session took place after a one week washout with the beverage reversed and the participants performing the same exercise protocol as the third session.

### Basis anthropometric measurement

Participant height and weight (to the nearest 0.1 kg) were measured using a digital weighing scale SOEHNELE 7307 (Leifheit, Nassau, Germany). Percent body fat (%) BF was determined using bio-impedance analysis (InBody 720, InBody, Seoul, South Korea). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

### Materials and Methods

#### Participants

Twelve, healthy, non-smoking sport science students (Table 1) were recruited for the study. 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, Palacky University Olomouc (reference number 75/2017) and all participants provided written informed consent in accordance with the ethical standards [23]. In addition, to the best of our knowledge, no side effects during or after the HRW administration have been previously reported [3, 22] or were reported in the present study.

### Table 1 Characteristics of male participants (n = 12).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.1 ± 4.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.3 ± 7.6</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>183.0 ± 5.6</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>23.1 ± 1.9</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>11.6 ± 2.7</td>
</tr>
<tr>
<td>VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>51.1 ± 6.1</td>
</tr>
</tbody>
</table>

SD – standard deviation; BMI – body mass index; VO₂max – maximal oxygen consumption.

---

**Table 1** Characteristics of male participants (n = 12).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.1 ± 4.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.3 ± 7.6</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>183.0 ± 5.6</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>23.1 ± 1.9</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>11.6 ± 2.7</td>
</tr>
<tr>
<td>VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>51.1 ± 6.1</td>
</tr>
</tbody>
</table>

SD – standard deviation; BMI – body mass index; VO₂max – maximal oxygen consumption.
Graded test to exhaustion

Maximal oxygen uptake (VO₂ max) and maximum heart rate (HR max) were obtained during a stepwise incremental test protocol performed on a bicycle ergometer (ER 900, Ergoline, Bitz, Germany). The exercise protocol consisted of a 6 min warm-up (3 min at 1.0 W.kg⁻¹, and 3 min at 2.0 W.kg⁻¹) where all participants were required to maintain their cadence between 60–70 RPM. This was followed by an increase in cadence to 80–90 RPM with power increased by 25 W.min⁻¹ until voluntary exhaustion. Ventilation and gas exchange were recorded continuously using 30 s averaging and with processing and analyses performed by Blue Cherry software (Geratherm Respiratory, Bad Kissinger, Germany). The criteria for attaining VO₂ max was defined as reaching one of the following: a) respiratory exchange ratio of > 1.11 [24]; b) VO₂ plateau defined as no increase in VO₂ in response to an increase in work rate [25]. VO₂ max was considered the highest VO₂ value in the final 30 s [26]. The HR response was measured continuously using a chest strap (Polar, Kempele, Finland).

HRW and placebo preparation

Both HRW and Placebo were prepared at Palacký University Olomouc, with participants and researchers blinded until after completion of the experiment and statistical analyses of the data. Standard bottled drinking water was used as the base for both the HRW and Placebo. The drinking water bottles, determined either for further preparing HRW or Placebo, were left in the laboratory overnight so that the water could stabilize and reach room temperature. The HRW was prepared from the bottled ‘base’ water using a commercially available generator “TIENS Hyper-H Health Cup” manufactured by Tianjin Tianshi Biological Engineering, Tianjin, China that is available in the European Union commercial market and meets necessary certification regulations. This generator produces H₂ gas through an electrolytic and dissolving process in the water. The freshly prepared HRW was administered immediately to the participants. The physico-chemical properties of both HRW and Placebo (Table 2) were determined using the pH/ORP/Temperature-meter (AD14, Adwa Instruments, Szeged, Hungary). The dissolved hydrogen concentration was determined using H₂Blue reagent (H2 Sciences, Henderson, NV, USA) according to the manufacturer instructions. In addition, there were no additional preparations of the bottled water for the Placebo.

