Drinking Hydrogen Water Enhances Endurance and Relieves Psychometric Fatigue: Randomized, Double-blind, Placebo-controlled Study

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| Complete List of Authors: | Mikami, Toshio; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
Tano, Kohei; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
Lee, Hosung; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
Lee, Hyowon; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
Park, Jonghyuk; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
Ohta, Fumiaki; Nippon Medical School - Musashisakai Campus, Department of Health and Sports Science  
LeBaron, Tyler; Institute for Heart Research, Slovak Academy of Sciences  
Ohta, Shigeo; Juntendo University School of Medicine Graduate School of Medicine, Neurology Medicine |
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Drinking Hydrogen Water Enhances Endurance and Relieves Psychometric Fatigue: 
Randomized, Double-blind, Placebo-controlled Study

Toshio Mikami1*, Kohei Tano2,7, Hosung Lee1, Hyowon Lee1, Jonghyuk Park1, Fumiaki Ohta3, 
Tyler W. LeBaron4,5, Shigeo Ohta6*

1Department of Health and Sports Science, Nippon Medical School, 1-7-1 Kyounan-cho, 
Musashino, Tokyo 180-0023, Japan, 2Fitness club, Asahi Big S Mukogaoka, 1998-1, Noborita, 
Tama-ku, Kawasaki-city, Kanagawa pref., 214-0014 Japan, 3Hydrogen Health Medical 
Laboratory, Co., Ltd., 3-6-1 Machiya, Arakawa-ku, Tokyo, 116-0001, Japan, 4Slovak Academy 
of Sciences, Centre of Experimental Medicine, Institute for Heart Research, Dúbravská cesta 9, 
Bratislava, Slovak Republic, 5Molecular Hydrogen Institute, Uthah, USA, 6Department of 
Neurology Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, 
Bunkyo-ku, Tokyo 113-8421, Japan.

7Present address: Dandelion Gymnagium 104 Gureisu Mukoiugaoka104, 3-1-5 Masugata, 
Tama-ku, Kawasaki-city, Kanagawa-pref., 214-0032, Japan

*Corresponding authors.

Shigeo Ohta, Department of Neurology Medicine, Juntendo University Graduate School of 
Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan E-mail; ohta@nms.ac.jp 
Tel and Fax; +81-44-434-2336

Toshio Mikami, Department of Health and Sports Science, Nippon Medical School, 1-7-1 
Kyouan-cho, Musashino, Tokyo 180-0023, Japan. E-mail; mikami@nms.ac.jp 
Tel; +81-422-34-3394. Fax; +81-422-34-3451
ABSTRACT

Acute physical exercise increases reactive oxygen species in skeletal muscle, leading to tissue damage and fatigue. Molecular hydrogen (H₂) acts as a therapeutic antioxidant directly or indirectly by inducing anti-oxidative enzymes. Here, we examined the effects of drinking H₂ water (H₂-infused water) on psychometric fatigue and endurance capacity in a randomized, double-blind, placebo-controlled fashion. In experiment 1 all participants drank only placebo water in the first cycle ergometer exercise session, and for comparison they drank either H₂ water or placebo water 30 min before exercise in the second examination. In these healthy non-trained participants (n=99), psychometric fatigue judged by visual analogue scales (VAS) was significantly decreased in the H₂ group after mild exercise. When each group was divided into two subgroups, the subgroup with higher VAS values was more sensitive to the effect of H₂. In experiment 2, trained participants (n=60) were subjected to moderate exercise by cycle ergometer in a similar way as in experiment 1, but exercise was performed 10 min after drinking H₂ water. Endurance and fatigue were significantly improved in the H₂ group as judged by maximal oxygen consumption and Borg’s scale, respectively. Taken together, drinking H₂ water just before exercise exhibited anti-fatigue and endurance effects.

Keywords: Borg’s scale, cycle ergometer, endurance, exercise, fatigue, hydrogen, hydrogen water, randomized clinical trial, VO₂ max, visual analogue score.

