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ARTICLE

Drinking hydrogen water enhances endurance and relieves psychometric fatigue: a randomized, double-blind, placebo-controlled study[1]

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Abstract: Acute physical exercise increases reactive oxygen species in skeletal muscle, leading to tissue damage and fatigue. Molecular hydrogen (H_2) acts as a therapeutic antioxidant directly or indirectly by inducing antioxidative enzymes. Here, we examined the effects of drinking H_2 water (H_2-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 H_2 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 was significantly decreased in the H_2 group after mild exercise. When each group was divided into 2 subgroups, the subgroup with higher visual analogue scale values was more sensitive to the effect of H_2. 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 H_2 water. Endurance and fatigue were significantly improved in the H_2 group as judged by maximal oxygen consumption and Borg's scale, respectively. Taken together, drinking H_2 water just before exercise exhibited anti-fatigue and endurance effects.

Key words: Borg's scale, cycle ergometer, endurance, exercise, fatigue, hydrogen, hydrogen water, randomized clinical trial, $\dot{V}O_2$ max, visual analogue score.

Résumé : L'exercice physique aigu entraîne une hausse des dérivés réactifs de l'oxygène dans le muscle squelettique, ce qui mène à des lésions tissulaires et à de la fatigue. L'hydrogène moléculaire (H_2) agit comme un agent thérapeutique antioxydant en entraînant directement ou indirectement la production d'enzymes antioxydantes. Ici, nous avons étudié les effets de l'H_2 dans l'eau potable (H_2 infusé dans de l'eau) sur la fatigue psychométrique et la capacité d'endurance dans un modèle avec répartition aléatoire, à double insu et contrôlé par placebo. Dans le cadre de l'expérience 1, tous les participants ont bu uniquement de l'eau placebo au cours de la première séance d'exercice sur bicyclette ergométrique, comparativement à la consommation d'eau H_2 ou placebo 30 minutes avant l'exercice physique au cours de la seconde évaluation. Chez ces participants en bonne santé et non entraînés (n = 99), la fatigue psychométrique jugée à l'aide d'échelles visuelles analogues diminuait de façon marquée dans le groupe H_2 après un exercice physique léger. Après avoir divisé chacun des groupes en deux sous-groupes, les participants du sous-groupe présentant les valeurs les plus élevées aux échelles visuelles analogues étaient plus sensibles à l'effet de l'H_2. Dans l'expérience 2, des participants entraînés (n = 60) étaient soumis à un exercice modéré sur bicyclette ergométrique de manière semblable à dans l'expérience 1, mais l'exercice physique était effectué 10 minutes après avoir bu de l'eau H_2. Nous avons observé des améliorations marquées quant à l'endurance et à la fatigue dans le groupe H_2 à l'aide de mesures de la consommation maximale d'oxygène et à l'échelle de Borg, respectivement. Dans l'ensemble, la consommation d'eau H_2 juste avant l'exercice physique a eu des effets contre la fatigue et en faveur de l'endurance. [Traduit par la Rédaction]

Mots-clés : échelle de Borg, bicyclette ergométrique, endurance, exercice physique, fatigue, hydrogène, eau hydrogénée, essai clinique avec répartition aléatoire, $\dot{V}O_2$ max, échelle visuelle analogue.

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

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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_2O_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, 2015). Numerous publications (more than 1000) 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 et al. 2018).

As part of the next step in hydrogen medicine, one question is whether H_2 benefits not only sick patients, but also healthy people in their daily life. Administration of H_2 water or H_2 saline decreased oxidative stress and exhibited anti-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 H_2 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 ($\dot{V}O_2$ 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 H_2 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 H_2 water. The H_2 concentration was measured with a hydrogen-specific sensor (Unisense, Aarhus N, Denmark). In Experiment 1, immediately before exercise, H_2 water was diluted with the placebo water to make the concentration of 0.8 $mg \cdot L^{-1}$ (0.8 ppm), and then subjects consumed 500 mL of H_2 water. In Experiment 2, commercial H_2 water (500 mL at 1.0 $mg \cdot L^{-1}$ (1.0 ppm)) was used without further treatment. In both experiments, the packages of H_2 and placebo water were identical to ensure study blindness.

