

Two-week continuous supplementation of hydrogen-rich water increases peak oxygen uptake during an incremental cycling exercise test in healthy humans: a randomized, single-blinded, placebo-controlled study

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Abstract

The various beneficial effects of the intake of molecular hydrogen (H₂) have been demonstrated in the field of sports science. Although supplementation of H₂ has been reported to increase mitochondrial metabolism in animal studies, the effects of the administration of H₂ on aerobic capacity during exercise in humans are still not clear. We investigated whether a single or 2-week continuous intake of H₂-rich water (HW) enhanced the aerobic capacity during incremental exercise in healthy humans. In this randomized, single-blinded, placebo-controlled experimental study, the participants performed an incremental cycling exercise to measure peak oxygen uptake and peak load before and after a single (500 mL) or a 2-week supplementation (total 5 L) of HW. In the latter experiment, the participants drank the 500 mL of HW on all weekdays (i.e., 10 times). The single intake of HW did not significantly increase peak oxygen uptake and peak load, and did not significantly alter the responses in oxidative stress, antioxidant activity, and lactate levels. However, importantly, the 2-week continuous consumption of HW significantly augmented peak oxygen uptake and tended to increase the peak load without any significant changes in lactate levels, oxidative stress, and antioxidant responses. In conclusion, the continuous supplementation of HW potentially augments the aerobic capacity, implying that continuous supplementation of H₂ might help improve aerobic exercise performance and physical health. This study protocol was approved by the Ethical Committee of Chubu University (approval No. 260086-2) on March 29, 2018.

Key words: aerobic capacity; antioxidant activity; blood lactate; mitochondrial metabolism; molecular hydrogen; oxidative phosphorylation; oxidative stress; reactive oxygen species

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INTRODUCTION

Many previous studies have shown the beneficial effects of the intake of hydrogen (H₂)-rich water (HW).^{1,2} For instance, the intake of HW was shown to stimulate lipid metabolism by inducing fibroblast growth factor 21 expression and/or peroxisome proliferator-activated receptor- γ coactivator-1 α expression in diabetic db/db mice.^{3,4} Furthermore, HW consumption improved lipid and glucose metabolism in patients with type 2 diabetes,⁵ and reduced the body fat percentage and serum triglyceride levels in middle-aged overweight women.⁶

Recently, the effectiveness of HW has been demonstrated by studies regarding exercise physiology and sports and health sciences.^{7,8} For example, Sha et al.⁹ revealed that a 2-month intake of HW enhanced the antioxidant activity in female soccer players. Ara et al.¹⁰ demonstrated that the increased antioxidative activities induced by loading of HW attenuated exercise-induced chronic fatigue in mice. Aoki et al.¹¹ indicated that the intake of HW suppresses acute fatigue as well as an increase in blood lactate (La) levels during exercise in elite athletes. Additionally, the findings that HW administration relieved exercise-induced psychometric fatigue¹² and

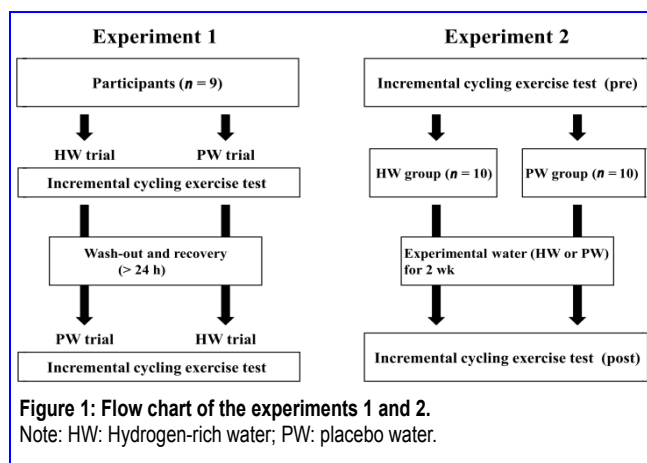
maintained the peak power output in repetitive sprints¹³ have also been reported in humans.