Experimental exercise protocol

The exercise protocol consisted of 34 min of exercise (Fig. 1). The first step served as a 10 min warm up, and was performed at 1 W.kg⁻¹, with all participants required to maintain their cadence at 60–70 RPM. Following, this warm up, steps at 2, 3 and 4 W.kg⁻¹ each lasted 8 min, and participants were asked to maintain their cadence between 70–80 RPM. During the incremental exercise protocol, ventilation and gas exchange were recorded continuously (breath by breath) with 30 s averaging and analyzed by BlueCherry software (Geratherm Respiratory, Bad Kissinger, Germany). Heart rate (HR) was measured continuously using a chest strap (Polar, Kempele, Finland). Blood lactate and 6–20 RPE scale [27] were assessed in the last minute of each 8 min step. Specifically, immediately when RPE was scored, blood lactate was sampled. Before collecting the sample, the finger was cleaned using an alcohol wipe in order to make the area clean and free of sweat. The skin was punctured with a lancet and the first blood drop was wiped away, while the second drop was analysed using a blood analyser Lactate Scout+ (EKF Diagnostics, Cardiff, United Kingdom). The instrument’s accuracy was checked before sampling according to the manufacturer’s guidelines.

Statistical analysis

All data are presented as arithmetic mean ± standard deviation (SD). Statistical significance was set to α = 0.05. Normality and homoscedasticity of data were checked using the Kolmogorov-Smirnov test and the Levene’s test, respectively. The hypothesis of homoscedasticity was rejected for some variables (p > 0.05), therefore the use of ANOVA was not appropriate which was overcome using a set of 3 separate paired Students’ t-tests. One paired t-test was used to evaluate the difference between HRW and Placebo for each exercise intensity. The Bonferroni adjustment (significance level set to
αBon = 0.05/3) was used to control the Type 1 statistical error. The effect size was evaluated using Cohen’s d according the formula $d = m_2 - m_1 / SD_{p}$, where $m_2$ is mean and $SD_p$ is the standard deviation of the differences between HRW and placebo $(\Delta = x_{HRW} - x_{Placebo})$. The following threshold values for the effect size were adopted [28]: < 0.2 (trivial), ≥ 0.2 (small), ≥ 0.6 (moderate), ≥ 1.2 (large), ≥ 2.0 (very large), ≥ 4.0 (extremely large). Statistical analyses were performed using MATLAB 8.4 with Statistics Toolbox 9.1 (MathWorks, Natick, MA, USA).

A sensitivity power analysis was performed using G*Power version 3.1.9.2 software. The calculation was performed using a two-tailed paired t-test with 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 $d = 0.89$.

## Results

The anthropometric and physiological characteristics of participants are presented in Table 1. Changes in body mass between the two experimental exercise protocols were not significant $(\Delta = 0.11 \pm 0.58 \text{kg}, p = 0.528, d = 0.19, \text{trivial effect})$. Therefore, the absolute intensities for both the HRW and Placebo trials were the same. The properties of the HRW and Placebo are presented in Table 2. Considering the H2 concentration, 600 ml of HRW contained 150 μmol of dissolved H2, representing a 1.96 ± 0.19 μmol. kg$^{-1}$ dose relative to body mass. The effects of HRW are displayed in Fig. 2. At the exercise intensity of 4 W.kg$^{-1}$, HRW compared with Placebo significantly reduced blood lactate concentration $(p = 0.006, d = 0.98, \text{moderate effect})$, minute ventilation (VE), $p = 0.011, d = 0.88, \text{moderate effect}$, ventilatory equivalent for oxygen $(VE/VO_2), p = 0.009, d = 0.92, \text{moderate effect}$, and RPE $p = 0.012, d = 0.87, \text{moderate effect}$. For the other variables, namely heart rate, oxygen uptake $(VO_2)$, and respiratory quotient $(RQ)$, no significant differences were found and the effect sizes were predominantly small. At the exercise intensity of 3 W.kg$^{-1}$, HRW significantly reduced lactate $(p = 0.007, d = 0.96, \text{moderate effect})$. However, no significant differences $(p > 0.339)$ between HRW and Placebo were found for the other variables and the effect sizes were primarily small. At the exercise intensity of 2 W.kg$^{-1}$, there were no significant differences $(p > 0.458)$ between HRW and Placebo and the effect sizes were trivial for all variables except for lactate, where there was a small effect size.