Measurement of H_2 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 H_2 concentration was firstly measured at baseline (7–20 ppm, *v*/*v*) by gas chromatography (Teramecs, Kyoto, Japan). The volunteers ingested 0.012 $mg \cdot (kg\ body\ mass)^{-1}$ by drinking 1.2 $mg \cdot L^{-1}$ (1.2 ppm) H_2 water. Immediately after drinking, their expired gases were collected at the indicated time and the H_2 concentration was measured. The resulting expired levels of H_2 due to ingestion of H_2 water was obtained by subtracting the baseline values.

Protocol of Experiment 1

NTTcom, Online Marketing Solution Co. Ltd. (Tokyo, Japan) enrolled 131 healthy volunteers (40–70 years old) who had normal blood pressure (< 140/90 mm Hg), had not consumed H_2 water for at least 3 months, and had no habit of extensive physical training; however, 18 volunteers declined to participate. Blood pressure, mass, and height of the participants were examined before exercise, and 9 participants who had blood pressure above 140/90 mm Hg were excluded from the study. The remaining 104 participants were divided into 2 groups by the stratified randomization method followed by dynamic allocation, considering age and sex by a specialist in statistics, T. Kumagai (Fermlab Inc., Tokyo, Japan). Data of 2 participants were not available by unknown errors. Furthermore, blood pressures of 3 participants were above 140/90 mm Hg before the second examination and so were excluded from further examination. A total of 99 volunteers (52 H_2 group; 47 placebo group) completed the study. All the participants drank 500 mL of placebo water and the exercise started 30 min after drinking H_2. Participants were subjected to exercise for $\dot{V}O_2$ 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 H_2 water (0.8 $mg \cdot L^{-1}$) or placebo water, and then were subjected to the second identical examination at 30 min after drinking H_2. The differences in each participant between the first and second examinations were calculated and analyzed.

Protocol of Experiment 2

Volunteers who had not consumed H_2 water for at least 3 months were recruited from the members of the Fitness Club, Asahi Big S Mukogaoka. 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 K. Tano. 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 an 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 mass 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 $mg \cdot mL^{-1}$ (1.0 ppm) H_2 water or placebo water. Ten minutes after drinking, the same examinations were performed. The differences in each subject between the first and second examinations were calculated and analyzed.

Measurement of $\dot{V}O_2$ max

$\dot{V}O_2$ 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, $\dot{V}O_2$ 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. $\dot{V}O_2$ 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 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).

VAS

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 2 end-points (Reips and Funke 2008; Lee et al. 1991).

Statistical analysis

Data are shown as the mean ± the standard error of the mean (SEM) or standard deviation (SD). The statistical significance for the comparison of 2 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 Tukey–Kramer's test. $P < 0.05$ was considered significant.

Results

Retention of H_2 in the body

We first examined how long H_2 remains in the body after drinking H_2 water by monitoring the H_2 concentration of expired gas. Figure 1 shows that H_2 reached a peak level within 10–20 min, and remained above baseline for 30–40 min. The retention time of H_2 appears longer in a body-mass-dependent manner (Fig. 1).

Experiment 1

Baseline measurements of 99 participants in Experiment 1 are reported in Table 1. No differences between H_2 and placebo groups are observed.

Figure 2A shows no difference in the change of $\dot{V}O_2$ max between H_2 and placebo groups. Figure 2B shows a slight but significant difference in the change of heart rate after exercise between H_2 and placebo groups.

Compared with placebo, H_2 resulted in a significant improvement (decrease) in VAS (Fig. 2C) and a non-significant improvement (decrease) in Borg's RPE scale (Fig. 2E). When each group was further divided into 2 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 (Figs. 3D and 3F). Thus, the effects of H_2 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 H_2. Baseline measurements of 60 participants from member of the fitness club are shown in Table 1. There were no significant differences in baseline between H_2 and placebo groups (Table 1). The average $\dot{V}O_2$ 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 H_2 group had a significant improvement (increase) in $\dot{V}O_2$ max (increase) and Borg's RPE (decrease) between the first and second examinations (Figs. 3A and 3C). Figure 3B indicates the relative change in $\dot{V}O_2$ max. When we estimated $\dot{V}O_2$ max and the Borg's scale in the generation-dependent subgroups from 20s to 60s, there was no significant change (Supplementary Fig. S1[2]) .