The supplementation of HW enhances mitochondrial ATP production, suggesting that intake of HW increase aerobic metabolism.¹⁴ Therefore, it can be expected that HW supplementation would increase the aerobic capacity. In fact, LeBaron et al.¹⁵ speculated that the intake of HW improved oxygen extraction and utilisation in active skeletal muscles based on the decreased heart rate (HR) observed during the same exercise intensity as a placebo trial in humans. However, the effects of HW on aerobic capacity during exercise are not clear. The purpose of the present study, therefore, was to clarify the effects of a single or a 2-week continuous supplementation of HW on aerobic capacity during an incremental cycling exercise in humans.

PARTICIPANTS AND METHODS

Experimental design

This study completed at our laboratory in Chubu University consisted of two experiments with a single-blind method design (**Figure 1**). In the first experiment, participants ingested, at random, HW or placebo water (PW) before performing an



exercise test to investigate the effects of single supplementation of HW on aerobic capacity (experiment [Exp] 1). In the second experiment, we randomly divided the participants into the following two groups: HW and PW groups. The participants performed the same exercise test as Exp 1 before and after 2 weeks of HW intake to elucidate the chronic effect of HW on aerobic capacity (Exp 2).

Participants and ethical approval

We orally recruited healthy participants at Chubu University, who were able to perform an incremental cycling exercise test. Six male and three female university students volunteered to participate in Exp 1 (Table 1). Twenty male university students participated in Exp 2, and were divided into two experimental groups: HW ($n = 10$) and PW groups ($n = 10$) (Table 1).

Each participant was informed of the experimental protocol and the possible risks involved in this study before providing written consent. This study protocol was approved by the Ethical Committee of Chubu University (approved No. 260086-2) on March 29, 2018.

Experimental protocol

In Exp 1, the participants came to our laboratory and drank 500 mL of HW or PW. After 30 minutes of seated rest, they performed an incremental cycling exercise test. They underwent the exercise test twice at random, i.e., HW and PW trials, with at least 24 hours between trials for recovery.

In Exp 2, the participants performed the exercise test twice, once before and once again at 2 weeks after the intake of HW or PW. The participants drank 500 mL of HW or PW on all weekdays, i.e., they drank HW or PW 10 times with a total volume of 5 L. The post exercise test was performed at 30 minutes after drinking the experimental water, as in Exp 1.

Preparation of HW

A stick-shaped H_2 generator (Hydrogen Water 7.0; MiZ Co. Ltd., Kanagawa, Japan) and 500-mL plastic bottles were used to prepare HW. HW was administered in the laboratory at room temperature for 24 hours after the H_2 generator was immersed in the bottle containing water. HW was stirred just before drinking to dissolve the H_2 , and the same procedure was done for PW to preserve the single-blind experimental design. The concentration of dissolved H_2 in water was measured by

Table 1: Characteristics of participants in experiments 1 and 2

	Height (cm)	Weight (kg)	Age (yr)
Experiment 1 ($n=9$)	169.6 \pm 3.0	63.7 \pm 4.1	19.9 \pm 0.4
Experiment 2			
HW ($n=10$)	175.5 \pm 1.5	69.9 \pm 2.3	20.3 \pm 0.4
PW ($n=10$)	172.6 \pm 2.1	67.8 \pm 1.4	20.4 \pm 1.5

Note: Values are expressed as the mean \pm SE. HW: Hydrogen-rich water; PW: placebo water.

titration with a dissolved H_2 reagent methylene blue kit (MiZ Co. Ltd., Kanagawa, Japan), as described previously.¹⁶ The measured concentration was 4.3 ± 0.9 ppm in Exp 1 and 5.9 ± 0.2 ppm in Exp 2.

Measurement of peak oxygen uptake and peak load

All participants underwent a practice session in advance to become accustomed to the exercise test. We adopted an incremental cycling exercise test using a bicycle ergometer (Aerobike 75XLIII; Combi Wellness Corporation, Tokyo, Japan). The workload was gradually increased by 20 W every 1 minute. As shown in Figure 2, the participants performed the exercise after a 3-minute warm up at 0 W following a 5-minute rest on the ergometer with a respiratory mask. The participants kept the pedalling cadence of 60 r/min during the exercise and performed the exercise until they could not maintain a pedalling rate of 50 r/min. We informed the participants that they were unable to return the cadence to 60 r/min regardless of the experimenters' verbal exhortation.

