Impact of hydrogen-rich gas mixture inhalation through nasal cannula during post-exercise recovery period on subsequent oxidative stress, muscle damage, and exercise performances in men

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Abstract

Molecular hydrogen has been suggested to have a cytoprotective effect on the whole body and to enhance exercise performances. However, the effect of hydrogen-rich gas mixture (HG) inhalation on physiological responses has been poorly investigated. We examined the impact of acute HG inhalation on subsequent oxidative stress, muscle damage, and exercise performances during the recovery period after a strenuous exercise. This is a two-trial, double-blind, crossover, repeated measures study. Eight physically active male volunteers inhaled HG (estimated fraction of inspired oxygen and hydrogen were 21.57 and 4.08% at most, respectively) or normal gas (placebo, ambient air 400 m above sea level) during a 60-minute recovery phase after oxidative stress-inducing exercise) completion comprising 30-minute treadmill running at an intensity corresponding to 75% of maximal oxygen uptake and squat jumps (5 sets × 10 repetitions). Before oxidative stress-inducing exercise and 10 minutes after the post-exercise gas inhalation, blood and urine samples were obtained and exercise performances (jumping ability; pedaling power output; muscle strength) were evaluated. Post-exercise HG inhalation attenuated the increase in urinary 8-hydroxydeoxyguanosine excretion rate ($P < 0.05$), a DNA oxidation marker, and the reduction in the countermovement jump height ($P < 0.05$), compared with Placebo inhalation. Other exercise performances and blood oxidative stress and muscle damage markers did not differ between HG and Placebo inhalation. Moreover, the increase in urinary 8-hydroxydeoxyguanosine excretion rate was significantly associated with countermovement jump performance reduction ($r = –0.78$, $P < 0.01$). These findings suggested that HG inhalation during post-exercise recovery period might improve exercise performance via reducing systemic oxidative damage. The study was approved by the Human Research Ethics Committee of the University of Yamanashi (approval No. H29-006) on June 28, 2017.

Key words: 8-hydroxydeoxyguanosine; endurance running; inhalation; molecular hydrogen; muscle fatigue; oxidative stress; sprint cycling; squat jumps

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Introduction

Strenuous exertion is sometimes required in various activities of daily living, such as competitive sports, camping, and unaccustomed physical training. In these situations, excessive physiological stress might strain the whole body, leading to various cell or tissue damages. As a result, these responses raise the possibility of muscle fatigue, tissue injury, and diseases. Reactive oxygen species (ROS) is considered to be one of the causes of these detrimental phenomena. In general, ROS is usually scavenged by various endogenous antioxidant activities (e.g., superoxide dismutase, reduced glutathione, vitamin C, etc.) and redox balance is maintained through homeostatic regulation. However, the overproduction of ROS by vigorous exercise can rapidly surpass the body's antioxidant system, leading to oxidative injury and its subsequent deteriorative consequences.[1-3] Thus, a counterplan against exercise-induced excessive ROS generation should be sought to maintain physical function and exercise performances.

In previous studies, molecular hydrogen has been clinically suggested to have a therapeutic potential because of its antioxidative and anti-inflammatory effects, making it an attractive tool in sports science-related fields.[2,4] Indeed, our and other research groups have reported that molecular hydrogen-rich water ingestion or gas inhalation attenuated strenuous exercise-induced oxidative damages in human,[5,6] rodents,[7,8] and horses.[9,10] However, the exact underlying molecular mechanisms in such hydrogen-mediated physiological effect remain elusive. If molecular hydrogen directly reduces the level of toxic ROS (i.e., hydroxyradicals) as previously suggested,[11] the effect of hydrogen might appear in a dose-dependent manner. Theoretically, the administered dose of hydrogen by inhalation of a hydrogen-rich gas mixture (HG) is considered to be greater than other ways including drinking a hydrogen-rich solution.[2] In addition, there is no study to observe the effect of HG inhalation on exercise-induced oxidative stress in humans.

Together, we hypothesized that HG inhalation during the post-exercise recovery period can decrease exercise-induced

oxidative stress and its related muscle damage in humans. Consequently, exercise performances after HG inhalation might be greater than those of normal gas (ambient air). In this study, we therefore examined the effect of 60-minute HG inhalation after intense exercise on subsequent oxidative stress, muscle damage, and exercise performance in healthy young males.