The effects of H_2 exhibited similar trends in Experiments 1 and 2, and were more noticeable with greater exercise intensity.

Fig. 1. Monitoring H_2 in the expiration after drinking H_2 water. Four volunteers ingested 0.012 mg·(kg body mass)$^{-1}$ by drinking 1.2 mg·L^{-1} 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, Kyoto, Japan). The body masses of the volunteers were ~80 kg (open circles), ~70 kg (filled triangles), ~50 kg (open triangles), and ~45 kg (filled circles). The data was obtained after the subtraction from baseline.

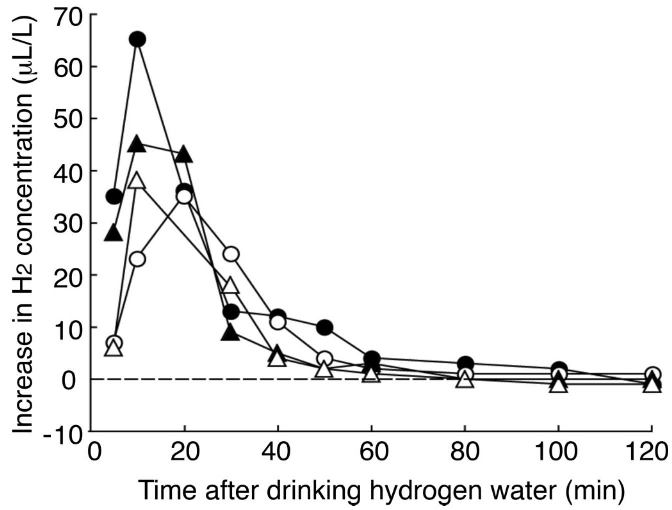


Table 1. Baseline characteristics of participants.

	Experiment 1		Experiment 2	
	Placebo	H_2 group	Placebo	H_2 group
Participants (*n*)	47	52	30	30
Men (*n*)	20	23	15	15
Women (*n*)	27	29	15	15
Age (years)	51.5±7.9	51.2±6.9	43.2±14.4	43.6±13.3
Height (cm)	164.2±7.6	164.7±9.2	165.3±8.8	162.9±8.7
Mass (kg)	60.7±14.9	59.6±11.9	62.0±12.7	63.5±11.1
BMI (kg·m⁻²)	22.0±3.3	22.4±4.3	22.5±3.0	23.9±3.2
Systolic BP (mm Hg)	118.4±11.6	117.5±13.3	121.0±12.8	120.8±14.3
Diastolic BP (mm Hg)	74.6±9.8	72.2±9.9	74.5±8.9	71.6±11.26
Resting HR (bpm)	71.2±12.1	70.2±10.1	71.6±8.8	71.1±12.0
$\dot{V}O_2$ max (mL·kg⁻¹·min⁻¹)	30.6±6.2	31.0±6.8	38.7±9.7	36.2±9.0
Borg's scale	12.6±2.2	13.4±1.9	11.8±1.78	12.0±1.4
VAS	3.7±2.2	4.3±2.0		

Note: Means ± SD are shown. BMI, body mass index; BP, blood pressure; HR, heart rate (bpm, beat per minute), $\dot{V}O_2$ max, maximum oxygen consumption; VAS, visual analogue scale.

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjpp-2019-0059.

Fig. 2. Change in each subject between the first and second 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 2 examinations was obtained. The changes of maximum oxygen consumption ($\dot{V}O_2$ max) (A), heart rate (B), visual analogue scale (VAS) (C), and Borg's scale (E) in H_2 and placebo groups are shown. (D, F): H_2 and placebo groups were divided into 2 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 is shown. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively, by paired Student's t test (B and C), and paired one-way ANOVA, followed by Tukey–Kramer's test (D and F). (B) $P = 0.015$, (C) $P = 0.046$, (D) **$P = 0.0084$, *$P = 0.0063$, (F) **$P = 0.0084$. [Colour online.]