We measured the oxygen uptake (VO_2), carbon dioxide output (VCO_2), minute ventilation (V_E), and HR on a breath-by-breath basis using a metabolic gas analyser (AE-310S; Minato Medical Science, Osaka, Japan). The respiratory exchange ratio (RER) was calculated from the ratio of VO_2 and VCO_2 . VO_2 was averaged every 20 seconds and peak VO_2 (VO_{2peak}) was defined as the peak value of the averaged VO_2 during the exercise. The peak values of other respiratory and circulatory parameters were calculated in the same way. We also recorded the rate of perceived exertion (RPE) using the Borg Scale¹⁷ for every minute during the exercise. The workload at the end of the exercise was determined as the peak load. In the present study, VO_{2peak} divided by body mass and peak load were used as parameters of aerobic capacity.

Evaluation of blood La, oxidative stress, and antioxidant activity

We obtained a blood sample from the participants' fingertips before, during (at 150 W), and immediately after the exercise to evaluate the La concentration, oxidative stress, and antioxidant activity. The La concentration was measured using a portable lactate measuring device (Lactate pro2; Arkray, Kyoto, Japan). To assess the oxidative stress and antioxidant activity, plasma was obtained by centrifugation, and the Free Radical Elective Evaluator (FREE® Carpe Diem; Wismerll, Tokyo, Japan) was used to assess the diacron reactive oxygen metabolites (d-ROMs), which optically measures the blood concentration of hydroperoxides according to the optical measurement method.¹⁸ The biological antioxidant potential

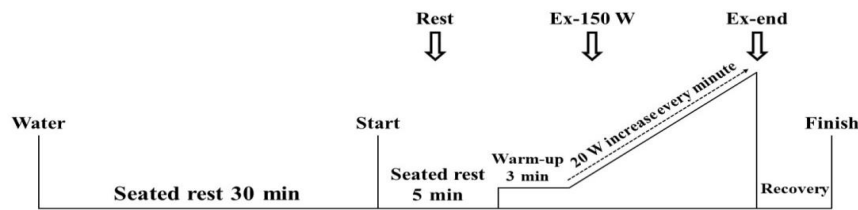


Figure 2: Experimental protocol of the incremental cycling exercise test.

Note: All participants were first required to drink the experimental water. They started to perform the exercise test after a seated rest for 30 minutes. They underwent an incremental cycling exercise using a ramp load method after a 3-minute warm up at 0 W. Recovery means that the participants rested in a sitting position on the bicycle ergometer after the cycling exercise. Peak oxygen uptake (VO_{2peak}) and peak load were used as parameters of aerobic capacity. White arrows indicate the time points of blood sampling to evaluate lactate levels, oxidative stress, and antioxidant activity. Ex: Exercise.

(BAP) was also assessed, which evaluates the antioxidant activity by measuring the capacity to reduce Fe^{3+} to Fe^{2+} .^{19,20} The values of d-ROMs are expressed in UCARR, which is an arbitrary unit (1 UCARR corresponds to 0.08 mg/dL H_2O_2).²¹ The details of the mechanisms and procedures of d-ROMs and BAP tests have been previously described.²²

Statistical analysis

Sample size calculation was performed by using the G* Power 3.1.9.7 software (Heinrich-Heine-Universität, Düsseldorf, Germany). The primary outcome variable in this study was the change in VO_{2peak} , peak load, and oxidative stress by drinking HW. A minimal sample size of eight participants in Exp 1 and ten participants in each group in Exp 2 was respectively needed for a statistical power of 80% ($1 - \beta$), effect size of 0.35, and an α error rate of 0.05 in the case of using a two-way repeated measures analysis of variance (ANOVA).

In Exp 1, a paired *t*-test was used to compare peak loads, respiratory and circulatory parameters, and RPE between the HW and PW trials. A two-way repeated measures ANOVA was used to assess the changes in La, d-ROMs, and BAP responses to the exercise. If a significant interaction was observed, an analysis of the simple main effect was conducted, and then, Bonferroni's test for multiple comparisons was further used to identify the specific differences. When only the main effects were significant, the Bonferroni's test for multiple comparisons was performed.