Participants and Methods

Participants

Eight healthy males who were able to perform high-intensity cycling exercise were recruited in the present study (**Table 1**). The participants did not have any history of cardiovascular or respiratory diseases. After receiving a detailed explanation of the experimental procedure, each participant singed an informed consent form. All experimental procedures were approved by the Human Research Ethics Committee of the University of Yamanashi (approval No. H29-006) on June 28, 2017, and were performed in accordance with the guidelines on the *Declaration of Helsinki*. This study followed the CONsolidated Standards Of Reporting Trials (CONSORT) statement.

Table 1: Physiological characteristics in the eight healthy male participants

Variables	Data
Age (yr)	20.9±0.3
Height (cm)	171.8±1.6
Weight (kg)	61.6±1.5
VO_2max (mL/kg per min)	53.7±2.9
v75%VO_2max (km/h)	12.7±0.5

Note: Values are mean ± standard error. v75%VO_2max: Treadmill speed corresponding to 75% of VO_2max; VO_2max: maximal oxygen uptake.

Study design

In order to examine whether HG inhalation during post-exercise recovery period had a significant effect on subsequent oxidative stress, muscle damage, and exercise performances, a double-blind, crossover, repeated measures design was adopted in this study (**Figure 1**). Considering the order effect, the participants were randomly allocated into two groups through the permuted block method. In the first trial, one group ($n = 4$) was assigned to the HG intervention; the other group ($n = 4$) was assigned to the normal gas (placebo) intervention. After more than a week, the participants of both groups performed the respective conditions of the second trial. The participants were instructed not to take antioxidant supplements and alcohol, not to perform strenuous exercise, and not to receive any specific recovery treatments 48 hours before the exercise test.

Preliminary experiment for aerobic capacity test

At first, all participants performed incremental running test to determine their maximal oxygen uptake and running speed in preliminary trials. They ran on a treadmill (T7000; Johnson, Co., Ltd., Tokyo, Japan) in the multiple-stage incremental exercise test composed of a 4-minute constant speed with 1-minute rest between stages. In each rest intervals, their fingertip blood samples were undertaken and blood lactate

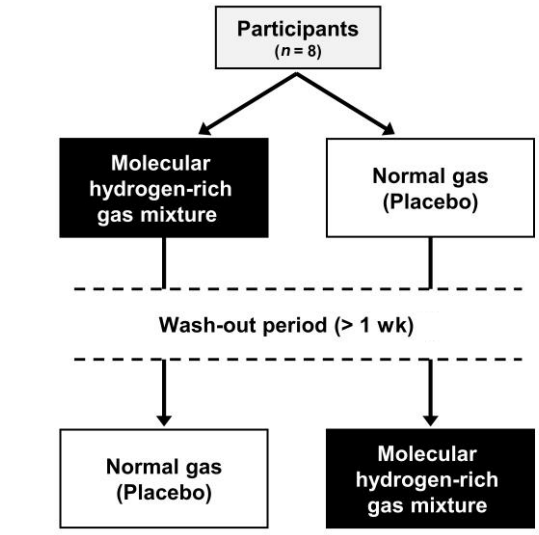

Figure 1: Research design.

concentrations were determined using a lactate analyzer (Lactate Pro 2; Arkray, Tokyo, Japan). The 4-minute running stages started from 10 km/h and increased by 1 km/h per stage until blood lactate concentration exceeded 4 mM (onset of blood lactate accumulation). After a 5-minute rest period after onset of blood lactate accumulation was obtained, they ran again for 3 minutes at onset of blood lactate accumulation speed without treadmill inclination. Thereafter, the angle of inclination was increased by 1% every minute until subjects reached volitional exhaustion. The criteria for exhaustion were as follows: (1) oxygen uptake reached plateau, (2) respiratory exchange ratio exceeded 1.2, and (3) heart rate was increased to 90% of the age-predicted maximum value (220 − participant's age).[12] When participants met at least two of the aforementioned criteria, the aerobic capacity test was terminated. Respiratory gas was measured through breath-by-breath method using an automatic gas analyzer (AE-300S; Minato Medical Science Co., Ltd., Osaka, Japan).