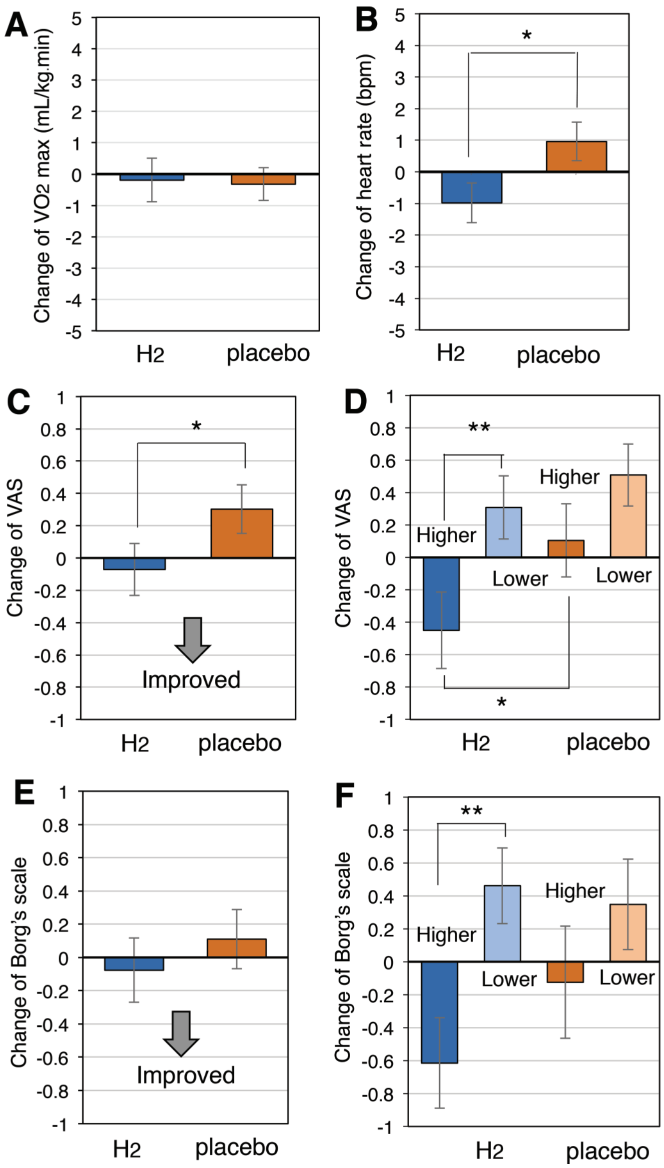
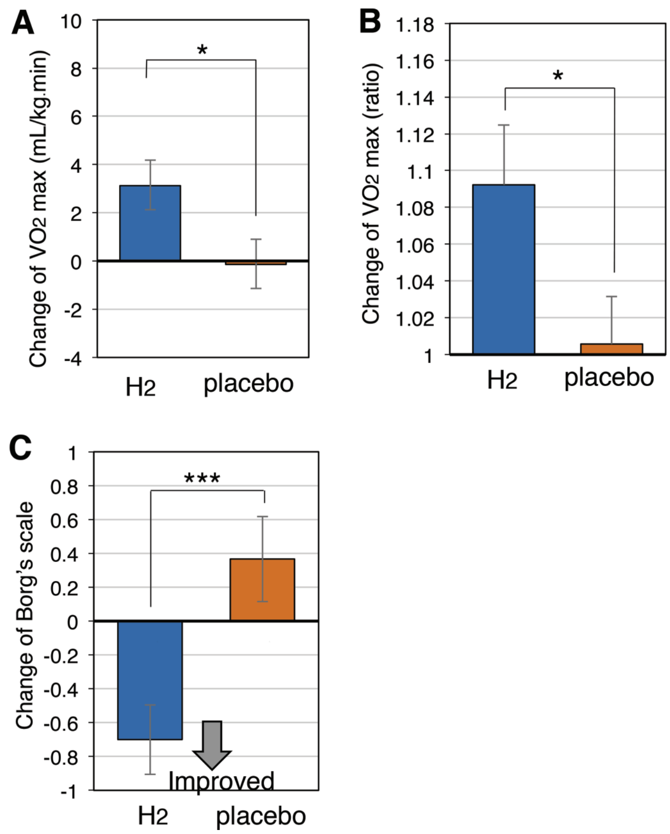