## Discussion

The primary aim of this study was to assess the physiological and perceptual responses to a stepwise incremental exercise protocol after consumption of 600 ml HRW. The primary findings of the study were as follows: a) during the 8th minute of exercise at 3 and at 4 W.kg$^{-1}$, there were significantly lower levels of blood lactate for HRW compared with Placebo, b) when HRW was administered, both VE and VE/VO$2$ were significantly lower compared with Placebo in the 8th minute of exercising at 4 W.kg$^{-1}$, and c) RPE was significantly reduced in HRW compared with Placebo in the 8th minute at 4 W.kg$^{-1}$. There were no significant differences between HRW and Placebo for VO$2$, RQ, and HR during any of the exercise intensities.

To the best of our knowledge, this study is the first to show that pre-exercise administration of 600 ml HRW with comparable pH to Placebo elicited a significant reduction (~16%) in blood lactate, specifically in the 8th minute of stage exercise at 3 W.kg$^{-1}$, and at 4 W.kg$^{-1}$. This result could be potentially explained by the effect of H2 on the resynthesis of ATP. During increasing exercise intensities, ATP starts to be resynthesized through the anaerobic breakdown of carbohydrates (glucose and glycogen, respectively) with the final metabolic product, lactic acid, causing an H$^+$ accumulation and a decrease in muscle pH [20]. An increase in muscle acidosis is accepted as an important factor contributing to peripheral muscle fatigue development, because acidosis is associated with the inhibition of both ATP resynthesis and muscle contractions [29]. Lactate production may serve as a biochemical indicator of anaerobic contributions to ATP production [30], however, lactate itself does not cause muscle fatigue [31], and moreover, lactate has been shown to be the preferred energy source for ATP resynthesis in long term exercise [32, 33]. In the context of the current lactate results, it is unclear whether HRW reduced anaerobic ATP production (reflected by lowered lactate) and/or improved mitochondrial aerobic-related lactate oxidation [34]. However, a recent in vitro H2 study [35] reported an increased mitochondrial membrane potential, the enhancement of mitochondrial activity and ATP production, indicating that H2 treatment activates OXOPHOS probably via an excessive calcium signalling pathway [36]. Therefore, it is tenable that in the current study, HRW may have had a stimulating effect on mitochondrial respiration or mitochondrial function performance resulting in either enhanced aerobic ATP production or lactate oxidation.

Recent literature has shown H2 to be a strong and selective antioxidant [2, 17, 37] that may potentially protect mitochondria function against damage induced by ROS, specifically, against the hydroxyl free radical during exercise and/or post-exercise. A study by Ostojic et al. [15] reported significantly lower blood lactate concentration whilst running at a critical speed (~13 km.h$^{-1}$) during maximal exercise in 11 athletes who ingested 11 of HRW 7 days prior the experiment. Unfortunately, detailed information about the HRW properties (dissolved H2 or ORP value) were not reported. In contrast, Da Ponte et al. [14] showed that 21 per day of HRW for 2 weeks, before exercise, improved performance in prolonged repetitive all-out sprints performed on a bicycle in 8 trained male cyclists. Interestingly, there were no significant differences in blood lactate concentration between HRW and Placebo $(p > 0.05)$. The discrepancy in blood lactate concentrations during exercise between our study and Da Ponte et al. [14] may be explained by the differences in type of exercise (continual versus intermittent), also in the level of dissolved H2 and pH in HRW. In our study, the H2 in the HRW was consistently at 0.5 ppm and pH = 7.4, while Da Ponte et al. [14] reported their HRW had dissolved H2 ranging from 0.15–0.45 ppm, and pH = 9.8. Variation in both available dissolved H2 and pH potentially plays a role in its effectiveness. An additional study reported significantly reduced post-exercise lactate level in ten soccer players after consuming 1.5l of HRW $(H_2 = 0.9–1.0 \text{ppm})$ within 8 hours before exercise, specifically 30 min cycling at exercise intensity 75% of VO2max, followed by muscle activity throughout 100 repetitions of maximal knee isokinetic extension [16]. It is well known that increasing the exercise load stimulates minute ventilation to help increase oxygen delivery to contracting mus-
cle and remove the waste product of aerobic metabolism, carbon dioxide [29]. Based on our results, it is evident that HRW administration elicited a significantly lower (~10%) minute ventilation and VE/VO2 at the highest exercise intensity. VE/VO2 is an accepted marker of ventilatory efficiency [20]. We propose that the decreased ventilatory drive during high intensity exercise after HRW supplementation may be due to either enhanced mitochondrial lactate oxidation or lower muscle H+ accumulation, less buffering and hence lower H2CO3 and dissociation to CO2 in the blood, which would typically drive minute ventilation. Based on this result, we propose that HRW intake within 30 min before exercise appears to be beneficial for performance in that it improves ventilatory efficiency, particularly at near maximum exercise intensities.