Experimental procedure

The overview of the experimental procedure is illustrated in **Figure 2**. The participants entered our laboratory after an overnight fast, other than the ingestion of tap water to eliminate the potential influence of diet on the physiological responses. Prior to the start of the exercise performance test (1st P-test) on the experimental day (Pre), blood and urine samples were collected. Then participants completed the 1st P-test consisting of countermovement jump, maximum voluntary isometric contraction (MVIC) of knee extensors, and 10-second and 30-second sprint cycling tests, as baseline values. Ten minutes after 1st P-test, subjects performed an oxidative stress-inducing exercise (OSE) protocol composed of 30-minute treadmill running (at 75% of the participant's predetermined maximal oxygen uptake) and squat jump (5 sets at 10 repetitions, with a 10-second rest interval between sets). The adopted OSE protocol was based on previous studies,[5,13,14] in which moderate running and eccentric jumping exercise increased oxidative damage. Over a 1-hour recovery period after OSE, participants sat on a comfortable chair and

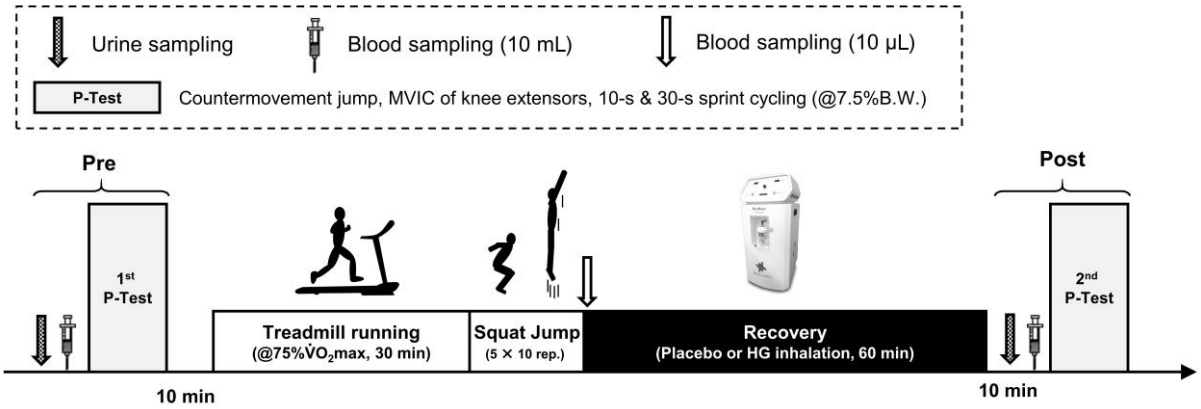

Figure 2: Overview of the experimental procedure.
Note: B.W.: Body weight; HG: molecular hydrogen-rich gas mixture; MVIC: maximal voluntary isometric contraction; Placebo: normal gas; P-test: exercise performance test; rep.: repetitions; VO_2max: maximal oxygen uptake.

continued to inhale Placebo or HG through a nasal cannula connected to gas generators. After the recovery period (Post), blood and urine samples were collected again, and participants subsequently (10 minutes after recovery period) underwent the 2nd P-test. The room temperature of the laboratory where all experiments were conducted was maintained at 24 ± 1°C throughout the present study.

Experimental gas

We prepared HG by using a hydrogen gas generator Hycellvator ET100 (Helix Japan, Co., Ltd., Tokyo, Japan). The product specification was previously described.[15] This apparatus can generate 30.0 mL/s gas mixture, consisting of 68.0% hydrogen (hydrogen purity, 99.99%) and 32.0% of oxygen analyzed by gas chromatography (Kureha Analyze Centre Co., Ltd., Fukushima, Japan; **Table 2**). All these gases were supplied through a nasal cannula connected to the gas generators. Although we could not measure directly the hydrogen and oxygen concentrations in the inspired gas due to the technical limitations, we could mathematically estimate it. Judging from the assumption that spontaneously breathing healthy young male typically shows an average inspiratory flow rate of 500 mL/s at rest, the hydrogen concentration in the inspired gas must have been around 4.08% at most. In other words, since the inspiratory flow rate far exceeded the flow rate of gas (30.0 mL/s) coming from the nasal cannula, ambient air entrained, which diluted the concentration of inspired hydrogen. Similarly, the oxygen from the nasal cannula was considered to be extremely minor, and inspired oxygen concentration must have reached no more than approximately 21.57% at most. We have also utilized another gas generator that has the same outer shape as the Hycellvator ET100 to produce Placebo (30.0 mL/s, ambient air 400 m above sea level) consisting of 0.00005% of hydrogen and 20.9% of oxygen.