In Exp 2, a two-way repeated measures ANOVA was performed to compare peak load, respiratory and circulatory parameters, and RPE before and after 2 weeks of intake of experimental water between the HW and PW groups. When a significant interaction was observed, an analysis of the simple main effect was performed. Bonferroni's test for multiple comparisons test was used when only the main effects were observed. Furthermore, an unpaired *t*-test was used to compare delta changes from before to 2 weeks after continuous intake of experimental water between the HW and PW groups. A two-way repeated measures ANOVA was also used to evaluate the changes in La, d-ROMs, and BAP during the exercise test, separately for each group. The *post hoc* analysis was the same as that of Exp 1.

Statistical analyses were carried out by using StatView 5.0 (SAS Institute, Cary, NC, USA) and SPSS 24.0 for Windows software (IBM, Armonk, NY, USA). The significance level was defined as $P < 0.05$. All values are presented as the mean \pm standard error (SE).

RESULTS

Characteristics of participants in this study

The characteristics of participants in Exp 1 and 2 are shown in **Table 1**.

Effects of the single supplementation of HW (Exp 1)

Table 2 shows VO_2 , VCO_2 , RER, V_E , and HR at rest for each trial. There were no significant differences in these parameters between the two trials ($P > 0.10$). There were no significant differences in the peak values of VCO_2 , RER, V_E , HR, and RPE (**Table 2**) between the trials either ($P > 0.10$). As shown in **Figure 3**, HW did not significantly increase VO_{2peak} ($P = 0.30$) and peak load ($P = 0.58$). The exercise significantly increased La levels and BAP ($P < 0.01$) but not d-ROMs ($P = 0.24$); however, the significant effects of HW were not observed in each parameter ($P > 0.10$; **Table 3**).

Effects of 2-week continuous supplementation of HW (Exp 2)

An unpaired *t*-test showed no significant difference in height ($P = 0.28$), weight ($P = 0.54$), age ($P = 0.87$; **Table 1**), and baselines peak loads ($P = 0.84$) and VO_{2peak} ($P = 0.43$) before starting the intake of the experimental water (Pre) between the HW and PW groups. As shown in **Table 4**, there were no significant differences in the resting respiratory and circulatory parameters between the two groups. The continuous intakes of HW did not significantly change these parameters at rest. The peak values of VCO_2 , RER, V_E , and HR were also not significantly changed after 2 weeks of intake of experimental water (**Table 4**).

The peak load was significantly elevated after the 2-week intake of experimental water, regardless of the experimental group ($P < 0.01$; **Figure 4A**). Importantly, the interaction tended to be significant ($P = 0.067$), suggesting that an increase in peak load from pre to post HW intake was potentially higher than that of PW intake. The difference in peak loads from before to after 2-week intake of experimental water also tended to be higher in the HW group than in the PW group ($P = 0.075$; **Figure 4B**). Because the body mass had changed over 2 weeks, we demonstrated VO_{2peak} divided by body mass (**Figure 4C**). VO_{2peak} of the PW group did not significantly change ($P = 0.73$), whereas that of the HW group was significantly elevated ($P < 0.01$). We also confirmed the presence of a significant interaction (water-by-time, $P < 0.05$; **Figure 4C**). In addition, the net increase in VO_{2peak} from before to after the 2-week treatment in the HW group was significantly higher than that in the PW group ($P < 0.05$; **Figure 4D**).



Table 2: Effects of a single intake of hydrogen-rich water on resting and peak respiratory and circulatory parameters during an incremental cycling exercise test in healthy humans (Experiment 1)

	Hydrogen-rich water	Placebo water	P-value
Oxygen uptake (mL/min)			
Rest	279±20	282±17	0.67
Peak value	2819±195	2813±183	0.90
Carbon dioxide output (mL/min)			
Rest	241±19	249±16	0.44
Peak value	3654±248	3629±238	0.78
Respiratory exchange ratio			
Rest	0.85±0.01	0.88±0.02	0.32
Peak value	1.49±0.06	1.53±0.06	0.59
Minute ventilation (L/min)			
Rest	9.6±0.5	10.0±0.4	0.25
Peak value	118.0±8.0	114.0±8.0	0.26
Heart rate (beat/min)			
Rest	78±5	84±8	0.39
Peak value	185±3	185±2	0.91
Rate of perceived exertion			
Rest	–	–	–
Peak value	18.8±0.6	19.1±0.5	0.20

Note: Regarding the rate of perceived exertion, we failed to collect the data of one participant; thus, the data from only eight participants are shown. Values are expressed as the mean ± SE ($n = 9$), and were analyzed by paired *t*-test.