Introduction

Acute and intense exertion during acute physical exercise results in an increased production of reactive oxygen species (ROS) in skeletal muscle, leading to oxidative...
stress-related tissue damages, microinjury, inflammation, muscle weakness, and fatigue. Dietary antioxidant supplementation can reduce ROS levels and muscle fatigue, as well as enhance exercise recovery (Steinbacher and Eckl 2015; He et al. 2016). On the other hand, various health benefits from regular exercise are mediated by exercise-induced ROS, and can be negated via conventional antioxidant supplementation (Niess and Simon 2007; Merry and Ristow, 2016). Thus, it is important to reduce oxidative stress without impairing important ROS signaling.

Molecular hydrogen (H$_2$) was considered to be a nonfunctional inert molecule in our body for a long time; however, we overturned this concept by demonstrating that H$_2$ acts as an antioxidant (Ohsawa et al. 2007). H$_2$ has several advantages with extensive effects: H$_2$ rapidly diffuses into tissues and cells, and it is mild enough to neither disturb metabolic redox reactions nor to affect signaling ROS such as H$_2$O$_2$; therefore, there should be little or no adverse effects of H$_2$. There are several methods to administer H$_2$; inhaling H$_2$ gas, drinking H$_2$-dissolved water (H$_2$ water), injecting H$_2$-dissolved saline (H$_2$ saline), taking an H$_2$ bath, or dropping H$_2$ saline into the eyes (Ohta 2014; Ohta, 2015). The numerous publications (more than 1,000) on its biological and medical benefits have revealed that H$_2$ can reduce oxidative stress not only by direct reactions with strong oxidants, but also indirectly by regulating various gene expressions. Modification of free radical chain reaction by H$_2$ may influence signal transduction, which subsequently regulates gene expressions (Iuchi et al. 2016; Kamimura et al. 2016).

In addition to the growing evidence obtained by ~170 animal disease models, ~40 clinical examinations were performed or are under investigation (Nicolson et al. 2016). For example, drinking H$_2$ water improved cognition in subjects with mild cognitive
impairment having a specific genotype (Nishimaki et al. 2018). Moreover, inhalation of hydrogen gas was therapeutic for patients with cerebral (Ono et al. 2017) and cardiac infarction (Katsumata et al. 2017), post-cardiac arrest syndrome (Tamura et al. 2016), and Alzheimer’s disease (Ono H et al. 2018).

As part of the next step in hydrogen medicine, one question is whether H₂ benefits not only sick patients, but also healthy people in their daily life. Administration of H₂ water or H₂ saline decreased oxidative stress and exhibited ant-fatigue effects in mice and horses (Ara et al. 2018; Tsubone et al. 2013; Yamazaki et al. 2015), and elite soccer athletes (Aoki et al. 2012). Here, we investigated the ergogenic and anti-fatigue effects of drinking H₂ water in healthy people performing mild and moderate exercise.

Materials and methods

Approval of the present protocol

The protocols for Experiments 1 and 2 were approved by the ethics committees of Nippon Medical School (Tokyo, Japan) and the committee of Fitness Club, Asahi Big S Mukogaoka (Kawasaki, Kanagawa, Japan) as a usual physical performance test, respectively. The study was registered in the university hospital medical information network (UMIN) as ID: UMIN000029062. Written informed consent was obtained from all participants.

The primary outcomes are the improvement of endurance and psychometric fatigue assessed by maximum oxygen consumption (VO₂ max) and the Borg's scale and/or visual analog scale (VAS), respectively, after bicycle-exercise with an ergometer.

Hydrogen and placebo water
Placebo water and H₂ water were donated by Medisol Inc., (Izumo-city, Shimane, Japan) and Blue Mercury Inc., (Tokyo, Japan) for Experiments 1 and 2, respectively. Placebo water was the same water used in making H₂ water. The H₂ concentration was measured with a hydrogen specific sensor (Unisence, Aarhus N, Denmark). In Experiment 1, immediately before exercise, H₂ water was diluted with the placebo water to make the concentration of 0.8 mg/L (0.8 ppm), and then subjects consumed 500 mL of H₂ water. In Experiment 2, commercial H₂ water (500 mL at 1.0 mg/L (1.0 ppm)) was used without further treatment. In both experiments, the packages of H₂ and placebo water were identical to ensure study blindness.