Oxidative stress and muscle damage markers

We analyzed serum diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) as one of the blood oxidative stress markers.[6,9,10,16] The principle of this method was previously described.[10] We calculated the serum BAP to d-ROM ratio (BAP/d-ROMs) as the values of total antioxidant potential.[6,9,16] We also evaluated 8-hydroxydeoxyguanosine (8-OHdG), which is an oxidized DNA nucleotide and cellular oxidative stress biomarker generally excreted in the urine.[5,16] The urinary 8-OHdG excretion rate was measured by enzyme-linked immunosorbent assay and standardized by urine flow rate at a laboratory (Nikken Seil Co., Ltd., Shizuoka, Japan).

Serum creatine kinase and lactate dehydrogenase activities and number of white blood cells were assessed as muscle damage and damage-related inflammation markers[17-19] at a clinical laboratory (Kofu Medical Association, Medical Technology Center, Kofu, Japan). These markers were assayed by enzymatic method, Japan Society of Clinical Chemistry standardized method and flow cytometry respectively.

Table 2: Experimental gas composition

	Placebo	HG
Oxygen (%)	20.9	32.0
Hydrogen (%)	0.00005	68.0
Nitrogen + carbon dioxide (%)	78.03	0.0001

Note: HG: molecular hydrogen-rich gas mixture; Placebo: normal gas.

Exercise performances

To evaluate exercise performances, two-time P-tests were carried out, before OSE (1st P-test, Pre) and after a 1-hour recovery period after OSE (2nd P-test, Post).

The jumping ability was evaluated as the height of countermovement jump using a digital jump tester (T.K.K. 3406 Jump MD; Takei Scientific Instruments, Co., Ltd., Niigata, Japan).[20] We used the averaged data of the three repeated measurements. Quadriceps muscle strengths in both sides were assessed by 3-second MVIC of knee extensors using a handheld dynamometer (m-Tas F-1, Anima Co., Ltd., Tokyo, Japan).[21] Participants were instructed to hold a sitting position with 90-degree hip and knee joint angles and a force sensor was placed on the anterior surface of the lower leg 10 cm above the malleolus. The MVIC value was determined as the average of six measurements (both sides [left and right] × 3 repeated measurements).

The cycling performance was evaluated by 10-second and 30-second maximal pedaling using an electromagnetically

braked cycle ergometer (Powermax V, Combi, Tokyo, Japan).[22] The applied load was equivalent to 7.5% of the participants' body weight in both sprint cycling tests. Power output during maximal pedaling was recorded and its mean and peak values were evaluated.

Statistical analysis

All data were expressed as mean ± standard error. For all data, the change ratio after gas inhalation (Post) relative to the baseline (Pre) value was indicated as well as their absolute values. Two-way repeated measures analysis of variance was performed for the main effects (gas and time) and the interaction (gas × time). If the analysis of variance confirmed significant interaction of gas and time, paired *t*-test was performed to compare between the two trials within the same time point and between time points within the same trial. For comparison of the relative changes in each variable after gas inhalation (Post) from baseline (Pre) between the two conditions, paired *t*-test was utilized. We performed all statistical analysis using R ver. 3.5.3. A value of $P < 0.05$ was considered statistically significant.

Results

Physiological responses against OSE

Blood lactate concentrations after OSE were not significantly different between placebo and HG trials (placebo, 10.6 ± 1.2 mM; HG, 10.9 ± 0.8 mM; $t = 0.34$, $P = 0.74$). This result indicates that OSE stimulus itself might be equal between placebo and HG trials.

Reponses in oxidative stress and muscle damage markers of eight healthy males

In serum d-ROMs, BAP, and BAP/d-ROMs, no significant interaction and main effects of gas and time were observed, respectively (**Table 3**). In contrast, a significant main effect of time was detected in urinary 8-OHdG excretion rate, but no significant differences were observed between placebo and HG inhalation within the same time point (**Table 3**). The relative changes of these oxidative stress responses at Post from Pre (baseline) are shown in **Figure 3**. There were no significant differences in serum d-ROMs, BAP, and BAP/d-ROMs (**Figure 3A–C**), whereas the change ratio of the urinary 8-OHdG excretion rate was significantly lower in HG than placebo inhalation ($t = 2.45$, $P < 0.05$; **Figure 3D**).