The exercise significantly increased the La levels, d-ROMs, and BAP in both groups, but HW did not significantly influence the response of these parameters to exercise ($P > 0.10$; **Figure 5**).

DISCUSSION

The major findings from the present study are as follows. First, no significant effects of HW on responses in blood La, oxidative stress, and antioxidant were observed. Second, a single intake of HW did not significantly increase peak load and VO_{2peak} ; however, a 2-week continuous supplementation significantly increased VO_{2peak} and tended to augment peak load. The present study suggests that continuous intake of HW enhances aerobic capacity in humans. Interestingly, similar results have also been reported in the previous meeting report.²³

Possible mechanisms underlying the improvement of aerobic capacity by continuous intake of HW

Although causation cannot be determined from our results, we can speculate the potential mechanisms that could underlie the increase in VO_{2peak} during an incremental cycling exercise test by continuous intake of HW. Maximal VO_2 (VO_{2max}) or VO_{2peak} is assumed to be mainly determined by 1) cardiopulmonary function that transports oxygen to the active muscle and 2) mitochondrial oxygen consumption (oxygen extraction and utilisation).²⁴⁻²⁶ To the best of our knowledge, it has not been reported that continuous intake of HW improves cardiopulmonary function during exercise in healthy humans. Therefore, HW is unlikely to affect the oxygen supply system.

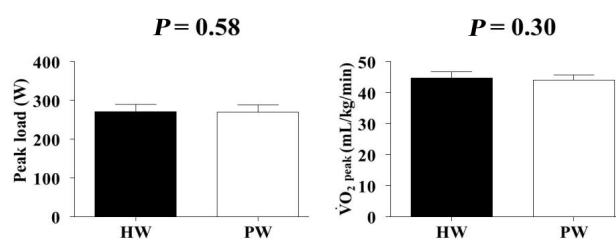


Figure 3: Effects of a single intake of hydrogen-rich water on the peak load and peak oxygen uptake during an incremental cycling exercise test in healthy humans (Experiment 1).

Note: Values are expressed as the mean ± SE ($n = 9$), and analyzed by paired *t*-test. HW: Hydrogen-rich water; PW: placebo water; VO_{2peak} : peak oxygen uptake.

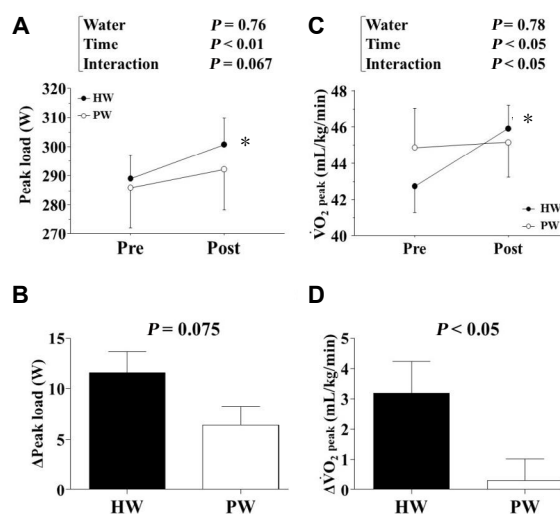


Figure 4: Effects of the 2-week continuous intake of hydrogen-rich water on the peak load and peak oxygen uptake during an incremental cycling exercise test in healthy humans (Experiment 2).

Note: Pre and Post mean before and after 2 weeks of continuous intake of hydrogen-rich water (HW) or placebo water (PW). Δ means each difference in peak load and peak oxygen uptake (VO_{2peak}) from before to after 2-week intake of experimental water. An analysis of the simple main effect was performed in VO_{2peak} since the interaction was significant. Values are expressed as the mean ± SE ($n = 10$ in each group), and were analyzed by either a two-way repeated measures analysis of variance followed by analysis of the simple main effect (A and C), or an unpaired *t*-test (B and D). * $P < 0.01$, vs. Pre.