In addition, our results also indicate a lower RPE score at the end of the highest intensity interval with HRW compared with Placebo. Borg’s RPE should be understood as complex information which is modulated by many physiological variables, including HR rhythm, ventilatory and breathing frequency response, and/or peripheral (muscle and joint), and central fatigue [27]. The lower perceived effort with HRW intake may be related to HRW-induced lower muscle acidosis that consequently induced, throughout lower CO2 production by buffering system, a lower ventilation response. From the central fatigue point of view, another advantage of H2 is its abil-
ity to move across the blood-brain barrier [38] where H₂ as signal molecule [7] may also act as a central anti-fatigue and/or the stimulating agent. Based on this, it is tenable that HRW supplementation before exercise may establish subjectively more tolerance to vigorous exercise. On the other hand, we believe that practical consideration of mentioned differences in RPE level should not be overestimated in this case. The findings of our study are in agreement with previous findings by Ostojic et al. [15]. In this study, participants reported a lower RPE during near-maximal effort running after ingesting 11 of an alkaline negative oxidative reduction potential formulation drink per day for seven consecutive days.

From a practical perspective, HRW shows promise as a beneficial hydration strategy for endurance athletes due to its ability to lower lactate and RPE and enhance ventilatory efficiency during moderate to high intensity exercise. From performance point of view, HRW has already been associated with anti-fatigue [17] and ergogenic properties [14, 15].

There remains some limitations and issues regarding HRW application for athletic performance. The dosage of H₂ was constant per person and was not adjusted to body mass. Ostojic [7] indicated that there is no clearly defined dose-response curve and therefore, the optimal dose of H₂ to induce physiological and performance changes remains undetermined. In addition, it has been shown that after 3 min of ingestion of HRW, the blood concentration of H₂ increased 7.5 fold [39], reaching its peak level in breath after 10 min, and then decreased to its baseline level within 60 min [40]. Therefore, it is important to perform research examining the timing of the administration of HRW, before or during the exercise, to ensure performance benefits. Another limitation of this study was the low sample size (n = 12).

In conclusion, based on the findings of this study, HRW consumption of 600 ml in total before exercise (300 ml 30 min before and 300 ml 1 min before) decreases blood lactate concentration especially at higher exercise intensities together with improved exercise-induced strain perception, and ventilatory efficiency in healthy, young males.

Acknowledgements

This work was supported in part by grant IGA_FTK_2019_002 from Palacký University Olomouc – Effect of hydrogen rich water on body response during laboratory tests in elite athletes. We thank to Miroslav Žák for helping us to collect data during laboratory testing. A partial results of this study were presented by Michal Botek at a congress of Nutrition Society of Australia in Canberra 2018.

Conflict of Interest

Authors declare that they have no conflict of interest.

References


[32] Brooks GA. Lactate doesn’t necessarily cause fatigue: Why are we surprised? J Physiol 2001; 536: 1