All parameters indicating muscle damage and related inflammations (i.e., serum creatine kinase and lactate dehydrogenase activities and the number of white blood cells) were significantly increased at Post compared with Pre (all $P < 0.01$), whereas no significant differences with respect to these

Table 3: Oxidative stress and muscle damage responses of eight healthy males at Pre- and Post-exercise performance test

			Two-way analysis of variance		
	Pre-test	Post-test	Gas × Time	Gas	Time
Oxidative stress parameters					
Serum d-ROMs (Caratelli unit)			n.s.	n.s.	n.s.
Placebo	187.4±11.3	196.8±12.1			
HG	187.5±14.9	196.4±13.4			
Serum BAP (μM)			n.s.	n.s.	n.s.
Placebo	2177.8±173.1	2164.9±177.7			
HG	2123.8±105.1	2229.6±185.3			
Serum BAP/d-ROMs (A.U.)			n.s.	n.s.	n.s.
Placebo	12.0±1.2	11.4±1.3			
HG	12.1±1.2	12.3±1.8			
Urinary 8-hydroxydeoxyguanosine excretion rate (ng/kg per hour)			n.s.	n.s.	$P < 0.01$
Placebo	4.0±1.0	18.9±2.3			
HG	7.7±1.6	22.8±2.0			
Muscle damage parameters					
Serum creatine kinas activity (U/L)			n.s.	n.s.	$P < 0.01$
Placebo	125.1±4.8	196.3±34.9			
HG	117.0±5.2	216.0±18.0			
Serum lactate dehydrogenase activity (U/L)			n.s.	n.s.	$P < 0.01$
Placebo	186.9±10.7	215.1±11.1			
HG	183.4±8.8	221.0±11.9			
Number of white blood cells (cells/mL)			n.s.	n.s.	$P < 0.01$
Placebo	6002.5±697.5	8737.5±1172.1			
HG	5665.0±525.5	7536.3±643.2			

Note: Values are mean ± standard error, and were analyzed by two-way repeated measures analysis of variance. A.U.: Arbitrary unit; BAP: biological antioxidant potential; BAP/d-ROMs: systemic total antioxidant capacity; d-ROMs: diacron reactive oxygen metabolites; HG: molecular hydrogen-rich gas mixture; n.s.: not significant; Placebo: normal gas; Post: before 2nd P-test; Pre: before 1st exercise performance test (P-test).

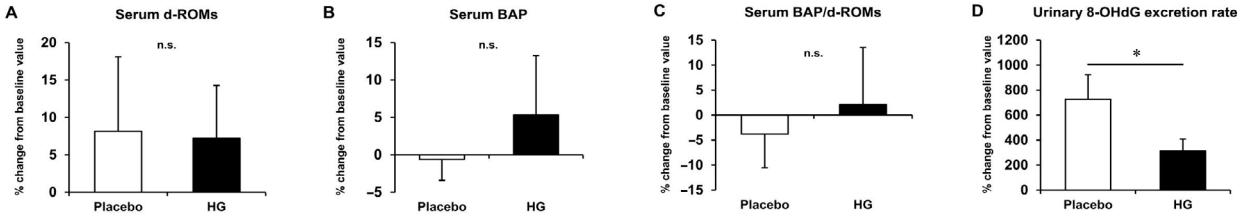

Figure 3: The relative changes in oxidative stress biomarkers from baseline values of 8 healthy males.
Note: (A–D) Serum d-ROMs, serum BAP, serum BAP/d-ROMs, and urinary 8-OHdG excretion rate. Values are expressed as mean ± standard error. *$P < 0.05$ (paired *t*-test). 8-OHdG: 8-Hydroxydeoxyguanosine; BAP: biological antioxidant potential; d-ROMs: diacron-reactive oxygen metabolites; HG: molecular hydrogen-rich gas mixture; n.s.: not significant; Placebo: normal gas.

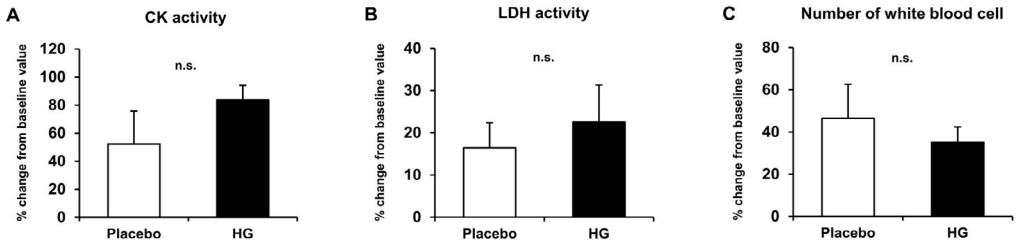

Figure 4: The relative changes in blood muscle damage biomarkers from baseline values of eight healthy males.
Note: (A–C) Serum CK activity, serum LDH activity, and number of white blood cell. Values are expressed as mean ± standard error, and were analyzed by paired *t*-test. CK: Creatine kinase; HG: molecular hydrogen-rich gas mixture; LDH: lactate dehydrogenase; n.s.: not significant; Placebo: normal gas.