As for the latter determinant affecting VO_{2max} , i.e., mitochondrial function, mitochondrial reactive oxygen species have been suggested to impair mitochondrial activities.^{27,28} Given that molecular H_2 has been suggested to directly and/or indirectly decrease oxidative stress,²⁹⁻³² it is possible that HW attenuated the decline in mitochondrial function evoked by exercise-induced oxidative stress.⁷ However, supplementation of HW did not reduce oxidative stress or increase antioxidant activity in this study. Therefore, it is logical to conclude that the H_2 scavenging reactive oxygen species mechanism did not operate in the present situation.

Recent studies have reported that HW enhances energy metabolism by inducing the expression of fibroblast growth factor 21 and/or peroxisome proliferator-activated receptor- γ coactivator-1 α .^{3,4} Moreover, Sobue et al.³³ proposed that H_2 can induce biological effects through the activation of a mitochondrial unfolded protein response via epigenetic histone modification and gene expression modification. Murakami et al.³¹ suggested that mild oxidative stress caused by H_2 enhanced


Table 3: Effects of a single intake of hydrogen-rich water on changes in blood levels during an incremental cycling exercise test in healthy humans (Experiment 1)

	Rest	Ex-150 W	Ex-end	Main effect		
				Water	Load	Interaction
Lactate (mM)				<i>P</i> =0.44	<i>P</i> < 0.01	<i>P</i> =0.75
HW	1.4±0.2	2.5±0.2**	10.8±0.7**			
PW	1.3±0.1	2.6±0.4**	11.2±0.9**			
d-ROMs (UCARR)				<i>P</i> =0.69	<i>P</i> =0.24	<i>P</i> =0.96
HW	254±20	255±13	270±13			
PW	255±22	260±16	281±32			
BAP (μM)				<i>P</i> =0.43	<i>P</i> < 0.01	<i>P</i> =0.80
HW	2133±38	2311±121	2778±55**			
PW	1862±188	2188±128	2720±44**			

Note: The blood samples were collected before (at rest), during (at 150 W), and immediately after the end of the exercise (Ex). Values are expressed as the mean ± SE (*n* = 9), and were analyzed by a two-way repeated measures analysis of variance followed by Bonferroni's test for multiple comparisons. ***P* < 0.01, vs. rest. 1 UCARR corresponds to 0.08 mg/dL H₂O₂. BAP: Biological antioxidant potential, which is an index of antioxidant activity; d-ROMs: diacron reactive oxygen metabolites, which is an index of oxidative stress level; HW: hydrogen-rich water; PW: placebo water.

Table 4: Effects of the 2-wk continuous intake of hydrogen-rich water on resting and peak respiratory and circulatory parameters during an incremental cycling exercise test in healthy humans (Experiment 2)

	Pre	Post	Main effect		
			Water	Time	Interaction
Rest					
Oxygen uptake (mL/min)			<i>P</i> =0.86	<i>P</i> =0.25	<i>P</i> =0.52
HW	304±14	287±9			
PW	296±9	291±12			
Carbon dioxide output (mL/min)			<i>P</i> =0.81	<i>P</i> =0.11	<i>P</i> =0.55
HW	280±16	254±9			
PW	277±18	265±15			
Respiratory exchange ratio			<i>P</i> =0.61	<i>P</i> =0.23	<i>P</i> =0.72
HW	0.92±0.03	0.89±0.02			
PW	0.93±0.04	0.91±0.03			
Minute ventilation (L/min)			<i>P</i> =0.95	<i>P</i> =0.67	<i>P</i> =0.48
HW	10.2±0.3	9.9±0.2			
PW	10.0±0.7	10.0±0.5			
Heart rate (beat/min)			<i>P</i> =0.92	<i>P</i> =0.08	<i>P</i> =0.90
HW	72±4	69±4			
PW	73±3	69±3			
Peak value					
Oxygen uptake (mL/min)			<i>P</i> =0.86	<i>P</i> =0.06	<i>P</i> =0.099
HW	2981±103	3202±78			
PW	3032±145	3095±137			
Carbon dioxide output (mL/min)			<i>P</i> =0.91	<i>P</i> =0.47	<i>P</i> =0.21
HW	4077±119	4255±115			
PW	4147±192	4106±224			
Respiratory exchange ratio			<i>P</i> =0.98	<i>P</i> =0.12	<i>P</i> =0.92
HW	1.37±0.04	1.32±0.03			
PW	1.37±0.03	1.32±0.04			
Minute ventilation (L/min)			<i>P</i> =0.97	<i>P</i> =0.06	<i>P</i> =0.78
HW	129±7	136±8			
PW	130±6	135±7			
Heart rate (beat/min)			<i>P</i> =0.50	<i>P</i> =0.71	<i>P</i> =0.64
HW	191±3	191±2			
PW	189±3	189±3			
Rate of perceived exertion			<i>P</i> =0.27	<i>P</i> =0.06	<i>P</i> =0.39
HW	18.8±0.5	19.2±0.4			
PW	17.8±0.6	18.8±0.5			