Fig. 3. Change in each subject between the first and second examination in Experiment 2. Participants were subjected to the first (with placebo water) and second (H_2 or placebo water) examinations, and the change in each participant between the 2 examinations was obtained. The changes of maximum oxygen consumption ($\dot{V}O_2$ max) (A), and Borg's scale (C) in H_2 and placebo groups are shown, and the relative change is shown (B). Mean ± SEM is shown. * and *** indicate $P < 0.05$ and $P < 0.001$, respectively, by paired Student's t test. (A) $P = 0.015$, (B) $P = 0.019$, (C) $P = 0.00078$. [Colour online.]



Discussion

This study showed that drinking H_2 water alleviated psychometric fatigue after mild and moderate exercise by bicycle ergometer, and enhanced endurance capacity as judged by measurement of $\dot{V}O_2$ max in moderate exercise. A recent publication agrees that acute administration of H_2 provides beneficial effects on exercise (LeBaron et al. 2019). Notably, healthy people usually perform exercise of the same intensities that were applied to the participants in our study. Thus, drinking H_2 water is suggested to have beneficial effects for healthy people.

The dwelling time of H_2 was examined by measuring the H_2 concentration in expired gas. After drinking H_2 water, most H_2 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 mass.

In the present study, we used 3 methods for evaluations, $\dot{V}O_2$ max, Borg's RPE, and VAS, for evaluating the effects of drinking H_2 water. In mild exercise by untrained participants (Experiment 1), the $\dot{V}O_2$ max values were significantly not different between the H_2 and placebo groups possibly because the exercise was too mild for H_2 to elicit a significant change. Additionally, the exercise started at 30 min after drinking H_2 water, when most hydrogen would have been expired as shown in Fig. 1. On the other hand, in VAS evaluation, H_2 significantly relieved the psychometric fatigue. Notably, the participants who felt more fatigue were more sensitive to the effects of H_2. 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 H_2 in the mild exercise experiment.

We considered that because the subgroup with higher VAS (felt more fatigue) in Experiment 1 exhibited more improvement in psychometric fatigue, more intense exercise might elicit the effects of H_2. In moderate exercise (Experiment 2) by the members of a fitness club, drinking H_2 water significantly improved $\dot{V}O_2$ 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 H_2 water. In addition, compared with Experiment 1, H_2 was at a higher level in the body during exercise in Experiment 2 becuase participants started the exercise test 10 min instead of 30 min after drinking H_2 water. Therefore, it is difficult to clarify the reasons for the discrepancy. More analyses will be required to confirm the effects of H_2; however, the current study strongly suggests that H_2 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. Because most H_2 was expired from the body prior to exercise as shown in Fig. 1, it may be difficult to elucidate that such small amounts of remaining H_2 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 H_2 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 H_2 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, 2013; Hyspler et al. 2015). Thus, the possibility that the remaining H_2 could directly reduce oxidative stress cannot be ruled out.

Recent progress indicates that H_2 exerts antioxidative and the other functions through the regulation of various kinds of gene expressions (Murakami et al. 2017: Kura et al. 2019; Nogueira et al. 2018). These data are consistent with the notion that H_2 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 H_2 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 H_2 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 H_2 water (Aoki et al. 2012). Therefore, it is possible that H_2 reduced oxidative stress directly or indirectly to suppress the increase in blood lactate during exercise, relieving psychometric fatigue.

Recently, H_2 water was shown to improve mood, anxiety, and autonomic nerve function in normal daily life (Mizuno et al. 2017). This study also supports that H_2 has beneficial effects in the daily life of healthy people.

Conflict of interest

K.T. and S.O. are inventors of a patent (WO2018-021435, an application patent on beneficial effects of molecular hydrogen after exercise) currently under application, and F.O. serves as a consultant involved in hydrogen. The other authors declare no conflict of interest.

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