variables between Placebo and HG inhalation were observed. Moreover, relative changes in these muscle damage markers did not differ between the two conditions (**Figure 4**).

Exercise performances of eight healthy males

The absolute values of exercise performances (i.e., height of countermovement jump, MVIC of knee extensors, and mean and peak power output during 10-second and 30-second sprint cycling) at Pre (1st P-test) and Post (2nd P-test) are shown in **Table 4**. A significant interaction of gas and time was detected with regard to the height of countermovement jump ($F_{1,7} = 5.89$, $P < 0.05$, $\eta^2_p = 0.46$). Compared to Pre test, the height of countermovement jump at Post test was significantly decreased in placebo trial ($P < 0.05$), but not in HG. Peak power output in 30-second sprint cycling was significantly decreased at Post test irrespective of the kind of inhaled gas, while the other cycling performances and MVIC of knee extensors showed no significant changes.

Figure 5 shows the relative changes in the exercise performances from Pre to Post values. No significant differences in MVIC of knee extensors and all cycling performances were observed between two trials (**Figure 5B–F**), whereas the change ratio of the height of the countermovement jump was significantly higher in HG than placebo ($t = 2.45$, $P < 0.05$, **Figure 5A**).

Additionally, we identified a significant correlation coefficient between the change ratio in urinary 8-OHdG excretion rate and that in height of countermovement jump, when data (2 conditions × 8 participants) were pooled ($n = 16$, $r = –0.78$, $P < 0.01$; **Figure 6**).

Discussion

To the best of the author's knowledge, this is the first study to examine the effects of HG inhalation during the recovery period after OSE on oxidative stress, muscle damage, and exercise performances. A main finding of this study was that HG inhalation after intense exercise attenuated the increase in urinary 8-OHdG excretion rate and the reduction in the height of countermovement jump. Moreover, we also found that the increase in urinary 8-OHdG excretion rate was associated with the reduction in countermovement jump performance.

Urinary 8-OHdG excretion rate was increased by OSE irrespective of the kind of inhaled gas during the recovery phase. A previous research reported that an acute exhaustive exercise increased urinary 8-OHdG excretion rate 1 hour after exercise compared with baseline values by threefold.[16] In this study, urinary 8-OHdG excretion rate approximately 1 hour after exercise was sevenfold greater than preexercise values in the placebo trial. That is, our OSE protocol must have been intense enough to increase oxidative damage. Contrary to urinary 8-OHdG excretion rate, serum d-ROMs, BAP, and BAP/d-ROMs did not differ between Pre and Post. Since previous studies demonstrated that molecular hydrogen significantly changed these variables,[6,9,10] we also measured those in this study. Sugita et al.[16] demonstrated that exercise-induced changes in the levels of d-ROMs and BAP returned to baseline values within 1 hour after exercise. In contrast, our recent results suggested that three consecutive days of intense exercise decreased BAP/d-ROMs even 16 hours after exercise.[6] Together, the time course changes in serum d-ROMs and BAP after exercise remain to be elucidated; therefore, future studies should examine them in more detail.

In our previous study, hydrogen saturated alkaline electrolyzed water attenuated a single bout of severe exercise-induced elevation in urinary 8-OHdG accumulation.[5] Similarly, a recent animal study demonstrated that acute strenuous exercise under exposure to hydrogen-rich gas reduced postexercise oxidative stress.[8] In accordance with these previous results, HG inhalation inhibited the increase of urinary 8-OHdG excretion rate compared with placebo. Moreover, although HG inhalation