Note: Pre and Post are defined as before and after 2 weeks of continuous intake of HW or PW. Values are expressed as the mean ± SE (*n* = 10 in each group), and were analyzed by a two-way repeated measures analysis of variance followed by Bonferroni's test for multiple comparisons. HW: Hydrogen-rich water; PW: placebo water.

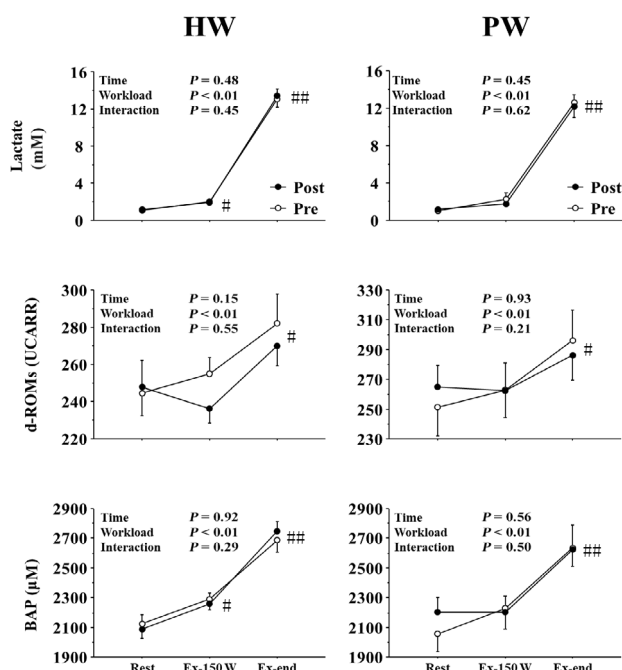


Figure 5: Effects of the 2-week continuous intake of hydrogen-rich water on changes in blood levels during an incremental cycling exercise test in healthy humans (Experiment 2).

Note: Pre and Post mean before and after 2 weeks of continuous intake of hydrogen-rich water (HW, left) and placebo water (PW, right). The blood samples were collected before (at rest), during (150 W), and immediately after the end of the exercise (Ex). 1 UCARR corresponds to 0.08 mg/dL H_2O_2 . Values are expressed as the mean \pm SE ($n = 10$ in each group), and were analyzed by a two-way repeated measures analysis of variance followed by Bonferroni's test for multiple comparisons. # $P < 0.05$, ## $P < 0.01$, vs. rest. BAP: Biological antioxidant potential, which is an index of antioxidant activity; d-ROMs: diacron reactive oxygen metabolites, which is an index of oxidative stress level.

mitochondrial oxidative phosphorylation. Taking together our findings and those of previous studies, we speculate that continuous intake of HW might increase mitochondrial energy production via the expression of these genes and proteins, thereby increasing the VO_{2peak} during incremental exercise.

In addition to the possibility that HW augmented mitochondrial energy production during the exercise, continuous intake of HW might have influenced mitochondrial biogenesis. The activation of adenosine monophosphate-activated protein kinase (AMPK) is known to facilitate mitochondrial biogenesis.³⁴ In fact, a previous investigation showed that the AMPK activator 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR) augmented oxygen consumption rates and endurance capacity in mice without any physical training.³⁵ In addition, daily intake of AICAR increased the mitochondrial enzymes in skeletal muscle.³⁶ Toedebusch et al.³⁷ showed that continuous AICAR injections delayed the initial decline in lifetime-apex VO_{2peak} with ageing in rats. Importantly, HW could activate AMPK³⁸; hence, it is plausible, although highly speculative, that the continuous intake of HW might also augment VO_{2peak} by enhancing mitochondrial biogenesis through AMPK stimulation.