**Measurement of H₂ in expired gas**

Expired gas from 4 volunteers was collected in a closed plastic bag equipped with a special apparatus for the measurement of expired gas. The H₂ concentration was firstly measured at baseline (7-20 ppm, volume/volume) by gas chromatography (Teramecs, Kyoto, Japan). The volunteers ingested 0.012 mg·kg⁻¹ body weight by drinking 1.2 mg/L (1.2 ppm) H₂ water. Immediately after drinking, their expired gases were collected at the indicated time and the H₂ concentration was measured. The resulting expired levels H₂ due to ingestion of H₂ water was obtained by subtracting the baseline values.

**Protocol of Experiment 1**

NTTcom, Online Marketing Solution Co. Ltd. (Tokyo, Japan) enrolled 131 healthy volunteers between 40-70 years old who: have normal blood pressure <140/90 mmHg, have not consumed H₂ water for at least 3 months, and have no habit of extensive
physical training; however, 18 volunteers cancelled the participation. Blood pressure, weight and height of the participants were examined before exercise, and 9 participants who had blood pressure above 140/90 mmHg were excluded from the study. The remaining 104 participants were divided into two groups by the stratified randomization method followed by dynamic allocation, considering age and sex by a specialist in statistics, Dr. T. Kumagai (Fermlab Inc., Tokyo, Japan). Data of two participants were not available by unknown errors. Furthermore, blood pressures of three participants were above 140/90 mmHg before the second examination and so were excluded from further examination. A total of 99 volunteers (52 H₂ group; 47 placebo group) completed the study. All the participants drank 500 mL of placebo water and the exercise started 30 min after drinking H₂. Participants were subjected to exercise for VO₂ max examination using AeroBike 75XLIII (Konami Sports Club Co., Ltd. Tokyo, Japan) for approximately 10 min with step-wise loading until the heart rate reached 75% of predictive max heart rate (220-age). They were asked Borg’s scale and VAS immediately after the mild exercise. Two weeks after the first examination, the participants drank either 500 mL of H₂ water (0.8 mg/L) or placebo water, and then were subjected to the second identical examination at 30 min after drinking H₂. The differences in each participant between the first and second examinations were calculated and analyzed.

**Protocol of Experiment 2,**

Volunteers who have not consumed H₂-water for at least 3 months were recruited from the members of the Fitness Club, Asahi Big S. In grouping, 12 participants were selected in each age block, 20-29, 30-39, 40-49, 50-59, and 60-69 years old. Each block
included 6 men and 6 women. The participants in each block were divided into either H$_2$ group or placebo group by the permuted block randomization method by Tano, K. The total number of the participants was 60, and all completed the study.

The participants were trained as the fitness club members. Thus, we consider that the members can receive a shorter resting time after drinking water, more intense exercise and shorter period from the first to the second examination. The participants drank 500 mL of placebo water, and 10 min later, were subjected to the examination using a cycle ergometer of Senoh Cordless Bike V70i (Tokyo, Japan) in moderate exercise for 11 min with step-wise increased loading towards the predictive max heart rate (220-age) in accordance with a program equipped with the ergometer, and were asked the Borg’s scale. Because the average body weight in the participants of Experiment 2 was heavier than that in Experiment 1, a slightly higher concentration of H$_2$ water was provided. After 1 week, participants drank either 500 mL of 1.0 mg/mL (1.0 ppm) H$_2$ water or placebo water. Ten min after drinking, the same examinations were performed. The differences in each subject between the first and second examinations were calculated and analyzed.