Table 4: Pre- and Post-exercise performances of eight healthy males

			Two-way analysis of variance		
	Pre	Post	Gas × Time	Gas	Time
Height of vertical jump (cm)			$P < 0.05$	–	–
Placebo	50.7±1.4	47.5±1.7*			
HG	49.9±1.5	49.1±1.7			
Maximal voluntary isometric contraction for knee extensors (N)			n.s.	n.s.	n.s.
Placebo	268.3±22.6	249.4±13.4			
HG	273.6±19.0	256.1±14.5			
10-s sprint cycling					
Mean power output (W)			n.s.	n.s.	n.s.
Placebo	673.4±28.4	663.1±36.9			
HG	672.0±29.0	678.8±25.5			
Peak power output (W)			n.s.	n.s.	n.s.
Placebo	732.6±29.7	719.7±36.9			
HG	727.7±30.0	736.9±27.2			
30-s sprint cycling					
Mean power output (W)			n.s.	n.s.	n.s.
Placebo	574.1±21.5	576.1±28.3			
HG	581.1±27.1	573.4±26.9			
Peak power output (W)			n.s.	n.s.	$P < 0.01$
Placebo	712.2±30.7	690.8±30.2			
HG	707.4±27.4	693.3±26.7			

Note: Values are mean ± standard error, and were analyzed by two-way repeated measures analysis of variance. *$P < 0.05$, *vs.* Pre. HG: Molecular hydrogen-rich gas mixture; n.s.: not significant; Placebo: normal gas; Pre: before 1st exercise performance test (P-test); Post: before 2nd P-test.

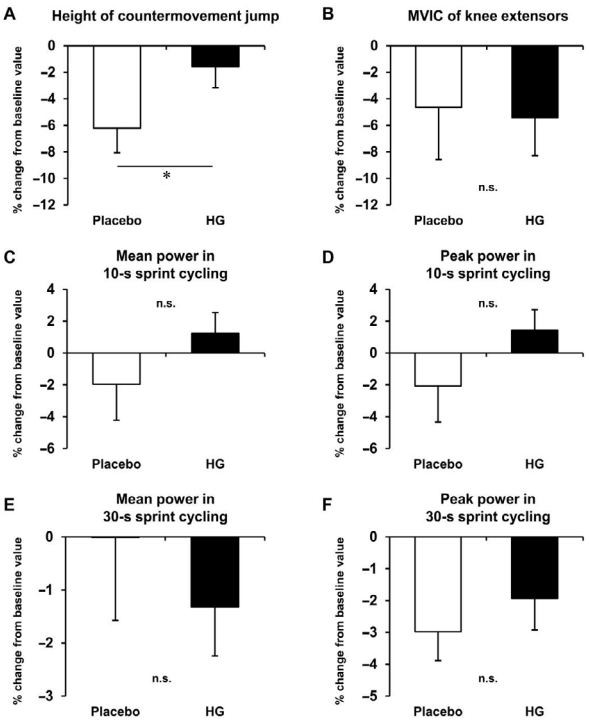

Figure 5: The relative changes in exercise performances from baseline values of eight healthy males.

Note: (A) Height of countermovement jump; (B) MVIC of knee extensors; (C, D) mean and peak power output in 10-second sprint cycling; (E, F) mean and peak power output in 30-second sprint cycling. Values are expressed as mean ± standard error. *$P < 0.05$ (paired *t*-test). HG: Molecular hydrogen-rich gas mixture; MVIC: maximum voluntary isometric contraction; n.s.: not significant; Placebo: normal gas.

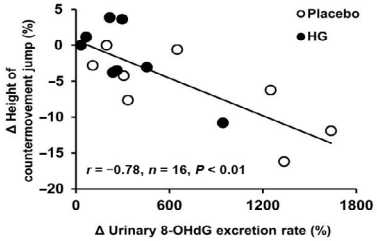

Figure 6: The relationship between change ratio in urinary 8-OHdG excretion rate and change ratio in height of countermovement jump from baseline values.

Note: Plotted data represent all subjects (n = 8) in two trials (placebo and HG inhalation). 8-OHdG: 8-Hydroxydeoxyguanosine; HG: molecular hydrogen-rich gas mixture; n.s.: not significant; Placebo: normal gas.

adopted in the present study might have slightly increased fraction of oxygen in inspired gas, no evidences reported that normobaric hyperoxic gas inhalation changed oxidative damages.[23-25] These findings suggested that the attenuation of urinary 8-OHdG increase by postexercise HG inhalation might be mainly due to the antioxidant potential of molecular hydrogen.