In the present study, although continuous intake of HW significantly increased the VO_{2peak} , a single intake did not. The activation of mitochondrial metabolism and biogenesis are thought to require long-term administration of H_2 ,^{3,4} or at

least for more than a few hours.^{31,33,38} It is reasonable to presume that a single supplementation with HW was not enough to induce those effects.

Regardless of the experimental group, the peak load during incremental exercise in the current study was significantly elevated after 2 weeks of treatments. This result could be attributed to an adaptation to the repeatedly performed exercise test. Importantly, the increase in peak load from pre to post HW tended to be higher than that with PW. The augmentation in peak load could be due to the elevation in VO_{2peak} , namely HW-induced increase in oxidative energy metabolism.

It is interesting to speculate if the continuous intake of HW facilitated anaerobic metabolism during exercise, thereby increasing the peak load, as Aoki et al.¹¹ reported that supplementation by 1500 mL of HW suppressed exercise-induced La production. In the present study, the La response to exercise was not significantly different between the two groups, which was in accordance with a previous study,¹³ suggesting that HW did not affect anaerobic metabolism during the exercise, at least in the present situation. However, a further study is required, because the effects of the intake of HW on La response to exercise are still contradictory.^{11,13}

Practical and clinical implications

H_2 has a safety advantage as it is not cytotoxic, even at high concentrations.¹ Drinking HW and inhalation of H_2 gas are two known administration methods.^{1,8} In the present study, we adopted HW instead of gas since it is more easily and safely administered, and thus more practical for use in daily life.^{1,8} It is well known that aerobic performance is strongly related to VO_{2max} or VO_{2peak} ,^{39,40}; therefore, this study supports the possibility that drinking HW benefits aerobic exercise performance.¹⁵ Cardiorespiratory fitness is an independent and strong predictor of all-cause and disease-specific mortality.⁴¹ Therefore, the present study showing that the continuous supplementation of HW enhanced aerobic capacity also implies that HW might contribute to maintaining and improving health.

Limitations

We acknowledge that the present study did not show any direct evidence to reveal the mechanism by which HW elevated VO_{2peak} . Additionally, the present study should be treated as a pilot study because we did not adopt a double-blind method when performing the experiments and did not determine the VO_{2max} . However, this study could be valuable as it showed the possibility that H_2 can be used as a supplement that enhances aerobic capacity in healthy humans.

Conclusion

The present study demonstrated that 2-week continuous supplementation of HW significantly augmented the VO_{2peak} and tended to increase the peak load in healthy individuals. These results suggest that continuous intake of HW potentially enhances the aerobic capacity.

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Author contributions

AH, SS, MI, and NH: decided conception and design of research; AH and NH: performed experiments and analyzed data; AH, SS, RK, SH, MI, and NH: interpreted results of experiments; AH and NH: prepared figures; AH, MI, and NH: drafted manuscript. All authors approved the final version of manuscript for publication.

Conflicts of interest

RK and SH are employees of MiZ Company Limited. For NH, a resource was provided by MiZ Company Limited, which manufactures and markets hydrogen generators. For AH, SS, and MI, there are no conflicts of interest, financial or otherwise.

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None.

Institutional review board statement

This study protocol was approved by the Ethical Committee of Chubu University (approved No. 260086-2) on March 29, 2018.

Declaration of participant consent

The authors certify that they have obtained participants' consent forms. In the form, participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials not be published and due efforts will be made to conceal their identity.

Reporting statement

The writing and editing of the article were performed in accordance with the CONSORT Statement.

Biostatistics statement

The statistical methods of this study were conducted and reviewed by the biostatistician of Chubu University, Japan.

Copyright transfer agreement

The Copyright License Agreement has been signed by all authors before publication.

Data sharing statement

Datasets analyzed during the current study are available from the corresponding author on reasonable request.

Plagiarism check

Checked twice by iThenticate.

Peer review

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