**Measurement of VO$_2$ max**

VO$_2$ max is the maximum rate of oxygen consumption measured during exercise of increasing exercise intensity. Maximal oxygen consumption reflects the cardiorespiratory fitness of an individual, which is a powerful modifiable determinant of life expectancy and endurance capacity. Instead of direct measurement of the actual oxygen consumption, VO$_2$ max was estimated by the submaximal cycle ergometer protocol in which exercise intensity is progressively increased while monitoring heart
rates with a sensor equipped on the earlobe. VO\textsubscript{2} max values were obtained automatically according to the program equipped with the cycle ergometer as described in previous publications (Akalan et al. 2008; Beltz et al. 2016).

**Borg’s scale**

The rating of perceived exertion (RPE), as measured by the Borg’s rating of perceived exertion scale (RPE scale), is used for quantitative measure of perceived exertion during physical activity. The scale rated exertion on a scale of 6-20 in questionnaires: for example, the number 6 indicates no exertion at all, 9 is very light, 13 is somewhat hard, 17 is very hard, and 20 is maximal exertion (Borg 1982; Buckley and Borg 2011).

**Visual analogue scale**

VAS was used for a psychometric fatigue scale that can be used in questionnaires. When responding to a VAS item, respondents specify their level of psychometric fatigue of exercise by indicating a position along a continuous 10 cm line between two end-points (Reips and Funke 2008; Lee et al. 1991).

**Statistical analysis**

Data is shown as the mean ± the standard error of the mean (SEM) or standard deviation (SD). The statistical significance for the comparison of two groups was evaluated by paired Student’s t-test, and analyses among subgroups were performed by paired one-way analysis of variance (ANOVA) followed by Turkey-Kramer test. $P<0.05$ was considered significant.
Results

Retention of H₂ in the body

We first examined how long H₂ remains in the body after drinking H₂-water by monitoring the H₂ concentration of expired gas. Figure 1 shows that H₂ reached a peak level within 10-20 min, and remained above baseline for 30-40 min. The retention time of H₂ appears longer in a body weight dependent manner (Figure 1).

Experiment 1

Baseline measurements of 99 participants in Experiment 1 are reported in Table 1. No differences between H₂ and placebo groups are observed.

Figure 2A shows no difference in the change of VO₂ max between H₂ and placebo groups. Figure 2B shows a slight but significant difference in the change of heart rate after exercise between H₂ and placebo groups.

Compared to placebo, H₂ resulted in a significant improvement (decrease) in VAS (Figure 2C) and a non-significant improvement (decrease) in Borg’s RPE scale (Figure 2E). When each group was further divided into two subgroups according to their score in the first examination (half of higher or lower), only the subgroups with higher scores showed a significant difference in the change from the first to the second examination (Figure 3D and F). Thus, the effects of H₂ water were best observed in the subgroup in which participants were psychometrically more fatigued.

Experiment 2

Because the subgroup with higher VAS values in Experiment 1 exhibited more
improvement on psychometric fatigue, more intense exercise was considered to be required to elicit the effects of H₂. Baseline measurements of 60 participants from member of the fitness club are shown in Table 1. There were no significant differences in baseline between H₂ and placebo groups (Table 1). The average VO₂ max of the participants in Experiment 2 was 36.2 mL·kg⁻¹·min⁻¹, which is much higher than those in Experiment 1 (P=0.0000017). Thus, more intense exercise could be performed by the participants in Experiment 2.

The H₂ group had a significant improvement (increase) in VO₂ max (increase) and Borg’s RPE (decrease) between the first and second examinations (Figure 3A and C). Figure 3B indicates the relative change in VO₂ max. When we estimated VO₂ max and the Borg's scale in the generation-dependent subgroups from 20s to 60s, there was no significant change (Supplementary Figure 1).

The effects H₂ exhibited similar trends in Experiments 1 and 2, and were more noticeable with greater exercise intensity.

Discussion

This study showed that drinking H₂ water alleviated psychometric fatigue after mild and moderate exercise by bicycle ergometer, and enhanced endurance capacity as judged by measurement of VO₂ max in moderate exercise. A recent publication agrees that acute administration of H₂ provides beneficial effects on exercise (LeBaron et al. 2019). Notably, healthy people usually perform exercise of the intensities that were applied to the current participants in our daily life. Thus, drinking H₂ water is suggested to have beneficial effects for healthy people.