Surprisingly, reductions in countermovement jump performance by the OSE protocol were diminished after HG inhalation, which were significantly associated with the increase in urinary 8-OHdG excretion rate. Previous reviews demonstrated that excessive ROS generation inhibited muscle contraction, thereby impairing exercise performance.[26,27] Muscle damage and its related inflammatory responses also induce muscle fatigue,[28] while the changes in these markers (i.e., creatine kinase, lactate dehydrogenase, and number of white blood cells)

were not different between Placebo and HG inhalation. These observations support the possibility that the improvement in countermovement jump performance after HG inhalation might be due to the reduction in oxidative damage.

However, a question remains on why HG inhalation attenuated fatigue just in the countermovement jump, but not MVIC of knee extensors and sprint cycling performances. Although we can only speculate, one possible explanation might be involved in the exercise mode of P-test. One previous study reported that excessive eccentric training increased various oxidative damages and decreased muscle strength, countermovement jump performance, and mean power in 10-second sprint cycling.[29] The authors also suggested that the reduction in countermovement jump performance was highly relevant to the changes in various oxidative stress biomarkers compared with muscle strength and cycling performance.[29] Moreover, the mode of countermovement jump was almost similar to the repetitive squat jump in OSE, so that countermovement jump performance was likely to be influenced by changes in oxidative damage compared with other performance indexes in P-test. Indeed, we examined the correlation analysis between cycling performance (i.e., mean and peak power in 10-second sprint cycling) and urinary 8-OHdG, and we found that the relationships between these exercise performances and the oxidative damage were marginally significant, respectively (mean power: $r = –0.44$, $P = 0.09$; peak power: $r = –0.49$, $P = 0.06$). Collectively, the attenuation in 8-OHdG formations might be linked to the improvement in countermovement jump performance.

It is also suggested that hyperoxic gas inhalation during the post-exercise recovery period enhanced arterial oxygen saturation, which attenuated muscle fatigue via activating muscle cell activity and removing metabolic waste accumulation.[30-32] However, theoretically, HG inhalation through a nasal cannula has been considered to have minor effects on the increased oxygen concentration in inspired gas as described in the "Participants and Methods" section. Therefore, the hypothesis that inhaled hyperoxic gas strongly contributes to the improvement of exercise performance in this study must be denied.

The present study has several limitations. First, the hydrogen gas generator utilized in this study (Hycellvator ET100) leads to a secondary, but inevitable production of oxygen. Even if the level of increase in oxygen concentration in inspired gas was minor, we could not completely distinguish the single and synergistic effect of molecular hydrogen and oxygen on the results. Second, we assessed the oxidative stress responses and exercise performances just once only after 60-minute HG inhalation. This sampling time referred preceding researches that utilize a 1-hour hyperbaric oxygen inhalation to investigate its physiological or ergogenic influence.[30,33,34] However, a previous animal study demonstrated that an exposure to HG during intense exercise significantly reduced inflammation or subsequent oxidative damages at a plurality of points of time during recovery.[8] Accordingly, the exercise performances and physiological responses to HG inhalation should be assessed at more time points in the future study. Finally, we recruited the generally small sample size ($n = 8$) of males. Although our results clearly demonstrated significant differences and large effect size in changes of the urinary 8-OHdG excretion rate and countermovement jump performance, future studies are required with a large population, including a consideration of the difference in gender, age, training status and so on.

In conclusion, HG inhalation during post-exercise recovery period attenuated the reduction in subsequent exercise performance via reducing oxidative damage. HG inhalation might be useful as one of the rapid recovery strategies to maintain exercise performance in sports in which repetitive competitions are conducted within the same day, such as judo, track and field athletes, and so on. Furthermore, this type of recovery might be applied not only to athletes but also to various people who exercise for health promotion, in view of protecting their physical condition from exercise-induced excessive oxidative damages.

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Author contributions
TA, TF, and KK organized the study. YS, SD, and KK designed the experiments. TA and TF prepared the specific gas generator. YS performed the data collection. YS, SD, and KK interpreted and analyzed the data. SD prepared all figures and tables. YS, SD, and KK drafted the manuscript. All authors approved the final version of the manuscripts.

Conflicts of interest
Helix Japan, Co., Ltd. (Tokyo, Japan) provided funding for the study and provided the Hycellvator ET100 hydrogen gas generator.

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This study was approved by the Human Research Ethics Committee of the University of Yamanashi (approval No. H29-006) on June 28, 2017.

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 will not be published.

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

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