The dwelling time of H₂ was examined by measuring the H₂ concentration in
expired gas. After drinking H₂ water, most H₂ seemed to maintain for 30-40 min. Although more subjects are necessary to obtain a conclusion, there was a trend that the dwelling time depended upon the body weight.

In the present study, we used three methods for evaluations, VO₂ max, Borg’s RPE, and VAS, for evaluating the effects of drinking H₂ water. In mild exercise by untrained participants (Experiment 1), the VO₂ max values were significantly not different between the H₂ and placebo groups possibly because the exercise was too mild for H₂ to elicit a significant change. Additionally, the exercise started at 30 min after drinking H₂ water, when most hydrogen would have been expired as shown in Figure 1. On the other hand, in VAS evaluation, H₂ significantly relieved the psychometric fatigue. Notably, the participants who felt more fatigue were more sensitive to the effects of H₂. It is reported that the VAS performed best in terms of sensitivity and reproducibility for breathlessness and general fatigue (Grant et al. 1999). It is therefore reasonable that Borg’s scale showed only a trend, while VAS showed significant effects of H₂ in the mild exercise experiment.

We considered that because the subgroup with higher VAS (felt more fatigue) in Experiment 1 exhibited the more improvement in psychometric fatigue, more intense exercise might elicit the effects of H₂. In moderate exercise (Experiment 2) by the members of a fitness club, drinking H₂ water significantly improved VO₂ max (increased) and Borg’s scale (decreased).

There are several different factors between Experiments 1 and 2, such as trained or untrained subjects with different intensities of load, different starting time, and different concentration of H₂ water. In addition, compared to Experiment 1, H₂ was at a higher level in the body during exercise in Experiment 2 since participants started the
exercise test 10 min instead of 30 min after drinking H2 water. Therefore, it is difficult to clarify the reasons for the discrepancy. More analyses will be required to confirm the effects of H2; however, the current study strongly suggests that H2 provides beneficial effects during mild and moderate exercise in healthy people.

In this study, we could not provide physiological information to verify the molecular mechanism. Since most H2 was expired from the body prior to exercise as shown in Figure 1, it may be difficult to elucidate that such small amounts of remaining H2 can directly influence oxidative molecules such as hydroxyl radicals in the body especially in Experiment 1. Although the reaction rate constant in an aqueous homogeneous solution between H2 and the hydroxyl radical is too slow to outcompete the more abundant cellular biomolecules for the hydroxyl radicals (Buxton et al. 1988; Wood and Gladwin 2007), the microenvironment inside living cells is different from an aqueous and homogeneous solution. Additionally, considerable amounts of H2 might maintain in the lipid and glycogen phases for a longer period as shown previously (Iuchi et al. 2016; Kamimura et al. 2011). In fact, the decreases in the level of hydroxyl radicals have been reported in many papers in cell cultures (Ohsawa et al. 2007; Yu et al. 2017; Chen et al. 2018), tissues (Oharazawa et al. 2010; Chuai et al. 2012; Igarashi et al. 2016; Zhang et al. 2017), and body (Shimouchi et al. 2012; Shimouchi et al. 2013; Hyspler et al. 2015). Thus, the possibility that the remaining H2 could directly reduce oxidative stress cannot be ruled out.

Recent progress indicates that H2 exerts anti-oxidative and the other functions through the regulation of various kinds of gene expressions (Murakami et al. 2017; Kura et al. 2018; Nogueira et al. 2018). These data are consistent with the notion that H2 plays a key role in decreasing exercise-induced inflammation, oxidative stress, and
cellular stress; however, changes in gene expression may not explain the benefits in the current study because H₂ water was administered shortly before the exercise, which may not provide enough time to modify signal transduction and subsequently regulate gene expression. Thus, it is difficult to obtain the final conclusion how H₂ provides these and other beneficial effects from the aspect of the regulation of gene expression.

A previous report showed a decrease in blood lactate during hard exercise as an effect by drinking H₂ water (Aoki et al. 2012). Therefore, it is possible that H₂ reduced oxidative stress directly or indirectly to suppress the increase in blood lactate during exercise, relieving psychometric fatigue.

Recently, H₂ water was shown to improve mood, anxiety, and autonomic nerve function in normal daily life (Mizuno et al. 2017). This study also supports that H₂ has beneficial effects in the daily life of healthy people.
Conflict of Interest

KT and SO are inventors of a patent under the application, and FO serves as a consultant involved in hydrogen. The others declare no conflict of interest.

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References


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**Table 1. Baseline characteristics of participants**

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BMI: body mass index
BP: blood pressure (mmHg)
HR: heart rate (bpm: beat per minute)
VO₂ max (mL·kg⁻¹·min⁻¹)
VAS: visual analogue scale
Means ± SD are shown.
**Figure legend**

**Figure 1.** Monitoring H$_2$ in the expiration after drinking H$_2$ water.

Four volunteers ingested 0.012 mg·kg$^{-1}$ bodyweight by drinking 1.2 mg/L of H$_2$ water. Expiration was collected in a specific closed plastic bag at the indicated time, and H$_2$ concentration in the expiration was measured by gas chromatography (Teramecs, Kyoyo, Japan). The body weights of the volunteers were ~80 kg (open circle), ~70 kg (closed triangle), ~50 kg (open triangle), and ~45 kg (closed circle). The data was obtained after the subtraction from baseline.

**Figure 2.** Change in each subject between the first (placebo only) and second (H$_2$ or placebo water) examination in Experiment 1.

Participants were subjected to the first (with placebo water) and second (with H$_2$ or placebo water) examinations, and then change in each participant between the two examinations was obtained. The changes of VO$_2$ max (A), heart rate (B), VAS (C) and Borg’s scale (E) in H$_2$ and placebo groups are shown. $P=0.015$, $P=0.046$ (D, F): H$_2$ and placebo groups were divided into two subgroups according to the VAS value (D) or the Borg’s scale (F) that was obtained in the first examination. “Higher” and “Lower” in panels indicate the subgroups of higher (felt more tired) and lower (felt less tired) scores. The changes are shown in the subgroup of VAS (D) and Borg’s scale (F). Mean ± SEM was shown, and *, and ** indicate $P<0.05$, and $P<0.01$, respectively by paired
Student’ t-test (B and C), and paired one-way ANOVA, followed by Turkey-Kramer test (D and F). (B) \( P=0.015 \), (C) \( P=0.046 \), (D) \( **P=0.0084 \), *\( P=0.0063 \), (F) \( **P=0.0084 \)

**Figure 3.** Change in each subject between the first (placebo only) and second (\( \text{H}_2 \) or placebo water) examination in Experiment 2.

Participants were subjected to the first (with placebo water) and second (\( \text{H}_2 \) or placebo water) examinations, and the change in each participant between the two examinations was obtained. The changes of \( \text{VO}_2 \text{max} \) (A), and Borg’s scale (C) in \( \text{H}_2 \) and placebo groups are shown. (B) indicates the relative change. Mean \( \pm \) SEM was shown, and *, and ** indicate \( P<0.05 \), and \( P<0.01 \), respectively by paired Student’s t-test. (A) \( P=0.015 \), (B) \( P=0.019 \), (C) \( P=0.00078 \).

**Captions for Supplementary Material**

**Supplementary Figure 1.** Change in each subject between the first (placebo only) and second (\( \text{H}_2 \) or placebo water) examination in Experiment 2 in generation-dependent subgroups from 20s to 60s. Participants were subjected to the first (with placebo water) and second (\( \text{H}_2 \) or placebo water) examinations, and the change in each participant between the two examinations was obtained. The changes of \( \text{VO}_2 \text{max} \) (upper panel), and Borg’s scale (lower panel) in \( \text{H}_2 \) and placebo groups are shown. There was no significant difference among the subgroups in one-way ANOVA. Note that decreases in the Borg's scale suggest the improvement in the lower panel.
Hydrogen water

Anti-fatigue

Endurance